

The use of complementary and integrative medicines and exploring natural health product-drug interactions *in vitro* in the management of pediatric attention-deficit hyperactivity disorder

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ABSTRACT

This thesis applied a novel interdisciplinary approach for pharmacovigilance to examine the use of complementary and integrative medicine (CIM), focusing on herbal remedies, to manage pediatric attention-deficit hyperactivity disorder (ADHD). The safety and potential risk of herb-drug interactions in ADHD management were first evaluated through an assessment of available information on the safety and efficacy of natural health products (NHPs) commonly used by ADHD patients as a means of identifying knowledge gaps. A clinical questionnaire was administered to caregivers of pediatric patients with ADHD to determine the factors and related outcomes of CIM use, including adverse events. A systematic search was conducted to further identify clinical adverse events involving herbal remedies and ADHD drugs to determine causal links to herb-drug interactions. *In vitro* analysis of identified herbal remedies was conducted to determine their potential for pharmacokinetic interactions, specifically on carboxylesterase-1 (CES1) mediated metabolism.

The presented research builds on otherwise scarce evidence of the safety of herbal remedies for ADHD, particularly with respect to herb-drug interactions and adverse events (AEs) associated with concurrent use of NHPs and ADHD prescription drugs. Beyond studies conducted on the pharmacokinetic safety of herbal remedies through the cytochrome P450 pathways that metabolize some ADHD drugs, including amphetamine, atomoxetine and guanfacine, few data were available for CES1, which metabolizes methylphenidate, the first line of drug used to manage ADHD. The clinical questionnaire revealed that 40% of patients had used CIM and confirmed the use of a variety of CIM. Moreover, the majority of CIM users were also concurrently taking ADHD medication, and eight mild adverse events were self-reported. The systematic search on the adverse event reporting system highlighted a potential NHP-drug

interaction between methylphenidate and St. John's wort, and the overall poor quality of NHP-related adverse event reports. As a follow-up from the adverse event results, various commercial St. John's wort products showed variable inhibition of recombinant human CES1 *in vitro*. Although the concentration of marker phytochemicals was not correlated to inhibition, hyperforin showed stronger activity than hypericin and quercetin. The preliminary *in vitro* investigation revealed that the herbal remedies used by ADHD patients have the potential to interact with CES1 mediated metabolism, with *Rhodiola rosea* identified as the most potent inhibitor. Further investigation on various commercial products of *Rhodiola rosea* revealed both reversible and irreversible inhibition of recombinant CES1. However, the inhibition was not dependent on the concentration of marker phytochemicals, and rosarin, rosavin, rosin, and salidroside were not potent inhibitors of recombinant CES1. Moreover, a commercial *Rhodiola rosea* extract showed concentration-dependent inhibition of human liver microsome mediated metabolism of methylphenidate.

Overall, results from this thesis suggest potential risk from use of NHPs concurrently with conventional medicine used to manage ADHD. Improved evidence and pharmacovigilance for the use of NHPs in a pediatric population is warranted.

RÉSUMÉ

Cette thèse a appliqué une nouvelle approche interdisciplinaire pour la pharmacovigilance afin d'examiner l'utilisation de la médecine complémentaire et intégrative (CIM), en se concentrant sur les remèdes à base de plantes, pour gérer le trouble d'hyperactivité avec déficit de l'attention (TDAH) pédiatrique. Pour identifier les lacunes dans les connaissances, la sécurité de CIM et le risque potentiel d'interactions herbe-médicament dans la prise en charge du TDAH ont été d'abord évalués par une enquête sur les informations disponibles à propos de la sécurité et l'efficacité des produits de santé naturels (PSN) couramment utilisés par les patients atteints de TDAH. Un questionnaire clinique a été administré aux soignants de patients pédiatriques ayant un TDAH afin de déterminer les facteurs et les résultats associés à l'utilisation des CIM, y compris les événements indésirables (EIs). Par la suite, une recherche systématique des rapports d'EI disponible au public a identifié davantage les EIs cliniques impliquant des remèdes à base de plantes et des médicaments pour le TDAH. Un ensemble d'EI cliniquement pertinentes ont été étudiées pour déterminer les liens de causalité avec les interactions médicament-herbe. Les expériences *in vitro* des remèdes à base de plantes ont été menées pour déterminer leur potentiel d'interactions pharmacocinétiques des enzymes métabolisent les médicaments de TDAH, en particulier sur l'activité de la carboxylestérase-1 (CES1).

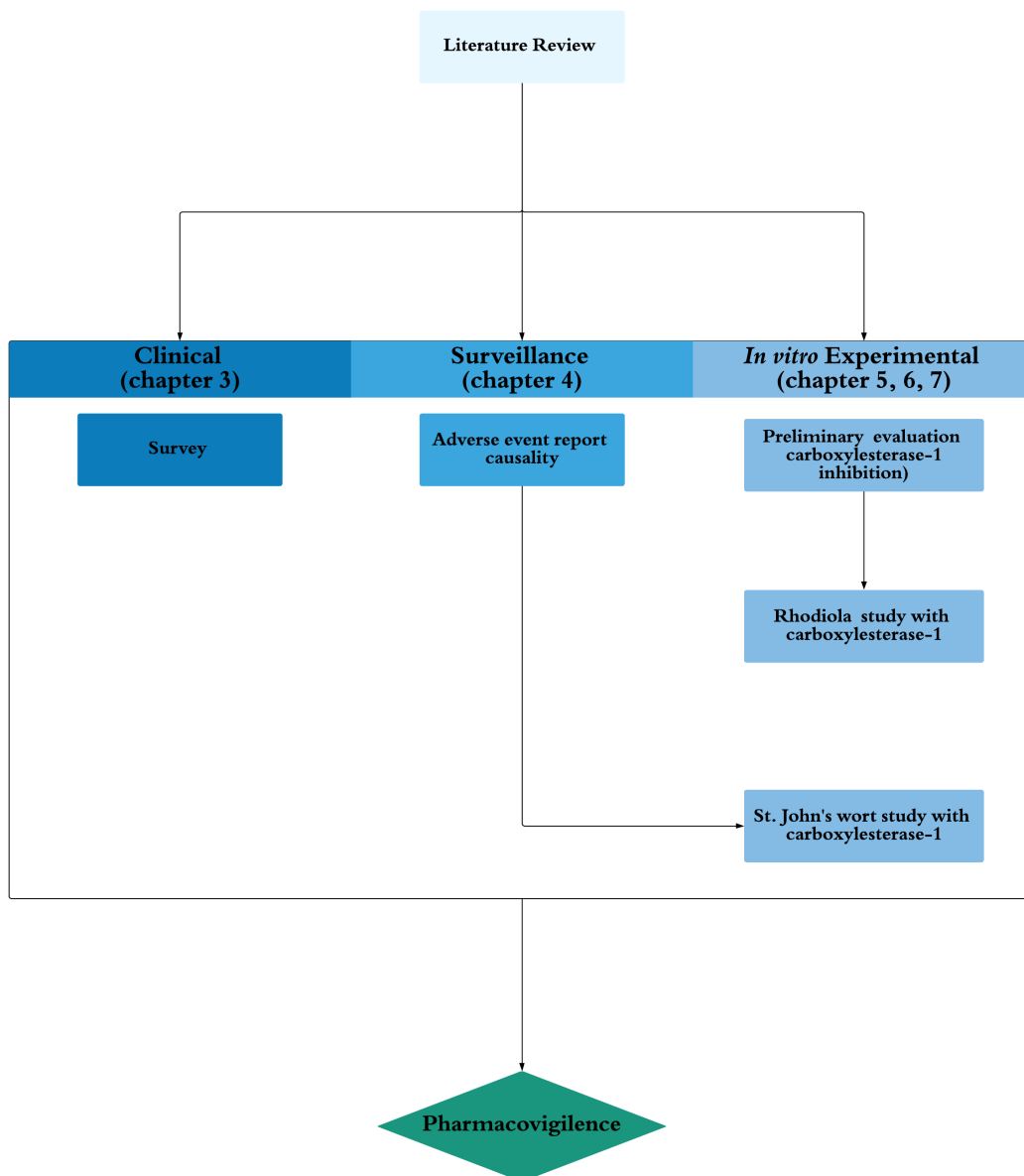
La recherche présentée s'appuie sur des preuves autrement rares de l'innocuité des remèdes à base de plantes pour le TDAH, en particulier en ce qui concerne les interactions médicament-herbe et les EIs associés à l'utilisation simultanée de PSN et de médicaments sur ordonnance pour le TDAH. Hormis les études sur la sécurité pharmacocinétique des plantes médicinales par les voies du cytochrome P450 qui métabolisent certains médicaments pour le TDAH, dont l'amphétamine, l'atomoxétine et la guanfacine, peu de données étaient disponibles

pour CES1, qui métabolise le méthylphénidate, la première ligne de médicaments utilisée pour gérer le TDAH. Le questionnaire clinique a révélé que 40% des patients avaient utilisé CIM et a confirmé l'utilisation d'une variété de CIM. De plus, la majorité des utilisateurs de CIM prenaient simultanément des médicaments pour le TDAH, et huit EIs bénins ont été auto-déclarés. La recherche systématique sur le système de déclaration des EIs a mis en évidence une interaction potentielle PSN-médicament entre le méthylphénidate et le millepertuis, et a souligné la mauvaise qualité globale des déclarations d'EIs liés aux PSN. À la suite de ces résultats, divers produits commerciaux à base de millepertuis ont montré une inhibition variable du CES1 humain recombinant *in vitro*. Les concentrations de composés phytochimiques marqueurs n'étaient pas corrélées à l'inhibition mais l'hyperforine a montré une activité plus forte que l'hypericine et la quercétine.

En regardant au-delà du millepertuis, l'enquête préliminaire *in vitro* a révélé que les remèdes à base de plantes utilisés par les patients atteints de TDAH ont le potentiel d'interagir avec le métabolisme médié par CES1. Avec l'activité inhibitrice la plus puissante, *Rhodiola rosea* a fait l'objet d'une enquête plus approfondie où les tests de divers produits commerciaux ont révélé une inhibition réversible ou irréversible du CES1 recombinant, selon le produit. Cependant, l'inhibition n'était pas dépendante de la concentration de composés phytochimiques marqueurs, et la rosarine, la rosavine, la colophane et le salidroside n'étaient pas des inhibiteurs puissants du CES1 recombinant. De plus, un extrait commercial de *Rhodiola rosea* a inhibé le métabolisme du méthylphénidate par les microsomes hépatiques humains d'une manière dépendante de la concentration.

Collectivement, les résultats de cette thèse suggèrent un risque potentiel lié à l'utilisation des PSN en même temps que la médecine conventionnelle utilisée pour gérer le TDAH.

L'amélioration des preuves et de la pharmacovigilance pour l'utilisation des PSN dans une population pédiatrique est justifiée.



Graphical schematic of thesis.

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LIST OF ABBREVIATIONS

4-NA	4-Nitrophenyl acetate
ACE inhibitors	Angiotensin-converting enzyme inhibitors
AChE	Acetylcholinesterase
ADHD	Attention-deficit hyperactivity disorder
AE	Adverse event
AER	Adverse event report
ANOVA	Analysis of variance
CAM	Complementary and alternative medicine
CES1	Carboxylesterase-1
CHEO	Children's Hospital of Eastern Ontario
CIM	Complementary and integrative medicine
CYP P450	Cytochrome P450
DIN	Drug identification number
DIN-HM	Homeopathic medicine number
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration
HM	Herbal medicine
HORN-DIPS	Horn Drug Interaction Probability Scale
HPLC-DAD	High Performance Liquid Chromatography with Diode Array Detection
IC ₅₀	Half maximal inhibitory concentration
K _i	Inhibitory constant

MPH	Methylphenidate
NADPH	Nicotinamide adenine dinucleotide phosphate
NHP	Natural health product
NPN	Natural Product Number
OROS	Osmotic-controlled release oral delivery system
P-gP	P-glycoprotein
SJW	St. John's wort
WHO	World Health Organization
WHO-UMC	World Health Organization Uppsala Monitoring Centre

Chapter 1
General Introduction

1.1 INTRODUCTION

Complementary and integrative medicine (CIM), an important and often underestimated part of healthcare, has an extensive history of use in health maintenance and in disease prevention and treatment, especially for chronic conditions (Qi 2013). CIM (also known as complementary and alternative medicine, CAM) encompasses a group of healthcare and medical systems, practices, and products that are not typically taught in conventional medicine training and includes natural health products (NHPs), mind-body practices, and other complementary health approaches (**Figure 1-1**) (National Center for Complementary and Integrative Health 2018). The out-of-pocket spending on CIM is significant, with Canadians spending \$8.8 billion on CIM in 2016 (Esmail 2017).

NHPs represent the most widely used form of CIM and include vitamins and minerals, herbal remedies (herbal medicine), homeopathic remedies, traditional medicine, probiotics, and other products such as amino acids and essential fatty acids (Health Canada 2015). NHPs are often perceived as “natural”, and therefore “safe”. However, NHPs, specifically herbal remedies, often contain a complex mixture of biologically active/or inactive substances, and can have inherent pharmacological activity (Kaufman et al. 2002). Herbal remedies may contribute to controlling symptoms for some patients. However, they are often used concurrently with conventional drugs, and may cause toxicity, or affect the safety and efficacy of other pharmacotherapy, causing herb-drug interactions leading to adverse events (Venkataramanan et al. 2006). Similar to drug-drug interactions, the interactions between NHPs and drugs (NHP-drug, herb-drug interactions) can result in pharmacokinetic and pharmacodynamic changes (**Figure 1-2**).

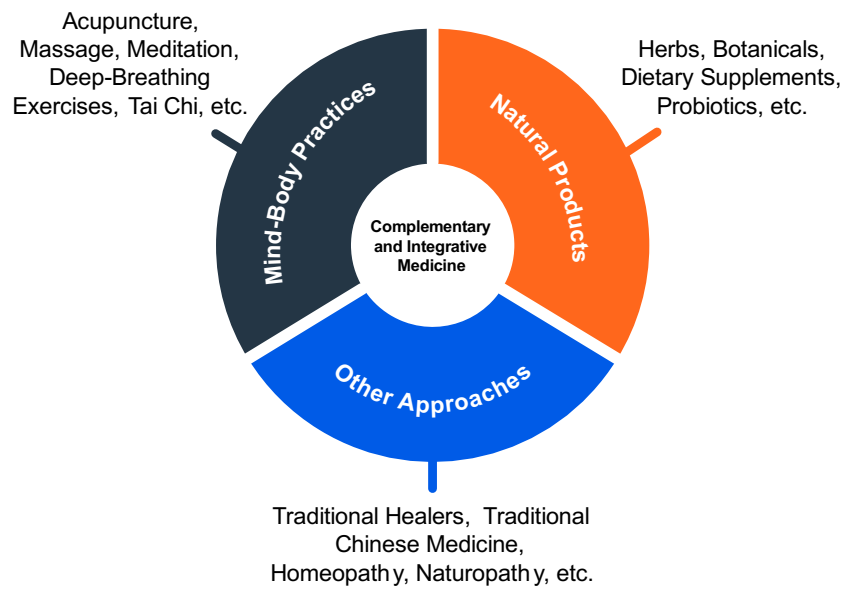


Figure 1-1 Modalities of complementary and integrative medicine, and common examples of each modality. Figure adapted from National Center for Complementary and Integrative Health (2016).

Pharmacodynamic interactions produce additive, synergistic, or antagonistic activity with conventional drugs with no change in plasma concentration of herbal remedy or drug. For example the herb kava (a central nervous system depressant), when taken with another central nervous system depressant alprazolam, can result in a semi-comatose state (Almeida et al. 1996).

Clinically significant interactions are most often due to interferences with pharmacokinetics (Huang et al. 2012). Once herbal remedies are ingested, various constituents of them are absorbed, distributed, metabolized, and eliminated by the body, often inhibiting or inducing metabolic enzymes or transporters (Venkataramanan et al. 2006; Noras et al. 2013). Drug metabolism can be divided into phase 1 and phase 2 metabolism. Phase 1 metabolism reactions include oxidation, reduction, hydrolysis, and hydration reactions resulting in a metabolite with increased polarity compared to the parent compound (e.g., CYP 450 isoenzymes, carboxylesterases, and alcohol dehydrogenases). Phase 2 metabolism consists of conjugation reactions (e.g., glutathione S-transferase, N-acetyltransferases, N-acyltransferases) (Gonzalez et al. 2006). Pharmacokinetic herb-drug interaction cause changes in the absorption, distribution, metabolism, or excretion, resulting in altered plasma levels of drugs or metabolites (Venkataramanan et al. 2006). For example, furanocoumarin components of grapefruit juice such as bergamottin and dihydroxybergamottin are potent irreversible inhibitors of CYP 3A4. Hence concurrent intake of grapefruit juice with various CYP 3A4 substrate drugs can result in an increase in the plasma concentration of the drug, leading to toxicity (Glaeser et al. 2007; Paine et al. 2005). The flavonoid components of grapefruit juice, kaempferol and naringenin, can also interact with esterase mediated metabolism of prodrugs lovastatin and enalapril, potentially affecting their efficacy (Li et al. 2007). Drug interactions from time-dependent inhibition (irreversible inhibition) pose a greater concern of risk— as the enzyme is deactivated, it must to

be synthesized *de novo* (Grimm et al. 2009), and resulting adverse events can have a delayed onset and are longer-lasting (Silverman 1995). Therefore, it is important to assess the safety of products, including the pharmacology of each ingredient and the product as a whole.

This thesis focuses on exploring the use and safety of CIM, specifically herbal remedies, in the context of attention-deficit hyperactivity disorder (ADHD).

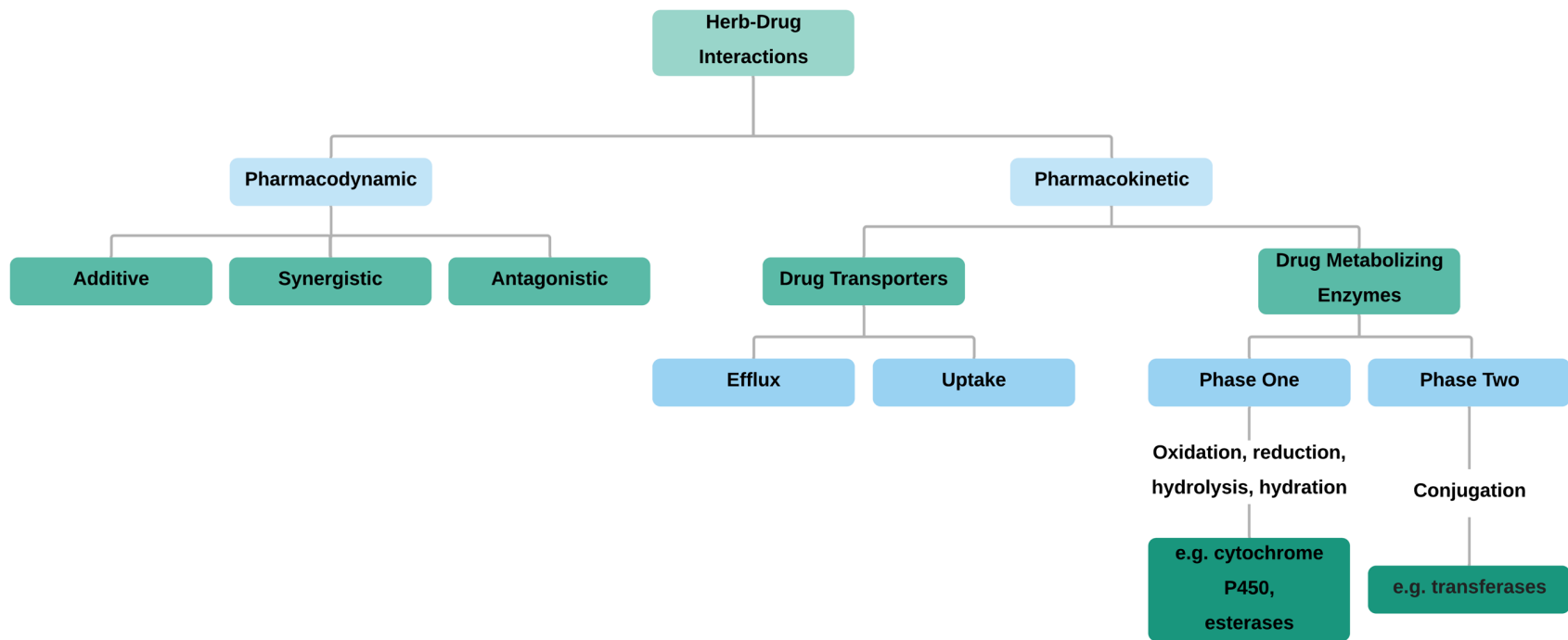


Figure 1-2 Illustration of major mechanisms of herb-drug interactions. Figure adapted from Oga et al. (2016).

ATTENTION-DEFICIT HYPERACTIVITY DISORDER

ADHD is a clinically heterogeneous neurodevelopmental disorder affecting 5% of children and adolescents globally (Polanczyk et al. 2007). ADHD is characterized as a chronic disorder, with only 15% of children exhibiting complete remission, both in terms of symptoms, and functional impairment in young adulthood (Faraone et al. 2006). The key characteristics of ADHD include a persistent pattern of inattention (e.g., difficulties sustaining focus, and wandering off task), and/or hyperactivity-impulsivity (e.g., excessive motor activity, fidgeting, talkativeness, and interrupting others) that interfere with functioning and/or development. Symptoms of ADHD affect multiple domains including social and family, school, and occupational settings (American Psychiatric Association 2013). Subtypes of ADHD include predominantly hyperactive-impulsive, predominantly inattentive, and combined presentation. In total, 50% of all referrals of children to mental health clinics are related to ADHD (Waschbusch et al. 2002).

Etiology and Neurobiology

To date, a clear etiology for ADHD has not been defined. ADHD is strongly heritable (Stawicki et al. 2006), however, environmental factors play a role, including diet (Howard et al. 2011), additives in food (Stevenson et al. 2010), chemical contamination from cigarettes (Nomura et al. 2010), prenatal alcohol exposure (O'Callaghan et al. 2007), neurotoxin exposure (Nicolescu et al. 2010), and low birth weight (Mick et al. 2002).

Although the neurobiology of ADHD has not been thoroughly understood, research suggests that imbalances in the dopaminergic and noradrenergic pathways are involved in the core symptoms characterizing ADHD (Pliszka 1998; Zametkin et al. 1987). The fronto-

subcortical region of the brain, rich in catecholamines, is affected and involved in the mechanism of action of stimulant drugs used to treat ADHD (Alexander et al. 1986; Biederman 2005).

Treatments

In general, a multi-modal treatment is recommended including behavior and school-based interventions, social skills training, and psychotherapy, in combination with pharmacotherapy. Moreover, use of polypharmacy is common in ADHD patients (Greenhill et al. 2004; Pliszka et al. 2006; Pliszka et al. 2007).

Pharmacotherapy

Changes in the dopaminergic and noradrenergic systems are associated with the clinical efficacy of pharmacotherapy for ADHD. Psychostimulants, such as methylphenidate (Ritalin ®, Concerta ®) and mixed salts of amphetamines (Adderall ®), are the first-line of pharmacotherapy prescribed to patients with ADHD as both have similar structures to endogenous catecholamines (**Figure 1-3**), and effectively reduce the severity of core ADHD symptoms (Atkinson et al. 2010; Canadian Attention Deficit Hyperactivity Disorder Resource Alliance 2011).

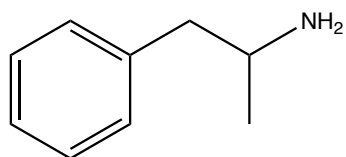
Methylphenidate (MPH) is a central nervous system stimulant, first synthesized in 1944 (Panizzon 1944), and used clinically since 1955 for various ailments. It is the most commonly used pharmacotherapy to treat ADHD worldwide (John S Markowitz et al. 2003). MPH inhibits dopaminergic and noradrenaline reuptake transporters, thereby, increasing the level of dopamine and noradrenaline in the synaptic cleft (Kuczenski et al. 1975; Arnsten et al. 2011). The major metabolic pathway of MPH is hepatic de-esterification via CES1 to form the pharmacologically inactive metabolite, ritalinic acid (Patrick et al. 1987; Sun et al. 2004).

Amphetamine (dextroamphetamine, and lisdexamfetamine salts) was the first psychostimulant used to treat children with behavioral disorders (Bradley 1937). Although more potent than methylphenidate at symptom alleviation, amphetamines are used less often due to concerns about abuse potential, and are not available in many countries for prescription (Faraone 2019a). In addition to blocking dopamine and noradrenaline reuptake transporters, amphetamine stimulates the direct release of dopamine through reverse transport, and increases the levels of dopamine and noradrenaline in the synaptic cleft (Kuczenski et al. 1975; Arnsten et al. 2011; Fleckenstein et al. 2007; Robertson et al. 2009; Heal et al. 2013). While the enzymes that metabolize amphetamine are not clearly elucidated, CYP 2D6 is involved in the formation of 4-hydroxy-amphetamine, an inactive metabolite (Bach et al. 1999).

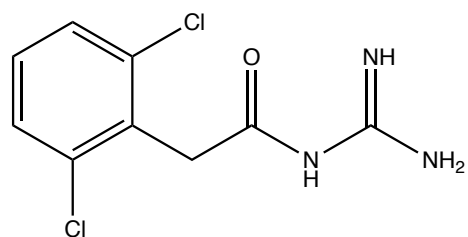
Between 10-30% of children do not respond to psychostimulants, and non-stimulants may be prescribed. Atomoxetine was the first approved specifically for use in ADHD as a non-stimulant in 2002. Atomoxetine is a noradrenaline reuptake inhibitor and blocks noradrenaline transporters, and increases synaptic noradrenaline (Christman et al. 2004; Swanson et al. 2006). Atomoxetine is primarily metabolized by CYP 2D6 to its active metabolite, 4-hydroxyatomoxetine, which is subsequently glucuronidated to 4-hydroxyatomoxetine-*O*-glucuronide (Chalon et al. 2003). Guanfacine is an α_2 -agonist, initially marketed as an anti-hypertensive, but often used as an off-label therapy to treat ADHD symptoms since 2009 (Jann et al. 2016). Guanfacine is mainly metabolized by CYP 3A4 to 3-hydroxyguanfacine (Wayne n.d.; Cruz 2010).

The efficacy of psychostimulants and non-stimulants has been well-established through various clinical trials (Storebø et al. 2015; Cortese et al. 2018; Padilha et al. 2018; Chan et al. 2016). However, parents show pervasive concern for administering pharmacotherapy for ADHD,

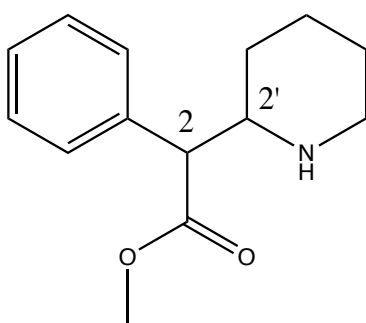
in part due to short- and long-term adverse effects from the use (Hansen et al. 2006; Adler et al. 2010; Johnston et al. 2008). Internationally from 1968-2010, adverse reactions reported from the use of ADHD medicines accounted for 14% of all reports from children between 2 to 11 years, and were also common for adolescents aged 12 to 17 years (Star et al. 2011). Common side-effects of psychostimulants include trouble sleeping, poor appetite, nervousness, agitation, anxiety, nausea, vision problems, and hypertension (Ahmann et al. 1993; Efron et al. 1997). Less common side-effects from psychostimulant use include motor and vocal tics, aggressiveness, and psychosis (Faraone 2019a). The side-effects of non-stimulants include headache, weight loss, somnolence, sedation, dizziness, and nausea (Biederman et al. 2008; Kratochvil et al. 2011).



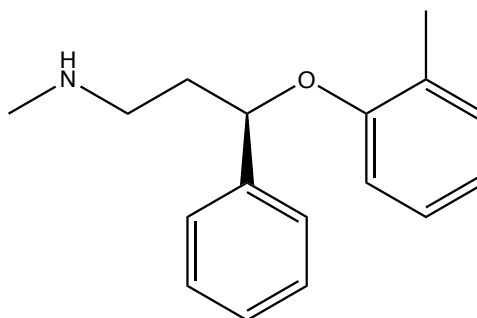
Amphetamine



Guanfacine



Methylphenidate



Atomoxetine

Figure 1-3 Structures of psychostimulants (methylphenidate and amphetamine), and non-stimulants (atomoxetine and guanfacine) commonly prescribed for attention-deficit hyperactivity disorder management. Methylphenidate has four stereoisomers: the *d-threo* ($2R$, $2'R$) and *l-threo* ($2S$, $2'S$) isomers, which are useful for the treatment of ADHD; and the *d-erythro* ($2R$, $2'S$) and *l-erythro* ($2S$, $2'R$) isomers.

Potential of risk of herb-drug interactions amongst ADHD patients

The chronic nature and associated comorbidities with ADHD, and the adverse event profile of conventional pharmacotherapy, lead patients and families to seek CIM therapies for managing ADHD (Stubberfield et al. 1999; Sinha et al. 2005). Based on the results from a pediatric neurology clinic which reported almost half of the patients who used CIM did so while using a prescription medicine, it is likely that pediatric patients with ADHD do so as well (Galicia-Connolly et al. 2014). Although using herbal remedies can be efficacious, the risk of interactions leading to adverse events are more likely in the case of polypharmacy (Wimmer et al. 2015). Herbal remedies are not as rigorously regulated as conventional drugs, and are often self-prescribed, and used off-label, specifically for children (Knopf et al. 2013). Moreover, there is a general lack of evidence of safe use of herbal remedies in children. Therefore, it is important to review the safety and efficacy of herbal remedies in the context of pediatric ADHD. In ADHD management, clinically relevant pharmacokinetic herb-drug interactions can occur through interferences with CYP enzymes, and/or CES1.

Cytochrome P450

Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing proteins responsible for the metabolism of endogenous substances including steroids and fatty acids, and xenobiotics including various therapeutic drugs (Nebert et al. 2002; Guengerich 1999). In humans, 57 functional genes have been identified, and grouped according to sequence similarity into 18 families and 44 subfamilies (Nelson et al. 2004). However, CYP1, CYP2, and CYP3 families contribute primarily to the metabolism of therapeutic drugs (Wilkinson 2005), including CYP 1A2, CYP 2C9, CYP 2C19, CYP 2D6, and CYP 3A4 (Sikka et al. 2005).

CYP enzymes are highly expressed in the liver, but are also present in the small intestine, lung, brain, and kidney (Krishna et al. 1994; Wrighton et al. 1992). CYP 3A4 and CYP 2D6 are responsible for metabolism of 38% and 12% of marketed drugs, respectively (Shimada et al. 1994). CYP P450 enzymes mainly catalyze oxidative phase 1 reactions, to create polar metabolites. This reaction requires nicotinamide adenine dinucleotide phosphate (NADPH) as a source of electrons (Guengerich 2007). Individual CYP P450 isoenzymes have unique substrate specificities, however, significant overlap may be present (Wilkinson 2005). Therefore, many substances are susceptible to interaction through the CYP P450 pathway, and early pharmacological and toxicological screening is an important process of drug development (Delgoda et al. 2004).

Carboxylesterase-1

Carboxylesterase-1 (CES1) is a serine hydrolase, belonging to the multi-gene family of α - β hydrolase fold proteins (Cygler et al. 1993) and is responsible for metabolism of endogenous and exogenous substances including esters, amides, thioesters, and carbamates (Casey Laizure et al. 2013; Satoh et al. 1998). CES1 has ubiquitous expression and is primarily expressed in the liver, but also in the intestine, kidneys, and lungs to a lower extent (Sanghani et al. 2009). CES1 is one the major carboxylesterase enzymes. CES1 has a large flexible pocket and a small rigid pocket in the active site, hence, many compounds are capable of binding to CES1. However, good substrates include those with small alcohol groups, or large acyl groups (Imai, 2006). Two variants of CES1 gene have been identified as “slow metabolizers”, both leading to elevated serum concentration of MPH (up to 7-fold increase). There is a potential for increased risk of adverse events for individuals with this polymorphism as it affects the pharmacokinetics and drug response (Zhu, Patrick, et al. 2008).

The effects of some herbal remedies are well known for CYP 450 enzymes and summarized in Chapter 2 of this thesis. Moreover, several comprehensive reviews have examined time-dependent inhibitors of CYP P450 isoenzymes (Zhou et al. 2005; Zhou et al. 2007; Venkatakrishnan et al. 2007; Johnston et al. 2008). CES1 remains an enzyme poorly characterized for herb-drug interactions. However, pharmacokinetic interactions through CES1 mediated metabolism have been reported *in vitro* and *in vivo*. For example, aripiprazole is a potent inhibitor of CES1 mediated metabolism of MPH *in vitro* and in animal studies (Zhu et al. 2010). Also, ethanol is a known inhibitor of CES1 substrate drugs both *in vitro* and in humans (Parker et al. 2015). Moreover, some chemical inhibitors of CYP 450 are capable of inhibiting CES1 activity (Polsky-Fisher et al. 2006). With the lack of evidence of CES1 mediated herb-drug interactions, the experimental Chapters (Chapters 5-7) of this thesis will focus on CES1.

1.1 HYPOTHESIS

The overall hypothesis guiding this thesis was that popular commercial herbal products may pose a risk to patients using them concurrently with drugs used for ADHD. The specific predictions are presented below for each of the experimental Chapters (Chapter 5, 6, and 7), and aim to bridge knowledge gaps highlighted in the descriptive study chapters (Chapters 2, 3, and 4).

1.2 OBJECTIVES, SPECIFIC HYPOTHESES & PREDICTIONS

The overall objective of this thesis was to take an interdisciplinary approach to evaluate the use and safety of CIM, specifically herbal remedies in pediatric ADHD.

Chapter 2

Objectives: Conduct a literature review to synthesize available data on the safety and efficacy of herbal medicines commonly used by pediatric ADHD patients.

- I. Determine herbal medicines commonly used by pediatric patients with ADHD
 - a. Determine evidence of efficacy from published clinical trials
 - b. Determine evidence of safety through:
 - i. *In vitro* herb-drug interaction studies in CYP 3A4, CYP 2D6, and CES1
 - ii. Clinical adverse events from concurrent use

Chapter 3

Objectives: Evaluate the use of CIM in pediatric ADHD in Eastern Ontario by formulating and administering a patient survey to:

- I. Document the prevalence of use of CIM for ADHD, comorbid disorders, disease prevention, and health maintenance
 - a. Determine factors (e.g., demographic, subtype of ADHD, comorbid conditions, and parental use of CIM) that may play a role in patients using CIM for ADHD
 - b. Determine the perceived helpfulness of CIM for the use
- II. Determine the parental/family perceived level of knowledge of CIM
 - a. Determine the preferred knowledge resources on CIM for families
- III. Gauge the level of communication about CIM between families and healthcare practitioners
- IV. Highlight the safety risks of CIM based on self-reported adverse events from CIM use.

Chapter 4

Objectives: To gauge the frequency of reported clinical adverse events related to the concurrent use of ADHD medication and herbal medicines by pediatric patients. Specifically:

- I. To identify adverse event reports (AERs) involving commonly used herbal remedies and ADHD prescription medicines
 - a. Evaluate the quality of collected AERs
 - b. Assess whether herb-drug interactions can be causally linked to reported adverse events

Chapter 5

Based on the literature review (Chapter 2), herbal remedies identified for use in ADHD management modulated the activity of various CYP P450 enzymes.

Hypothesis: The herbal remedies identified for use in ADHD management would also modulate CES1 activity *in vitro*.

Predictions: Herbal remedies will have inhibitory effects on the metabolism of a probe substrate by recombinant CES1.

Objectives: Evaluate the inhibitory effects of commonly used herbal medicines used by ADHD patients on the activity of recombinant CES1 mediated metabolism. Specifically:

- II. Screen extracts of commonly used herbal medicines for inhibition potential *in vitro* in enzyme inhibition assays
 - a. Determine IC₅₀ values to measure relative potency of selected extracts
 - b. Determine whether select extracts exhibit time-dependent inhibition
- III. Perform a phytochemical analysis on selected extracts to determine presence of marker phytochemicals
 - a. Identify marker phytochemicals in commercial extracts using chromatographic and spectroscopic techniques
 - b. Test marker compounds *in vitro* using CES1 enzyme inhibition assay

Chapter 6

In the preliminary evaluation (Chapter 5), an extract of a commercial *Rhodiola rosea* L. (Crassulaceae) product exhibited strong inhibitory effects on recombinant CES1 mediated metabolism. Though the marker phytochemicals displayed weak inhibition (tested at 11-16 µM),

the effects of varying concentrations of marker phytochemicals in the products, and their relative inhibitory potency for recombinant CES1 were not known. Moreover, the direct effects of *Rhodiola rosea* on MPH metabolism were not known.

Predictions: Extracts of different commercial products will display varying inhibitory potencies on CES1. Based on the preliminary evaluation, the marker phytochemicals will have weak inhibitory potency, and the concentration of marker compounds will be not linked to inhibition. *Rhodiola rosea* will inhibit HLM mediated metabolism of MPH.

Objectives: To evaluate the effects of extracts of commercial *Rhodiola rosea*, on the activity of CES1 mediated metabolism

- I. Screen extracts for inhibition potential *in vitro* using enzyme inhibition assays
 - a. Determine IC₅₀ values to determine relative potency of extracts
 - b. Determine whether select extract exhibit time-dependent inhibition
 - c. Determine potency of marker compounds *in vitro* in CES1 enzyme inhibition assay
- II. Perform a phytochemical analysis on commercial extracts to determine presence of marker compounds
 - a. Identify and quantify marker compounds in extracts
- III. Examine the impact of select extracts on ADHD drug metabolism using an *in vitro* human microsome metabolism assay
 - a. Test extracts in a co-incubation assay with methylphenidate to determine direct effects on biotransformation

Chapter 7

Based on Chapter 4, two clinical adverse events reports involving the use of methylphenidate and St. John's wort (*Hypericum perforatum* L., Hypericaceae) were identified to have a causal link for herb-drug interaction. Although the mechanisms of the pharmacological interaction are not known, St. John's wort modulates the activity of other drug-metabolizing enzymes (such as CYP P450) *in vitro*.

Hypothesis: The interactions between methylphenidate and St. John's wort can be explained by the ability of St. John's wort and its marker constituents (hyperforin, hypericin, and quercetin) to modulate CES1 activity.

Predictions: St. John's wort, and its marker constituents, will inhibit recombinant CES1 activity as well as HLM mediated metabolism of methylphenidate *in vitro*. Moreover, higher concentration of marker compounds in the extracts of the commercial products will result in higher CES1 inhibition

Objective: To evaluate the effects of commercial extracts of St. John's wort, identified as having a causal link for herb-drug interaction to reported adverse events in Chapter 4, on the activity of carboxylesterase-1 mediated metabolism

- I. Screen extracts of commercial products for inhibition potential *in vitro* enzyme inhibition assays
 - a. Determine IC₅₀ values to gauge relative potency of extracts
 - b. Determine whether select extract exhibit time-dependent inhibition
 - c. Determine potency of marker compounds *in vitro* in CES1 enzyme inhibition assay

- II. Perform a phytochemical analysis on commercial extracts to determine presence of marker compounds
 - a. Identify and quantify marker compounds in extracts
- III. Examine the impact of select extracts on ADHD drug metabolism using an *in vitro* human microsome metabolism assay
 - a. Test extracts in a co-incubation assay with methylphenidate to determine direct effects on biotransformation

1.3 ETHICAL CONSIDERATIONS

The clinical survey in Chapter 3 of this thesis was approved by the Children's Hospital of Eastern Ontario Research Ethics Board, and the University of Ottawa Research Ethics Board (**Appendix A**).

Chapter 2

Complementary and alternative medicine use in pediatric attention-deficit hyperactivity disorder (ADHD): Reviewing the safety and efficacy of herbal medicines

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Author's Contributions

BCF, CSH, and HM contributed to the design of the study. Data collection and analysis was performed by CSH, EFH, and HM. BCF, CSH, EFH and HM drafted, revised, and approved the final manuscript.

2.1 ABSTRACT

Natural Health Products (NHP), including medicinal herbs, are a modality of Complementary and Alternative Medicine (CAM) commonly used by pediatric patients with Attention-Deficit Hyperactivity Disorder (ADHD). Most families of pediatric patients find NHP treatment to be beneficial, however, clinical evidence of efficacy remains weak or lacking. Evidence of medicinal herb safety is similarly scarce, particularly with respect to NHP-drug interactions and adverse events (AEs) associated with concurrent use of NHPs and ADHD prescription drugs. To support both families and physicians managing ADHD care, this review focuses on integrating available data on the safety and efficacy of medicinal herbs commonly used by pediatric ADHD patients. In addition to discussing results from clinical trials, patient surveys and experimental studies relating to commonly used medicinal herbs, the paper summarizes adverse event reports involving concurrent use of herbs and ADHD drugs, identified through the FDABable database. While NHPs and other CAM offer patients alternative treatment options with potential benefits as well as risks, additional research is needed to support open discussion and evidence-based decision making by families and physicians.

2.2 INTRODUCTION

According to the National Centre for Complementary and Alternative Medicine, complementary and alternative medicine (CAM) represents a group of diverse health and medical systems, practices, and products that are not part of conventional (allopathic) medicine but used alongside or in place of conventional medicine (National Center for Complementary and Alternative Medicine 2004). Accordingly, CAM serves as an umbrella term encompassing various categories: biologically based practices or natural health products (NHP) (e.g., herbal medicines, vitamins), mind-body medicine (e.g., yoga, relaxation), manipulative and body-based practices (e.g., chiropractic, osteopathy), energy medicine (e.g., Qigong, magnets), and whole medical systems (e.g., homeopathy, naturopathy) (National Center for Complementary and Alternative Medicine 2004; Goldrosen et al. 2004).

The use of CAM is not only widespread among adults but also children. Based on recent studies, up to 40% of healthy children and up to 75% of children with chronic disorders utilize one CAM modality or another, with some using 2 or more simultaneously (Armishaw et al. 1999; Ball et al. 2005; Barnes et al. 2008; Loman 2003; McCann et al. 2006; Ottolini et al. 2001; Post-White et al. 2009; Sanders et al. 2003; Sawni-Sikand et al. 2002; Spiegelblatt et al. 1994; Vohra et al. 2009; Vohra et al. 2012; Zorzela et al. 2014). CAM use is especially high in pediatric patients with developmental disorders, a trend due, in part, to the chronic nature of disease and treatment, the occurrence of comorbid conditions, the desire of parents to try anything that may help their child, and concerns about adverse events related to prescribed pharmacotherapy (Stubberfield et al. 1999; Sinha et al. 2005; Chan et al. 2003). Among this population, Attention-Deficit Hyperactivity Disorder (ADHD) patients are among the most common users of CAM practices (Huang et al. 2013).

CAM use in pediatric psychiatry and ADHD

Surveys aiming specifically to identify determinants of CAM use among pediatric ADHD patients, though few in number, reveal that 54% - 68% of families report giving their child at least one type of CAM over the previous year (Stubberfield et al. 1999; Chan et al. 2003), or over their lifetime (Sinha et al. 2005). Children with an ADHD diagnosis are also more likely to receive CAM treatment than children in whom ADHD is suspected or display problematic behaviors, as indicated by parents (Bussing et al. 2002). The most commonly reported CAM modalities were vitamins and dietary supplements, herbal medicines, sensory integration, occupational therapy, art, music, special exercises, relaxation, prayer, biofeedback, chiropractic, massage and hypnosis (Stubberfield et al. 1999; Sinha et al. 2005; Chan et al. 2003). When asked why they opted for CAM, families most frequently referred to preference for more natural therapy, desire for control over treatment, minimization of symptoms, cultural/family tradition, recommendation by friends or family, concerns regarding side-effects of conventional drugs, hope for cure, and recommendation by physicians or CAM practitioners (Stubberfield et al. 1999; Sinha et al. 2005; Chan et al. 2003). Since these studies focused on ADHD symptom management, however, they may not capture CAM use for treatment of comorbid conditions, disease prevention, or health maintenance and consequently underestimate overall use.

Observing more broadly the pediatric psychiatric population, the use of CAM again appears prevalent, as does its combination with pharmacotherapy. A recent study in Canada identified that 48% - 78% patients had used CAM – with most finding it helpful – and that 46% had done so concurrently with conventional medicine (Galicia-Connolly et al. 2014). Whereas many patients and families report CAM-related benefits, adverse events (AE) are also well documented. Among 80 AEs reported by pediatric specialty outpatients – 40% of which were

self-reported as moderate or severe – 56% involved concomitant use of CAM with prescription drugs (Adams et al. 2013). An Australian study of CAM-related pediatric AE reported 39 relevant cases; 64% were severe, life threatening or fatal and 77% were considered probably or definitely related CAM use (Lim et al. 2011). The 4 fatalities were deemed to have resulted from failure to use conventional medicine in favor of CAM.

The use of CAM in pediatric ADHD is clearly widespread, whether for symptom management, health maintenance, or prevention of concomitant conditions such as sleep problems, depression, anxiety, or conduct disorders (Birdee et al. 2010). Despite this prevalence, only 30%-65.2% of families discuss pediatric CAM use with a physician (Galicia-Connolly et al. 2014; Adams et al. 2013; Cala et al. 2003; Chan 2002). Clinicians, on the other hand, may be similarly disinclined to discuss CAM use due to their personal beliefs or lack of knowledge about the topic; indeed, less than 5% of pediatricians report feeling “very knowledgeable” about CAM therapies (Chan 2002; Kemper et al. 2004). This lack of communication is of particular concern since ADHD drugs are administered over long periods of time, at times in cocktails. The addition of CAM to the mix can potentially improve symptoms or general health but can also increase the risk of drug interactions and AEs (Adams et al. 2013; Spiteri Staines 2011; Phua et al. 2009). Accordingly, both patients and practitioners must be well informed and maintain an open dialogue about alternative treatments.

Unfortunately, the evidence to support conversations about CAM safety and efficacy in ADHD is generally dispersed, weak or incomplete. As an initial step toward filling this gap, this review focuses on herbal medicines, synthesizing available data regarding the safety and efficacy of botanicals commonly used by pediatric ADHD patients.

2.3 HERBAL MEDICINES IN ADHD

Why focus on herbal medicines?

Herbal medicines (HM) are often perceived as “natural” and “safe”, leading to fewer AEs compared to conventional medicine (Birdee et al. 2010). Similar to prescription drugs but unlike many types of CAM, HM and other NHPs contain raw or processed medicinal and non-medicinal substances, many of which are bioactive and can elicit pharmacokinetic and pharmacodynamic responses (World Health Organization 2005a). Once consumed, such substances are absorbed, distributed, metabolized, and eliminated by the body, often inhibiting or inducing metabolic enzymes or transporters. Many of these substances also possess bioactivity as ligands for receptor targets leading to molecular and physiological effects (Phua et al. 2009). Whereas herbal medicines may contribute to symptom control for some patients, they may also lead to toxicity or affect the safety and efficacy of other medicines (Noras et al. 2013).

Given that HM are amongst the most common form of CAM used in combination with both psychostimulants and non-stimulants, the potential for herb-drug interactions is prevalent (Galicia-Connolly et al. 2014). Because most HM are chemically complex and, in some jurisdictions, are not rigorously regulated for quality control, the chemistry, potency, and safety of any given HM can vary from one product to the next. Differences in plant chemistry may arise due to genetics, geography, and environmental factors as well as harvesting, storage, manufacturing, and formulation practices (Gardner 2002). This variability can influence patient response and health (World Health Organization 2005a; Chan et al. 2000; Organization 2005; Ekor 2014) more frequently if the product is adulterated, used inappropriately, long-term, or concurrently with prescription drugs (especially those with narrow therapeutic indices) (Phua et al. 2009).

Commonly used herbal medicines by ADHD patients

Among pediatric ADHD and other pediatric psychiatry patients, some of the most commonly used HM include St. John's wort (*Hypericum perforatum* L., Hypericaceae); chamomile (*Matricaria chamomilla* L., Asteraceae); rhodiola (*Rhodiola rosea* L., Crassulaceae); valerian (*Valeriana officinalis* L., Valerianaceae); bacopa (*Bacopa monnieri* L., Plantaginaceae); pycnogenol (*Pinus pinaster* Ait., Pinaceae); kava kava (*Piper methysticum* G. Forster., Piperaceae); ginkgo (*Ginkgo biloba* L., Ginkgoaceae), ginseng (*Panax ginseng* C.A. Meyer., Araliaceae); evening primrose oil (*Oenothera biennis* L., Onagraceae); lemon balm (*Melissa officinalis* L., Lamiaceae); echinacea (*Echinacea angustifolia* DC., Asteraceae. *Echinacea purpurea* L. Moench., Asteraceae); goldenseal (*Hydrastis canadensis* L., Ranunculaceae); peppermint (*Mentha x piperita* L., Lamiaceae); rosemary (*Rosmarinus officinalis* L., Lamiaceae); green tea (*Camellia sinensis* L., Theaceae); garlic (*Allium sativum* L., Alliaceae); eleuthero (*Eleutherococcus senticosus* Ruprecht et Maximowicz., Araliaceae); linden (*Tilia cordata* Miller., Tiliaceae); skullcap (*Scutellaria lateriflora* L., Lamiaceae); ginger (*Zingiber officinale* Roscoe., Zingiberaceae); and passion flower (*Passiflora incarnate* L., Passifloraceae) (Chan et al. 2003; Chan 2002; Galicia-Connolly et al. 2014; Cala et al. 2003; Chan et al. 2000; Pellow et al. 2011). Whereas many of these HM are utilized for ADHD-specific symptom management, others (e.g., ginseng, goldenseal) are considered adaptogens useful in health maintenance and disease prevention. Cala et al. (2000) reported that treatment of ADHD symptoms accounts for only 35% of medicinal herb consumption by pediatric ADHD patients, citing treatment of colds, rashes, fevers, asthma, insomnia, and allergies, as well as the maintenance of general health as other reasons for HM use. More studies examining this type of CAM use are needed to better understand if these results extend to other populations (Cala et al. 2003).

Evidence of herbal medicine efficacy in ADHD

Traditional uses of HM usually provide the basis of evidence for their utility in the management of ADHD symptoms. For example, restlessness, poor concentration, and sleep difficulties commonly seen in ADHD patients may improve with the use of a sedative herb such as kava kava, chamomile, or valerian, which possess anxiolytic properties (Chan 2002).

Evidence from randomized placebo-controlled trials, a current requirement for determining the therapeutic efficacy of drugs, is scarce with only a few popular herbs evaluated to this standard for ADHD. Most clinical trials examining the efficacy of HM in ADHD, whether yielding positive or negative results, suffer from inadequate trial design (e.g., small subject sample size, short duration, inadequate dose) (Gardiner et al. 2013), incomplete reporting and risk of bias (e.g., poor or no subject blinding) (Wong et al. 2012). Other limitations may include the lack of a placebo control group, (undocumented) concurrent use of ADHD pharmacotherapies, and few examples of head-to-head trials with approved drugs (Snyder et al. 2012; Searight et al. 2012; Sarris et al. 2011; Calixto 2000).

The effectiveness of HM (and other CAM) for ADHD symptom management has been reviewed elsewhere (refer to Snyder et al. 2012; Searight et al. 2012; Sarris et al. 2011; Calixto 2000). To focus on pediatric research, **Table 2-1** summarizes the results of identified clinical trials using herbs for ADHD in children, highlighting reported results as well as study weaknesses. ginkgo, for example, was effective at reducing inattention scores of subjects compared to placebo in one randomized, placebo-controlled, double-blind study (Shakibaei et al. 2015) but less effective at reducing symptoms in a separate parallel-group, randomized, double-blind study (Salehi et al. 2010). Both studies had a short duration of intervention (6 weeks) and a small subject sample size. In a small ($n = 18$) four-week, randomized double-blind, double-

crossover trial, evening primrose oil showed no significant differences in symptoms relative to D-amphetamine but also placebo (Arnold et al. 1989). A fifteen-week randomized, placebo-controlled, double-blind trial of evening primrose oil with 132 subjects reported significant medium to strong effects in reducing ADHD symptoms compared to placebo (Sinn et al. 2007). Although the sample size in this study was larger, the subjects did not show severe ADHD symptoms at baseline. Overall, evidence for the efficacy of HM in the treatment of pediatric ADHD in well-designed trials is limited and generally weak.

Despite the lack of clinically proven efficacy, HM may offer benefits to some patients and serve as an option for the 10-30% of ADHD patients who do not respond to stimulant drugs or who experience intolerable AE (Spencer et al. 2004). As stated, many patients also use HM to treat comorbid conditions and symptoms that extend beyond core symptomology and thus available clinical trial data. Importantly, ADHD patients who use HM (and CAM) generally perceive them to be beneficial. For example, in three independent pediatric neurology studies, HM were reportedly perceived by families to be helpful for 50-77.8% of children who use them (Soo et al. 2005; Cala et al. 2003; Adams et al. 2013). Given the popularity of HM and their perceived benefits, it is crucial to examine the evidence of safety and risk.

Evidence of herbal medicine safety and risk

As summarized in **Table 2-1**, most clinical trial results of HM used for pediatric ADHD report few mild to moderate AE, generally comparable to placebo, if AE are reported at all. Notably, an eight-week randomized, controlled, double-blind study examining Ningdong granule reported fewer and milder AE compared to methylphenidate as well as one severe AE (Li et al. 2011). The high degree of reported HM tolerability when administered alone, in moderation, over the short term suggests but does not confirm safety and the frequent failure to report AE

may reflect either poor safety data or poor attention to monitoring and reporting. Importantly, in addition to potentially toxic effects at high doses, HM pose a risk of AE due to interactions with prescribed drugs.

Table 2-1 Summarized results of clinical trials of herbal medicines used for pediatric ADHD.

Natural Health Product (Reference)	Methodology/ Sample Size	Duration of Treatment	Results	Limitations	Adverse Events
Bacopa (Dave et al. 2014)	Open label/ 31	6 months	Effective for ADHD symptoms and well tolerated by children	SS, LP	N/A
Chamomile (<i>Matricaria chamomilla</i>) (H Niederhofer 2009)	PC/ 3	4 weeks	Improvements in mean scores for hyperactivity, immaturity, and inattention	SS, SD	N/A
Evening primrose oil (Arnold et al. 1989; Sinn et al. 2007)	R, DB, DCO/ 18	4 weeks	No difference compared to placebo or D-Amphetamine	SS, SD	N/A
	R, PC, DB/ 132	15 weeks	Significant medium-strong positive treatment effects of core ADHD symptoms compared to placebo	Symptoms not severe at baseline	N/A
<i>Ginkgo biloba</i> (Shakibaei et al. 2015; Salehi et al. 2010)	PG, R, DB/ 50	6 weeks	Less effective than MPH for ADHD	SD, SS LP	10 AE: mild-moderate, no significant difference between control and experimental
	R, PC, DB/ 66	6 weeks	Effective and safe complementary therapy	SD, SS No drug free follow-up	Mild, no significant difference between experimental and placebo
Ginseng (Ko et al. 2014)	DB, R, PC/ 72	8 weeks	Safe and effective alternate therapy for hyperactivity, impulsivity symptoms	SS, SD ADHD NOS only	No significant difference between experimental and placebo

Lemon balm Valerian (Gromball et al. 2014)	PR, MC, NI/ 169	7 weeks	Reduced symptoms of restlessness, concentration difficulties and impulsivity	SD, LP, Sample did not meet ADHD criteria	Mild AE in 2 (1.18%) of patients
Ningdong granule (Li et al. 2011)	R, C, DB/ 72	8 weeks	Effective and safe short term	SS, SD, LP	Mild, Fewer AE compared to MPH, 1 severe AE
Passion flower (Akhondzadeh et al. 2005)	PG, R/ 34	8 weeks	No difference between placebo and MPH	SS, SD, LP, Fixed dose	No significant difference between experimental and MPH
Pycnogenol (Trebatická et al. 2006)	R, DB, PC/ 61	4 weeks	Significant reductions in hyperactivity, visual motor coordination, attention, and concentration	SS, SD	Mild to moderate in 2/61 patients
St. John's wort (Weber et al. 2008)	DB, R, PC/ 54	8 weeks	Did not improve symptoms	SD SS	No significant difference between experimental and placebo

PG=Parallel group, R=Randomized, DB=Double blind, DCO=Double cross over, PC=Placebo controlled, PR=Prospective, MC=Multicenter, NI=Non-interventional, C=Controlled, SD=Short duration, SS=Small sample size, LP=Lack of placebo, nos=Not Otherwise Specified, N/A=Not available, MPH=methylphenidate.

Mechanisms of herb-drug interactions

Similar to typical drug-drug interactions (Spiteri Staines 2011), HM (or components thereof) can interact with drugs at the pharmacodynamic level, with one acting synergistically or antagonistically to the other. For example, combining *Ephedra* (which contains ephedrine) with another stimulant will enhance effects on heart rate and blood pressure as well as risk of related AEs. More frequently, however, herb-drug interactions (as well as other NHP-drug interactions) occur at the pharmacokinetic level. As substrates, inhibitors, and inducers of drug-metabolizing enzymes and transporters, NHPs can increase or decrease drug bioavailability and alter efficacy and safety by proxy (Venkataramanan et al. 2006). Such interactions may not always equate to a clinically significant drug interaction and depends largely on the therapeutic index of the drug and degree of change in systemic exposure (Chavez et al. 2006; Venkataramanan et al. 2006).

Both acute and chronic use of HM can impact the disposition of prescribed drugs. Acute administration may cause AEs through increased or decreasing drug metabolism and clearance but chronic administration may lead to a biphasic response where both toxicity and efficacy may decrease (Bauer et al. 2003).

Experimental evidence of risk

Knowledge about the clearance of ADHD pharmacotherapies is fundamental to examining related risks of herb-drug interactions. Considering the importance of the hepatic cytochrome P450 (CYP) system in drug metabolism, drug-drug and herb-drug interactions, there is a dearth of available information on the CYP profiles of several approved ADHD drugs, with few comprehensive published data for either methylphenidate or amphetamine currently available in the public domain.

The metabolism of the stimulant medications is mostly poorly understood. Briefly, methylphenidate (MPH) is extensively metabolized by carboxylesterase-1A1 (Sun et al. 2004), an enzyme whose substrate/inhibitor profile is poorly characterized (Casey Laizure et al. 2013), but other enzymes may also be involved. Amphetamine is metabolized extensively by CYP 2D6 in mice and rats, but to a minor extent in humans (Law et al. 2000; Green et al. 1986; Dring et al. 1970), where it may also act as a weak inhibitor (Wu et al. 1997). Lisdexamfetamine, an amphetamine pro-drug, does not appear to be metabolized by human liver homogenate, hepatocytes, or microsomes (Pennick 2010), nor does it significantly interfere with the metabolism of specific CYP substrates (Krishnan et al. 2007). The metabolism of atomoxetine is better characterized, with CYP 2D6 generating the primary metabolite (Ring et al. 2002) and a corresponding bimodal distribution of pharmacokinetic parameters reflecting CYP 2D6 poor- and extensive-metabolizers (Farid et al. 1985; Sauer et al. 2003). Another major non-stimulant medication, guanfacine, is metabolized primarily via CYP 3A4 according to the product monograph (Shire Pharma Canada ULC 2015).

Several HM commonly used in ADHD management are known to inhibit or induce phase 1 and/or 2 enzymes involved in drug metabolism (**Table 2-2**). There is a lack of evidence supporting direct effects of HM in the pharmacokinetics of ADHD drugs specifically, with most evidence derived from *in vitro* studies targeting CYP isozyme activity and inhibition. The impact of HM on carboxylesterases responsible for methylphenidate metabolism remains unclear. Many herb-drug interactions observed *in vitro*, however, are not confirmed *in vivo* (Spiteri Staines 2011). *In vitro* approaches using microsomal assays and primary cultures of human hepatocytes provide mechanistic insight on potential herb-drug interactions that can inform human studies

and clinical practice (Venkataramanan et al. 2006). Standing alone, *in vitro* data (**Table 2-2**), suggest a potential risk (or lack thereof) that requires experimental or clinical corroboration.

Clinical evidence of risk

Adverse events, specifically drug interactions are a major cause of patient morbidity and mortality. An estimated 1.5 million adults in the U.S.A. are at risk for possible AEs resulting from interactions between prescription drugs and NHPs, and these statistics are unknown for pediatric populations (Eisenberg et al. 2001). In a large pediatric emergency room study in Canada, approximately 20% of pediatric patients used medications with 1 or more NHP and, based on 35 reported NHP-drug interactions, most were pharmacokinetic in nature (e.g., modified drug absorption) (Goldman et al. 1998). Looking specifically at herbal medicines, two pediatric studies of CAM-related adverse events found that HM were linked to 1 of 19 moderate AE (21) and 12 of 39 total AE, respectively. Given their high frequency of concurrent drug and HM use by pediatric ADHD patients, the risk of herb-drug interactions and related AE in this context warrants further investigation and monitoring.

Notably, less than 10% of all AEs are reported. AEs involving both NHPs and prescription drugs are reported even less frequently, estimated at <1% of their occurrence (U.S. Department of Health and Human Services 2004). With regard to this scarcity of NHP-related AEs relative to pharmaceuticals, several factors contribute to the underreporting of such events: healthcare practitioners may be unaware of patients' NHP use or unaware of a patient experiencing an AE unless it is serious enough to demand clinical attention; exacerbation of an AE by an NHP may simply be attributed to the uncertainty about causality; time pressure in the workplace; or lack of patient awareness about NHP use and risks (Walji et al. 2009; Murty et al. n.d.; Chavez et al. 2006; Lim et al. 2011; Vohra et al. 2012)

Table 2-2 *In vitro* NHP-drug interaction studies for medicinal herbs causing adverse events with ADHD drugs in a pediatric population

Natural Health Product (Reference)	Enzymes tested (CYP)	Conclusions
Bacopa (Ramamamy et al. 2014)	3A4** , 2C9, 2C19, 1A2, 2D6*	Moderate inhibition with 3A4, 2C9 and 2C19, 1A2 Use with caution when administered concurrently
Echinacea (Gurley et al. 2005)	1A2, 2D6 ,2E1, 3A4	Slight modulatory effect on 2D6 and 2E1 Minor effect on 1A2 and 3A4 Concomitant administration with conventional drugs not recommended
Eleuthero (Guo et al. 2014)	2C9, 2D6 , 2E1 and 3A4	No effects on 2D6, 3A4 Weak inhibitory effect on 2C9 and 2E1
Evening primrose oil (Zou et al. 2002)	1A2,2C9,2C19, 2D6 3A4	Potential to inhibit the metabolism of coadministered medications
Garlic (HO et al. 2010)	2C9, 3A4	Suppresses CYP 2C9 and CYP 3A4 not affected Concurrent administration with CYP 2C9 substrates may cause adverse drug reactions
Ginkgo (von Moltke et al. 2004)	1A2, 3A ,2C9	Enzymes not significantly inhibited by major components of preparations in clinical use Significant inhibitors include flavonol aglycones, biflavonol amentoflavone and other non-glycosidic constituents
Ginseng (Gurley et al. 2005)	1A2, 2D6 ,2E1, 3A4	Clinically significant interactions are unlikely
Goldenseal (Gurley et al. 2005)	1A2, 2D6 , 2E1, 3A4	Strong inhibition of 3A4 and 2D6 substrates Serious adverse events may occur from concurrent use with substrates
Green tea (Misaka et al. 2012)	2B6, 2C8, 2C19, 2D6 , 3A	Catechins have potential for clinically relevant interactions for 2B6, 2C8, and 3A4 substrates

Kava kava (Mathews et al. 2002)	1A2, 2C9, 2D6 , 3A4 , 4A9/11, 2A6, 2C8, 2E1	High potential for drug interactions
Peppermint oil (Dresser et al. 2002)	3A4	Moderate and reversible inhibitor
Rhodiola (Xu et al. 2013; Hellum et al. 2010)	2D6	Rhodosin and rhodionin non-competitive inhibitors Potential for drug interactions
	3A4	Potential for clinically relevant drug interactions
St. John's wort (Obach 2000; Komoroski et al. 2004)	1A2,2C9,2C19, 2D6 , 3A4 .	High inhibition potential for 3A4, 2C9, and 2D6
	1A2, 2C9, 2D6 , 3A4	Inhibition and induction potential for 3A4
Valerian (Gurley et al. 2005)	3A4/5 , 1A2, 2E1, 2D6	Typical doses of valerian are unlikely to cause clinically significant effects on metabolism of 2D6 and 3A4/5

*-CYP 2D6 is the metabolizing enzyme of amphetamine and atomoxetine,

** -CYP 3A4 is the metabolizing enzyme of guanfacine

Searching the FDable Adverse Events Database

Given the widespread use of HM among pediatric ADHD patients yet the dearth of published AE studies in North America, the FDable Adverse Events Database was examined to gauge the frequency and severity of AEs involving the concurrent use of HM and ADHD pharmacotherapy, including potential herb-drug interactions. We systematically searched the database (<http://www.fdable.com>) for reports involving one (or more) of 22 different HM commonly used by ADHD patients, specifically, and children, more generally. Using the advanced search option, we targeted each herbal by name (common and scientific) and recorded the total number of AE reported for patients of 0-18 years (**Figure 2-1**). From these reports, we identified those that also involved ADHD medications (methylphenidate: Concerta, Metadate, Ritalin, Focalin, Quillivant, Daytrana, and Methyllin; amphetamine: Adderall, Dexedrine, ProCentra, Zenzedi, and Dextrostat; methamphetamine: Desoxyn; lisdexamfetamine: Vyvanse; guanfacine: Intuniv; atomoxetine: Strattera). Note that, due to the limited data available for each report, we are unable to determine any degree of causality relating to potential herb-drug interactions.

Among the 22 targeted HM, 167 adverse event reports (AERs) were identified for pediatric patients (**Figure 2-1**). No AERs were found for bacopa, eleuthero, linden, rosemary, and skullcap. Goldenseal, lemon balm, pycnogenol and rhodiola had 1 AER and none of these involved ADHD drugs. Approximately 12% of the identified AE reports involved both a HM and an ADHD drug. Echinacea, ginkgo, garlic, St. John's wort, evening primrose oil, and ginseng were involved in AERs with ADHD drugs. Methylphenidate was a suspect drug with 11 of the 20 AERs and was involved with each of the medicinal herbs mentioned above. Atomoxetine and amphetamine were the suspect drugs in 7 and 2 reports respectively. The most

frequent herb-drug AER combinations were evening primrose oil with methylphenidate, and ginseng with atomoxetine (3 cases each). In most cases, additional substances were reported beyond medicinal herb and ADHD drug. Although causality cannot be determined based on the FDable data, potential HM-drug interactions identified through the database (e.g., evening primrose oil with methylphenidate and ginseng with atomoxetine) warrant further investigation, particularly since all six of the HM involved in AE related to ADHD drugs (**Figure 2-1**) are known to modulate CYP activity *in vitro* (**Table 2-2**).

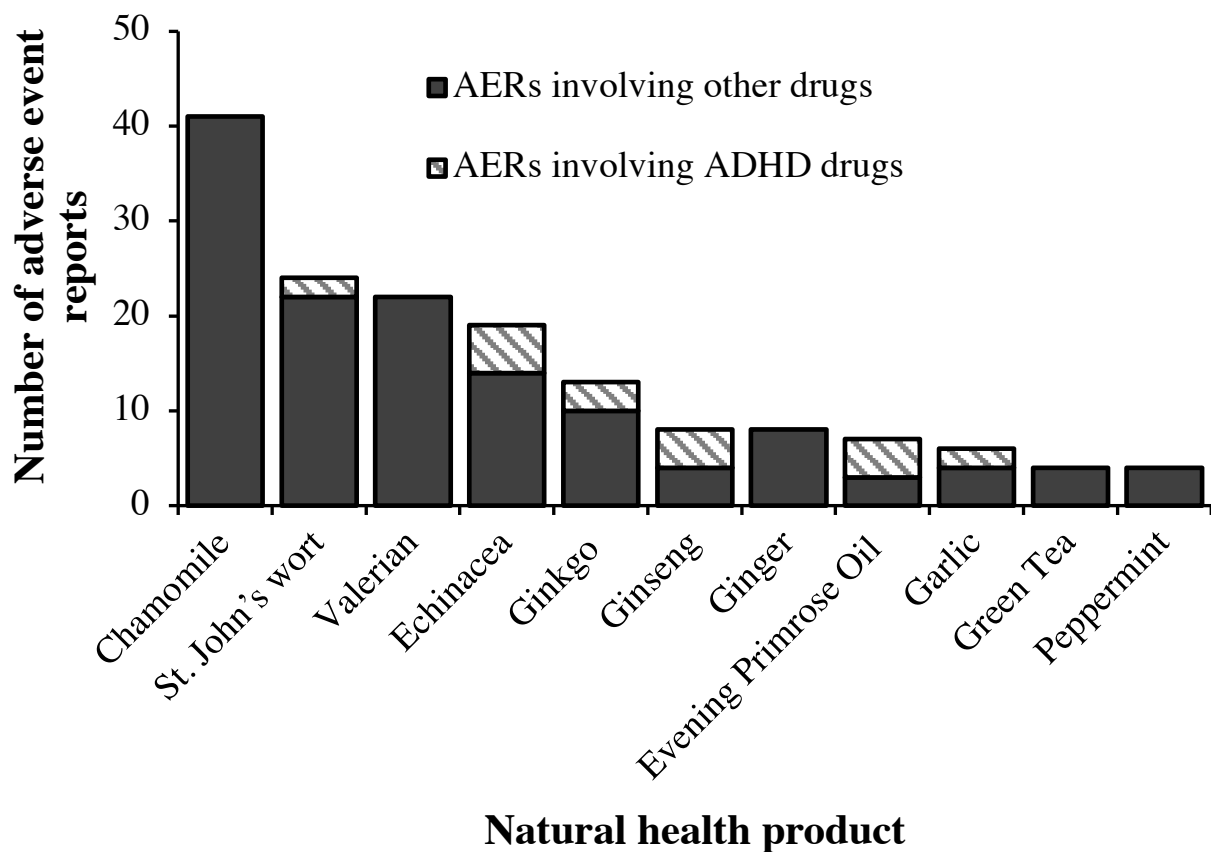


Figure 2-1 Total adverse event reports (AERs) and adverse event reports involving ADHD drugs for common herbal medicines used by pediatric ADHD patients (age 0-18 years), as identified through the FDable database (<http://www.fdable.com>).

2.4 CLOSING REMARKS

Knowledge gaps in the field

Numerous knowledge gaps obscure our understanding of the potential benefits and risks of HM use in pediatric ADHD yet highlight needed avenues of future research. The need for more clinical evidence of efficacy and safety in high quality pediatric ADHD trials is paramount, including comparative trials with standard ADHD pharmacotherapies. Adverse events are broadly underreported and those involving HM and other NHP can be more difficult to report and interpret since information about ingredients or formulations is not always readily available. Regulatory standards and frameworks to facilitate accurate reporting of AE involving HM and CAM, in general, must be implemented and supported to strengthen existing monitoring and surveillance program (Vohra et al. 2009). While tools like the FDABle AER database offers access to standardized case data, individual reports lack sufficient detail to evaluate any degree of causality or interaction.

The direct effects of herbal medicines on ADHD drugs in clinical and experimental models are also urgently needed. Whereas indirect *in vitro* approaches using marker probe substrates provide insight on potential risk, the effect of HM on ADHD drug metabolism remains mostly unstudied. *In vitro* models, although informative, do not always reflect specific effects on different drugs or, more importantly, the potential clinical significance of observed interactions. A more complete understanding of ADHD drug metabolism is therefore also needed.

Doctor-patient communication

As with any treatment, the use of HM by children with ADHD should be monitored by families and practitioners to evaluate safety and efficacy, especially when stimulant or non-stimulant medications are or will be used as well. Open communication between patients,

families, and physicians is an essential component of mitigating risk and benefit. However, while healthcare providers are their preferred and most trusted source of medical advice, the majority of families do not discuss the concurrent use of CAM and prescription medicines with a medical doctor (Galicia-Connolly et al. 2014).

Research indicates that most families expect physicians to ask them about CAM use in a non-judgmental fashion. Families also do not expect physicians to be experts in the field of CAM, but rather be open to discussion and provide advice on efficacy and safety (Cui 2013). In order to better advise families, physicians should be aware of available tools to facilitate communication and access information about commonly used NHPs and the possible interactions with prescription drugs (Cui 2013; Shelley et al. 2009; Galicia-Connolly et al. 2014). As is often available for prescription drugs, physicians could offer educational resources regarding CAM to patients and families in the clinic (brochures, direction to credible websites, publications, etc.). In doing so, patients can be well informed and may feel more comfortable initiating a discussion about CAM with their physician.

2.5 CONCLUSION

Patients and families using or interested in using HM and other NHP or CAM should not be dismissed or discouraged blindly from doing so; potential benefits and risks should be discussed and weighed – keeping in mind potential interactions with substances taken concomitantly. If not yet offered by patients or families, healthcare providers should, whenever possible, initiate a conversation about CAM. With the high prevalence of HM use in pediatric ADHD, limited clinical evidence yet family-reported benefits, and risks of herb-drug

interactions, more research is needed in order to support evidence-based decision making and effective communication between patients, families and doctors.

Chapter 3

Evaluating the use and knowledge of complementary and alternative medicines (CAM) in pediatric attention-deficit hyperactivity disorder

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3.1 ABSTRACT

Focusing on children and adolescents with ADHD, this study aimed to determine: i) the prevalence of the use of Complementary and Integrative Medicine (CIM) for ADHD, comorbid disorders, disease prevention and health maintenance; (ii) the perceived level of knowledge of CIM among parents/families; (iii) how families and healthcare practitioners communicate about CIM; (iv) perceived helpfulness of CIM; and (v) self-reported adverse events related to CIM use. We developed a cross-sectional questionnaire carried out at the ADHD clinic at the Children's Hospital of Eastern Ontario in Ottawa between August 2016 and May 2019. The sample was drawn from patients under the age of 18 years with an ADHD diagnosis. Caregivers completed a self-administered questionnaire regarding their sociodemographic information, their child's ADHD, and their use and experiences with CIM. A total of 74 survey were completed with a response rate of 51%. The mean child age was 10.7 years (range: 4-18 years), and 80% were male. Our results indicate that 40% of patients had used some form of CIM, and 57% of CIM users had used herbal medicines. The most commonly used forms include nutrient and dietary supplements, in general, as well as melatonin specifically, herbal medicines, physical therapy, elimination diet, and homeopathy. CIM were perceived as most helpful for general health maintenance, physical health conditions, mental health conditions, and to a lesser extent for ADHD. Only 6 (8%) of the families reported feeling knowledgeable about CIM, and 73% reported discussing concurrent use with their child's physician. Of the 92 CIM use reports, 8 mild adverse events were reported. Various modalities of CIM are commonly used by children with ADHD. Healthcare practitioners should initiate conversation about the use of CIM with families, provide reliable resources for information about safety and efficacy, and aid families in making an informed decision about the use of CIM.

3.2 INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder, defined by The Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) as “a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with function or development” (American Psychiatric Association 2013). Worldwide, ADHD affects an estimated 5-7% of children and youth (Polanczyk et al. 2007; Thomas et al. 2015). In pediatrics, common psychiatric disorders comorbid with ADHD include oppositional defiant disorder, conduct disorder, mood and anxiety disorder and learning disorder (Pliszka 1998; Spencer et al. 1999). Stimulant medications such as methylphenidate and salts of amphetamine are the first line of pharmacotherapy prescribed and used by pediatric patients with ADHD. These medications are effective but associated with a variety of side-effects and adverse events (Stevens et al. 2013). Most patients taking stimulants for ADHD experience some form of side-effects (Elia et al. 1991) including weight loss, abdominal pain, sleep disturbances, headaches, irritability, depressed mood, appetite loss, anorexia, motor tics, nausea, and fatigue (Ahmann et al. 1993; Efron et al. 1997). Twenty to thirty percent of children with ADHD do not respond to treatment with stimulant medication (Goldman et al. 1998). Non-stimulants such as atomoxetine and guanfacine are the second-line of pharmacotherapy prescribed to pediatric patients with ADHD. However, side-effects are still common with non-stimulants and include abdominal pain, vomiting, decreased appetite, dizziness, fatigue, irritability, and somnolence (Wilens et al. 2015; Cheng et al. 2007). Adverse effects contribute to pervasive concern about the use of stimulants and non-stimulants—specifically in children—and lead patients and families to look for alternative therapies (Sarris et al. 2011; Sinha et al. 2005).

According to the National Center for Complementary and Integrative Health (2018), Complementary and Integrative Medicine (CIM) includes a group of healthcare and medical systems, practices, and products not traditionally taught in conventional medical care, or that may have origins outside of Western medical practice. CIM includes natural health products (“dietary supplements” in the United States e.g., vitamins, minerals, herbal remedies, probiotics, amino acids and essential fatty acids), mind body practices (e.g., yoga, chiropractic and osteopathic manipulation, meditation), and other complementary health approaches (e.g., traditional and Indigenous healers, Ayurveda, Traditional Chinese Medicine, homeopathy, naturopathy) (National Center for Complementary and Integrative Health 2018). The use of CIM is widespread among children as studies indicate that 11%-33% of general pediatric population (Loman 2003; Ottolini et al. 2001; Sawani-Sikand et al. 2002; Spiegelblatt et al. 1994) and 12%-78% of children with chronic disorders use at least one form of CIM (Post-White et al. 2009; Galicia-Connolly et al. 2014; Adams et al. 2013; Gross-Tsur et al. 2003; Bussing et al. 2002; Ball et al. 2005; Soo et al. 2005; Hagen et al. 2003).

Worldwide, only a few studies have explored the determinants of CIM use among pediatric ADHD patients. Those published in Australia (Concannon et al. 2005; Sinha et al. 2005; Stubberfield et al. 1999), Israel (Gross-Tsur et al. 2003), and U.S.A. (Chan 2002; Huang et al. 2013; Bussing et al. 2002) report 12-71% of families report giving their child at least one CIM. Most families (58%-92%) perceived the CIM treatments to be helpful for their child (Sinha et al. 2005; Huang et al. 2013). These studies focused on ADHD symptom management and may not include the use of CIM for treatment of comorbid disorders, disease prevention or health maintenance, and therefore may underestimate the overall use of CIM. Though the frequency of CIM use has not been determined directly in pediatric patients with ADHD in Canada, the

frequency is likely high due to the chronic nature of the condition, occurrence with comorbid conditions, parental concerns about adverse events from conventional drugs, and desire for a natural or holistic approach (Kemper et al. 2013; Wang et al. 2018; Chan et al. 2003; Huang et al. 2013; Sinha et al. 2005; Stubberfield et al. 1999).

Natural health products (NHPs) and other CIM practices that use biological- or chemical-based products contain raw or processed substances that can modulate metabolic and physiological function. When NHPs are used concurrently with conventional drugs, they can lead to NHP-drug interactions that are mediated by pharmacokinetic and/or pharmacodynamic mechanisms (World Health Organization 2018).

The concurrent use of CIM (including NHPs) with prescription drugs has been reported frequently in pediatric psychiatry patients. A Canadian study identified 46% of pediatric neurology patients had used CIM concurrently with prescription drugs (Galicia-Connolly et al. 2014). Many of these families do not disclose their child's CIM use with their healthcare practitioners (Cala et al. 2003; Chan et al. 2003; Sinha et al. 2005). The risk of NHP-drug interactions may be prevalent for children with ADHD given that various types of NHPs (including vitamins and herbal medicines) are commonly used alongside psychostimulants on a regular and often long-term basis, without the prescribing physicians' knowledge (Galicia-Connolly et al. 2014). Systematically searching in the FDA database of adverse event reports, our group found 120 clinical adverse events involving commonly used herbal medicines in pediatric patients, and 19% of the reports involved both an herbal medicine and an ADHD drug. Based on expert adjudication using validated causality tools, the likelihood of an NHP-drug interaction was found possible and probable in two cases involving methylphenidate and St. John's wort (Mazhar et al. 2019). Therefore, research identifying NHPs used by ADHD patients,

their relative prevalence, and information on clinical adverse events is needed to guide future pre-clinical and clinical research targeting ADHD drug metabolism.

To our knowledge, research on determinants of CIM use, including concurrent use of pharmaceuticals, in pediatric patients with ADHD has not been conducted in Canada.

Accordingly, our objective was to perform a questionnaire-based study targeting children and adolescents with ADHD to evaluate: (i) the prevalence of the use of CIM for ADHD, comorbid disorders, disease prevention and health maintenance; (ii) the parental/family perceived level of knowledge of CIM; (iii) communication about CIM between families and healthcare practitioners; (iv) perceived helpfulness of CIM; and (v) self-reported adverse events from CIM use.

3.3 METHODS

The current study was approved by the Children's Hospital of Eastern Ontario Research Institute Research Ethics Board and the University of Ottawa Research Ethics Board.

3.3.1 Pilot

The questionnaire was piloted by 14 colleagues to estimate length, readability, and highlight any errors. On average, the survey took approximately 20 minutes to complete.

3.3.2 Sample

This research was based on a convenience sample of pediatric patients and caregivers who visited the Children's Hospital of Eastern Ontario (CHEO) ADHD Clinic between August 2016 and May 2019. Participants were eligible for the study if: 1) their child was under 18 years of age and 2) the child had an ADHD diagnosis according to the Diagnostic and Statistical

Manual 5 (DSM-V) guidelines. Both new and existing patients at the CHEO ADHD Clinic were eligible. Informed consent was obtained from all participants.

3.3.3 Questionnaire Design

The study was a one-time electronic questionnaire built and housed using the REDCap platform (REDCap 9.2.1- © 2019 Vanderbilt University) and was derived from a previous questionnaire by Adams et al. (2013). However, the questionnaire was modified and adapted for the purposes of this study. The questions in the survey (**see Appendix B for inactive survey link**) were binary (e.g., yes/no), scaled (e.g., Likert), fixed option (e.g., specific options plus “other”), and short open-ended questions (for demographics). The questionnaire (available in both French and English) contained contingency questions.

The caregivers completed a self-administered questionnaire regarding their demographic information, and their child’s ADHD (such as time since diagnosis, subtype of ADHD, prescribed medication, comorbid conditions).

The caregivers responded to questions about their child’s use and experiences with CIM. A general question about CIM use was followed by questions in each use report about specific modalities including nutrient and dietary supplements, elimination diet, homeopathic treatments, Aboriginal healing practices, traditional Chinese medicine and acupuncture, physical therapy, and herbal medicine. To obtain more information about herbal medicine use, we included products identified from the literature as used by pediatric ADHD and neurology outpatients (Mazhar et al. 2016), including echinacea, evening primrose oil, ginkgo, ginseng, goldenseal, kava, lemon balm, passion flower, pycnogenol, rhodiola, St. John’s wort, and valerian (Huang et al. 2013; Galicia-Connolly et al. 2014; Chan et al. 2000; Chan 2002; Cala et al. 2003; Pellow et al. 2011). In addition, participants could list up to 5 other forms of CIM that were not present in

the survey but that their child had used. Each question about CIM use was followed by contingency questions asking specific details about the use, including when it was used, reason for use, perceived helpfulness, adverse events, and recommendation of the product. Participants were given an open-ended question at the end to list any additional CIM not mentioned in the study (without additional detailed contingency questions about use).

3.3.4 Recruitment Procedure

The clinic schedule was examined on Epic Health Information Systems (© 2019 Epic Systems Corporation, Verona, Wisconsin, U.S.A.) to check when eligible patients had their appointments. The list of patients was provided to the front desk reception staff. During the patient's appointment reminder call, the front desk staff requested the family to come in 20 minutes early for a research study.

On the day of the appointment, the family was directed to the research assistant by the front desk staff. The research assistant gave background information and methodology about the study and asked if they would like to participate in the study. If the family agreed to participate, they were given the questionnaire on a tablet device. If participants were unable to complete the study before their medical appointment, the research assistant took a record of the return code and obtained the family's email address using the Consent Form for Disclosure of and Use of Email form. The survey link and return code were sent to the family to continue the survey. The electronic link to the survey housed in REDCap was sent to participants through REDCap (from a CHEO email address) using the "Participant List" feature. No identifiers (e.g., name) were entered into REDCap to maintain anonymity. The informed consent was obtained on the first page of the survey, and participants could choose to withdraw from the study at any time if they

felt uncomfortable with the questions being asked. There was no time limit for completing the survey, and it was completed on a voluntary basis with no compensation.

3.3.5 Data handling and record keeping

Data were stored securely on mobile devices (laptops and USB keys). Study data stored on mobile devices were de-identified, and electronic files were password-protected and encrypted. The data files will be retained for 7 years after the study closure, as recommended by CHEO Research Institute Privacy and Confidentiality Statement.

Email addresses of participants in REDCap were not stored with the study data. Individual responses were not linked to the email address to remain anonymity. Personal information (e-mail address collected to send the survey link) was kept strictly confidential. Communication on REDCap was encrypted using secure socket layer (SSL) with 256-bit encryption or higher.

3.3.6 Statistics

Statistical analysis was performed on SPSS (IBM Corp. Released 2017. IBM SPSS Version 25.0. Armonk, NY). Descriptive statistics including mean, median, frequencies, and percentages were calculated. Frequencies were presented where participants were able to pick multiple answers, and percentages do not equal 100% where participants could choose more than one response. Groups (CIM users and non-users) were compared using Chi-squared analysis or Fisher's exact test and $P < .05$ was considered statistically significant.

3.4 RESULTS

A total of 157 families were approached to participate in the study between August 2016 and May 2019, and 145 (92%) agreed to participate. Of the 145 survey links sent, 74 completed the questionnaire (response rate: 51%). Sixty-five families did not return the survey, and 6 left incomplete responses.

3.4.1 Parent/Caregiver Demographics

The informant was a parent in 90% of the cases. Four families completed the questionnaire in French. The caregivers were mostly female (87%) with an average age of 42.2 ± 7.7 years (**Table 3-1**). Caregivers reported their highest level of education as college (35%), university (26%), post-graduate degree (24%), high school (7%), and registered or trade apprenticeship (3%). The annual household income reported by families was <\$10,000 (4%), \$10-19,999 (7%), \$20-39,999 (11%), \$40-79,000 (13%), and >\$79,000 (27%). About 38% of families chose not to disclose their income. Caregiver culture group was self-identified as British Isle (40%), other (24%), European (8%), unknown (4%), Caribbean (3%), Aboriginal (1%), Arab (1%), Latin (1.3%), and South Asian (1.3%). However, differences in sociodemographic status of caregivers were not found to be factors associated with the child's CIM use.

3.4.2 Demographic and Descriptive Data for Child

The mean age \pm standard deviation of the patients was 10.7 ± 3.2 years (range: 4-18) and 80% were male (**Table 3-1**). Most of the patients were taking a stimulant including methylphenidate ($n = 35$ or 47%) or salts of amphetamine ($n = 30$ or 40%) at the time of participation. Overall, 50% of the patients were taking stimulants alone, 28% were taking both a stimulant and non-stimulant and 15% were taking non-stimulants alone (**Table 3-2**). Most patients (58%) had been diagnosed with ADHD for >3 years and were either combined subtype

(34%) or hyperactive impulsive subtype (30%). Ten (6%) of the patients surveyed did not have any self-reported comorbidities with other psychiatric disorders. The most common comorbid psychiatric disorder reported by parents were anxiety (49%), other disorders (40%), oppositional defiant (35%), and learning (31%). About 79% of patients were visiting the clinic for a follow-up with a treatment. The child's age ($P = .54$), sex ($P = .71$), ethnic/cultural group ($P = .57$), ADHD drug being taken ($P = .69$), time since diagnosis of ADHD ($P = .92$), clinical subtype ($P = .71$), comorbid conditions ($P = .37$), and reasons for visit to the clinic ($P = .23$) were not associated with the child's CIM use.

Table 3-1 Demographic information for the child and caregiver.

Characteristics	Total Frequency (%) <i>n</i> =74	CIM users Frequency (%) Total=30	CIM non-users Frequency (%) Total=44
Child			
Sex ¹			
Male	59 (80)	24 (80)	35 (80)
Female	12 (16)	4 (17)	8 (16)
Herbal medicine use	17 (23)	17 (57)	0
Parent/caregiver sociodemographic			
Highest level of education ¹			
High school	5 (7)	2 (7)	3 (7)
Registered apprenticeship/trade	2 (3)	0	2 (4)
College, CEGEP, non-university	26 (35)	7 (23)	19 (43)
Undergraduate university	19 (26)	11 (37)	8 (18)
Post-graduate university	18 (24)	9 (30)	9 (20)
Household Income ¹			
Less than \$10,000	3 (4)	0	3 (7)
\$10,000 to \$19,999/	5 (7)	2 (7)	3 (7)
\$20,000 to \$39,000	8 (11)	2 (7)	6 (14)
\$40,000 to \$79,000	10 (13)	5 (17)	5 (11)
Greater than \$79,000	20 (27)	12 (40)	8 (18)
Not sure	7 (9)	2 (7)	5 (11)
Ethnic/Cultural Group ¹			
Aboriginal	1 (1)	0	1 (2)
Arab	1 (1)	0	1 (2)
British Isle	30 (40)	15 (50)	15 (34)
Caribbean	2 (3)	0	2 (4)
European	6 (8)	2 (7)	4 (9)
Latin, Central, and South American	1 (1)	1 (3)	0
Other	18 (24)	8 (27)	10 (23)
South Asian	1 (1)	1 (3.3)	0
Unknown	3 (4)	2 (7)	1 (2)
CIM use ²	38 (51)	29 (97)	9 (20)
Herbal medicine use ²	28 (38)	23 (77)	5 (11)

¹ Total percentage may not total to 100% as some participants clicked “choose not to answer”

² Statistically significant ($P < .05$)

Table 3-2 Child's clinical and diagnostic information ¹.

Information	Total Frequency (%) <i>n</i> = 74	CIM users Frequency (%) Total=30	CIM non-users Frequency (%) Total=44
Time since ADHD diagnosis			
0-4 months	2 (3)	1 (3)	1 (2)
4-12 months	8 (11)	4 (13)	4 (9)
1-2 years	20 (27)	9 (30)	11 (25)
More than 3 years	43 (58)	16 (53)	27 (61)
Not sure	1 (1)	0	1 (2)
Subtype of ADHD ²			
Combined	25 (34)	12 (40)	13 (29)
Predominantly Hyperactive-Impulsive	22 (30)	9 (30)	13 (29)
Predominantly Inattentive	8 (11)	2 (7)	6 (14)
Not sure	17 (23)	7 (23)	10 (23)
Medication prescribed ²			
Not prescribed	4 (5)	2 (7)	2 (4)
Amphetamine	30 (40)	13 (43)	17 (39)
Atomoxetine	10 (13)	4 (13)	6 (14)
Clonidine	7 (9)	3 (10)	4 (9)
Guanfacine	10 (13)	5 (17)	5 (11)
Methylphenidate	35 (47)	14 (47)	21 (48)
Other	12 (16)	5 (17)	7 (16)
Comorbidity ²			
Anxiety disorder	36 (49)	17 (57)	19 (43)
Learning Disorder	23 (31)	10 (33)	13 (29)
Mood disorder	13 (18)	4 (13)	9 (20)
Oppositional Defiant Disorder	26 (35)	9 (30)	17 (39)
Sleep problems	16 (22)	10 (33)	6 (14)
Other	30 (40)	14 (47)	16 (36)
None	10 (13)	4 (13)	6 (14)
Not sure	3 (4)	1 (3)	2 (4)

¹No statistically significant difference were observed between CIM users and CIM non-users

²Total percentage may not total to 100% as some participants clicked "choose not to answer", and/or participants could choose multiple answers.

3.4.3 Use of CIM and Perceived Helpfulness

Thirty of seventy-four (40%) patients had used some form of CIM. Among these participants, the average number of CIM taken by patients was 3 ± 1.8 (range: 1-6) and the most commonly used treatments (**Table 3-3**) were nutrient/dietary supplements (90%), followed by herbal remedies (57%): echinacea (13%), evening primrose oil (13%), physical therapy (43%), elimination diet (37%), homeopathy (30%), Aboriginal healing practices (7%) and blue green algae (3%). Additional specific CIM reported as “other” included melatonin by 14 participants (47%), with 6 providing details about their use. Omega-3-rich fish oil was listed by 7 participants, with 3 providing details on use. Iron, Equazen™ (source of essential fatty acids), Natural Calm® (magnesium supplement), chlorella, lavender oil, eucalyptus oil, energy, light, meditation, relaxation, and yoga, were each listed once alongside details of use. A total of 17 specific CIM treatments were reported in the open-ended question (without specific questions about use) (**Appendix C, Table C-1**).

Thirty-eight (51%) caregivers reported use of CIM for themselves and children were more likely to use CIM if their caregiver reported use as well ($P < .0001$). Amongst CIM users, 97% of the caregivers were also using CIM, whereas only 20% of the caregivers used CIM in the CIM non-user group. Specifically, about 38% of the caregivers reported using herbal medicine, and caregiver herbal medicine use was associated with the child’s herbal medicine use ($P = .004$).

Although reason for use varied when asked about specific (**Table 3-3**) considering CIM use for all modalities reported (92 reports), treating ADHD symptoms was mentioned 47 times (51%), treating symptoms of other mental health conditions was mentioned 46 times (50%), maintaining general health was mentioned 44 times (48%), and treating physical health problems

(including colds, flu, and allergy symptoms) was mentioned 30 times (33%). Perceived helpfulness of CIM varied among specific CIM treatments. Among families who used CIM for general health, for other mental health conditions, or physical health, 68%, 53% and 54% respectively reported CIM to be “pretty helpful” or “very helpful”. In contrast, 70% of families who used CIM for ADHD reported it to be “a little helpful” or “not at all helpful”.

Across the 92 CIM use reports, participants reported taking the product or seeking therapy following the recommendation of a physician (39%), a CIM practitioner (22%), other parents (20%), a pharmacist (12%), community members (12%), or teachers (9%). Interestingly, 34% of participants reported that the product/therapy was self-recommended by the caregiver. About 60% of CIM users and 23% of CIM non-users agreed that CIM is in line with their beliefs, and these differences were statistically significant ($P = .008$) between CIM users and non-users.

Overall, 64 of the 92 (70%) use reports stated using some modality of CIM concurrently with prescription drugs for ADHD. Specifically, caregivers reported (**Figure 3-1**) their child using Aboriginal healing practices (100% or 2 of 2), other CIM (85% or 11 of 13), nutrient and dietary supplements (82% or 22 of 27), physical therapy (77% or 10 of 13), elimination diet (73% or 8 of 11), homeopathy (56% or 5 of 9), herbal medicine (35% or 5 of 11), concurrently with ADHD drugs. For CIM users, 15 (50%) reported the prescribing physician to be favorable of CIM use concurrently with prescribed medication, 5 (16.6%) reported physician to be neutral, and 8 (27%) reported not discussing the concurrent use with the prescribing physician.

Table 3-3 Total frequency and reasons for use for various modalities of Complementary and Integrative Medicine by pediatric patients with ADHD based on completed use reports for 30 CIM users.

	Reason for CIM use ¹												
	Total Frequency of use (n)	ADHD			Other Mental Health Conditions			Physical Health			General Health Maintenance		
		Perceived Helpfulness			Perceived Helpfulness			Perceived Helpfulness			Perceived Helpfulness		
		Frequency of use (n)	Not at all/ a little (n)	Pretty or very (n)	Frequency of use (n)	Not at all/ a little (n)	Pretty or very (n)	Frequency of use (n)	Not at all/ a little (n)	Pretty or very (n)	Frequency of use (n)	Not at all/ a little (n)	Pretty or very (n)
Aboriginal Healing Practices	2	1	0	1	2	0	2	2	0	2	2	0	2
Echinacea	4	0			0			3	1	2	2	1	1
Elimination Diet	11	9	5	4	6	2	4	2	0	2	4	1	3
Evening primrose oil	4	1	1	0	1	1	0	1	1	0	2	2	0
Homeopathy	9	2	1	1	4	3	1	6	4	2	3	0	3
Nutrient and Dietary Supplements	27	13	11	2	12	5	7	7	2	5	21	6	15
Physical Therapy	13	9	5	4	5	1	4	5	4	1	4	2	2
Lavender Oil	2	1	1	0	2	1	1	0			0		
Melatonin	6	2	1	1	5	2	3	0			1	0	1
Omega fish oils	3	3	2	1	0			0			1	0	1
Other single use reports ²	11x1	6	6	0	9	6	3	4	2	2	4	2	2
Total	92	47	33	14	46	21	25	30	14	16	44	14	30

¹ Respondents could choose multiple responses

² Other single CIM reported include: chlorella, energy, Equazen, eucalyptus oil, iron, light, meditation, Natural Calm, relaxation, blue-green algae, and yoga

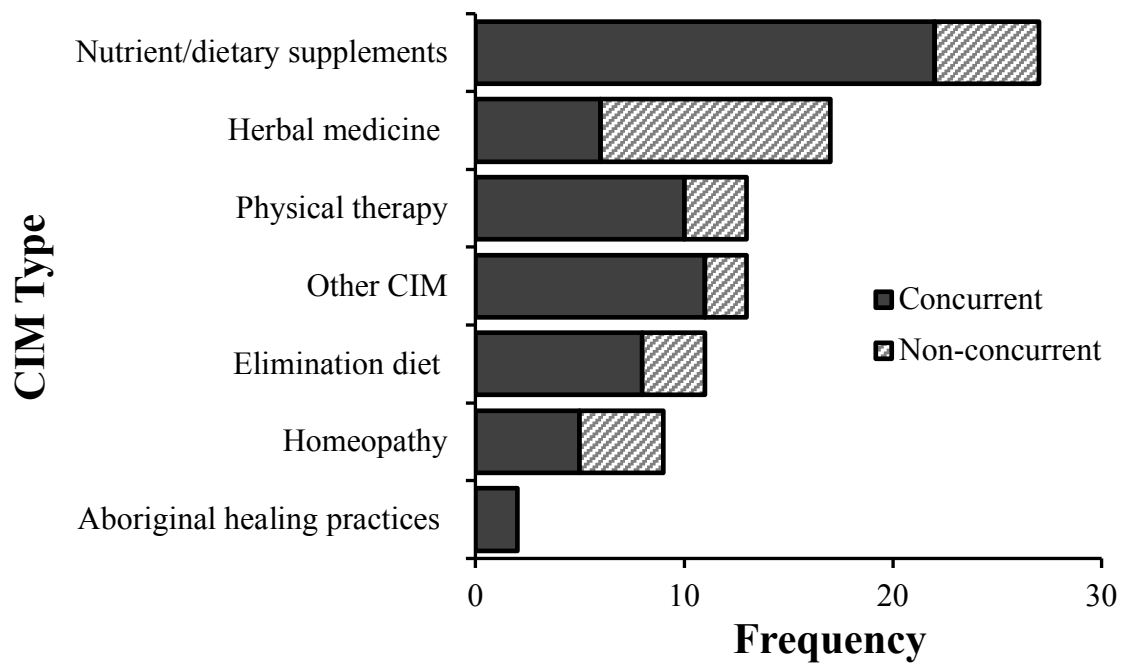


Figure 3-1 Frequency of complementary and integrative medicine (CIM) used concurrently with ADHD prescription medicine by pediatric patients.

3.4.4 Adverse events

Eight mild adverse events were self-reported among 92 specific use reports provided by CIM users. These events were associated with use of nutrient and dietary supplements ($n = 2$), elimination diet ($n = 3$), physical therapy ($n = 1$), melatonin ($n = 1$), and Natural Calm TM ($n = 1$). In seven of these reports, caregivers reported concurrent use with ADHD medication.

3.4.5 Barriers for CIM use

The most common barrier(s) to using CIM reported by participants was lack of knowledge (49%), followed by concerns about lack of efficacy (35%), lack of insurance coverage (28%), potential negative effects (22%), excessive cost (20%), lack of access (8%), that conventional medicine is sufficient (3%), and that CIM are not in line with beliefs (1%) (**Figure 3-2**). Lack of knowledge ($P = .030$) and concerns about potential negative effects ($P = .45$) were barriers to CIM use that were significantly different between CIM users and non-users, and both were more often reported by non-CIM users.

3.4.6 Discussion about CIM with Healthcare Practitioners

CIM users reported most frequently discussing the use/potential use of CIM (**Figure 3-3**) with physicians (73%), followed by family or friends (60%), teachers (57%), other parents (47%), pharmacist (37%), other healthcare professionals (33%), CIM practitioner (27%), and with no one (10%). Participant who did not use CIM generally did not discuss potential use with anyone (68%), and less often with a physician (18%), and other parents (15%).

When asked if they feel comfortable talking to their child's physician about CIM, 70% of CIM users, and 47% of CIM non-users, agreed with the statements. Moreover, 63% of CIM users and 45% of CIM non-users agreed that their child's physician's opinion on CIM could influence their child's CIM use. There was no significant difference between CIM users and non-

users regarding degree of comfort in talking about CIM ($P = .42$) or influence of physician opinion on CIM ($P = .41$). Topics most often reported as “pretty helpful” to “very helpful” to discuss with healthcare practitioners by all participants included efficacy (70%), potential negative effects (68%), and concurrent use with drugs (68%).

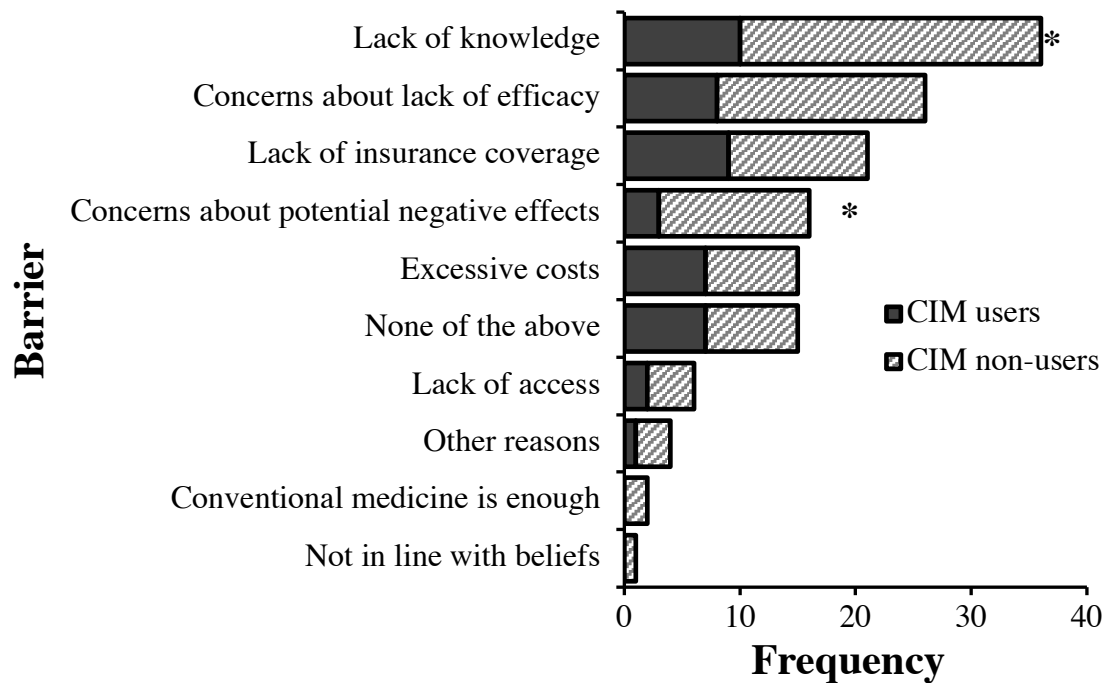


Figure 3-2 Barriers to Complementary and Integrative Medicine (CIM) use as reported by CIM users and non-users from the caregiver's perspective. Statistically significant ($P < .05$) differences are marked by *, as determined by Fisher's exact test. Overall percent does not equal 100% as respondents could choose multiple responses.

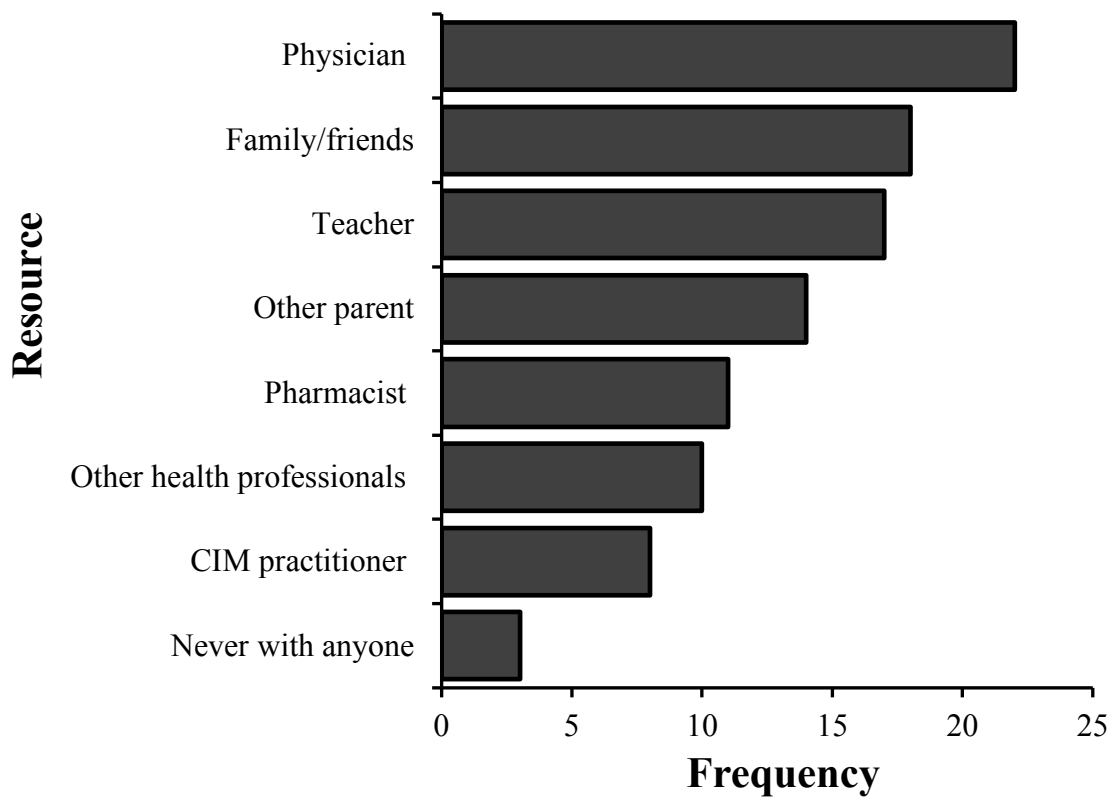


Figure 3-3 Frequency of reports by caregivers of CIM users about discussions of their child's use and/or potential use of Complementary and Integrative Medicine (CIM) with various sources. Overall percent does not equal 100% as respondents could choose multiple responses.

3.4.7 Information Sources for CIM

The sources of information about CIM most commonly consulted by all participants ($n = 74$) (**Figure 3-4**) include physicians (35%), friends or family (30%), other internet websites (27%), books/magazines (22%), and pharmacist (22%). Thirty-two (3 CIM users, 29 CIM non-users) participants did not consult any resources. Information sources rated most often as “pretty helpful” to “very helpful” include CIM practitioner (88%), health food store (75%), books/magazines (75%), and other internet websites (74%). Interestingly, physicians (46%), television (40%), and government and official websites (30%) were rated least often as “pretty helpful” to “very helpful” for CIM information.

Five (17%) CIM users and one (2%) non-user agreed that they felt knowledgeable about CIM. In contrast, one third (33%) of CIM users and more than half (52%) of non-user disagreed or strongly disagreed with feeling knowledgeable about CIM. No significant differences were found between CIM users and non-users regarding perceived knowledge of CIM.

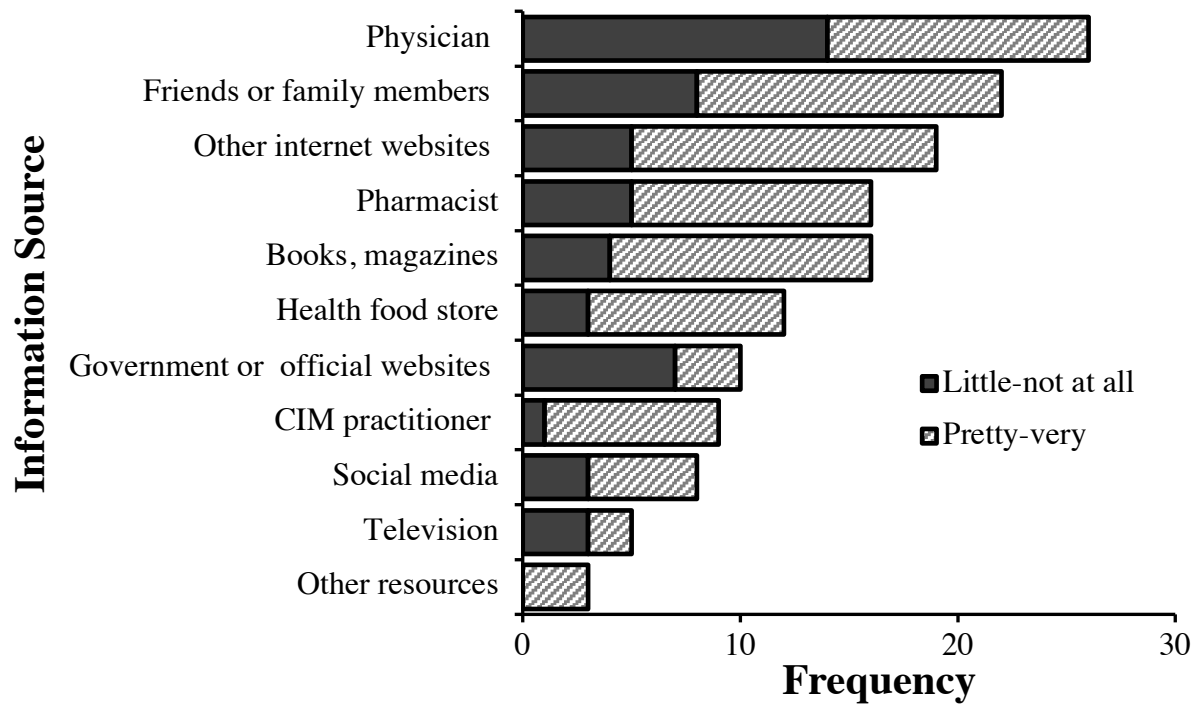


Figure 3-4 Information sources consulted about Complementary and Integrative Medicine (CIM) and their perceived helpfulness (little to not at all, or pretty to very helpful), by all participants. Overall percent does not equal 100% as respondents could choose multiple responses.

3.5 DISCUSSION

To our knowledge, this is the first survey evaluating CIM use in Canada at an ADHD outpatient clinic in Canada. Our study provides novel information on the use of CIM in ADHD pediatric patients including specific reasons for uses, and perceived helpfulness for each use.

This study found 40% of children with ADHD attending CHEO ADHD outpatient clinic had ever used some modality of CIM. This is within the range of use (12-54%) (Bussing et al. 2002; Huang et al. 2013; Chan et al. 2003) in pediatric ADHD studies in the United States, however, lower than similar studies in Australia (64-71%) (Sinha et al. 2005; Stubberfield et al. 1999; Concannon et al. 2005).

We saw higher rates of CIM use than studies conducted in North America in general pediatric settings (11%-31%) (Loman 2003; Ottolini et al. 2001; Barnes et al. 2008; Spiegelblatt et al. 1994; Sawni-Sikand et al. 2002). The higher rates of CIM use in ADHD compared to general pediatric population could be due to the reported comorbidities with other conditions, side-effects from conventional medicines, and the chronic nature of ADHD. However, CIM use in our study was lower than some studies in pediatric chronic disease (61-78%) (Post-White et al. 2009; Adams et al. 2013; Ball et al. 2005; Hagen et al. 2003). Variances in rates CIM use could be due to differences in study population, sample size, prevalence measures (e.g., ever used vs. use in the past year), and CIM definition (some studies exclude dietary supplements). On the other hand, our results were similar to two separate studies with a CHEO subpopulation, (neurology and speciality outpatient) demonstrating 42% (Adams et al. 2013) and 48% (Galicia-Connolly et al. 2014) of CIM use.

Socioeconomic variables in our study did not influence CIM use. The lack of association between CIM use and socioeconomic variables could present difficulty in identifying ADHD

patients more likely to use CIM. A possible explanation for socioeconomic variables not being predictors for CIM use could be that caregivers tend to use CIM for the most complex cases (those with comorbidities and low general health), those who had delays in healthcare due to accessibility to specialists, those who delayed diagnosis/treatment by replacing conventional medicine with CIM (Birdee et al. 2010). However, children were more likely to use CIM if their parents used CIM. This association is evident in other studies that explored CIM use in pediatric populations in general (Loman 2003; Ottolini et al. 2001; Sawni-Sikand et al. 2002), as well as ADHD specific studies (Sinha et al. 2005), and pediatric neurology studies (Soo et al. 2005). On the other hand, a study by Spiegelblatt et al. (1994) at a Canadian pediatric outpatient clinic in Quebec found that older children (>1 years), with highly educated mothers were more likely to use CIM. However, their findings relied on data for infants, and our population tended to be school aged patients (4 years or older).

The most popular therapies reported in our study were nutrient and dietary supplement, herbal medicines, physical therapy, elimination diet, and homeopathy. These are consistent with most reported modalities of CIM in other ADHD studies in U.S.A. (Chan et al. 2003) and Australia (Sinha et al. 2005), and a neurology study in Canada (Galicja-Connolly et al. 2014). Compared to Cala et al. (2003), we saw lower prevalence of use for specific herbal medicines such as echinacea, ginkgo, St. John's wort, ginseng, chamomile, valerian, ginger, and peppermint. Although our study examined CIM use including herbal medicine, Cala et al. (2003) specifically focused on herbal remedies. Cala et al. (2003) also examined herbal medicine use in ADHD or depression, and the specific uses were undistinguished for each condition. This likely overestimated CIM use for ADHD, and therefore complicates comparison with our study.

Although not specifically included in our study, melatonin and omega fish oils were frequently mentioned as other specific treatments used by patients. Melatonin (Bendz et al. 2010; Abdelgadir et al. 2018) and omega fish oils (Bloch et al. 2011; Hawkey et al. 2014) are well-tolerated and efficacious treatment options for symptoms observed in children with ADHD. There is evidence for a pharmacokinetic interaction between melatonin and citalopram (an antidepressant). Melatonin is a substrate (CYP 1A2, CYP 2C19) and inhibitor (CYP 1A2, CYP 2C19, CYP2D6, CYP 3A4 and CYP 3A7) for various CYP 450 enzymes *in vitro* (Ma et al. 2005; Foster et al. 2015). Although there are no known interactions between omega-3-rich fish oils and ADHD drugs, omega-3-rich fish oils inhibit CYP 2D6, CYP 3A4, and CYP 2C19 *in vitro* (Strandell et al. 2004). There warrants further study of both melatonin and omega-3-rich fish oils in the context of ADHD, as adverse events could likely occur in the cases of polypharmacy.

The majority of the families found CIM to be helpful for general health maintenance, for physical health conditions, and for other mental health conditions. However, only 30% of those using CIM for ADHD found them to be helpful for treating ADHD symptoms. Previous studies found most families found CIM to be helpful (Sinha et al. 2005; Soo et al. 2005; Galicia-Connolly et al. 2014), however, these studies did not differentiate between specific reasons of use or how the treatment helped. Cala et al. (2003) reported herbal remedies to be the most helpful for ADHD, but since the questionnaire is not available for viewing, it can be assumed this comparison was only made with children with depression. Nonetheless, prevalence of use is likely to remain high due to perceived helpfulness in one or more health categories in ADHD.

Concurrent use of some CIM (specifically natural health products) can pose a risk for interactions with conventional drugs or other natural health products. For example, evidence

from *in vitro* (Komoroski et al. 2004; Obach 2000) and *in vivo* (J S Markowitz et al. 2003) studies demonstrate that St. John's wort, a herbal medicine used for depression, modulates cytochrome P450 activity. St. John's wort has been found to clinically interact with several central nervous system drugs, some of which include anxiolytics (Dannawi 2002) and antidepressants (Prost et al. 2000). Preliminary *in vitro* research in our group found several herbal medicines (including rhodiola, rosemary, peppermint, ginkgo, and ginger) to inhibit carboxylesterase-1 mediated activity of marker substrates (Mazhar et al. 2019-unpublished work presented in Chapter 5 of this thesis). Accordingly, concurrent use of NHPs with drugs can pose a risk for patients taking ADHD drugs including stimulants and non-stimulants.

In the current study, concurrent use of CIM and conventional ADHD medicine was common and comparable to the study at a neurology clinic at CHEO (67%) (Galicia-Connolly et al. 2014). Specifically, for natural health products, we found herbal remedies and nutrient and dietary supplements were used most often with ADHD drugs, which is also consistent with the study at an affiliated CHEO site by Galicia-Connolly et al. (2014). For CIM users, 27% did not disclose their child's concomitant use with prescription drugs to their physician. Although there were no serious adverse events reported, 8 mild adverse events related to the use of CIM were reported. Half of the adverse events reported were from the use of a natural health product. Similarly, a Canadian pediatric surveillance program survey found majority of the adverse events reports by pediatric CIM users to be associated with the use of natural health products (Vohra et al. 2009). The incidence of CIM related adverse events, coupled with both the lack of inquiry and disclosure of CIM use with physicians, can affect patient safety. Hence, it is imperative for healthcare practitioners to be aware of patient CIM use and foster a space that allows for open and non-judgemental discussion about CIM to avoid unfavorable interactions and adverse events.

Lack of knowledge of CIM was the most common barrier to CIM use reported among participants. The majority of the respondents did not feel knowledgeable about CIM and reported that knowledge about the efficacy, potential negative effects, and concurrent use with conventional drugs would be the most useful to discuss. Most CIM users reported consulting a physician as a source of information for CIM. A survey at a pediatric specialty outpatient clinic in Canada (Edmonton and Ottawa) identified other families as well as CIM and conventional healthcare practitioners to be most often sought and highly trusted sources of knowledge for CIM (Adams et al. 2013). It is evident that families consult and trust healthcare practitioners about CIM, however, approximately 50% of respondents reported physicians to be only “a little helpful” or “not at all helpful”, while sources such as health food stores, books/magazines, social media, and other internet websites (i.e., not government or official websites) were deemed more helpful. Interestingly, less than 5% of physicians feel knowledgeable about CIM (Kemper et al. 2004), and 88% agreed (or strongly agreed) that they needed to gain more knowledge to properly counsel patients on CIM (Patel et al. 2017). Other sources not found to be helpful were government or non-government official websites, and television. Though it is unclear why families find one information source more helpful than another, accessibility and/or promptness of information may play a role.

There is a lack of rigorous scientific research on CIM, especially regarding children. The quality of information from informal sources commonly accessed by patients, such as health food stores, social media, and non-official internet websites, can be highly variable and based on anecdotal evidence or testimonials (Kelner et al. 2003). Moreover, when the sources are directly associated with commercial products or websites, the provided information can be biased towards obvious financial interests. Hence, healthcare practitioners need improved knowledge on

CIM, including recognizing unsafe combinations, and support families to make decisions based on credible evidence.

Potential limitations in our study include low sample size and the non-completion rate, which limited statistical power for comparison between sub-populations. Notably, however, similar studies had comparable sample sizes and non-completion rates (Soo et al. 2005; Jean et al. 2007; Huang et al. 2013; Chan et al. 2003; Doering et al. 2013). Since the study was based on self-reporting and used parents as a proxy, there could be recall bias. The validity of the study could be strengthened by including a comparison group (e.g., non-ADHD children, or children with other conditions). Moreover, patients may have used other CIM treatments that were not specifically referenced in the survey. For example, melatonin, omega-3 fatty acids, and essential oils were not specifically included in our study, but were frequently added by respondents. This limitation, likely resulting in underestimates of CIM in the study, reflects the inherent difficulty in studying CIM, as its definition and scope varies across studies and regulatory districts.

3.6 CONCLUSION

In our study, 40% of pediatric patients with ADHD used some modality of CIM, and 57% of those using CIM used herbal medicine. Most families found CIM them helpful for general health maintenance, other mental health conditions, and physical health conditions. Parental CIM use was positively associated their child's use. Given the lack of evidence of efficacy and safety for CIM in pediatric population, this study highlights the necessity for improved communication about CIM between health care practitioners, and families. Future studies should investigate herb-drug interactions, and clinical adverse events for pediatric patients, and build a foundation of knowledge of safe and efficacious therapies for children.

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Chapter 4

Natural Health Product-Drug Interaction Causality Assessment in Pediatric Adverse Event Reports Associated with Attention-Deficit Hyperactivity Disorder Medication

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Author's Contributions

BCF, CN, CSH, HM, PMG, and PR contributed to the design of the study. Data collection and analysis was performed by BCF, CN, CSH, HM, PMG, and PR. HM drafted the manuscript.

BCF, CN, CSH, HM, PMG and PR revised, and approved the final manuscript.

4.1 ABSTRACT

Some pediatric patients with attention-deficit hyperactivity disorder (ADHD) use Natural Health Products (NHPs) such as herbal remedies. Although herbal remedies are generally considered to be safe when they are used appropriately, they may contain active components that can interact with medications being used concurrently, with potential for NHP-drug interactions leading to adverse events. The objective of this study was to: i) identify adverse event reports (AERs) involving commonly used herbal remedies and ADHD prescription medicines in children and adolescents; ii) to evaluate the quality of collected AERs; and iii) to assess whether NHP-drug interactions can be causally linked to reported adverse events. We systematically searched the FDABLE database (FDABLE.com) for herbal remedies commonly used by patients (4-18 years old) also taking ADHD drugs from 1997 to 2015. We assessed the completeness of the AERs and used three causality assessment tools modified for NHPs (Naranjo Adverse Drug Reaction Probability Scale, HORN Drug Interaction Probability Scale, and World Health Organization Uppsala Monitoring Centre Scale). Of the 23 identified AERs involving both an herbal remedy and an ADHD prescription medication, most involved multiple (>3) substances with inadequate detail to assess multiple potential interactions. Following data extraction and evaluation of completeness, five AERs involving only one herbal remedy and one ADHD medication were evaluated for causality. An NHP-drug interaction was assessed to be probable in one case and to be possible in another. Both these reports involved a methylphenidate formulation and St. John's wort. Eighteen of the 23 identified AERs involving both an herbal remedy and an ADHD drug also involved other multiple ingredient products. The reporting quality was poor for the five AERs examined. Further research is needed to study the interaction between St. John's wort and methylphenidate.

4.2 INTRODUCTION

Complementary and Integrative Medicine (CIM) encompasses a group of healthcare and medical systems, practices, and products that are not typically taught in conventional medicine training and includes natural health products (NHPs), mind body practices (e.g., yoga, chiropractic and osteopathic manipulation, meditation), and other complementary health approaches (e.g., traditional healers, Ayurveda, Traditional Chinese Medicine, homeopathy, naturopathy) (National Center for Complementary and Integrative Health 2018). NHPs, also known as dietary supplements in the United States, represent the most widely used form of CIM and include vitamins, minerals, herbal remedies, probiotics, amino acids, and essential fatty acids. In the U.S.A., they are regulated by the U.S. Food and Drug Administration (U.S. Food and Drug Administration 2018). In Canada, NHPs include vitamins and minerals, herbal remedies, homeopathic remedies, traditional medicine, probiotics, and other products such as amino acids and essential fatty acids and are regulated by the Natural and Non-prescription Health Products Directorate (NNHPD) of the federal government (Health Canada 2015).

In 2016, Canadians and Americans spent \$8.8 billion and \$28.3 billion, respectively, on CIM therapies out-of-pocket (National Center for Complementary and Integrative Health 2016; Esmail 2017). For children, an estimated \$1.9 billion was spent in 2016 by Americans (National Center for Complementary and Integrative Health 2016). This represents a large portion of out-of-pocket spending as the use of CIM becomes more mainstream. According to the 2010 Ipsos Reid survey, 73% of Canadians had used NHPs and 32% used them on a daily basis (Ipsos 2011). In the United States, 75% of adults had used dietary supplements (Council for Responsible Nutrition 2018).

Surveys investigating CIM use in North American pediatric populations suggest that up to 40% of healthy children (Barnes et al. 2008; Jean et al. 2007; Loman 2003; Ottolini et al. 2001; Spiegelblatt et al. 1994) and up to 75% of children with chronic illness use some form of CIM (Ball, Kertesz, and Moyer-Mileur 2005; Neuhouser et al. 2001; Post-White et al. 2009). The use of CIM in general tends to be higher in children with mental health conditions and amongst these children, those with attention-deficit hyperactivity disorder (ADHD) are the most common users of CIM (Adams et al. 2013; Birdee et al. 2010; Huang et al. 2013; Kemper, Gardiner, and Birdee 2013; Wang et al. 2018). CIM use for ADHD tends to be higher due to the chronic nature of the condition, occurrence with comorbid conditions, parental concerns about adverse events from conventional drugs, and desire for a natural or holistic approach (Kemper et al. 2013; Wang et al. 2018; Chan et al. 2003; Huang et al. 2013; Sinha et al. 2005; Stubberfield et al. 1999).

Among the various modalities of CIM, herbal remedies are one of the most commonly used NHPs by pediatric neurology patients and ADHD patients (Wang et al. 2018; Chan et al. 2003; Sinha et al. 2005). Though there is lack of research on the concurrent use of NHPs with conventional psychostimulants prescribed, herbal remedies have previously been reported to be used commonly alongside psychostimulants (Hazell et al. 1996; Galicia-Connolly et al. 2014). Herbal remedies or botanicals, are derived from plant materials or preparations from one or more plants, and have gained particular attention due to their complex nature (World Health Organization 2015; World Health Organization 2005a). While herbal remedies are generally safe when used properly, they are not risk free (Health Canada 2015) and can pose a risk of interactions with pharmaceutical drugs, potentially rendering these treatments ineffective or toxic, resulting in adverse events (Birdee et al. 2010; Noras et al. 2013; World Health Organization 2015). Herbal remedies, either as single entity or mixed formulations, can contain

raw, or processed medicinal and non-medicinal ingredients, that may be bioactive or non-active substances. They can act synergistically, antagonistically, or additively to each other, and to other substances being used concurrently, with potential for interactions. This may affect efficacy and/or result in adverse events (Benzie et al. 2011; Cheng et al. 2010).

According to the International Conference on Harmonization (ICH) (1994), an adverse event is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment”. Serious adverse events include those that require or prolong hospitalization, cause congenital anomalies, birth defects, or persistent or significant disability, are life threatening, or result in death. Health Canada and the U.S. Food and Drug Administration (FDA) requires all suspected serious or unexpected adverse events to be reported to the Canada Vigilance Program through MedEffect Canada or MedWatch FDA. These reports are then studied for pharmacovigilance activities including determining safety signals for products which can highlight potential associations between an adverse event and a health product (Health Canada 2018).

Adverse events are generally under reported, especially when they involve NHPs. Previous studies show that 91-99% of adverse events are not reported (Fletcher 1991; Martin et al. 1998; Hazell et al. 2006). One of the main reason behind underreporting of these events is that patients do not disclose the use of NHPs to their healthcare practitioners (Hussain 2011; Vohra et al. 2012). Other reasons include difficulty identifying adverse events related to NHPs, how to report NHP specific reactions, finding the reaction not severe enough to report, and occupational time constraints (Hohl et al. 2018; Barnes et al. 1998; Ide et al. 2016; Vickers et al. 2006). Various surveys about the use of CIM and NHPs show 56%-72% of patients did not disclose the use of

NHPs to their healthcare practitioners (Eisenberg et al. 2001; Jean et al. 2007; Jou et al. 2016; Kennedy 2005). For pediatric patients, only 30-65% of families discuss NHP use with healthcare practitioners (Adams et al. 2013; Cala et al. 2003; Chan 2002; Galicia-Connolly et al. 2014). Although some healthcare practitioners believe CIM can offer benefits, many are concerned about potential side-effects, the delay in use of approved treatments, patients taking CIM as an alternative to conventional medicine, and interferences with doctor-patient communication (Kemper et al. 2004; Wardle et al. 2014). Given the popular use of NHPs, specifically herbal remedies, alongside pharmaceuticals in pediatric ADHD and the lack of communication about NHPs between practitioners and families, the safety of concomitant use warrants more investigation, especially adverse events potentially caused by NHP-drug interactions.

Accordingly, the objective of this study was to identify adverse event reports (AERs) involving commonly used herbal remedies and ADHD prescription medicines, to evaluate the quality of collected AERs, and to assess whether NHP-drug interactions can be causally linked to reported adverse events.

4.3 METHODS

We searched the literature and identified 22 herbal remedies (**Table 4-1**) used by pediatric patients with ADHD or other neurological disorders (Cala et al. 2003; Chan 2002; Chan et al. 2000; Chan et al. 2003; Galicia-Connolly et al. 2014; Pellow et al. 2011). AERs involving the 22 herbal remedies were searched and identified on the FDABLE LLC database (Glastonbury, CT U.S.A., www.fdable.com). FDABLE contains data from FDA's drug Adverse Event Reporting System (FAERS). We chose to search on FDABLE as it contains reports submitted in the U.S.A., and internationally, and provides the most amount of open access information at the case level

compared to other adverse event reporting systems (Fouretier et al. 2016). Identified reports meeting the inclusion criteria were obtained from the U.S Food and Drug Administration Freedom of Information. An expert committee of adjudicators assessed these reports for quality, and probability of NHP-drug interaction using various completeness and causality scales. The researchers did not have access to patient or reporter identifiers such as name or contact information.

4.4 IDENTIFICATION OF ADVERSE EVENT REPORTS

We systematically searched the FDABLE LLC database using the advanced search option. (See **Figure 4-1** for systematic search flow chart) We searched for 22 herbal remedies used by patients (4-18 years old) from 1997 to 2015. Herbal remedies were searched using their common name, genus, and alternative names/spelling (if applicable) (**Figure 4-1**).

For each AERs identified for a given herbal remedy, a sub search was performed for reports that also included ADHD drugs (search terms included generic and brand names for different formulations): methylphenidate (Biphentin ®, Concerta ®, Daytrana ®, Focalin ®, Metadate ®, Methyllin ®, Quillivant ®, and Ritalin ®), amphetamine (Adderall ®, Dexedrine ®, Dextrostat ®, Procentra ®, Vyvanse ®, and Zenzedi ®), guanfacine (Intuniv ®) and atomoxetine (Strattera ®). Total number of reports for each herbal remedy, and number of reports with ADHD drugs was recorded. The case number, primary suspect drug(s), adverse reactions, patient outcomes, manufacturer sending report, age (years), sex, and event date were recorded and submitted to U.S. Food and Drug Administration Freedom of Information to obtain full copies of the report.

Table 4-1 Search terms including common name and genus used for the systematic search on FDable.com (http://fdable.com/advanced_aers_query) for herbal remedies used by pediatric patients (4-18 years).

Herbal remedy common name/alternative spelling (if applicable)	Genus name
Bacopa	Bacopa
Chamomile/camomile	Matricaria
Echinacea	Echinacea
Eleuthero	Eleutherococcus
Evening primrose oil	Oenothera
Garlic	Allium
Ginger	Zingiber
Ginkgo/ginkgo	Ginkgo
Ginseng	Panax
Goldenseal/golden seal	Hydrastis
Green tea	Camellia
Kava kava	Piper
Lemon balm	Melissa
Linden	Tilia
Passion flower	Passiflora
Peppermint	Mentha
Pycnogenol	Pinus
Rhodiola	Rhodiola
Rosemary	Rosmarinus
Skullcap/skull cap	Scutellaria
St. John's wort	Hypericum
Valerian	Valeriana

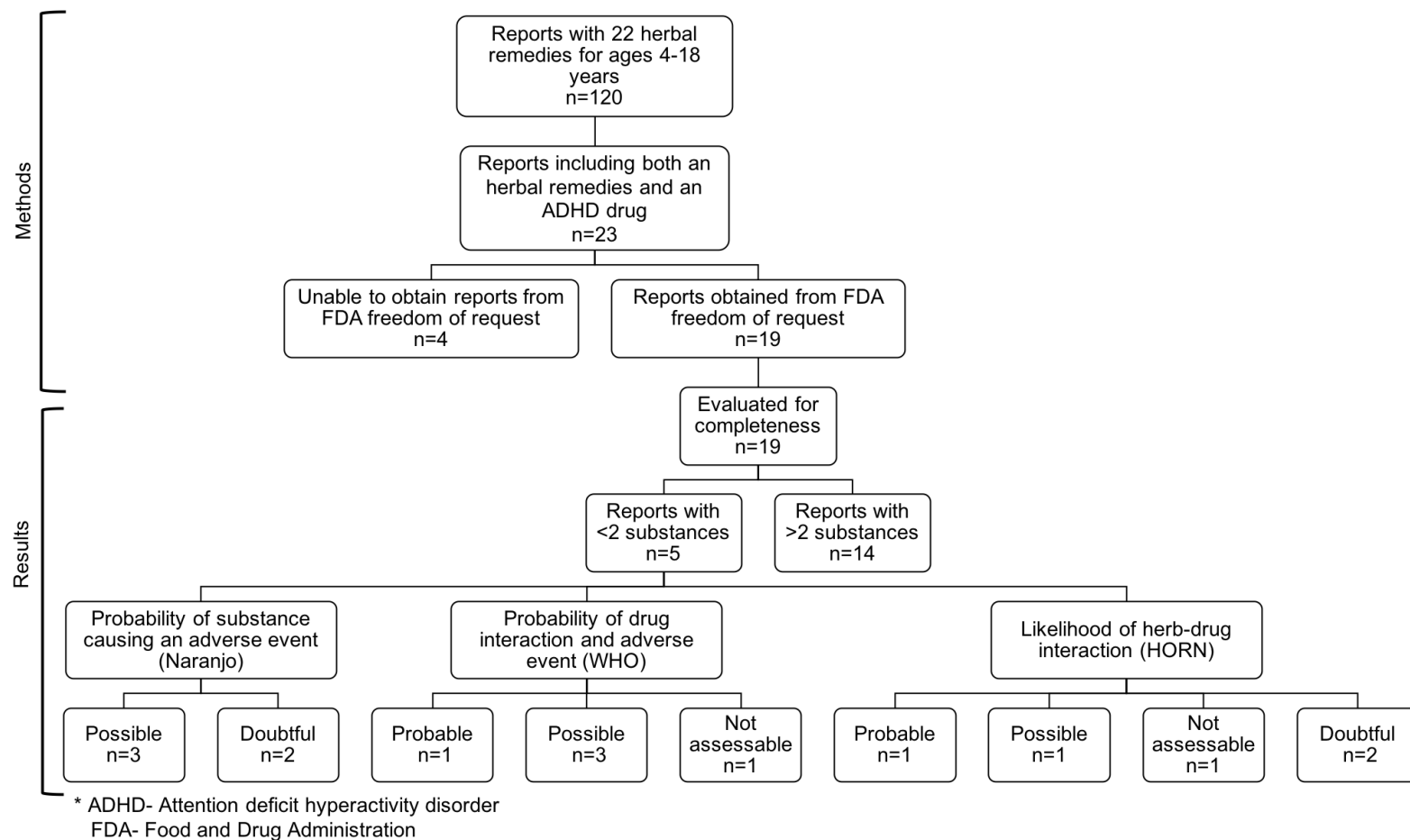


Figure 4-1 Flow chart of systematic search results from 1997-2015 from FDABLE of adverse event reports found for commonly used herbal remedies by children (4-18 years) (http://fdable.com/advanced_aers_query).

4.4.1 AER Data Extraction

We used the Gardiner Extraction Tool (Gardiner et al. 2013) to mine, collect, and organize data from each case report. The extraction form contained a total of 33 fields for patient information, adverse event, health information, lab tests, and herbal product information.

4.4.2 Completeness scales

Three completeness scales were used to assess the quality of the AERs. The Berman and Ernst Scale (Fugh-Berman et al. 2001) is a 10-point scoring system used to confirm whether reports contain adequate information to be further assessed for NHP-drug interaction causality. Reports with scores between 0-3 are unevaluable, 4-7 have some evidence upon which to evaluate an interaction, and 8-10 represent well-documented reports.

The Gardiner Adherence Scale, was developed and adapted for herbal remedies by Gardiner et al. (2013) and adhered with the Guidelines for Submitting Adverse Event Reports for Publication based on the International Society of Epidemiology criteria (Kelly et al. 2007). The scale shows the basic level of information that is necessary in pediatric case reports of adverse events involving an herbal remedy use. The total adherence score was out of 17.

Lastly, we used the Gardiner Extraction Tool as a secondary measure of completeness to provide specific details about missing information relevant to NHPs. Each field present was given a score of one, and absent fields were assigned a score of zero (Gardiner et al. 2013). A total score out of 33 was calculated for each report and percent completeness was calculated with each scale.

4.4.3 Causality Assessment and Assessment tools

Previously validated Naranjo Adverse Drug Reaction Probability Scale (Naranjo et al. 1981), HORN Drug Interaction Probability Scale (Horn et al. 2007) and World Health Organization Uppsala Monitoring Centre Causality Categories (World Health Organization 2015) scales were modified specifically for NHPs (Necyk 2013) and used to determine probability of NHP-drug interaction among the collected AERs. AERs involving one herbal remedy and one drug were used for evaluation. First, each report was assessed by four adjudicators (psychiatrist, Health Canada pharmacologist, pharmacist, and a PhD student). Packages containing the AERs, extraction form, and completeness scores were sent to adjudicators along with modified Naranjo, HORN and WHO drug interaction scales. Naranjo tool was to be filled out if the adverse reaction was thought to be due to a single substance, and HORN and WHO scales were to be completed for potential NHP-drug interactions.

Naranjo Adverse Drug Reaction Probability Scale: A tool that estimates the probability of a drug causing an adverse event using the known categories of definite, probable, possible and doubtful adverse drug reactions (Naranjo et al. 1981).

HORN Drug Interaction Probability Scale (HORN DIPS): The HORN DIPS uses a series of questions to help to determine a probability score for a drug-drug interaction (Horn et al. 2007).

World Health Organization Uppsala Monitoring Centre (WHO-UMC): WHO-UMC is a system created by the Programme for International Drug Monitoring and assesses case reports for drug interactions and adverse reactions using the causality category terms (i.e., certain, probable/likely, possible, unlikely, conditional/unclassified, unassessable/unclassifiable (World Health Organization 2004).

The independent assessments were then discussed amongst the adjudicators to determine a final assessment.

4.5 RESULTS

4.5.1 Identification of Adverse Event Reports

The systematic search of the FDABLE database identified 120 AERs including the 22 targeted herbal remedies. Observing the outcomes of the reports indicated that 38.3% of the adverse events resulted in hospitalization, 5.8% in deaths, 3.3% were life threatening, 5% resulted in disability, 31.7% were medically significant or other, and 15.8% of the reports did not report an outcome.

Twenty-three (19.2%) of the 120 AERs involved an ADHD prescription drug. In these 23 reports, eight herbal remedies were identified: echinacea (6), evening primrose oil (3), garlic (2), ginkgo (3), ginseng (4), green tea (1), St. John's wort (2), and valerian (2) (**Figure 4-2**). From the 23 reports involving ADHD prescription drugs, we identified five AERs that reported the use of only two products (one herbal remedy with one ADHD drug). These reports were subsequently evaluated for NHP-drug interaction potential. We did not evaluate reports with more than two products being used concurrently as it can be difficult to determine causality when a patient has consumed multiple medications due to the possibility of multiple interactions (both NHP-drug and drug-drug interactions). Among the other 18 reports (including the 4 reports we were unable to obtain from FDA freedom of request) involving the use of an ADHD drug, and two or more NHPs, most patients were taking between 4-10 different products.

4.5.2 Assessment of Completeness

Assessment of completeness was conducted on the reports obtained from the FDA freedom of request ($n = 19$) (**Figure 4-1**).

For the Berman and Ernst scale, the mean completeness score was 4.6 out of 10, meaning the reports provides some evidence for an interaction, but there may be other causes of the event. Six reports had a score between 0-3, 11 reports had a score between 4-7, and 2 reports had a score of 8. Information on patient history and concurrent diseases, conditions and medications were provided in most cases. The following categories were poorly described in the reports: description of interactors, obvious alternative explanations excluded, chronology, time sequence to drug administration and adverse event, challenge, and re-challenge information.

For the assessment of completeness using Gardiner Adherence Scale (Gardiner et al. 2013), the average adherence score was 6.2 out of 17 (range: 2-12). In general, the following categories were poorly reported: physical examination, patient disposition, herbal product information, dosage, duration of herbal remedy use before adverse event, concomitant therapies, and discussion. The patient demographics, health status, medical history and description of adverse event were almost always provided.

We also used the Gardiner Extraction Tool, which was developed for assessing the completeness of AERs involving NHP/supplements. A total score out of 33 was assigned to each of the 19 AERs obtained from FDA freedom of request, yielding an average score of 10.05 (30.4% completeness, range: 4-16). As illustrated in the heat map in (**Figure 4-3**), NHP product information and lab test results were rarely reported. Although the description of the adverse event was always reported, patient and health information were generally lacking in the reports. This information is necessary to better evaluate the adverse event for causality.

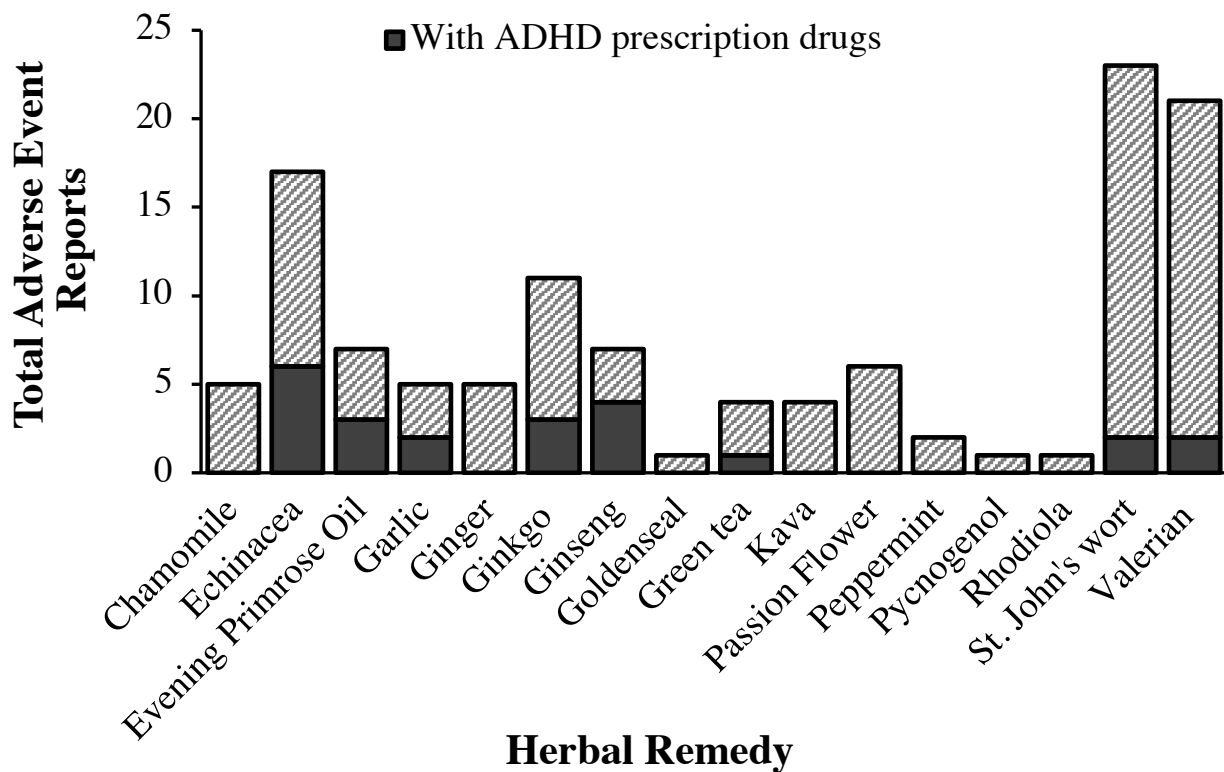


Figure 4-2 Total number of adverse event reports for commonly used herbal remedies by children, and adverse events with prescription drugs used for attention-deficit hyperactivity disorder for children aged 4-18 years from 1997-2015 using FDable advance search (http://fdable.com/advanced_aers_query).

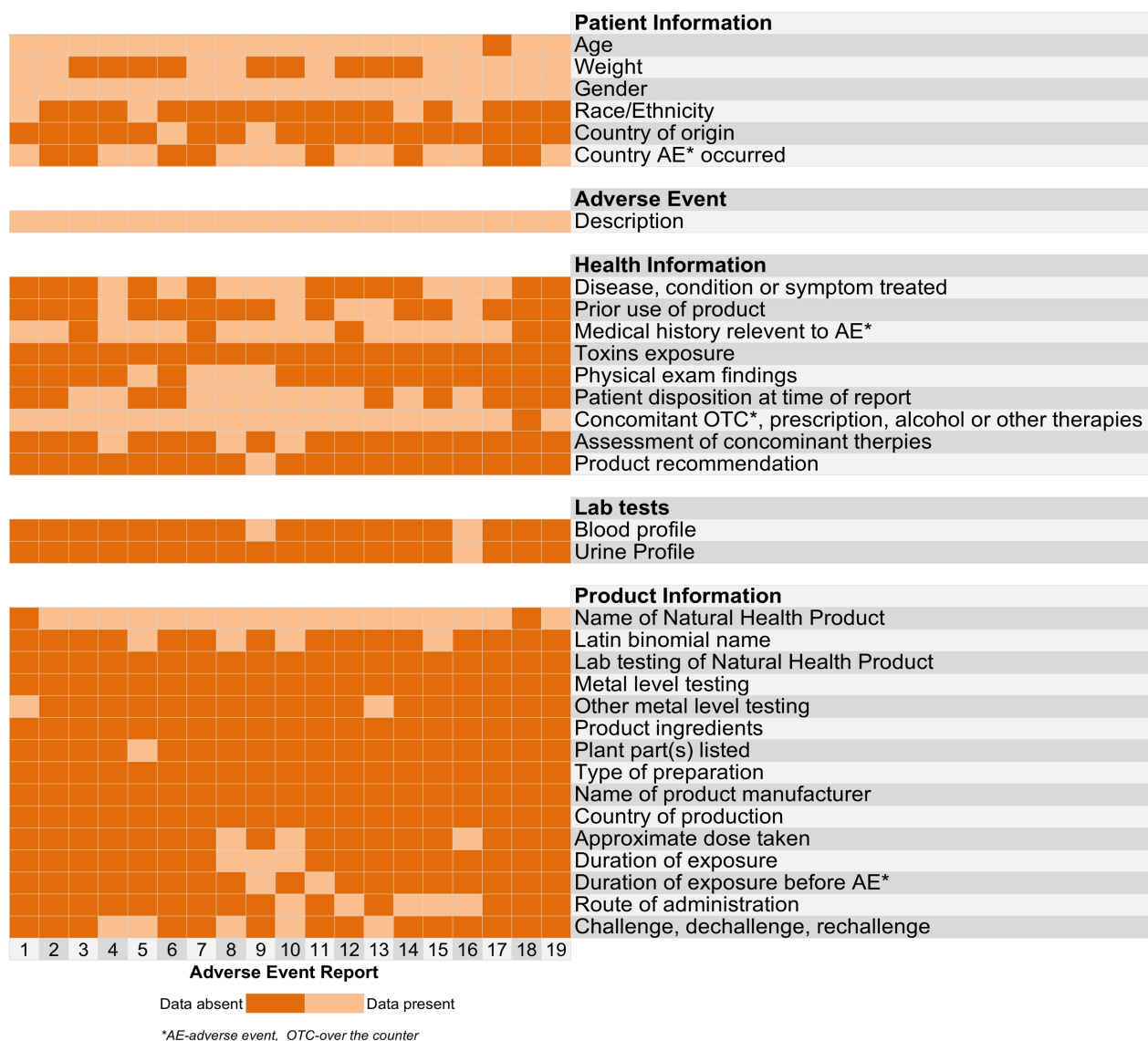


Figure 4-3 Heat map of information available in 19 pediatric adverse event reports involving commonly used herbal remedies by children and prescription drugs used for attention-deficit hyperactivity disorder. The categories on the y-axis were utilized from the Gardiner Extraction Tool (Gardiner et al. 2013).

4.5.3 AERs involving an ADHD drug and an herbal remedy

A summary of the five pediatric cases involving only an ADHD medication and an herbal remedy is presented below, based on all the information available in the AERs obtained from the FDA. Refer to **Table 4-2** for associated case number, case key features, completeness, and adjudication (see **Appendix D for full AERs retrieved from the FDA**).

- I. An 8-year-old male with a history of ADHD, astigmatism, conduct disorder, learning disorder and asthma was taking Strattera® 25 mg for ADHD from October 2008 to September 2009. He was taking *Ginkgo biloba* 85 mg daily from May 2001 to November 2010. After starting Strattera® patient experienced headache and eye pain that resulted in hospitalization. Ophthalmologic exam raised suspicion of glaucoma. The reporting child psychiatrist stated that the event was related to Strattera®.
- II. A 7-year-old female was treated with Ritalin® 10 mg/day orally for ADHD starting October 2001. Patient was also receiving Efalex evening primrose oil (supplementing fatty acids) since 2001. On March 7, 2002, she developed a tic involving both her arms, and it became more complex involving hands, fingers, wrists, neck, head and hips. Ritalin® was discontinued on March 7, 2002. After discontinuation, there was improvement in the movement of head, arm, neck, and legs but the movements were still present. There was no family history of tics or of a movement disorder. Prior to the tics, the patient experienced nightmares for one week. She also had a skin rash and dry skin around her mouth. The case was considered medically significant.
- III. An 11-year-old male was taking OROS (osmotic-controlled release oral delivery system) methylphenidate 36 mg daily orally for ADHD for a few years. The patient was also taking evening primrose oil for an unknown indication and duration. Patient was adopted and may have been exposed to drugs prenatally. He had a history of moderate to severe developmental delay and slow learning. On December 30, 2002 he ran out of OROS methylphenidate and had to be

taken to the emergency with severe torticollis, rolling arm movements, lip chewing, slight pharyngitis. On January 2, 2003 he presented to his pediatrician with the same symptoms and slurred speech, and not eating or drinking and was admitted to the hospital. The patient was also taking evening primrose oil for unknown indication and duration. Torticollis improved with intravenous cetirizine hydrochloride. Patient was further diagnosed with pharyngeal abscess.

IV. A 17-year-old female with history of ADHD and depression was treated with OROS methylphenidate for about a year. Concurrent medication included St. John's wort. Patient experienced psychotic symptoms on an unknown day. Patient was seeing and hearing things in the night which were not there and was disturbed. The outcome was deemed medically significant.

V. A 15-year-old male started Ritalin® 20 mg orally/day in 1998 for ADHD and tolerated it well. He had a period of sadness and took St. John's wort (5 drops) orally daily to treat his depression starting June 1, 2001. A few hours later, he presented agitation, unexplained weeping, aggressiveness alternating with depression and difficulty concentrating. On June 6, 2001, St. John's wort was discontinued, and these symptoms abated. Three weeks later St. John's wort was reinitiated, and the same symptoms occurred. St. John's wort was discontinued again, and the symptoms abated again. The reaction as considered medically significant.

Case I and II were adjudicated as doubtful for HORN DIPS, and possible for both WHO and Naranjo scale. Case III did not have adequate information on the timeline and dosage of evening primrose oil to determine an interaction with both HORN DIPS and WHO scale. For Naranjo scale, it was decided that although an interaction with methylphenidate (Concerta®) could not be completely ruled out, again due to lack of information the adjudicators decided on a doubtful possibility of a reaction with methylphenidate.

Case IV was adjudicated to have a possible likelihood of an interaction between St. John's wort and Concerta® for HORN DIPS and WHO scales. Although St. John's wort and Concerta® alone could be the cause of an acute psychotic reaction due to an increase in dopaminergic transmission (Naranjo-possible likelihood), St. John's wort is a known inhibitor of P-glycoprotein and could lead to an increasing concentration of methylphenidate in the brain (Zhu et al. 2006; Zhu, Wang, et al. 2008). However, the role of P-glycoprotein in the transport of *d*-methylphenidate is likely to be minor (Zhu, Wang, et al. 2008).

Case V was adjudicated as probable for HORN DIPS and Naranjo and probable/likely for WHO scale. Although we are unaware of previous reports of this interaction, excessive weeping, agitation, and aggressiveness can appear during Ritalin® treatment (Novartis Pharmaceuticals Canada Inc. 2017). Concomitant use of St. John's wort could exacerbate this as they share similar modes of action and St. John's wort is an inhibitor of P-glycoprotein which could result in higher plasma levels of Ritalin®. This case has a probable likelihood of an interaction as the adverse event appeared a few hours after administration of St. John's wort and the reaction stopped when St. John's wort was discontinued then reappeared upon rechallenge with St. John's wort

Table 4-2 Description of adverse event reports evaluated for causality of natural health product drug interactions and the outcome of the adjudication

CASE INFORMATION						COMPLETENESS SCALE (Total score)			ADJUDICATION		
Case #	Patient Information Age (years) Sex	Adverse Event	Drugs	Herbal Remedy	Outcome	Berman and Ernst Scale (10)	Guidelines for Adverse Event Reports for Publication (17)	Gardiner Tool (33)	HORN DIPS	WHO	Naranjo
(I)	8 Male	Glaucoma	Strattera®	<i>Ginkgo biloba</i>	Hospitalized	6	10	16	Doubtful	Possible	Possible
(II)	7 Female	Movement disorder tic, nightmares skin Rash	Ritalin®	Efalex Evening Primrose Oil	Medically Significant	5	7	12	Doubtful	Possible	Possible
(III)	11 Male	Not eating, drinking, torticollis rolling arm movements lip chewing pharyngitis slurred speech	Concerta®	Evening Primrose Oil	Hospitalized	3	6	8	Not assessable	Not assessable	Doubtful
(IV)	17 Female	Psychotic disorder, schizophrenia	Concerta®	St. John's wort	N/A	4	5	8	Possible	Possible	Possible
(V)	15 Male	Depression agitation aggressive, crying disturbed attention drug interaction	Ritalin®	St. John's wort	Medically Significant	8	12	12	Probable	Probable/ Likely	Doubtful

4.6 DISCUSSION

Our systematic search found 120 AERs involving commonly used herbal remedies by pediatric patients. Of these, 23 (19.2%) of the AERs involved an ADHD medication. With the widespread use of NHPs, including herbal remedies among pediatric patients across different clinical populations, the high relative prevalence of AERs involving ADHD drugs in the 120 reports we found may reflect a high prevalence of ADHD diagnoses in the United States. On the other hand, it may also suggest an elevated risk of interaction between phytochemicals and ADHD medications. Since AERs often go unreported, particularly those related to CIM, the collected AERs likely reflect a fraction of such events but nonetheless offer an important tool for monitoring and investigating potential NHP-drug interactions.

Of the 23 identified AERs involving both an herbal remedy and an ADHD drug, most involved multiple (>3) substances with inadequate detail to assess multiple potential interactions. Idiosyncratic reactions (NHP-NHP reactions) may play a role in AERs involving multiple products.

Following data extraction and evaluation of completeness, 5 AERs that involved only one herbal remedy and one ADHD drug were evaluated for causality. Based on consensus adjudication using the HORN scale, the likelihood of an NHP-drug interaction was assessed to be probable in one case and to be possible in another. Both these reports (IV and V) involved a methylphenidate formulation (Concerta ® and Ritalin ®) and St. John's wort.

St. John's wort is known to interact with various drugs through several mechanisms. In these cases, a possible mechanism could be that St. John's wort inhibits P-glycoprotein affecting the concentration of methylphenidate in the blood and the brain (Dürr et al. 2000; Perloff et al. 2001; Zhu et al. 2006). Additionally, St. John's wort is known to inhibit numerous drug-

metabolizing enzymes including the cytochrome P450 family (Komoroski et al. 2004; Obach 2000; J S Markowitz et al. 2003; Markowitz et al. 2000). Amphetamine and atomoxetine are metabolized by hepatic cytochrome P450 (CYP) enzymes, but methylphenidate is converted to inactive ritalinic acid by carboxylesterase-1 (CES1). Liver CES1 is responsible for the hydrolysis of both the *d-threo* and *l-threo* isomers of methylphenidate (the two components of Ritalin ® or the extended-release formulation Concerta ®), and for the resulting metabolism of the drug. Many natural inhibitors of CES1 and a few inducers are found in herbal products (Xu et al. 2018), but the effects of St. John's wort on methylphenidate metabolism and CES1 have yet to be studied.

It is vital to highlight the low quality of AERs examined in this study. Our investigation revealed an overall mean completeness score of 10.05 out of 33 using the Gardiner Extraction Tool and 6.2 out of 17 on the Gardiner Adherence Scale. As well, an average score of 4.6 out of 10 on the Berman and Ernst completeness scale suggests that reports provided some evidence for an interaction, but that other causes may underlie or have contributed to the adverse event. This degree of (in)completeness both complicated adjudication of potential herb-drug interactions and highlights poor reporting guidance with regard to NHPs. The most consistently missing information was details on the herbal remedies (such as Latin binomial name, product ingredients, plant parts, type of preparation, name of manufacturer, dose, country of production, duration of exposure, route of administration) and other concomitant therapeutics which are vital to determine an interaction. This makes it difficult to assess the likelihood of an NHP-drug interaction and decreases the reliability of the AERs.

When documented thoroughly, adverse event reports can be an important tool to determine the safety of a product post market. Well documented AERs can inform healthcare practitioners

about the possibility of the risk associated with a product alone, or in combination with others, and they can inform regulatory agencies to re-evaluate a product safety signal to determine if it will remain on the market. Overall, they can aid to prevent the reoccurrence of the adverse event in patients (Kelly et al. 2007) and to identify combinations of therapeutics that may present risk and require testing *in vitro* and *in vivo* to confirm NHP-drug interaction causality. Importantly, despite missing information, our study identified potential interactions that warrant further attention and investigation.

Given the popularity and out-of-pocket costs associated with CIM use, it is important for the public to have convenient access to evidence-based information for informed decision-making. Moreover, communication about NHPs between healthcare practitioners and families is important, and clinics and hospitals should provide regular educational seminars to healthcare providers about safety, especially in the case of polypharmacy. Improved communication with healthcare practitioners could also reduce instances of delays in diagnosis and in seeking approved treatment from CIM use and can improve adherence with conventional treatment. More awareness about pharmacovigilance is also important. One way to increase pharmacovigilance of NHPs is consumer education on awareness and reporting of adverse events, which in turn can increase the quality of consumer reports (Kelly et al. 2007; World Health Organization 2005b; World Health Organization 2015).

There were some limitations to this study. The AERs assessed were all in English language, therefore we were not able to capture if non-English AERs exhibit similar trends for quality and adverse events. The quality of AERs in general was low, and readers should exercise caution when interpreting the results. Other NHPs commonly used by pediatric ADHD patients that were not included in this study, but may pose similar risk of interaction, such as melatonin,

caffeine, blue green algae, and unsaturated fatty acid supplements, warrant investigation. The identified AERs may reflect off-label use, or the lack of evidence of safe use of NHPs in children, rather NHP-drug interactions. However, this caveat is difficult to gauge for children with ADHD because of the lack of clinical data on the use, efficacy, or safety in this population. Specifically, due to the prevalence of comorbid psychiatric disorders, it is not clear whether children taking NHPs do so to alleviate ADHD symptoms, symptoms from other conditions, or for maintaining general health.

4.7 CONCLUSION

We identified 23 AERs involving both an herbal remedy and an ADHD drug in a pediatric population. Overall, the quality of these reports was low. Eighteen of these reports involved multiple (> 3) substances with inadequate detail to assess multiple potential interactions. The five AERs examined for NHP-drug interaction causality adjudication using the HORN DIPS scale showed likelihood of an NHP-drug interaction to be probable in one case and to be possible in another. Both these reports (IV and V) involved a methylphenidate formulation (Concerta ® and Ritalin ®) and St. John's wort.

4.8 CLINICAL SIGNIFICANCE

Healthcare practitioners, particularly those treating pediatric ADHD patients, need to be aware of risks of NHP interactions and need to maintain a dialog about the use of these products. Given the demands and priorities set upon emergency room staff and other healthcare practitioners dealing with adverse events, establishing extensive NHP-specific protocols is impractical. However, since our results highlight the difficulty in assessing AERs involving

herbal remedies due to lack of information, regulating bodies such as the FDA could implement simple tools to improve report quality with limited impact on front-line resources. For example, a checklist of details required to assess AERs involving NHPs: a product name (brand/ingredients and manufacturer), dose, route, dates of duration of use, and indication for use. Additional information desired that could be helpful to assess causality of the reports includes: regulatory identification number (if available: such as in Canada, natural product number (NPN), drug identification number (DIN), or homeopathy drug identification number (DIN-HM)), Latin binomial name of ingredients, plant part, type of preparation, challenge/dechallenge/rechallenge information.

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Chapter 5

An *in vitro* evaluation of the inhibition of recombinant human Carboxylesterase-1 by commercial herbal extracts

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Author's Contributions

CSH, HM and PR contributed to the design of the study. HM conducted the experimental data collection. CSH and HM contributed to the data analysis. HM drafted the manuscript. CSH, HM and PR revised and approved the final manuscript.

5.1 ABSTRACT

Methylphenidate is a psychostimulant prescribed for symptoms of attention-deficit hyperactivity disorder (ADHD), and is metabolized by carboxylesterase-1 (CES1) enzyme. There is little information on herbal remedies used for ADHD and their interactions with CES1. An *in vitro* study was conducted with extracts of 21 herbal remedies using a recombinant human CES1 enzyme inhibition assay to determine if the extracts may influence enzyme activity. Extracts were preliminarily evaluated for their potential to inhibit CES1 metabolism of 4-nitrophenyl acetate using an *in vitro* microtiter plate enzyme inhibition assay. Select extracts were serially diluted to determine the half maximal inhibitory concentration (IC₅₀) and were tested for irreversible inhibition using time-dependence enzyme assay. Phytochemical characterization of selected extracts was performed, and marker compounds were evaluated for their potential to inhibit CES1-mediated metabolism of the probe substrate. Most of the extracts inhibited CES1 activity to some degree, with inhibition ranging from 15-95% at a supraphysiological screening concentration of 200 µg/mL. *Rhodiola rosea* was the most potent inhibitor of CES1 (IC₅₀= 4.7 µg/mL). No time-dependent inhibitors of CES1 were identified in this study. At 10 µg/mL, marker compounds of ginger, [8]-gingerol (60.3%, *P* = .005, 95% CI: -107.8 to -12.8) and [10]-gingerol (67.2%, *P* = .002, 95% CI: -114.7 to -19.7), showed significant inhibition of CES1 compared to vehicle control. Herbal remedies used by ADHD patients have the potential to interact with CES1 mediated metabolism. *In vivo* and clinical research are required to determine if the potential interactions would significantly affect safety and efficacy of methylphenidate at a clinical level.

5.2 INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is among the most prevalent childhood psychiatric disorders, globally affecting 5.3%-7.3% of children and youth (Polanczyk et al. 2007; Thomas et al. 2015). Psychostimulants such as methylphenidate and amphetamine are first-line of treatments and are generally effective at treating symptoms of ADHD but are associated with adverse events (Stevens et al. 2013).

Whereas amphetamine is metabolized by cytochrome P450 2D6 (Teva Canada Limited 2015), methylphenidate is metabolized by human carboxylesterase-1 (CES1) (Sun et al. 2004). CES1 is a serine hydrolase enzyme, belonging to the multi-gene family of α/β hydrolase fold proteins (Cygler et al. 1993), and is responsible for metabolism of endogenous and exogenous substances including esters, amides, and thioesters (Casey Laizure et al. 2013; Satoh et al. 1998). CES1 is primarily expressed in the liver, but also in the intestine, kidneys, and lungs to a lower extent (Sanghani et al. 2009), and selectively hydrolyses substrates with small alcohol groups, or large acyl groups (Imai 2006). CES1 converts methylphenidate to its inactive metabolite, ritalinic acid, in the liver (Sun et al. 2004) (**Figure 5-1**).

Considered and regulated as dietary supplements in the United States, natural health products (NHPs) are defined by Health Canada as vitamins and minerals, herbal remedies, homeopathic medicines, traditional medicines, probiotics, and other products such as amino acids and fatty acids (Health Canada 2015). Together with vitamins, minerals, other dietary supplements (e.g., omega-3-fatty acids) and homeopathic formulations, herbal medicines are the most common form of NHPs used by children with ADHD (Sinha et al. 2005). Herbal medicines are also among the most common NHPs used concurrently with psychostimulants (Galicia-Connolly et al. 2014). Like other NHPs, herbal medicines are chemically complex and contain

raw or processed medicinal and non-medicinal substances that, when used concurrently with prescription medication, can interfere with drug metabolism, transport, or action and lead to herb-drug interactions. Herb-drug interactions include pharmacokinetic or pharmacodynamic responses leading to potential adverse events (World Health Organization 2005a).

Several studies demonstrated that many herbal medicines commonly used to manage ADHD symptoms inhibit and/or induce drug-metabolizing enzymes such as the cytochrome P450 (CYP P450) enzyme family (Mazhar et al. 2016). For example, goldenseal, traditionally used as an anti-inflammatory for infections (Blumenthal et al. 2000), is an inhibitor of CYP 3A4 and CYP 2D6 *in vitro* and *in vivo* (Gurley et al. 2005). However, the impact of herbal medicines on CES1 activity remains largely unexplored, with only a few *in vitro* studies examining the inhibition of natural products. In one such study, Sun et al. (2016) reported that the constituents of Psoralea (used widely in Asia for asthma, diarrhea, and osteoporosis) were inhibitors of CES1 mediated metabolism *in vitro* in human liver microsomes. Among these, the flavonoid and their derivatives neobavaisoflavone, corylifolinin, coryfolin, and corylin inhibited CES1 non-competitively, with respective inhibitory constant (K_i) of 5.3, 9.4, 1.9, and 0.7 μM . Two naturally occurring triterpenoids, oleanolic acid and urosolic acid, widely distributed in herbal products, were identified as potent inhibitors of CES1 metabolism, with half maximal inhibitory concentration (IC_{50}) values of 0.28 and 0.24 μM , respectively (Zou et al. 2017). Furthermore, black cohosh extract competitively inhibits CES1 mediated metabolism of irinotecan in human liver microsomes (Gorman et al. 2013). Liu et al. (2010) reported extracts of goldenseal strongly inhibit (75%), and aqueous extracts of echinacea to mildly inhibit (18%) human liver mediated metabolism of oseltamivir. Human liver microsome mediated metabolism studies are generally specific to the substrate used, and may not be generalizable to other CES1 substrates. However,

from the limited number of studies, there is some evidence that medicinal plants can interfere with CES1, potentially altering the metabolism of methylphenidate, resulting in changes in efficacy or safety.

Time-dependent inhibition, also known as irreversible inhibition, occurs from the covalent binding of a chemically reactive intermediate to the enzyme resulting in loss of enzyme activity. Time-dependent inhibition results in an increase in inhibition when the inhibitor is incubated with the enzyme before the addition of substrate (Iwata et al. 2005; Grimm et al. 2009). Herb-drug interactions from time-dependent inhibition can have a delayed onset, persist after the herb treatment has stopped, and pose a greater concern for risk as the enzyme has to be synthesized *de novo*. A well-known example is the irreversible inhibition of CYP 3A4 by furanocoumarins found in grapefruit juice (6',7'-dihydroxybergamottin and bergamottin). The subsequent increase in blood levels of substrate drugs used concurrently with grapefruit juice caused by furanocoumarins in grapefruit juice has the potential to cause adverse events in patients, since the plasma level may move beyond the therapeutic dose into the toxic dose range (Paine et al. 2005). Though not widely studied with herbal medicines (Yan 2012), time-dependent inhibitors are common among serine hydrolases, and can cause serious adverse events in a clinical setting (Bachovchin et al. 2010). Consequently, due to complex chemical nature of herbal medicines, it is important to understand if and how they interact with CES1.

Although limited clinical research has been conducted on the use of herbal medicine specifically for ADHD, in a previous review (Mazhar et al. 2016), we identified 21 herbal medicines used by children with neurological disorders, including ADHD (Cala et al. 2003; Chan et al. 2003; Chan 2002; Chan et al. 2000; Galicia-Connolly et al. 2014; Pellow et al. 2011). Many of these herbal medicines have not been studied extensively in the context of the inhibition

of CES1 mediated metabolism, and hence their potential for NHP-drug interaction with methylphenidate is not known.

The main goal of this study was to assess the potential risk of herb-drug interactions related to concurrent use of herbal medicines with CES1 substrates, such as methylphenidate. Commercial herbal extracts were evaluated *in vitro* for inhibitory effects on the activity of CES1 enzyme. For the most active extracts, the inhibitory potency (IC_{50}) was determined, followed by assessment of inhibitory potential of their marker compounds. The mode of inhibition of these extracts was investigated using time-dependent assays and comparison to a known irreversible (JZL184) and reversible competitive inhibitor (oseltamivir) of CES1 enzyme (Crow et al. 2012; Shi et al. 2006).

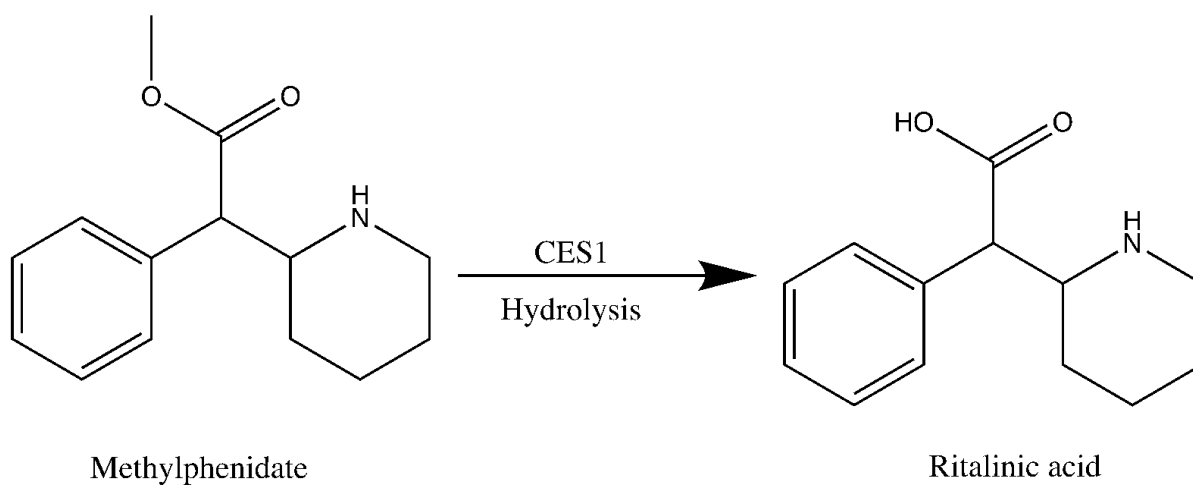


Figure 5-1 Major pathway of the metabolism of methylphenidate in humans.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals and Reagents

Recombinant human CES1 was purchased from Corning Life Sciences (Tewksbury, MA, U.S.A.). Oseltamivir, 4-nitrophenyl acetate (4-NA), and Tris were purchased from Sigma-Aldrich (Oakville, Ontario). JZL184 was obtained from Cayman Chemical Company (Ann Arbor, Michigan, U.S.A.). HPLC (high-performance liquid chromatography) grade acetone was purchased from Fisher Scientific (Ottawa, Ontario). Bilobalide, menthol, and rosmarinic acid were obtained from Sigma-Aldrich (Oakville, Ontario). Rosarin, rosavin, rosin, salidroside, and tyrosol were purchased from ChromaDex (Los Angeles, California, U.S.A.). [6], [8], and [10]-gingerol, ginkgolide A and ginkgolide B were purchased from Extrasynthese (Genay, France). All solvent used in chromatographic analysis were HPLC grade and purchased from Fisher Scientific (Ottawa, Canada).

5.3.2 Commercial Plant Extracts and Tinctures

A targeted selection of 21 commercial grade plant tinctures (**described in Table 5-1**) were generously provided by St. Francis Herb Farm (St. Francis Herb Farm 2704 Dafoe Road, Combermere, Ontario K0J 1L0), namely: bacopa (*Bacopa monnieri* L., Plantaginaceae); chamomile (*Matricaria chamomilla* L., Asteraceae); EchinAce (*Echinacea purpurea* L. Moench., Asteraceae and *Echinacea angustifolia* DC., Asteraceae); *Echinacea angustifolia*; Echinapure (*Echinacea purpurea*); eleuthero (*Eleutherococcus senticosus* Ruprecht et Maximowicz., Araliaceae); garlic (*Allium sativum* L., Alliaceae); ginger (*Zingiber officinale* Roscoe., Zingiberaceae); ginkgo (*Ginkgo biloba* L., Ginkgoaceae); goldenseal (*Hydrastis canadensis* L., Ranunculaceae); green tea (*Camellia sinensis* L., Theaceae); kava (*Piper methysticum* G. Forster., Piperaceae); lemon balm (*Melissa officinalis* L., Lamiaceae); linden

(*Tilia x europaea* Miller., Tiliaceae); passion flower (*Passiflora incarnate* L., Passifloraceae); peppermint (*Mentha x piperita* L., Lamiaceae); rhodiola (*Rhodiola rosea* L., Crassulaceae); rosemary (*Rosmarinus officinalis* L., Lamiaceae); skullcap (*Scutellaria lateriflora* L., Lamiaceae); St. John's wort (*Hypericum perforatum* L., Hypericaceae); and valerian (*Valeriana officinalis* L., Valerianaceae).

For each tincture, 30 mL was weighed then dried using a Labconco Centrивap Concentrator with an attached Centrивap Cold Trap to remove alcohol and an EC Modulyo Freeze Drier (Thermo Electron, Ottawa, ON, Canada) to remove remaining water. Dried extracts were weighed and stored in darkness at -20°C until use. A 10 mg aliquot of each dried extract was reconstituted in 20% acetone to obtain 10 mg/mL stock solutions. Each stock solution was vortexed and sonicated to ensure homogeneity immediately before use.

Table 5-1 Information for 21 commercial herbal products used in this study. Information such as common name, scientific name, common uses, and product details are provided.

Common name <i>Scientific name</i>	Common use(s), or purpose(s) (reference)	Product details*	
		Plant part	Standardized quantity per mL (Quantity crude equivalent ratio)
Bacopa <i>Bacopa monnieri</i>	Memory enhancement, learning, concentration, and anxiolytic ^{1, 2}	Whole plant	250 mg (1:4)
Chamomile <i>Matricaria chamomilla</i>	Gastrointestinal anti-inflammatory, calmative ^{3, 4}	Flower	250 mg (1:4)
Echinacea EchinAce <i>Echinacea angustifolia</i> <i>Echinacea purpurea</i> EchinaPura <i>Echinacea purpurea</i> Echinacea <i>Echinacea angustifolia</i>	Upper respiratory tract infections ^{4, 5}	Root and rhizome Tops and roots Flower, leaf and root Roots and rhizome	667 mg per 0.67 ml (1:1) 83 mg per 0.33 ml (1:4) 1000 mg (1:1) 250 mg (1:4)
Eleuthero <i>Eleutherococcus senticosus</i>	Fatigue, concentration, anti-inflammatory ^{4, 6}	Root	250 mg (1:4)
Garlic <i>Allium sativum</i>	Upper respiratory tract infections, hypertension, improves blood flow ^{3, 4, 6}	Bulb	250 mg (1:4)
Ginger <i>Zingiber officinale</i>	Motion sickness, nausea ^{6, 7}	Rhizome	1000 mg (1:1)
Ginkgo <i>Ginkgo biloba</i>	Memory, concentration, anti-depressant ⁸	Leaves	250 mg (1:4)
Goldenseal <i>Hydrastis canadensis</i>	Gastrointestinal anti-inflammatory ^{3, 6}	Roots and rhizome	200 mg (1:5)
Green tea <i>Camellia sinensis</i>	Antioxidant ^{9, 10}	Leaves	250 mg (1:4)

Table 5-1 Information for 21 commercial herbal products used in this study (continued).

Common name <i>Scientific name</i>	Common use(s), or purpose(s) (reference)	Product details*	
		Plant part	Standardized quantity per mL (Quantity crude equivalent ratio)
Kava <i>Piper methysticum</i>	Anxiolytic, cognitive enhancement ^{11, 12}	Dried rhizome	250 mg (1:4)
Lemon balm <i>Melissa officinalis</i>	Sleep aid, gastrointestinal complaints, appetite stimulant ¹³	Tops	250 mg (1:4)
Linden <i>Tilia x europaea</i>	Cold related symptoms, anxiolytic ^{3, 4}	Dried flower	250 mg (1:4)
Passion flower <i>Passiflora incarnata</i>	Anxiolytic, sleep aid ^{4, 14}	Tops	250 mg dry (1:4) or 1000 mg fresh (1:1)
Peppermint <i>Mentha x piperita</i>	Gastrointestinal complaints ^{3, 4}	Leaves	250 mg (1:4)
Rhodiola <i>Rhodiola rosea</i>	Adaptogen, antioxidant, cognitive functions ^{15, 16}	Root	250 mg (1:4)
Rosemary <i>Rosmarinus officinalis</i>	Anti-septic, stimulant, rheumatic conditions, anxiolytic ^{4, 15}	Leaves	250 mg (1:4)
Skullcap <i>Scutellaria lateriflora</i>	Calmative, sleep aid ^{6, 17}	Flowering tops	250 mg (1:4)
St. John's wort <i>Hypericum perforatum</i>	Depression, anxiolytic ^{4, 15}	Flowering tops	250 mg (1:4)
Valerian <i>Valeriana officinalis</i>	Anxiolytic, sleep aid, appetite stimulant ^{4, 6}	Root	250 mg dry (1:4) or 1000 mg fresh (1:1)

* Commercial tinctures (prepared in grain alcohol) used in the enzyme assays were provided by St. Francis Herb Farm.

(1) Chopra et al. 1958; (2) Mukherjee et al. 1966; (3) Bradley 1992; (4) Blumenthal et al. 2000; (5) Moerman 1998; (6) Mills et al. 2000; (7) Bradley 1937; (8) World Health Organization 1999 (9) Coimbra et al. 2006; (10) van het Hof et al. 1997; (11) Heinze et al. 1994; (12) Emser 1993; (13) European Medicine Agency 2014; (14) Godfrey et al. 2010; (15) Hoffmann 2003; (16) European Medicines Agency 2012; (17) Bradley 2006.

5.3.3 CES1 Inhibition Assay

Preliminary evaluation of herbal medicines

A microtiter plate assay was used to evaluate the inhibitory potential of commercial plant extracts towards recombinant CES1-mediated metabolism of 4-NA. The procedure from R&D Systems was adapted and modified to include herbal medicines as a test sample (R&D Systems, n.d.). The assays were performed in 96 well clear-bottom microtiter plates (Corning Costar) and absorbance was measured using a Cytation 3 cell Imaging Multi-Mode Reader (BioTek Instruments Inc., Winooski, VT, U.S.A.).

Samples were incubated in the presence (test) and absence (test-blank) of recombinant CES1 (6.5 nM) and 4-NA (1 mM) in 50 mM Tris buffer at a pH of 7.5 at 37°C. JZL184 (2 µM) was used as a positive control and 20% acetone was used as vehicle control (final well concentration: 2%). Wells were assigned as “control”, “blank”, “test”, and “test-blank”: control wells contained vehicle control, 4-NA, distilled water, and enzyme; blank wells contained vehicle control, 4-NA, distilled water, and Tris buffer. Test wells contained extract, distilled water, 4-NA, and enzyme; test-blank wells contained extract, distilled water, 4-NA, and buffer. The total assay volume was 100 µL. The plate was incubated for 15 minutes at 37°C after the addition of CES1 and absorbance readings were taken at 405 nm. All samples were tested in triplicate in three independent experiments. Percent inhibition values were measured using the following equation:

$$\% \text{ Inhibition} = \left[100 - \frac{(\text{Sample}_{T_{\text{final}}} - \text{Sample}_{T_{\text{initial}}}) - (\text{Sample blank}_{T_{\text{final}}} - \text{Sample blank}_{T_{\text{initial}}})}{(\text{Vehicle}_{T_{\text{final}}} - \text{Vehicle}_{T_{\text{initial}}}) - (\text{Vehicle blank}_{T_{\text{final}}} - \text{Vehicle}_{T_{\text{initial}}})} * 100 \right] * 100\%$$

Concentration-dependent response curves

Ginger, ginkgo, peppermint, rhodiola, and rosemary exhibited the strongest inhibition (>70%) in the preliminary evaluation (**Figure 5-1**), and were tested for concentration-dependent response analysis. Serial dilutions of stock solutions were performed for each of the 5 commercial tinctures to yield six to nine concentrations (200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.2 µg/mL, 3.1 µg/mL, 1.6 µg/mL, and 0.8 µg/mL) to evaluate concentration-dependent response and determine the half maximal inhibitory concentration (IC₅₀).

Irreversible Time-dependent kinetic activity

Irreversible inhibition was assessed *in vitro* using time-dependent assays with the pre-incubation method described by Yamamoto et al. (2002). To test for time-dependence of commercial extracts of ginger, ginkgo, peppermint, rosemary, and rhodiola towards CES1, two solutions were prepared. The pre-incubation solution contained sample (JZL184, oseltamivir, or plant extract) and CES1 (6.5 nM) in 50 mM tris buffer (pH: 7.5). The reaction solution contained 4-NPA (1 mM) in 50 mM tris buffer (pH: 7.5). After 0, 5, and 10, minutes of pre-incubation at 37°C, the reaction solutions were added to the pre-incubation solutions in a total volume of 100 µL. The absorbance from the metabolism of 4-NPA was measured after 15 minutes of incubation to determine the residual activity of CES1 relative to vehicle control. The kinetic response of each extract (40 µg/mL) was compared to JZL184 (0.5 µg/mL), and oseltamivir (1 mg/mL).

Evaluation of marker compounds for CES1 inhibition

Select standard marker compounds of rhodiola (rosarin, rosavin, rosin, salidroside, tyrosol), rosemary (rosmarinic acid), peppermint (menthol), ginger ([6]-gingerol, [8]-gingerol, [10]-gingerol), ginkgo (quercetin, bilobalide, ginkgolide A, and ginkgolide B) were evaluated at

10 µg/mL for their ability to inhibit CES1 mediated metabolism *in vitro* using the CES1 inhibition assay methods described above.

5.3.4 Phytochemical Analysis of extracts

High-performance liquid chromatography diode array detection (HPLC-DAD) was used to analyze extracts of ginger, rosemary, rhodiola, and ginkgo. An Agilent 1100 HPLC system with Chemstation software (Version B 3.02) was used for phytochemical analysis. The system consisted of an autosampler (G1313A with 100 µL loop), Quaternary pump (G1311A), a solvent degasser (G1322A), a column oven (G1316A), and a photodiode array detector (G1315A).

Separation of marker compounds in ginger, rosemary and rhodiola were performed on Phenomenex Synergi Max-RP (250 x 3 mm 4 µm particle size, Phenomenex Inc., Mississauga, Ontario). Mobile phase A consisted of 0.1% trifluoroacetic acid in water, and mobile phase B consisted of 0.1% trifluoroacetic acid in acetonitrile. The injection volume was 1 µL, and the column oven temperature was set at 55°C. The flow rate was 0.5 mL/minute and the DAD was set to 280 nm to 350 nm.

For ginger, the gradient initiated with 5% B for 1 minute, and increased to 100% in 17 minutes. The gradient was held at 100% B for 7 minutes, followed by a 5-minute re-equilibration period. For rosemary and rhodiola, the gradient initiated with 10% B, and increased to 50% B in 19 minutes. The gradient was increased to 100% B over 5 minutes, and kept at this condition for 5 minutes, followed 5 by minutes of re-equilibrium.

Separation of ginkgo was performed on Phenomenex Luna C18 column (150 x 2 mm, 3-micron particle size). Mobile phase A was water, and mobile phase B was methanol, with a flow rate of 0.3 mL/min. Mobile phase B was held at 25% for 1 minute, increased to 100% over 24 minutes, held at 100% for 10 minutes, and reduced to 25% at 35.1 minutes. The column was

equilibrated for 7 minutes. The detector was set to 210 nm-330 nm at 55°C. The injection volume was 10 μ L.

Gas chromatography (GC) was used to analyse extracts of peppermint. The method to identify menthol was adapted by Lee (2019) from Supelco Analytical Products (Munich, Germany). Briefly, 1 μ L of peppermint extract (1 mg/mL in 99% ethanol) was injected on Agilent 6890N Network Gas Chromatogram with an autosampler (7683 series) and dual injector (7683 series) with a flame ionization detector (FID) and SLB-5MS GC Column (30 m x 0.25 mm x 0.25 μ m). The injector temperature was 230°C. The oven was set to 60°C for 3.5 minutes, 3.5°C /minute to 155°C, 30°C/minute to 300°C with a post run at 340°C for 10 minutes. The carrier gas was hydrogen and was set at a flow rate of 1.4 mL/minutes. Methanol standard was prepared at 250 μ g/mL in 99% ethanol. The data was processed in ChemStation.

For both HPLC and GC, peak identification was confirmed through relative retention times of standard marker compounds with further confirmation through comparison of UV absorption spectra (for HPLC results only).

5.3.5 Statistics

A one-way ANOVA with Bonferroni's post hoc test was used to evaluate screening data (percent inhibition of CES1) by performing a comparison between extracts. IC₅₀ values were obtained by plotting percent inhibition against log-transformed concentration (mg/mL) using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0). A $P \leq .05$ was considered significant for all comparison. For the time-dependent experiments, a P -value of $\leq .05$ using an ANOVA with Dunnett's post hoc test indicated significant effects on the activity of CES1 when comparing 5- or 10-minute incubation times relative to the 0-minute incubation for each inhibitor.

5.4 RESULTS

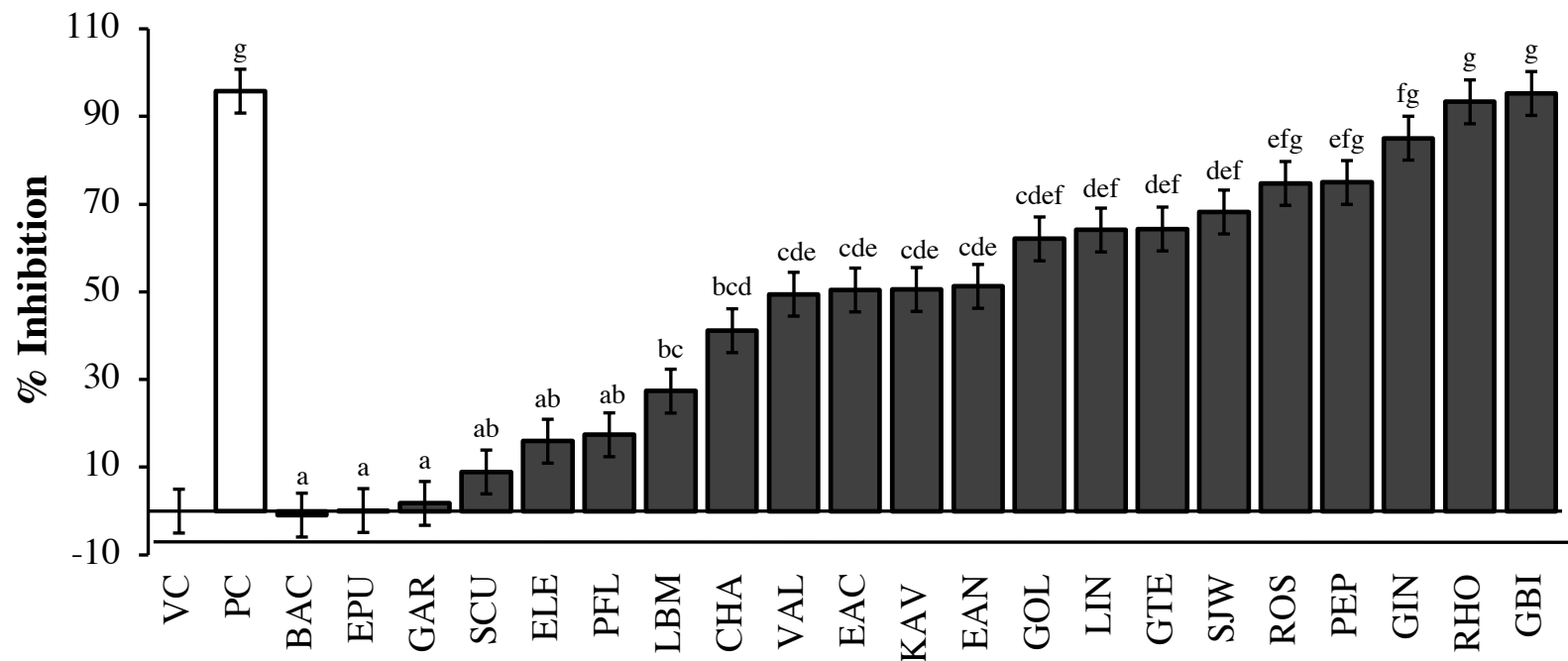
Preliminary evaluation of herbal medicines

Twenty-one targeted commercial herbal extracts were assessed for their ability to inhibit CES1 mediated metabolism of 4-NA at a supraphysiological concentration of 200 µg/mL.

Figure 5-2 displays the mean percent inhibition of CES1 for all the tested extracts. Five of the extracts elicited the strongest inhibition (>70%) including ginkgo, rhodiola, ginger, peppermint, and rosemary ($P < .05$). Nine extracts exhibited 30%-69.9% inhibition including St. John's wort, green tea, linden, goldenseal, *Echinacea angustifolia*, kava, EchinAce, valerian, and chamomile. Four extracts showed less than 30% inhibition including lemon balm, eleuthero, passion flower, and skullcap, with bacopa, garlic, and *Echinacea purpurea* extracts showing little to no inhibition (0%-2%).

Concentration-dependent responses

The five most active extracts were serially diluted and assayed to determine concentration-response relationships and IC₅₀ values. **Figure 5-3** displays IC₅₀ non-linear regression curves, and the IC₅₀ values (with 95% confidence intervals). For potency, an IC₅₀ of ≤9.9 µg/mL was considered potent; 10–99.9 µg/mL was mild; and ≥100 µg/mL was considered weak (Picking et al. 2018). Rhodiola was the most potent towards CES1 with an IC₅₀ of 4.3 µg/mL, followed by ginkgo (22.2 µg/mL), ginger (53.2 µg/mL), peppermint (70.9 µg/mL), and rosemary (74.2 µg/mL).



Herbal Medicine

Figure 5-2 The inhibitory effect of 21 extracts of commercial herbal tinctures (200 µg/mL) on carboxylesterase-1 mediated metabolism of 4-nitrophenyl acetate. JZL184 (2 µM or 1 µg/mL) was used as positive control (PC). Percent inhibition was calculated relative to vehicle control (VC-2% acetone final concentration). Means ± SEM are presented for three independent trials. Significant differences between extracts ($P < .05$) are denoted by letters, as determined by one-way ANOVA with Bonferroni post hoc test. (BAC) bacopa; (EPU) *Echinacea purpurea*; (GAR) garlic; (SCU) skullcap; (ELE) eleuthero; (PFL) passion flower; (LBM) lemon balm; (CHA) chamomile; (VAL) valerian; (EAC) EchinAce; (KAV) kava, (EAN) *Echinacea angustifolia*; (GOL) goldenseal; (LIN) linden; (GTE) green tea; (SJW) St. John's wort; (ROS) rosemary; (PEP) peppermint; (GIN) ginger; (RHO) rhodiola; and (GBI) ginkgo.

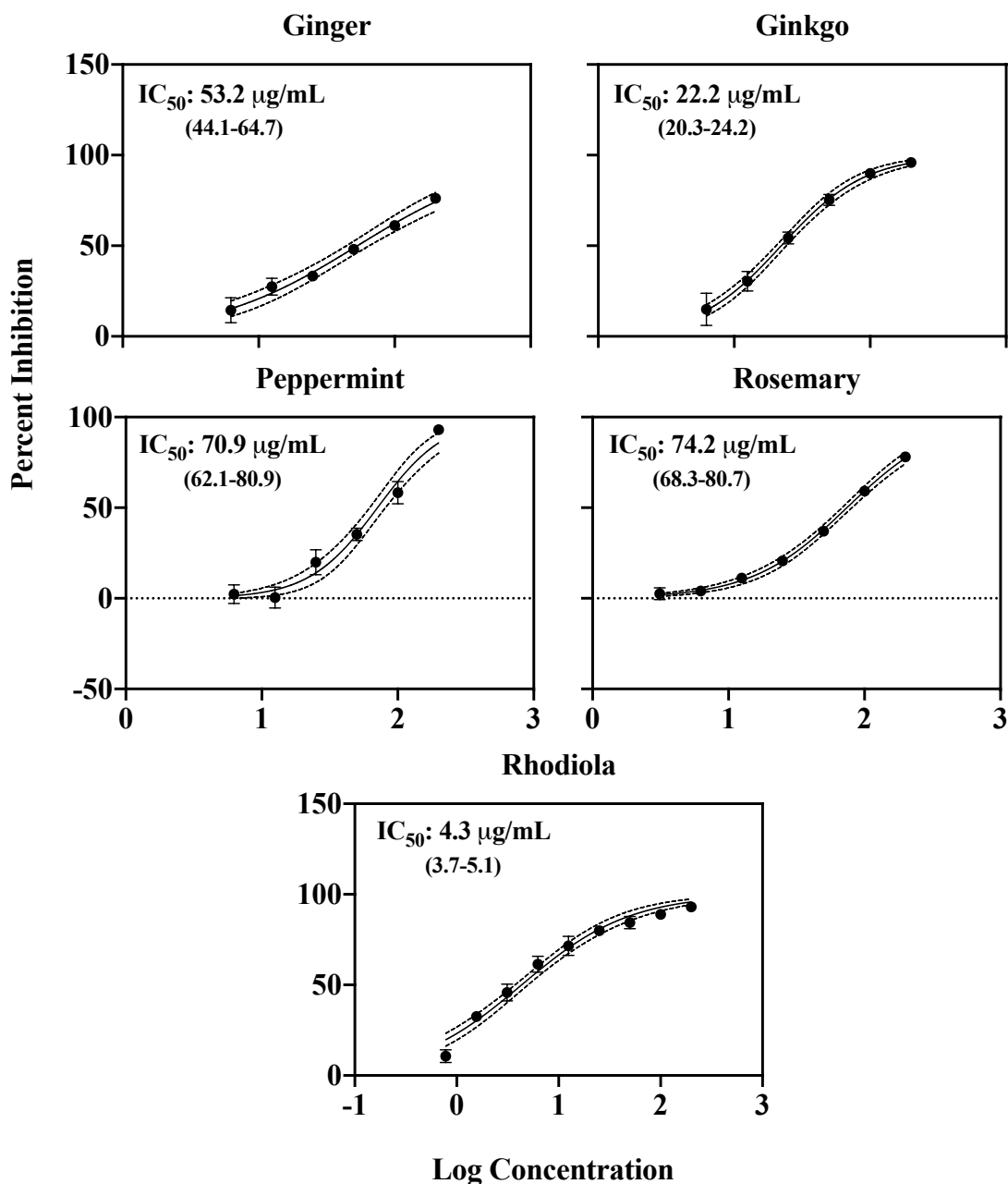


Figure 5-3 The mean median inhibitory concentration (IC_{50}) values and 95% confidence intervals for commercial extracts of ginger, ginkgo, rhodiola, rosemary, and peppermint on carboxylesterase-1 mediated metabolism of 4-nitrophenyl acetate. Concentrations were transformed into log form and percent inhibition was calculated relative to vehicle control (2% acetone final concentration), ($n = 3$). IC_{50} values were obtained using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0).

Time-dependent kinetic activity

In the time-dependent study (**Figure 5-4**), JZL184 displayed decreasing CES1 residual activity with increasing pre-incubation times, as expected. Significant differences were observed between the 0 and 5-minute ($P < .0001$, 95% CI: 9.2 to 28.4), 0 and 10-minute ($P < .0001$, 95% CI: 20.5 to 39.8), and 0 and 15-minute ($P < .0001$, 95% CI: 31.2 to 50.5) pre-incubation times for JZL184. In contrast and as expected, the residual activity of CES1 remained relatively constant with increasing time of pre-incubation with oseltamivir, a competitive inhibitor. No significant differences in CES1 activity were observed for ginger, peppermint, rhodiola, and rosemary extracts for pre-incubation times 5, 10, and 15 minutes relative to the 0-minute pre-incubation time. A significant increase ($P < .04$, 95% CI: -30.6 to 1.5) in CES1 residual activity was observed for ginkgo between the 0 and 10-minute pre-incubation times, but no significant differences were found between 5-minute pre-incubation times relative to 0 minutes for ginkgo. Overall, no herbal time-dependent inhibitors of CES1 were identified in this study.

Inhibition of CES1 by marker compounds

Marker compounds of ginger, ginkgo, peppermint, rosemary, and rhodiola were tested for their inhibitory potential against recombinant CES1 at 10 $\mu\text{g/mL}$ (**Figure 5-5**). Marker compounds of ginger, [8]-gingerol ($P = .005$, 95% CI: -107.8 to -12.8), and [10]-gingerol ($P = .002$, 95% CI: -114.7 to -19.7), showed significant inhibition compared to vehicle control. [6]-gingerol inhibited CES1 by 24% but this was not statistically significant ($P = .77$, 95% CI: -71.6 to 23.4). Marker compounds of ginkgo, rhodiola, rosemary and peppermint showed weak to zero inhibition. The positive control, JZL184, inhibited CES1 completely.

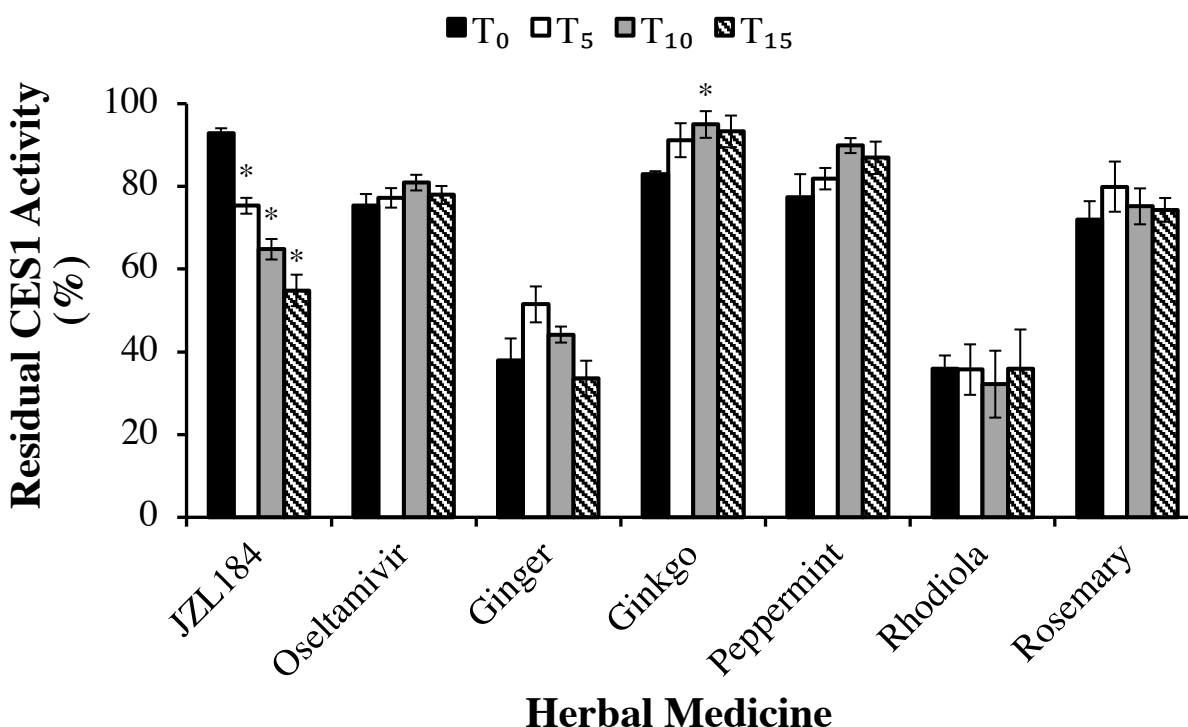


Figure 5-4 Determining mode of inhibition for commercial herbal extracts on carboxylesterase-1 (CES1) mediated metabolism of 4-nitrophenyl acetate using microtiter assay. The activity of CES1 after pre-incubation with extracts (0.04 mg/mL), JZL184 (0.5 µg/mL), and oseltamivir (1 mg/mL) were compared at 0, 5, 10, or 15-minute pre-incubation times. Values are shown as mean CES1 residual activity \pm SEM ($n = 3$). An ANOVA with Dunnett's post hoc was used to compare the differences in residual activity of time 0 vs. 5, 10 and 15-minute pre-incubation time for each control and extract. Significance is marked by* ($P < .05$).

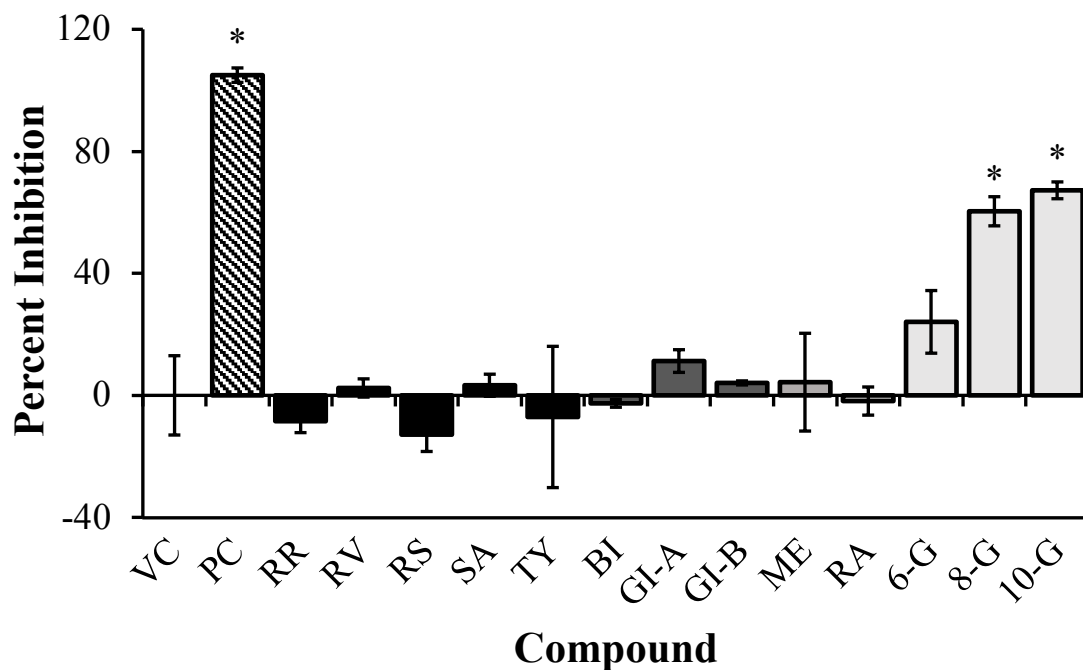


Figure 5-5 The inhibitory effect of marker compounds (10 $\mu\text{g/mL}$) of commercial herbal extracts on carboxylesterase-1 mediated metabolism of 4-nitrophenyl acetate. Means \pm SEM are presented for three independent trials. JZL184 (2 μM or 1 $\mu\text{g/mL}$) was used as a positive control (PC). (RR) rosin; (RV) rosin; (RS) rosin; (SA) salidroside; (TY) tyrosol; (BI) bilobalide; (GI-A) ginkgolide A; (GI-B) ginkgolide B; (ME) menthol; (RA) rosmarinic acid; (6-G) [6]-gingerol; (8-G) [8]-gingerol; (10-G) [10]-gingerol. A one-way ANOVA with Dunnett's multiple comparisons test was used to compare percent inhibition to vehicle control (2% acetone final concentration) * $P < .05$.

5.4.1 Phytochemistry marker compounds

Phytochemical profiling of extracts was performed to confirm the presence of marker compounds. The HPLC-DAD chromatograms in **Figure 5-6**, **Figure 5-7**, **Figure 5-8**, and **Figure 5-9** illustrate the presence of respective marker compounds in commercial extracts of ginger, rosemary, rhodiola, and ginkgo, respectively. Gas chromatography in **Figure 5-10** was used to confirm the presence of menthol in peppermint extracts as menthol cannot be detected through ultraviolet detection.

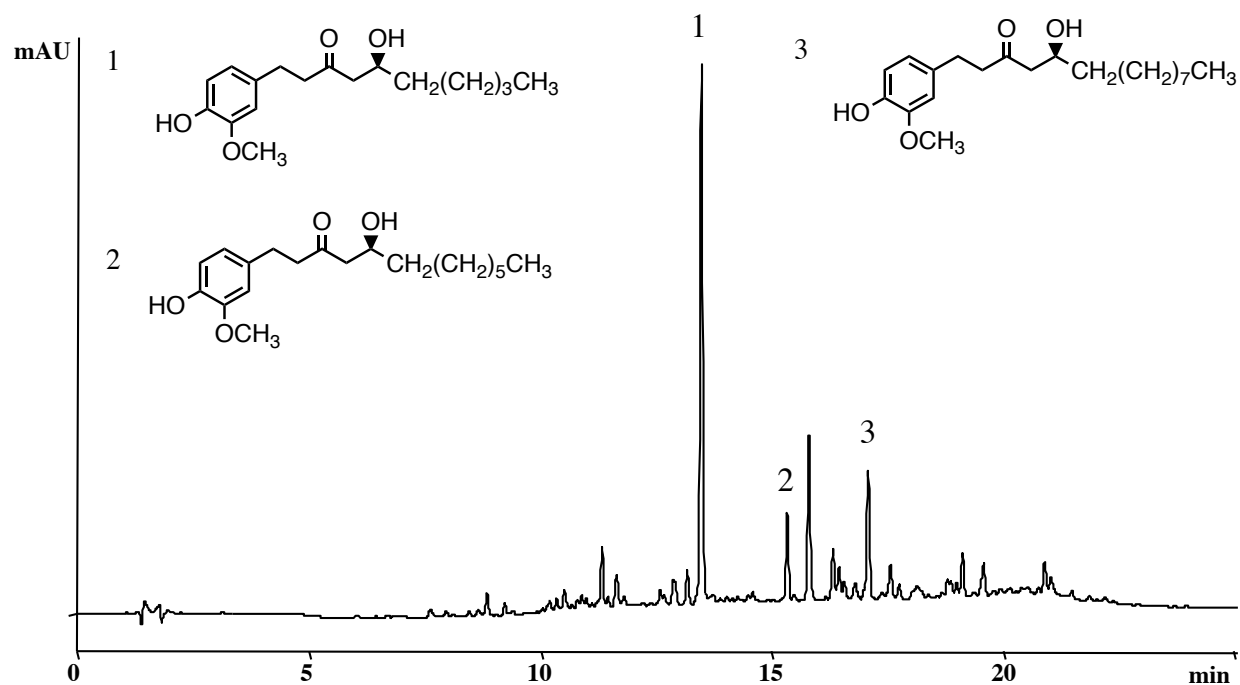


Figure 5-6 HPLC-UV chromatogram of 10 mg/mL extract of a commercial ginger product at 280 nm. Peaks of marker compounds are identified as (1) [6]-gingerol; (2) [8]-gingerol; (3) [10]-gingerol.

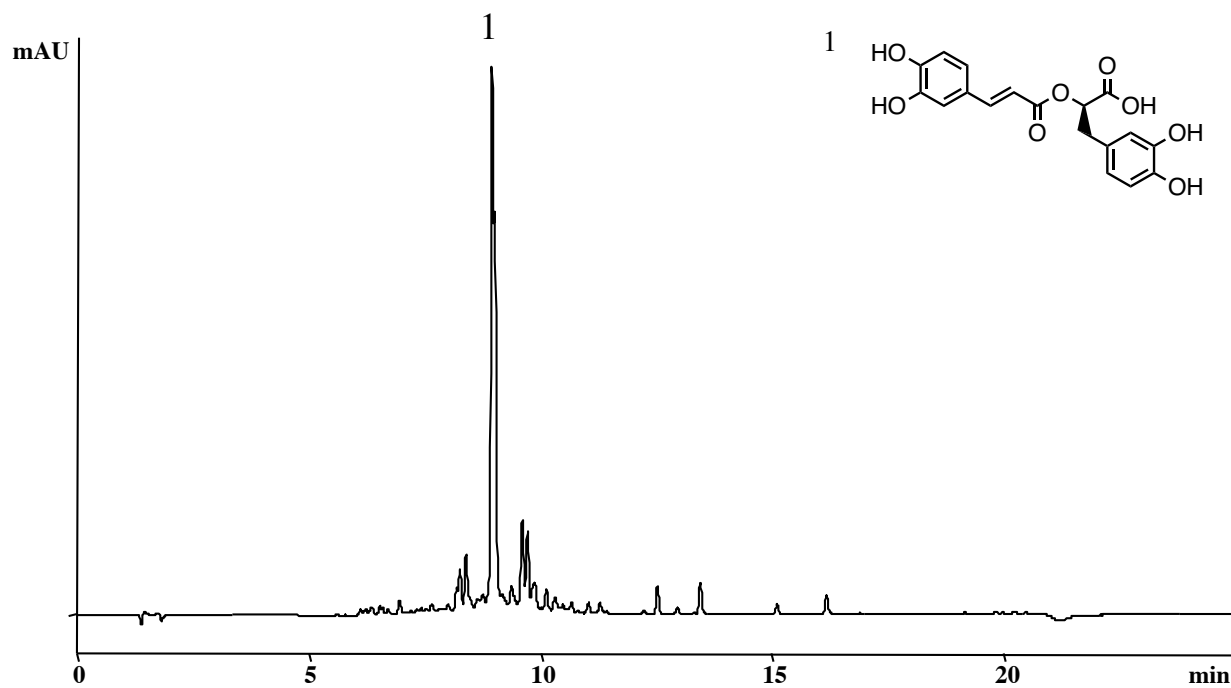


Figure 5-7 HPLC-UV chromatogram of 10 mg/mL extract of a commercial rosemary product at 350 nm. Peak of marker compound is identified as (1) rosmarinic acid.

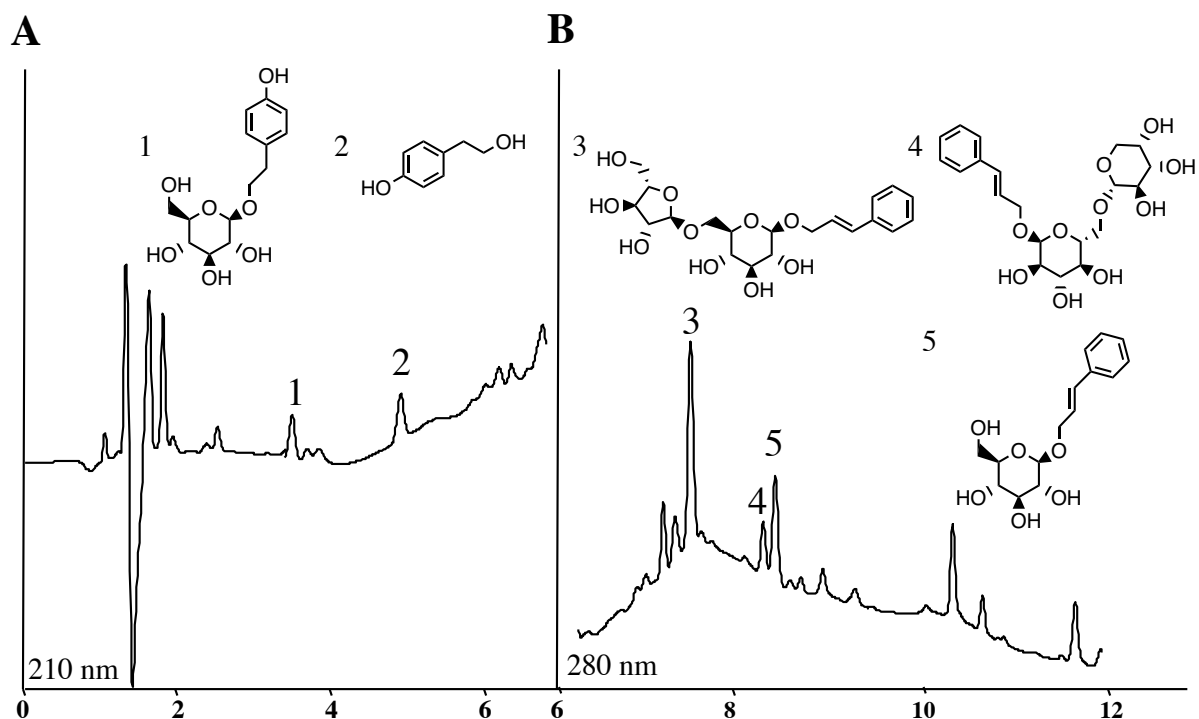


Figure 5-8 HPLC-UV chromatogram of 10 mg/mL extract of a commercial rhodiola product at A) 210 nm and B) 280 nm. Peak of marker compound is identified as (1) salidroside; (2) tyrosol; (3) rosarin; (4) rosavin; (5) rosin.

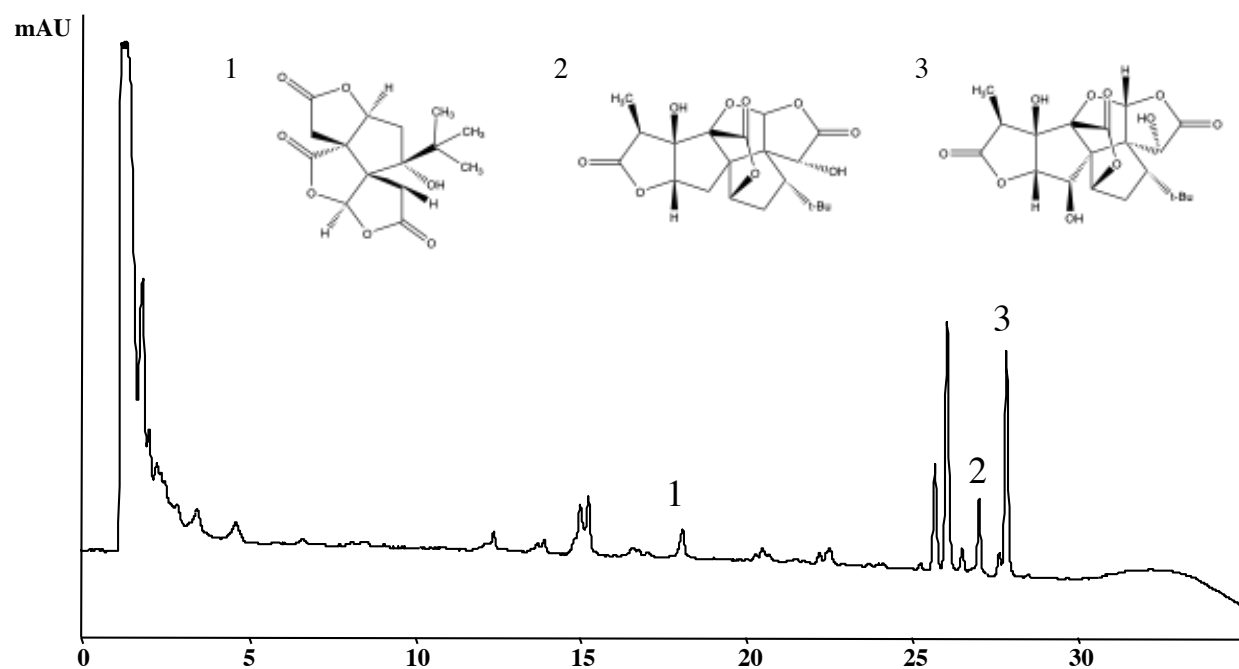


Figure 5-9 HPLC-UV chromatogram of 10 mg/mL extract of a commercial ginkgo product at 210 nm and 280 nm. Peak of marker compound is identified as (1) bilobalide; (2) ginkgolide A; (3) ginkgolide B.

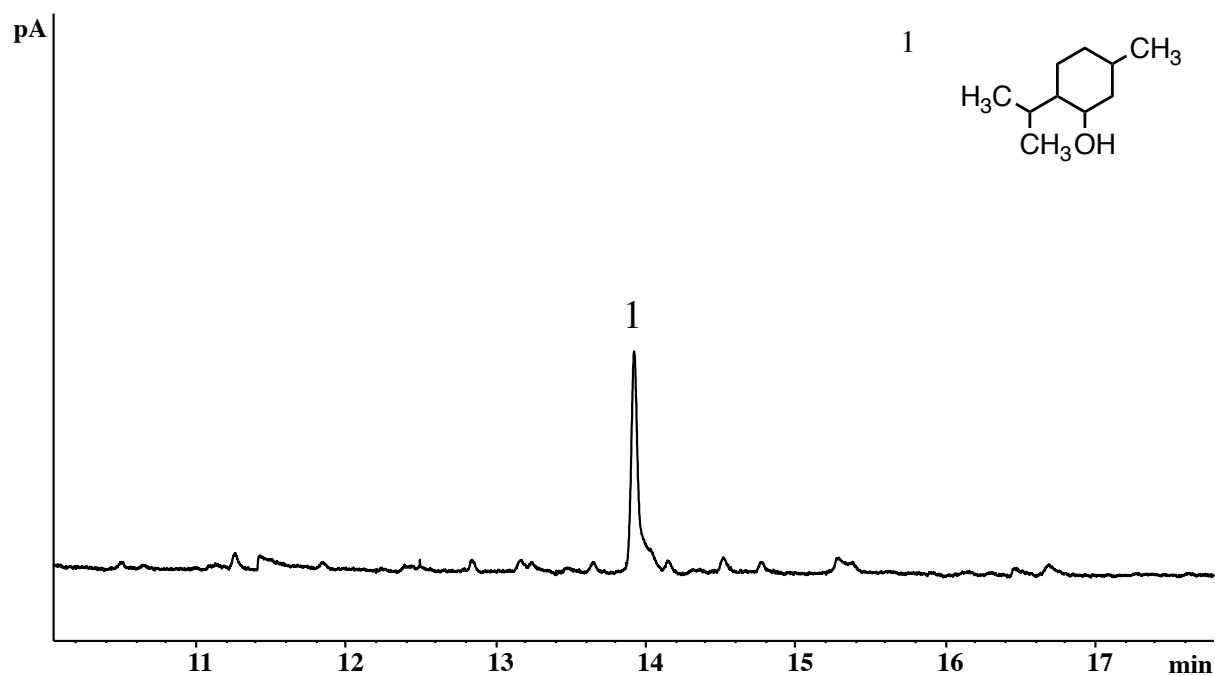


Figure 5-10 Gas chromatogram of 1 mg/mL extract of a commercial peppermint product.
Peak of marker compound is identified as (1) menthol.

5.5 DISCUSSION

The use of herbal medicine by children with ADHD, especially those concurrently using the psychostimulant methylphenidate, is common. Although licenced herbal medicines are generally considered safe to use, their safety profile when used concurrently with conventional drugs, especially those that are substrates of CES1, has not been studied extensively.

Our study demonstrated that the majority of the extracts from the 21 commercially available herbal extracts showed some inhibition of CES1 mediated metabolism of 4-NA at supraphysiological concentrations. Only a few other studies have examined the effects of these commercial herbal products in CES1 mediated metabolism. Gorman et al. (2013) found St. John's wort to be a weak inhibitor (49.4 mg/mL) of CES1 mediated metabolism of chemotherapeutic agent, irinotecan, in human liver microsomes. In the current study, St. John's wort displayed relatively moderate inhibition of recombinant CES1. However, in a passive monitoring of adverse events, Mazhar et al. (2019) found 2 reports involving St. John's wort and methylphenidate to be causality linked to be herb-drug interactions. Therefore, St. John's wort could have product-specific effects on CES1 inhibition, or could show varying inhibition based on the substrate, or enzyme source used. Liu et al. (2010) reported goldenseal to be a strong inhibitor (75% inhibition), and the aqueous extract of *Echinacea purpurea* extract (18% inhibition) to be a relatively mild inhibitor of CES1 mediated metabolism of the antiviral drug, oseltamivir, in human liver microsomes. Our study, the first to target CES1 specifically using a human recombinant enzyme, showed *Echinacea purpurea* to have negligible inhibition and goldenseal to have 62% inhibition at a single supraphysiological concentration (200 µg/mL). Differences in inhibition may reflect differences in experimental models or in phytochemistry of commercial extracts due to a host of intrinsic (plant species and source) and extrinsic factors

(environment and growth conditions, extraction protocols, product processing and storage, contamination or adulteration) (Foster et al. 2002; Zhang et al. 2011). Nonetheless, like drugs, the dose and rate at which herbal medicines are consumed could be a factor in their safety.

Ginger has previously been reported as a strong inhibitor of CES1 mediated metabolism of irinotecan in human liver microsomes, with an indeterminate mode of inhibition (Gorman et al. 2013). Our study showed ginger to be a reversible inhibitor of CES1. Moreover, we reported that marker compounds of ginger (between 29-33 μ M), [6]-gingerol, [8]-gingerol, and [10]-gingerol, showed 24%-67% inhibition of recombinant CES1 activity. The inhibition of CES1 by ginger-based products, as in our study, may be due to additive or synergistic inhibition by the marker constituents tested. To our knowledge, other herbal products examined in this study have not yet been studied for their effects on CES1 inhibition.

Many of the 21 herbal medicines investigated in the current study have been investigated *in vitro* for their effects on CYP 450 system. Particularly, CYP 2D6 and CYP 3A4 are important considering these enzymes are involved in the metabolic pathways of other therapeutic agents used for ADHD (amphetamine, atomoxetine, guanfacine) (Ring et al. 2002; Bach et al. 1999). Previous studies report rhodiola (Hellum et al. 2010; Xu et al. 2013), St. John's wort (Komorowski et al. 2004; Obach 2000), goldenseal (Chatterjee et al. 2003; Sevier et al. 2010) and kava (Mathews et al. 2002) to be moderate to strong inhibitors of both CYP 2D6 and CYP 3A4. Green tea (Misaka et al. 2012), and peppermint (Dresser et al. 2002) were moderate to strong inhibitors of CYP 3A4. In the current study, these products showed potential for interaction with CES1 substrate with inhibition ranging from 62%-93% in the initial evaluation of the extracts. Bacopa is a moderate inhibitor of CYP 3A4 (Ramasamy et al. 2014), but did not show any inhibition of

recombinant CES1. In general, herbal remedies known to inhibit CYP 3A4, and CYP 2D6 were among the moderate to stronger inhibitors of CES1 in the current study.

Subsequent analyses of concentration-dependent and time-dependent responses indicated that rhodiola was a reversible yet potent inhibitor (IC_{50} = 4.3 μ g/mL) of human CES1 at supraphysiological concentrations and may pose a risk to individuals concurrently using conventional drugs, such as methylphenidate, that are substrates of CES1. Moreover, we found ginkgo (22.2 μ g/mL), ginger (53.2 μ g/mL), peppermint (70.9 μ g/mL), and rosemary (74.2 μ g/mL) to be mild inhibitors of CES1 under these study conditions, that may also pose a risk of interaction when used concurrently with CES1-substrate drugs.

The HPLC-DAD profile of rhodiola extract in this study exhibited the presence of marker compounds that rhodiola is commercially standardized to, including salidroside, tyrosol, rosavin, rosin, and rosarin (Dimpfel et al. 2018). Considering the IC_{50} , rhodiola was the most potent commercial product identified in our study, however, its marker constituents showed little to no activity in CES1 at a single concentration. Moreover, rhodiola constituents did not show inhibition of various cytochrome P450 enzymes (Hellum et al. 2010; Thu, Nilsen, et al. 2016; Zhu et al. 2013). Hence, the tested marker compounds do not explain the observed CES1 inhibitory activity of the extract in this study. This is concerning as the level of risk (in terms of CES1 inhibition) cannot be determined based on the marker compounds labelled on the product. Additional research is needed to understand variation in inhibition potential between various products of rhodiola, and identifying phytochemicals contributing to the inhibitory activity.

CES1 activates several ester containing prodrugs (e.g., oseltamivir) and anti-hypertensive angiotensin-converting enzyme (ACE) inhibitor drugs (e.g., imidapril), and inactivates many other therapeutic agents including anti-epileptic drugs (rufinamide), and antithrombotic drugs

(e.g., clopidogrel) (Shi et al. 2006; T Williams et al. 2011; Takai et al. 1997; Tang et al. 2006).

Like methylphenidate, the risk of herb-drug interaction for patients taking these therapeutic agents is high, especially for those who are taking multiple therapeutic drugs, or herbal medicines. Patients practicing polypharmacy would be expected to be at a higher risk of experiencing clinically significant herb-drug interactions. Studies have reported CES1 polymorphisms resulting in reduced ability to hydrolyze CES1 substrates including methylphenidate, clopidogrel, and oseltamivir (Zhu, Patrick, et al. 2008; Zhu et al. 2009; Zhu et al. 2013). Reduction in enzyme activity can result in an increase in the concentration of active parent drugs leading to potential toxic effects, and poor efficacy for prodrugs requiring activation through CES1. Individuals with CES1 polymorphisms utilizing herbal medicines that we identified as CES1 inhibitors with CES1 substrates, may have an increased risk of changes in drug pharmacokinetics leading to clinically significant adverse effects.

Considering the overall observed CES1 inhibition by extracts, the concurrent use of methylphenidate (and other CES1 substrate drugs) with herbal remedies tested, in general, is likely safe. However, patients using higher or frequent doses of herbal remedies (especially of ginkgo, rhodiola, peppermint, rosemary, and ginger products) are at higher risk of herb-drug interactions and should monitor their adverse events. It is also fundamental for patients to communicate with their healthcare professionals about the use of herbal medicines, and for healthcare practitioners to routinely inquire about the use of these products. Healthcare professionals should gain familiarity about the effects of concurrent use of popular herbs with therapeutic drugs to provide evidence-based information to patients.

5.6 ACKNOWLEDGEMENTS

The authors would like to thank St. Francis Herb Farm for providing the herbal tinctures. We would like to thank Saipranay Guntaka, Evan Trofimchuk, and Mahdi Mahallati for the dried extract preparations. We would also like to thank Rui Liu for his help with training with the assay procedures and phytochemical analysis.

Chapter 6

In vitro* inhibition of Carboxylesterase-1 mediated metabolism by extracts of *Rhodiola Rosea

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Author's Contributions

CSH, HM, and PR contributed to the design of the study. HM and LOM performed data collection. Data analysis was performed by CSH, HM, and LOM. HM wrote the manuscript. CSH, HM, LOM and PR revised, and approved the final manuscript.

6.1 ABSTRACT

Rhodiola rosea L. (Crassulaceae) is a highly valued medicinal plant used for many centuries and is classified as an adaptogen. In a previous *in vitro* study, a commercial *R. rosea* product potentially inhibited the activity of carboxylesterase-1 (CES1), a serine hydrolase enzyme responsible for the metabolism of many clinically relevant drugs, such as methylphenidate. The goal of this study was to evaluate the effect of various commercial products containing *R. rosea* on CES1 enzyme *in vitro*. Commercial products of *R. rosea*, and its marker phytochemical constituents, were examined for their effects on recombinant CES1 mediated metabolism. Extracts were tested for irreversible (time-dependent) inhibition by pre-incubating extracts with CES1 prior to adding the substrate. Phytochemistry of extracts was characterized by HPLC-DAD with quantification of marker compounds. Select extracts were examined to determine effects on human liver microsomes mediated metabolism of methylphenidate, a psychostimulant. All commercial products of *R. rosea* showed inhibition of recombinant CES1 activity with the most potent samples eliciting IC₅₀ values of ~30 µg/mL. The same two samples, when pre-incubated with CES1 prior to adding substrate, showed time-dependent inhibition suggestive of irreversible inhibition. Phytochemical characterization showed varying concentrations of rosavins and salidroside across the commercial products. However, the tested phytochemicals did not show potent inhibition of recombinant CES1. When assayed using human liver microsomes, the selected *R. rosea* extracts showed dose-dependent inhibition of methylphenidate metabolism. Commercial *R. rosea* products can inhibit CES1 mediated metabolism *in vitro*. Follow-up research is warranted to confirm these interactions *in vivo*.

6.2 INTRODUCTION

In a national survey in 2001, about 57% of users reported using natural health products (NHPs) concurrently with conventional drugs (Singh et al. 2006). The use of NHPs is steadily increasing, with about 73% of Canadians reporting having used NHPs, (including vitamins and minerals, herbal remedies and homeopathic medicines) in 2010, compared to 71% in 2005 (Ipsos 2011). Parallel to the increasing use, the incidence of adverse reactions from the use of NHPs increased from 12% (in 2005) to 15% (in 2010) (Ipsos 2011). Unlike conventional drugs, NHPs, especially herbal remedies, are chemically complex and contain raw or processed medicinal and non-medicinal substances that, when used concurrently with conventional drugs, can interfere with drug metabolism, transport, or action and lead to NHP-drug interactions. NHP-drug interactions include pharmacokinetic or pharmacodynamic responses leading to potential adverse events (World Health Organization 2005a). Herbal constituents that are irreversible inhibitors of metabolic enzymes can exacerbate safety concerns further, as there is an increased risk of pharmacokinetic interactions when products are used over a long period of time (Orr et al. 2012). Therefore, it is important to study the safety profiles of commonly used herbal medicines, and to identify potential herb-drug interactions resulting from concurrent use with conventional drugs.

Rhodiola rosea L. (Crassulaceae), commonly known as Rhodiola, Golden Root, Arctic Root and Roseroot, is mainly distributed in low-arctic to high-temperature regions of Asia, Europe, Greenland, and North America (Brown et al. 2002). *Rhodiola rosea* (*R. Rosea*) is a highly valued medicinal plant in Eurasia, specifically in Central Asia, Russia, Siberia, and Scandinavian countries. The roots and rhizomes of *R. rosea* have been used medicinally for many centuries for various indications including depression, enhancing work performance, fatigue, nervous system stimulant, altitude sickness, anemia, impotence, gastrointestinal

disorders, infections, cold, and flu symptoms (Khanum et al. 2005; Brown et al. 2002). *R. rosea* is classified as an “adaptogen”, providing non-specific resistance against a variety of physical, chemical and biological stressors (Brown et al. 2002; Lazarev 1958). Among other affects, *R. rosea* has antioxidant (Calcabrini et al. 2010), anti-inflammatory (Bawa et al. 2009), anti-cancer (Liu et al. 2012), anti-depressant (Perfumi et al. 2007), anti-diabetic (Kwon et al. 2006), anxiolytic (Mattioli et al. 2009), psychostimulant (Spasov et al. 2000), and neuroprotective properties (Palumbo et al. 2012).

In terms of safety, several studies have evaluated the effect of commercial *R. rosea* on cytochrome P450 enzymes (CYP P450) *in vitro*. The IC₅₀ values in these studies ranged from 7.2-106.6 µg/mL for CYP 3A4, 13.0-186.1 µg/mL for CYP 2D6, 10.7-116.0 µg/mL for CYP 1A2 and 19.2 µg/mL for CYP 2C9 (Thu, Nilsen, et al. 2016; Thu et al. 2017), suggesting variable effects on CYP enzymes based on tested *R. rosea* material, and extraction solvents. Moreover, through time-dependent and NADPH-dependent assays, *R. rosea* was characterized as a reversible inhibitor of CYP 2C9 (Thu et al. 2017). *R. rosea* was evaluated *in vitro* using a cocktail approach to determine the effect of commercial products on CYP 1A2, CYP 2C9, CYP 2C19, CYP 2D6, and CYP 3A4 in humans. This study revealed a significant reduction in the drug/metabolite ratio, suggesting a significant inhibitory effect on CYP 2C9 in humans (Thu, Spigset, et al. 2016). Clearly, *R. rosea* has the capacity to inhibit CYP enzymes, hence the effects on other drug-metabolizing enzymes warrants investigation.

Around 140 compounds belonging to various biochemical classifications have been isolated from the roots and rhizomes of *R. rosea*, including monoterpene alcohols and their glycosides, cyanogenic glycosides, aryl glycosides, phenylethanoids, phenylpropanoids and their glycosides, flavonoids, flavolignans, proanthocyanidins, and gallic acid derivatives (Panossian et

al. 2010). While salidroside, a phenylethanoid compound, was previously considered the major marker compound (Brown et al. 2002; Wang et al. 2012), it is also present in other *Rhodiola* species. Hence, the phenylpropanoids collectively called “rosavins” (rosarin, rosavin, and rosin), in conjunction with salidroside (**Figure 6-1**) are now considered to be the major marker compounds of *R. rosea*, and are used to determine quality of commercial products (Rodin et al. 2012; Brown et al. 2002; USP 2020b). Commercial *R. rosea* products are generally standardized to about 1% salidroside content, and about 3% total rosavins (Dimpfel et al. 2018). The marker compounds of *R. rosea* do not show significant activity on CYP P450 enzymes *in vitro*, including CYP 2D6, CYP 3A4, CYP 3A5, and CYP 1A2 (Ahmed 2015; Xu et al. 2013; Thu, Nilsen, et al. 2016; Hellum et al. 2010).

Unlike cytochrome P450 enzymes, which are usually the focus of research on NHP-drug interactions, carboxylesterase mediated NHP-drug interactions have been largely overlooked. Carboxylesterase-1 (CES1) is a serine hydrolase, belonging to the multi-gene family of α/β hydrolase fold proteins (Cygler et al. 1993), and is responsible for metabolism of various endogenous and exogenous substances including esters, amides, thioesters, and carbamates (Sato et al. 1998; Casey Laizure et al. 2013). CES1 is primarily expressed in the liver, but also in the intestine, kidneys and lungs to a lower extent (Sanghani et al. 2009). CES1 is responsible for the hydrolysis of many clinically relevant therapeutic agents including antihypertensives (imidapril), chemo-therapeutic agents (irinotecan), psychostimulants (methylphenidate), anti-virals (oseltamivir), as well as some drugs of abuse such as cocaine and heroin (Geshi et al. 2005; Sanghani et al. 2004; Laizure et al. 2003; Srinivas et al. 1992; Kamendulis et al. 1996; Shi et al. 2006).

In vitro studies investigating inhibition of CES1 mediated metabolism reported significant inhibition of the enzyme with various therapeutic drugs (isradipine, tacrolimus, nelfinavir, simvastatin, lovastatin), suggesting the potential for clinical drug interactions with therapeutic agents that are also substrates of CES1 (Thomsen et al. 2014; Rhoades et al. 2012; Fukami et al. 2010). Some popular NHPs and their constituents have also been determined as inhibitors of CES1, suggesting risk of clinical NHP-drug interactions. For example, Liu et al. (2010) reported extracts of echinacea and goldenseal inhibit human liver microsome mediated metabolism of oseltamivir, reducing the formation of the active drug. Our preliminary research evaluated 21 herbal remedies commonly used by patients with attention-deficit hyperactive disorder (ADHD) and showed that an extract of a commercial *R. rosea* product was a potent inhibitor (IC₅₀: 4.3 µg/mL) of CES1 *in vitro* (Mazhar et al. 2019-Chapter 4 unpublished work).

To date, the effects of different commercial *R. rosea* products, and inhibitory effects of varying marker compounds on CES1 mediated metabolism have not been evaluated. The present study aims to evaluate the inhibitory potential of various commercially available *R. rosea* products on CES1 enzyme activity, and further elucidate the safety risk by detection of time-dependent (irreversible) inhibition. Phytochemical constituents of extracts of *R. rosea* commercial products were identified and quantified using HPLC-DAD (High Performance Liquid Chromatography-Diode Array Detection) with reference standards, which were subsequently examined for their inhibitory potential for CES1 enzyme.

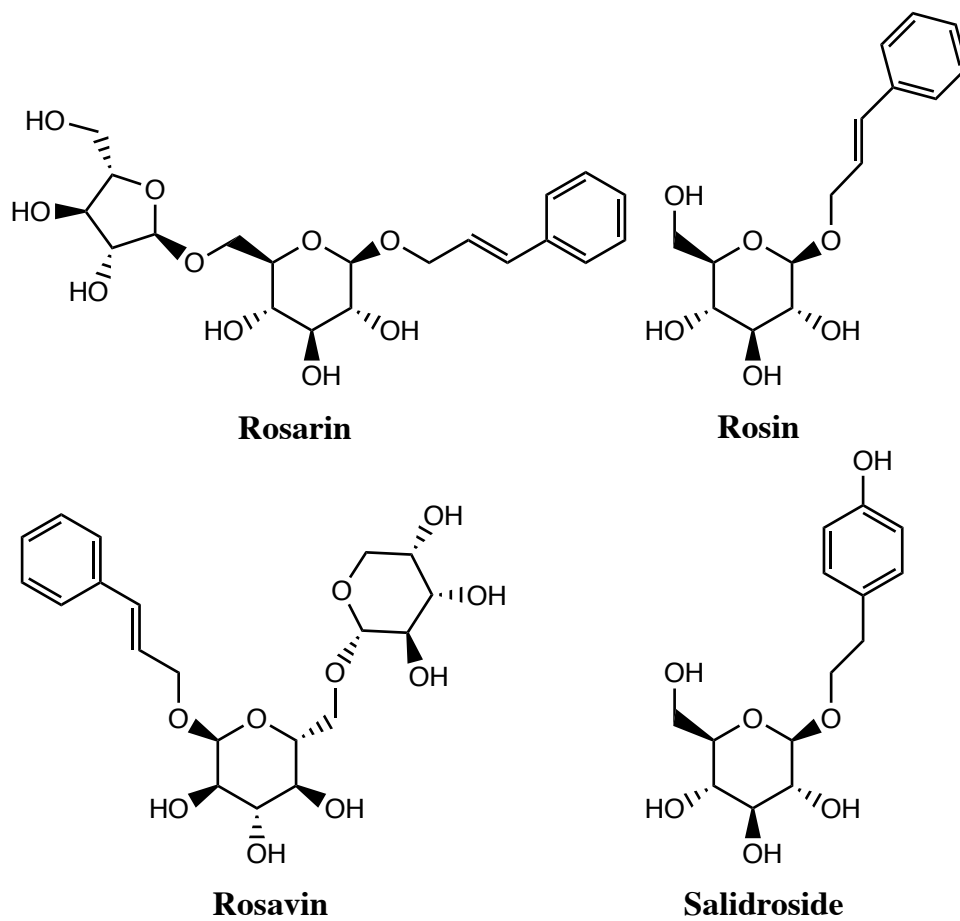


Figure 6-1 Chemical structures of marker phytochemicals of *Rhodiola rosea*.

6.3 METHODS

6.3.1 Chemical and reagents

Recombinant human CES1 and human liver microsomes (HLM) (Ultrapool™ HLM, 150 donors, mixed gender) were purchased from Corning Life Sciences (Tewksbury, MA, U.S.A.). 4-Nitrophenyl acetate (4-NA), oseltamivir, and Tris were purchased from Sigma-Aldrich (Oakville, Ontario). JZL184 was obtained from Cayman Chemical Company (Ann Arbor, Michigan, U.S.A.). HPLC grade acetone was purchased from Fisher Scientific (Ottawa, Ontario). Rosarin, rosavin, rosin, salidroside were purchased from ChromaDex (Los Angeles, California, U.S.A.). Methylphenidate and ritalinic acid were purchased from Toronto Research Chemicals (Toronto, Ontario). All solvents used in HPLC analysis were HPLC grade and purchased from Fisher Scientific (Ottawa, Canada). *R. rosea* products (**Table 6-1**) were purchased from local retailers in the Ottawa region.

6.3.2 Commercial Plant Extracts and Tinctures

Information about the *R. rosea* products (Nature's way, Orange, Starwest Botanicals) tested in this study can be found in **Table 6-1**. The dried plant material and capsules were extracted with 99% ethanol (and/or distilled water for aqueous extract of dried plant material) by gentle oscillation for 3 hours at room temperature. The ethanolic extracts of the dried material and capsules, and tincture were spun in Labconco Centrивap Concentrator with an attached Centrивap Cold Trap overnight to remove alcohol. All samples were lyophilized in an EC Modulyo Freeze Drier (Thermo Electron, Ottawa, ON, Canada) to remove water. Dried extracts were weighed and stored in darkness at -20 °C until use.

For the CES1 assay, 10 mg of each dried extract was dissolved in 20% acetone to obtain 10 mg/mL stock solutions. Each stock solution was vortexed and sonicated to ensure homogeneity immediately before serial dilution and experimental use.

6.3.3 CES1 inhibition assay

The CES1 inhibition assay procedure described in Chapter 5 (**Section 5.3.3**) was followed for the single concentration, concentration-dependent inhibition, inhibition of marker compounds, and the time-dependent inhibition (irreversible inhibition) studies.

Single concentration, and concentration-dependent inhibition

R. rosea extracts were evaluated at a single supraphysiological concentration of 200 µg/mL to gauge inhibitory potential. Subsequently, serial dilutions of stock solutions were performed to yield six to seven concentrations (400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.2 µg/mL) to evaluate concentration-dependent response and determine half maximal inhibitory concentrations (IC₅₀).

In vitro time-dependent Inhibition (irreversible inhibition)

Commercial *R. rosea* extracts (40 µg/mL) were assessed for potential time-dependent inhibition of CES1 *in vitro* using the pre-incubation method described in Chapter 5 (**5.4.3**). The kinetic response of *R. rosea* was compared to positive and negative controls including an irreversible inhibitor, JZL184 (0.5 µg/mL), and a reversible competitive inhibitor, oseltamivir (1 mg/mL), respectively (Crow et al. 2012; Shi et al. 2006).

Effect of marker compounds on CES1 in vitro

Standard marker compounds of *R. rosea* (rosarin, rosavin, rosin, salidroside) were tested at 4 to 5 concentrations (23-270 μ M) to evaluate their inhibitory potency for CES1 mediated metabolism using the CES1 inhibition assay.

Table 6-1 Product label information of commercial *Rhodiola rosea* L. natural health products (NHPs) examined in this study.

NHP	Medicinal Ingredient	Standardization	Formulation	Product claims	Suggested dose
RR-1	<i>R. rosea</i> root extract 250 mg	3% rosavins	Capsules	Support cognitive functions (mental focus and stamina)	1 capsule (250 mg) 2 times per day
RR-2	<i>R. rosea</i> root 250 mg	-	Tincture	Mental fatigue related to stress	1 mL 3 times per day
RR-3/4*	<i>R. rosea</i> root	-	Dried plant material	N/a	N/a

*(RR-3) ethanolic and (RR-4) water extracts of dried material

6.3.4 Phytochemical characterization and quantification of marker compounds in *R. rosea* commercial extracts

The HPLC-DAD (High-performance liquid chromatography diode array detection) analysis method was adapted from previously published methods (Ahmed 2015). Briefly, *R. rosea* extracts were reconstituted in methanol at 5 mg/mL, sonicated, and filtered using 0.2 µm PTFE filters. Agilent 1100 HPLC system with Chemstation software (Version B 3.02) was used for phytochemical analysis. The system consisted of an autosampler (G1313A with 100 µL loop), Quaternary pump (G1311A), a solvent degasser (G1322A), a column oven (G1316A), and a photodiode array detector (G1315A).

Separation of marker compounds was performed on Luna C18 (4.6 mm x 150 mm, 5 µm particle size, Phenomenex Inc., Mississauga, Ontario). Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B was methanol. The separation gradient consisted of 5-100% solution A for 25 minutes at 40°C. The injection volume was 10 µL, and the column oven temperature was set at 55°C. The flow rate was 0.5 mL/minute and the DAD was set to 280 nm to 350 nm. Phytochemical markers were identified in the extracts based on comparison to retention time of pure standard compounds. Quantification was performed using area under the peak and calculated based on the linear calibration curves of the pure compounds.

6.3.5 Inhibition of human liver microsome mediated metabolism of methylphenidate

Experiments for human liver microsome (HLM) mediated metabolism of methylphenidate (MPH) were modified from Ross et al. (2007). Two time points, 0 minutes (baseline) and 90 minutes (endpoint), were used to determine effects of *R. rosea* (sample RR-2) on microsomal CES1 mediated metabolism of MPH. For the 0- and 90-minute time points, the reaction mixture (total volume: 200 µL), consisted of 50 mM Tris (pH: 7.5), MPH (final

concentration: 1 μ M), and *R. rosea* RR-2 extract (50 μ g/mL and 100 μ g/mL). The reaction was initiated by adding HLM (final concentration: 1 mg/mL) to the mixture. The reactions were vortexed and incubated for 90 minutes at 37°C in a shaking incubator at 200 rpm. After 90 minutes, ice cold methanol (200 μ L) was added to the 90-minute reaction mixtures. Ice cold methanol (200 μ L) was added to the 0-minute reactions immediately after the addition of microsomes. After stopping the reaction with methanol, all the reactions (0-minute and 90-minute) were vortexed, then centrifuged at 10,000 x g, and the supernatant was filtered through 0.2 μ PTFE filters (Chromatographic Specialities Inc., Brockville, ON, Canada). JZL184 (1 μ M) was used as a positive control, and 20% acetone was used as a vehicle control. Two negative control were conducted, one without HLM and one without MPH. The supernatants were stored at -20°C until HPLC analysis.

HPLC analysis was carried out on Luna 3 μ C18 (2) (150 mm x 2 mm, 3 micron) column held at 25°C with a flow rate of 0.25 mL/min. Mobile phase A was water in 0.1% trifluoroacetic acid, and mobile phase B was acetonitrile in 0.1% trifluoroacetic acid. Mobile phase B was 5% at 0 minutes, increased to 50% at 10.1 minutes, 100% at 15 minutes, and held at 40% till 16 minutes. The column was equilibrated for 5 minutes after each sample, and the DAD was set to 210-330 nm. Percent inhibition was calculated by comparing the peak area of methylphenidate before (time 0) and after incubation with HLM exposed to *R. rosea*, vehicle or positive control.

6.3.6 Statistics

IC₅₀ values were obtained by plotting percent inhibition against log-transformed concentration (mg/mL) using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0). A one-way ANOVA with Bonferroni post hoc test was used to compare % inhibition from extracts and marker compounds, IC₅₀ values, and phytochemical

quantification of commercial *R. rosea* products. A one-way ANOVA with Dunnett's post hoc was used to compare HLM mediated metabolism of MPH, relative to vehicle control. An ANOVA with Dunnett's post hoc was used to compare the differences in residual activity of time 0-minutes vs. 5-, 10- and 15-minute pre-incubation time for each control and extract. A Pearson Correlation was performed to explore potential relationships between inhibition of extracts and the concentration of marker phytochemicals in extracts. A $P \leq .05$ was considered significant for all comparison.

6.4 RESULTS

6.4.1 CES1 Inhibition

Single concentration and concentration-dependent inhibition of CES1

A preliminary assessment (**Figure 6-2**) was conducted for all extracts of *R. rosea* at a single supraphysiological concentration (200 µg/mL) to determine the inhibitory potential for CES1 mediated metabolism of 4-NA. The inhibition of CES1 from the extracts ranged from 66.3-84.0%. RR-4 displayed weaker inhibition than RR-3 ($P < .02$, 95% CI: -32.1 to -3.2). The positive control, JZL184, inhibited CES1 at 91.1%.

The concentration-response curves, and corresponding IC₅₀ values of extracts of *R. rosea* on CES1 mediated metabolism of marker substrate are displayed in (**Figure 6-3**). RR-2 and RR-1 were the most potent ($P < .05$) among the investigated *R. rosea* extracts for CES1 enzyme with an IC₅₀ of 29.7 µg/mL and 30.5 µg/mL, respectively. RR-3 and RR-4 showed relatively weaker inhibition, with IC₅₀ values of 75.1 µg/mL and 108.7 µg/mL, respectively.

Time-dependent Inhibition (irreversible inhibition)

The *R. rosea* extracts were evaluated for time-dependent inhibition of CES1. As expected, JZL184 showed significant ($P < .05$) decreases in residual CES1 activity with increasing pre-incubation times (**Figure 6-4**). The residual activity of oseltamivir remained relatively consistent, as expected, with increasing pre-incubation times. RR-2 showed significant ($P < .05$) decreases in residual CES1 activity across all incubation times. Though RR-1 exhibited a general trend of decreasing residual CES1 activity across pre-incubation times, it only reached statistical significance ($P < .05$) with the 10- and 15-minute pre-incubation times. Neither RR-3 nor RR-4 appeared to have statistically significant time-dependent inhibition patterns.

Effect of pure compounds on CES1 activity

The marker phytochemicals rosarin, rosavin, rosin, and salidroside were evaluated for their inhibitory potency for recombinant CES1 at 4 to 5 concentrations (23-270 μM) *in vitro*. The marker compounds showed IC_{50} values ranging from 139-205 μM (**Figure 6-5**). The strongest inhibitory potency was exhibited by rosarvin, followed by rosarin, salidroside, and rosin.

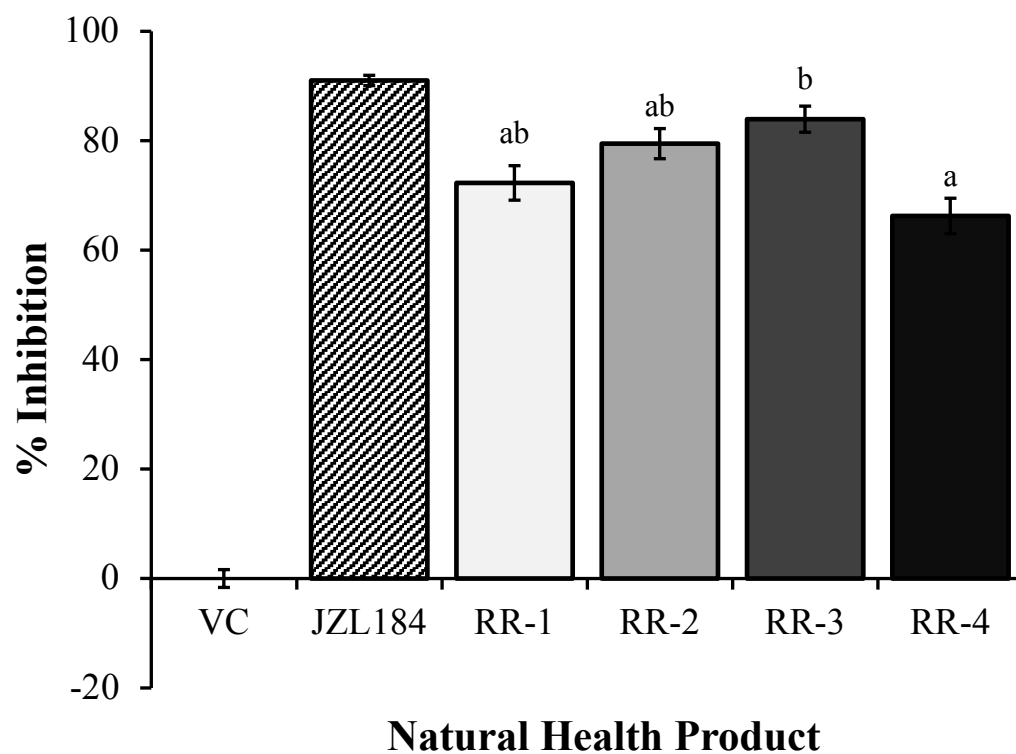


Figure 6-2 Mean percent inhibition of carboxylesterase-1 mediated metabolism of marker substrate by extracts of commercial *Rhodiola rosea* products (200 µg/mL) and positive control (JZL184, 2 µM or 1 µg/mL). Percent inhibition was calculated relative to vehicle control (VC-2% acetone final concentration), and presented as means \pm SEM of three independent experiments. Significant differences amongst the extracts ($P < .05$) are denoted by letters, as determined by one-way ANOVA with Bonferroni post hoc test.

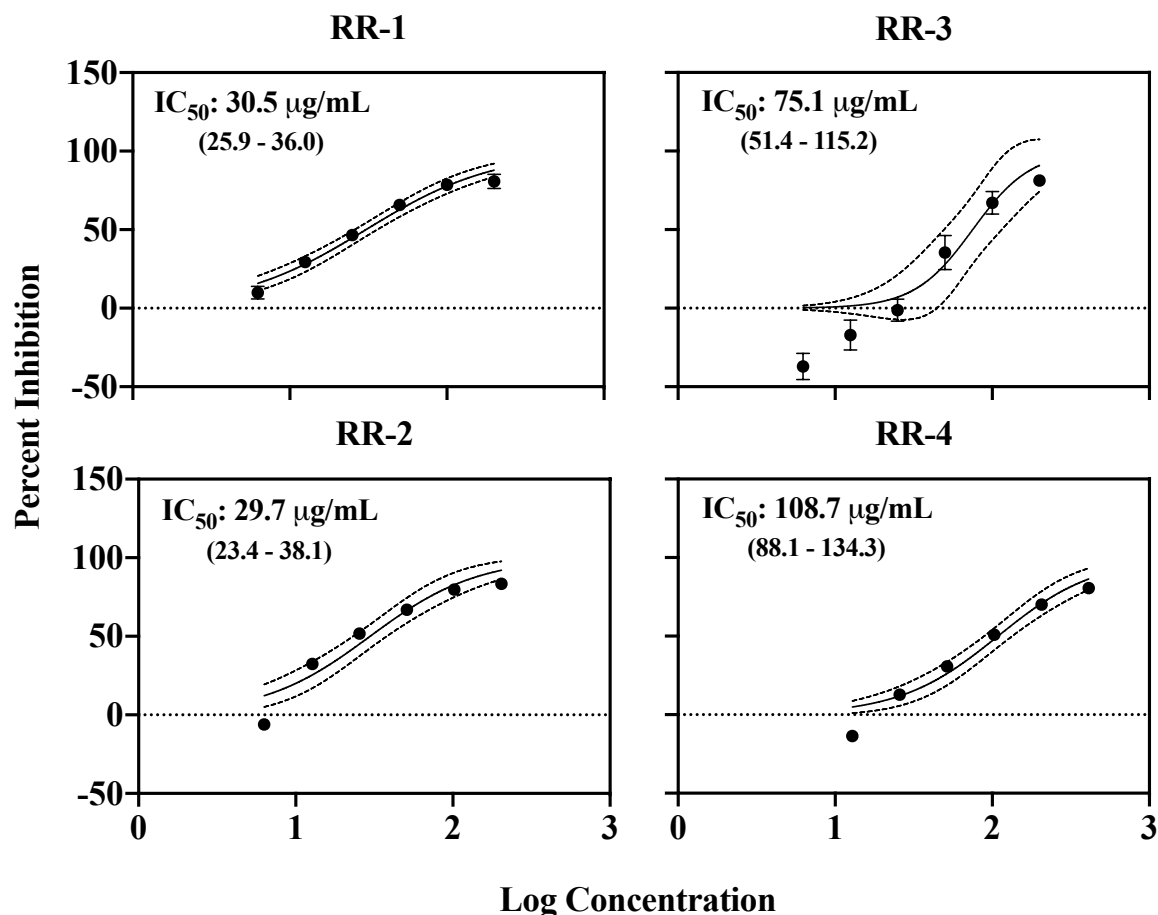


Figure 6-3 Mean inhibitory potency (IC₅₀), and concentration-response curves of extracts of *Rhodiola rosea* products on carboxylesterase-1 mediated metabolism *in vitro*. Extracts were tested at various concentrations (6.2-400 µg/mL). Mean IC₅₀ (95% confidence intervals) values are presented. The concentrations were transformed into log form and percent inhibition was calculated relative to vehicle control (2% acetone final concentration), ($n = 3$). IC₅₀ values were obtained using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0).

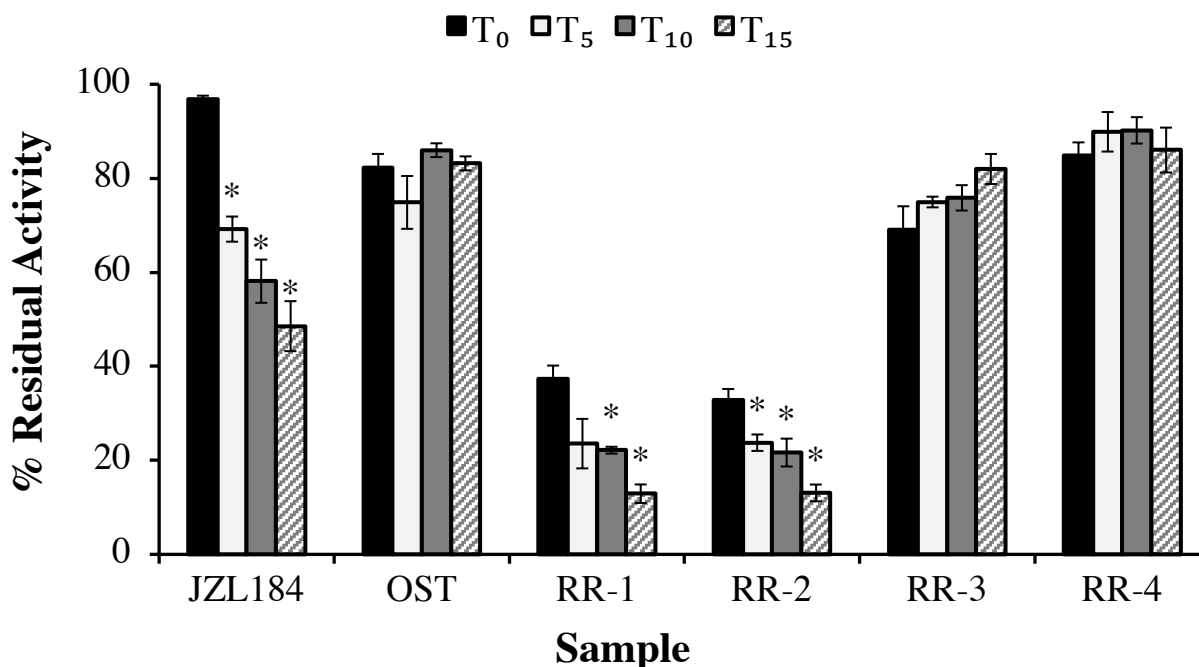


Figure 6-4 Mean percent residual activity of carboxylesterase-1 (CES1) after pre-incubation with each extract of *Rhodiola rosea* products (40 µg/mL) for 0, 5, 10, and 15 minutes. The results displayed are means of 3 independent experiments \pm SEM. Percent inhibition was calculated relative to vehicle control (2% acetone final concentration). JZL184 (0.05 µg/mL) and oseltamivir (1 mg/mL) were used as positive and negative control respectively. An ANOVA with Dunnett's post hoc was used to compare the differences in residual activity of time 0-minutes vs. 5-, 10- and 15-minute pre-incubation time for each control and extract. Significance is marked by* ($P < .05$).

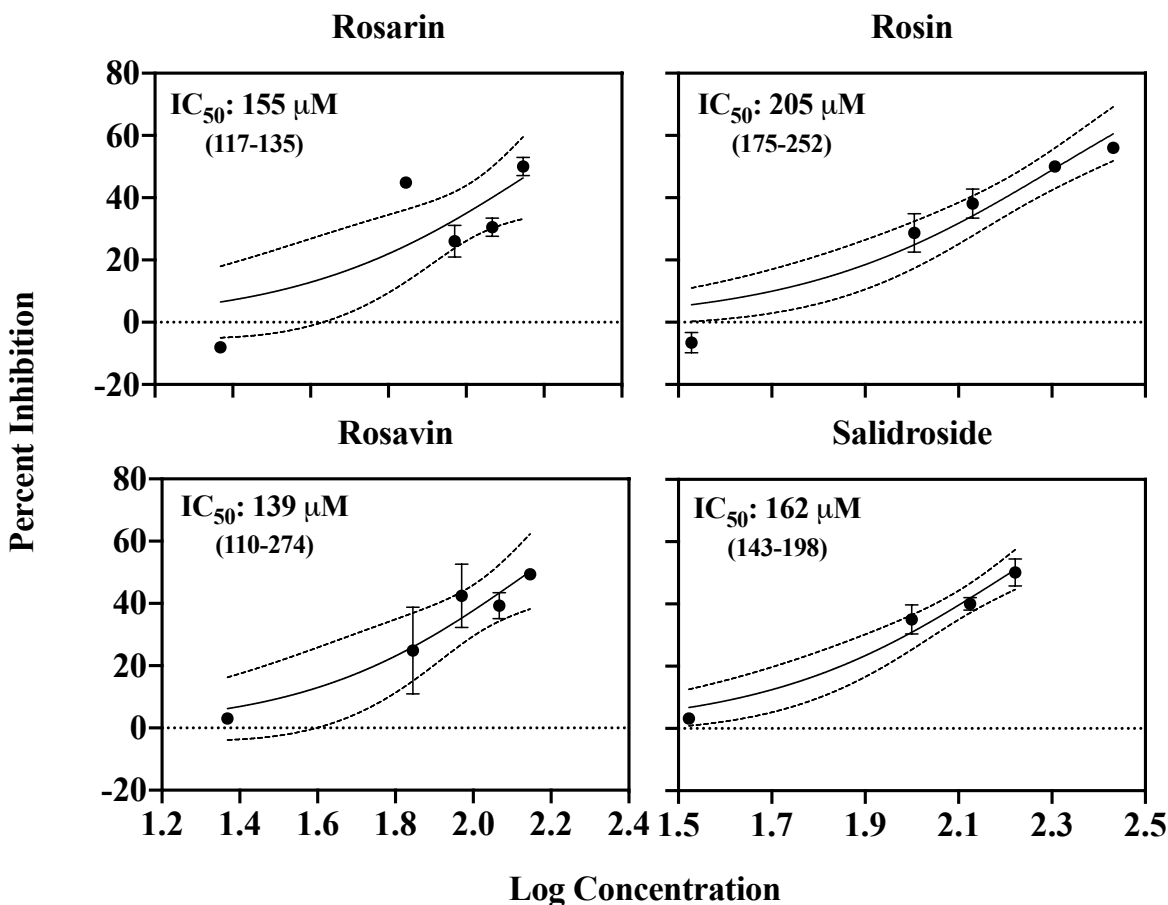


Figure 6-5 Mean inhibitory potency (IC_{50}), and concentration-response curves of marker compounds of *Rhodiola rosea* for carboxylesterase-1 mediated metabolism *in vitro*. Rosarin, rosavin, rosin, and salidroside were tested at various concentrations (23-270 μ M). Mean IC_{50} (95% confidence intervals) values are presented. The concentrations were transformed into log form and percent inhibition was calculated relative to vehicle control (2% acetone final concentration), ($n = 3$). IC_{50} values were obtained using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0).

6.4.2 Phytochemical Analysis

Phytochemical analysis of all extracts was performed using HPLC-DAD with respect to marker compounds, including salidroside, rosarin, rosavin, and rosin. **Table 6-2** displays phytochemical quantification of the extracts of *R. rosea*. The content of marker compounds was variable in each of the extracts. The variation of salidroside ranged from 2.9-114.1 µg/g, with the highest amount present in RR-1 ($P < .05$), and no detectable amount in RR-3. Rosarin, rosavin and rosin content ranged from 15.3-86.3 µg/g, 8.7-118.0 µg/g, and 3.9-43.7 µg/g, respectively. RR-1 had the highest concentration of rosavin and rosin, and RR-4 had the highest concentration of rosarin ($P < .05$). The total rosavin (rosarin, rosavin, and rosin) content varied as well (38.5-200.4 µg/g), with the highest total rosavin content found in RR-1 and lowest in RR-2 ($P < .05$). The concentration of marker phytochemicals in the extracts was not correlated with the observed inhibition.

6.4.3 Effect of *R. rosea* on HLM mediated metabolism of MPH

RR-2 (50 µg/mL and 100 µg/mL) extract exhibited concentration dependent inhibition of HLM-mediated metabolism of MPH (**Figure 6-6**). The 50 µg/mL extract of RR-2 showed 68.7% inhibition and the 100 µg/mL extract showed 88.9% inhibition relative to vehicle control. However, the inhibition was not statistically significant compared to vehicle control. The positive control, JZL184, exhibited 53.4% inhibition of MPH metabolism in HLM.

Table 6-2 Quantification of phytochemical marker compounds in extracts of commercial *Rhodiola rosea* natural health products (NHPs) using high performance liquid chromatography-diode array detection. The concentration of each compound in individual extracts is presented in $\mu\text{g/g}$ of extract. Means of three injections are presented \pm SEM. Significant differences within each marker compound ($P < .05$) are denoted by letters, as determined by one-way ANOVA with Bonferroni post hoc test.

	Total Rosavins*	Rosarin	Rosavin	Rosin	Salidroside
RR-1	200.3 ^c	38.6 \pm 2.1 ^b	118.0 \pm 10.5 ^c	43.7 \pm 3.8 ^c	114.0 \pm 2.1 ^b
RR-2	41.3 ^a	15.3 \pm 3.3 ^a	16.4 \pm 7.7 ^{ab}	6.7 \pm 2.8 ^{ab}	2.9 \pm 5.8 ^a
RR-3	93.4 ^b	32.3 \pm 2.6 ^b	44.2 \pm 5.8 ^b	16.9 \pm 2.1 ^b	N.d.
RR-4	98.9 ^b	86.3 \pm 0.1 ^c	8.7 \pm 1.1 ^a	3.9 \pm 0.4 ^a	9.5 \pm 4.7 ^a

N.d. -Not detected

* Total rosavins includes rosarin, rosavin, and rosin.

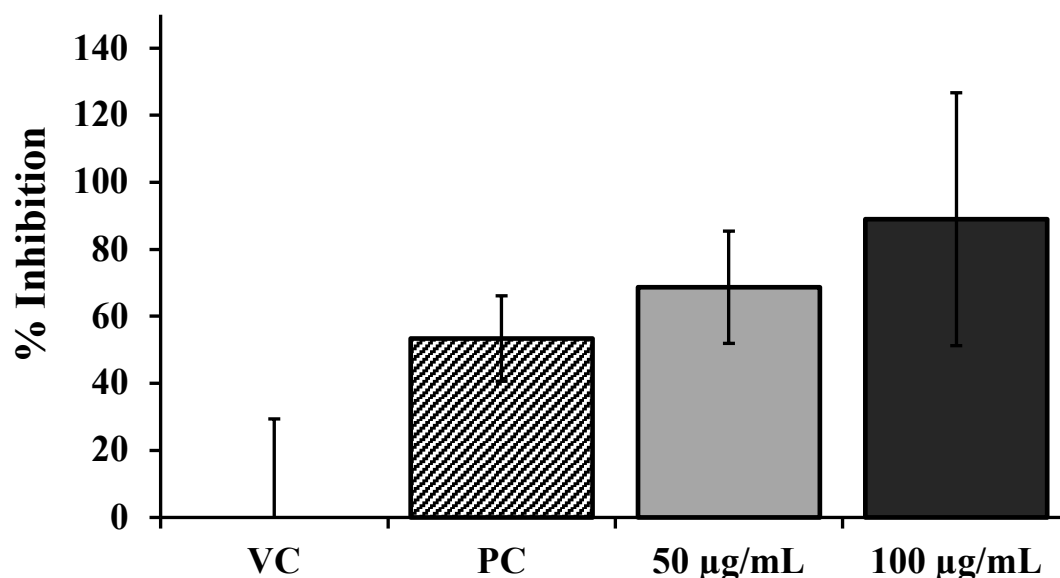


Figure 6-6 Mean percent inhibition of human liver microsome mediated metabolism of methylphenidate by RR-2, an extract of a commercial *R. rosea* product. Data presented as means (\pm SEM) of three independent experiments of difference in peak area of parent compound after 90 minutes of incubation, calculated relative to vehicle control (VC: 1% acetone final concentration). Positive control was JZL184 (1 μ M or 0.5 μ g/mL). A one-way ANOVA with Dunnett's post hoc test was conducted to compare all samples to vehicle control, with significant differences at $P < .05$.

6.5 DISCUSSION

R. rosea, a medicinal plant with adaptogen properties, is increasing in popularity amongst consumers worldwide (Cuerrier et al. 2014). In 2018, *R. rosea* was rated the 27th top selling herbal remedy in the United States (Smith et al. 2019). Previous research by our group identified an extract of a commercial *R. rosea* product to have a high risk of pharmacokinetic herb-drug interaction via recombinant CES1 mediated pathway *in vitro*. Though this extract of *R. rosea* demonstrated potent inhibition of CES1 ($IC_{50}=4.3 \mu\text{g/mL}$) (Mazhar et al. 2019- unpublished work Chapter 4), *R. rosea* products differ in phytochemistry (Wiedenfeld et al., 2007; Avula et al., 2009) and may present variable risk. The growing popularity of *R. rosea*, diversity of products, and risk of herb-drug interaction when used concurrently with prescription drugs thus motivated this evaluation of different *R. rosea* products. The current study examined the effect of the extracts of four *R. rosea* preparations, and their marker phytochemicals on *in vitro* CES1 mediated metabolism. The preparations included capsules, tinctures, and ethanolic and aqueous extracts of dried plant material. Ethanolic and aqueous extracts were included as *R. rosea* has traditionally been reported to be used as alcohol extracts (e.g., steeped in vodka), or as a tea (Länger 2012). The strongest inhibitory effect in recombinant CES1 in this study was exhibited by RR-1 and RR-2 ($IC_{50} \sim 30 \mu\text{g/mL}$). Extracts with IC_{50} ranges between 10-99.9 $\mu\text{g/mL}$ are considered moderate inhibitors based on published consensus (Picking et al. 2018). However, the concentrations used in this study were supraphysiological as different plant constituents have varying bioavailability and absorption, and these pharmacokinetic parameters are largely unavailable for many plant constituents (Awortwe et al. 2018).

Irreversible covalent binding of a reactive intermediate to an enzyme leads to loss of enzyme function and time-dependent inhibition, where longer pre-incubation of enzyme and

inhibitor results in lower residual activity when a substrate is introduced (Orr et al. 2012; Grimm et al. 2009). Herb-drug interactions from time-dependent inhibition can have a delayed onset and persist after the herb treatment has stopped, as enzyme activity is restored by *de novo* enzyme synthesis. In this study, two of four tested *R. rosea* extracts exhibited time-dependent (irreversible) decrease in CES1 activity whereas two showed only competitive CES1 inhibition. Therefore, some products (RR-1 and RR-2) may pose a greater risk of clinically relevant herb-drug interaction. It is possible that one or more of the phytochemical constituents, whether or not evaluated in this study, are time-dependent inhibitors of CES1.

Subsequent phytochemical characterization of the extracts showed variable concentration of rosavin, rosarin, rosin, and salidroside. Generally, commercial products of *R. rosea* are standardized to 3% rosavins, and 0.8-1% salidroside (Brown et al. 2002). However, various intrinsic (organ specificity, species differences, seasonal variation) and extrinsic factors (environment, harvest time, differences in techniques of drying, extraction, storage and manufacturing) can affect the level the marker compounds in a product (Ampong-Nyarko 2014; Benzie et al. 2011). In this study rosavin, rosarin, rosin and salidroside displayed weak inhibition of CES1 considering the general consensus of an IC_{50} of $>10\ \mu\text{M}$ is a weak inhibitor (White 2000). Although these compounds have pharmacological activities, they may not necessarily contribute to this bioactivity *in vitro*. Furthermore, in this study, differences in inhibition were not correlated to the quantity of marker compounds in the commercial extracts. Hence, it is likely that other compounds play a more significant role in the inhibition of CES1.

Besides the phenylethanoid and phenylpropanoid compounds, *R. rosea* contains phytochemicals from a variety of classes (Panossian et al. 2010) that may overlap with medicinal plants with similar properties. Phytochemicals in other adaptogens, such as *Panax ginseng* (i.e.,

Asian ginseng) and *Schisandra chinensis* (i.e., magnolia-vine) have been studied for their effects on CES1. Components of *Panax ginseng* including the triterpenes, dammarenediol II and 20S-*O*- β -(D-glucosyl)-dammarenediol II, potently inhibited CES1 mediated metabolism of probe substrate D-luciferin methyl ester (1.99 μ M and 1.76 μ M respectively) in HLM (Sun et al. 2019). Schisandrin B, a lignin found in *Schisandra chinensis* inhibited CES1 mediated hydrolysis of 2-(2-benzoyl-3-methoxyphenyl) benzothiazole by 80% in HLM (Fu et al. 2019). The pentacyclic triterpenoids, oleanolic acid and urosolic acid, found in many natural products (Fai et al. 2009) are potent inhibitors of CES1 mediated metabolism in HLM, with IC₅₀ values of 0.28 μ M and 0.24 μ M (Zou et al. 2017). It is possible that triterpenes and lignins, both found in *R. rosea* (Kurkin et al. 1991; Kurkin et al. 1985; Dubichev et al. 1991), could contribute to the inhibition of CES1.

A previous study using bioactivity-guided fractionation and purification identified hydroquinone as an active component in the chloroform fraction of *R. rosea* for inhibitory activity towards acetylcholinesterase (AChE), an enzyme also in the serine hydrolase family (IC₅₀: 9.8 μ g/mL) (Wang et al. 2007). Hillhouse et al. (2004) showed that alcohol extracts of *R. rosea* exhibited moderate (42%) inhibition of recombinant AChE. This fraction contained two flavanol glycosides, gossypetin-7-*O*-L-rhamnopyranoside and rhodioflavonoside, which inhibited AChE by 58% and 38% respectively. Although the inhibition of AChE can provide beneficial outcomes such as improvement in memory impairments for Alzheimer's disease, AChE inhibitors can also act as carboxylesterase inhibitors and lead to potential pharmacokinetic alterations of substrates of carboxylesterase (Tsurkan et al. 2013).

Inhibitory activity of RR-2 was further tested in HLM to determine direct effects on metabolism of MPH as this extract showed the strongest potential for herb-drug interaction in

recombinant enzyme assays. HLM contain a complex mixture of phase 1 enzymes, making them a more biologically relevant model for studying herb-drug interactions (Dudda et al. 2013; Bradshaw et al. 2018). *R. rosea* has previously been identified as an herbal remedy used by pediatric patients with ADHD, and other psychiatric disorders (Pellow et al. 2011; Sharma et al. 2015). Methylphenidate was chosen as the substrate for HLM mediated metabolism because *R. rosea* products may be used concurrently with methylphenidate, and the effects of NHPs on methylphenidate mediated metabolism are generally overlooked due to the drug's diverse adverse event profile (Efron et al. 1997). RR-2 product showed concentration-dependent effects of up to 88.9% inhibition of HLM mediated metabolism of MPH. As no cofactors (e.g., NADPH) were included, oxidation of MPH via cytochrome P450 pathways as an alternative mechanism can be excluded in our incubations. However, given the variation in the level of inhibition within samples and the vehicle control, it is plausible that other phase 1 enzymes (with broad substrate specificity) may play a role in the metabolism of MPH in HLM. Moreover, the *l*-enantiomer of MPH is metabolized six-fold more than the *d*-enantiomer by CES1 (Sun et al. 2004). In this study we used the racemic (*d,l*-threo) MPH, the preparation prescribed in clinical settings, which could contribute to the varying levels of hydrolysis in HLM.

R. rosea has been more extensively studied in CYP P450 enzyme systems, where reported results on CYP P450 activity are variable. In one study, ethanolic extracts of *R. rosea* showed potent inhibition of baculoviral expressed CYP 3A4 (IC_{50} = 1.7-3.1 μ g/mL) using testosterone as a substrate (Hellum et al. 2010). Thu, Nilsen, et al. (2016) used baculoviral expression with testosterone (CYP 3A4) and dextromethorphan (CYP 2D6) as substrates, and determined that commercial *R. rosea* variably inhibited CYP 3A4 (IC_{50} = 7.2-106 μ g/mL) and CYP 2D6 (13-186 μ g/mL). In both studies, the concentrations of marker phytochemicals

(salidroside, rosarin, rosavin, and rosin) were not correlated to inhibition of CYP enzymes. Scott et al. (2006) presented that *R. rosea* root extracts inhibited CYP 3A4 by 67%, and a positive relationship was found between rosarin concentration and inhibition of CYP3A4. A study performed in CYP 2D6 HLM mediated metabolism with flavonoid components of *R. rosea* found rhodiosin ($IC_{50}= 0.5 \mu\text{g/mL}$) and rhodionin ($IC_{50}= 0.2 \mu\text{g/mL}$) to be potent inhibitors. Although the phenylpropanoid ‘rosavins’ (rosarin, rosavin, and rosin), in conjunction with salidroside are used to determine quality of commercial products of *R. rosea*, it may be beneficial to perform further studies on the flavonoid constituents to determine if they should be standardized in *R. rosea* products to improve their safety profile. Furthermore, it is unlikely that rhodiosin and rhodionin are solely responsible for CYP 2D6 inhibition, and other components, including the excipients should be studied for inhibition potential.

CES1 is an important member of the serine hydrolase superfamily responsible for the metabolism of a variety of clinically important drugs (methylphenidate) and prodrugs (oseltamivir, imidapril, irinotecan) (Casey Laizure et al. 2013). To our knowledge, this is the first study examining and comparing extracts of various products of *R. rosea*, and their marker phytochemical constituents, for reversible and irreversible inhibition of CES1. Examining effects of commercial products on enzymes *in vitro* is a first stage assessment tool used for initial risk assessment of potential clinical interaction of medicinal plants with drugs. Risk assessments can identify products that require testing *in vivo*, and clinically through surveys and post-market surveillance. It is important to highlight that *in vitro* results do not always translate to clinically relevant interactions, but do demonstrate potential for risk.

Overall, our results indicate that commercial *R. rosea* products may pose a risk of herb-drug interaction with conventional drugs metabolized by CES1. Future studies should determine

natural pharmacophores, and use bioassay guided fractionation for CES1 to determine active fractions and compounds that may be time-dependent inhibitors. The risk of NHP-drug interactions should be investigated in vulnerable population of patients (e.g., pediatric). Healthcare professionals should frequently ask patients about the use of NHPs, and provide evidence-based advice, keeping in mind the risk to benefit ratio of NHPs.

6.6 ACKNOWLEDGEMENTS

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Chapter 7

Inhibition of carboxylesterase-1 mediated metabolism by commercial extracts of St. John's wort (*Hypericum perforatum*): Linking *in vitro* activity to clinical adverse events

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Author's Contributions

CSH, HM, and PR contributed to the design of the study. HM and LOM performed data collection. Data analysis was performed by CSH, HM, and LOM. HM wrote the manuscript. CSH, HM, LOM and PR revised, and approved the final manuscript.

7.1 ABSTRACT

St. John's wort (SJW) is one of the most commonly used herbal remedies among children with attention-deficit hyperactivity disorder (ADHD). Previous research identified 3 reports of possible herb-drug interactions leading to clinical adverse events involving SJW and methylphenidate (MPH), a psychostimulant. To identify possible mechanism of the interactions, our objective was to characterize the effects of commercial SJW products and select marker compounds on carboxylesterase-1 (CES1), a clinically relevant enzyme involved in xenobiotic metabolism. Products of SJW, marker phytochemical constituents (hyperforin and hypericin), and another abundant constituent, quercetin, were evaluated for their effects on recombinant CES1-mediated metabolism. Phytochemical quantification and time-dependent inhibition of the extracts were evaluated. Select SJW extracts were examined to determine effects on human liver microsome mediated metabolism of methylphenidate. The inhibitory potency for the extracts for ranged from 99.1 to 434.6 µg/mL. None of the commercial products were identified as time-dependent inhibitors. Phytochemical characterization showed varying concentrations of hypericin and quercetin, and relatively consistent concentrations for hyperforin across the extracts. Hyperforin was the most potent CES1 inhibitor (IC_{50} : 65.4 µM) among marker compounds. The ethanolic extract of a SJW product completely inhibited human liver mediated metabolism of methylphenidate. Commercial SJW products can interfere with the safety and efficacy of methylphenidate.

7.2 INTRODUCTION

Hypericum perforatum L. (Hypericaceae), commonly known as St. John's wort (SJW) is a perennial medicinal herb native to Europe, North Africa, and western Asia, and naturalized to Australia, and the Americas (Lawvere et al. 2005). In Canada and the United States, SJW is available to treat depression, anxiety, and sleep disturbances. It is also known for improving mental performance including concentration and memory (Laakmann et al. 1998; Schulz et al. 2001; Lehrl et al. 1993). In 2018, SJW was ranked the 36th top selling herbal remedy in the United States mainstream retail channel (supermarkets, drug stores, and mass market retailers) (Smith et al. 2019). SJW preparations consists of dried flowers and stems, and can be found as alcohol extracts, dry extracts in capsules or tablets, oil infusions, and aqueous extracts (i.e., tisanes) (Blumenthal et al. 2000).

The anti-depressant effects of SJW are well known, with numerous meta-analyses of randomized clinical trials available (Linde et al. 2005; Linde et al. 1996; Kim et al. 1999; Ng et al. 2017; Röder et al. 2004). However, research on the anti-depressant effect of its phytochemical constituents is conflicting. SJW contains several classes of phytochemicals including flavanols, flavanol glycosides, biflavones, naphthodianthrones, phloroglucinols, and phenylpropanes (Nahrstedt et al. 1997; Erdelmeier 1998). Commercial SJW products are generally standardized to hypericin and/or hyperforin, e.g., not less than 0.6% of hyperforin and not less than 0.04% of the combined total of hypericin and pseudohypericin, on the dried basis (USP 2020a).

While various studies have aimed to identify the pharmacologically active constituents of SJW, it remains unclear which constituents wholly or partially account for the anti-depressant activity. Hyperforin, a phloroglucinol derivative, has anti-depressant properties as demonstrated by experimental (Chatterjee, Bhattacharya, et al. 1998; Chatterjee, Nöldner, et al. 1998) and

clinical studies (Laakmann et al. 1998). Hyperforin inhibits the reuptake of synaptic serotonin, noradrenaline, and dopamine in animal models (Chatterjee, Bhattacharya, et al. 1998; Cervo et al. 2002). An early study shows that hypericin inhibits monoamine oxidase (Suzuki et al. 1984), however, subsequent studies refute this report (Demisch et al. 1989; Cott 1997). Thiede et al. (1994) show that the flavonoid components are responsible for monoamine oxidase inhibition.

More recently, SJW has been investigated for use by children with attention-deficit hyperactivity disorder (ADHD), with mixed findings on efficacy (Niederhofer 2010; Weber et al. 2008). SJW was effective for treating comorbid conditions with ADHD such as depression, autism, anxiety (Simeon et al. 2005; Hübner et al. 2001; Findling et al. 2003; Helmut Niederhofer 2009). Nonetheless, in the United States, SJW is one of the most commonly used herbal remedy among children with ADHD (Cala et al. 2003).

SJW is known to interact with several conventional medicines, and as a result, severe cases of herb-drug interactions have led to numerous *in vitro* and *in vivo* studies investigating its safety. SJW has known interactions with indinavir (Piscitelli et al. 2000), cyclosporine (Barone et al. 2000), and warfarin (Jiang et al. 2004), resulting in subtherapeutic concentrations of the drug, or toxic effects. The pharmacokinetic safety of SJW has been studied extensively. Hyperforin and hypericin are potent inhibitors of human recombinant CYP 2D6 and CYP 3A4 *in vitro* (Obach 2000). Quercetin is also an inhibitor of CYP 2D6 and CYP 3A4 in human liver microsome mediated metabolism of probe substrates (Rastogi et al. 2014). Commercial SJW products activate the pregnane X receptor, and induce CYP 3A4 *in vitro* in human hepatocytes (Wentworth et al. 2000; Moore et al. 2000). In humans, SJW induces CYP 3A4 (Roby et al. 2000), and intestinal P-glycoprotein (Dürr et al. 2000).

Psychostimulants are commonly used concurrently with herbal remedies for ADHD management (Galicia-Connolly et al. 2014). However, the safety of SJW for concurrent use with methylphenidate (MPH), a psychostimulant used for ADHD management, has not been established. Our previous research identified two clinical adverse event reports involving methylphenidate and SJW that were adjudicated as probable or possible for herb-drug interactions. The adverse events involved psychotic symptoms with hallucinations in one report, and agitation, aggressiveness, excessive weeping, depression, and difficulty concentrating in the other (Mazhar et al. 2019). Another case report describes the diminishing effects of MPH upon administration of SJW, with the patient becoming more inattentive, indicating a potential interaction (Niederhofer 2007). The mechanism of these reactions remains unclear.

As a step towards understanding the mechanisms, the objective of this research was to determine the potential for St. John's wort to inhibit human carboxylesterase-1 (CES1) mediated metabolism and determine the mode of inhibition through time-dependent enzyme kinetics assays. The extracts of St. John's wort were characterized for phytochemistry, and marker compounds were quantified and evaluated for inhibition potential for CES1 enzyme. One potent SJW extract was tested for direct effects of SJW on human liver microsome-mediated metabolism of MPH.

7.3 METHODS

7.3.1 Chemical and reagents

Recombinant human CES1 and human liver microsomes (HLM) (Ultrapool™ HLM, 150 donors, mixed gender) were purchased from Corning Life Sciences (Tewksbury, MA, U.S.A.). 4-Nitrophenyl acetate (4-NA) oseltamivir, and Tris were purchased from Sigma-Aldrich

(Oakville, Ontario). JZL184 was obtained from Cayman Chemical Company (Ann Arbor, Michigan, U.S.A.). HPLC grade acetone was purchased from Fisher Scientific (Ottawa, Ontario). Methylphenidate and ritalinic acid were purchased from Toronto Research Chemicals (Toronto, Ontario). Hypericin, hyperforin, and quercetin were purchased from ChromaDex (Los Angeles, California, U.S.A.). All solvent used in HPLC analysis were HPLC grade and purchased from Fisher Scientific (Ottawa, Canada). SJW products (Botanica, Orange, Starwest Botanicals) (**Table 7-1**) were purchased from local retailers in the Ottawa region.

7.3.2 Commercial Plant Extract and Tinctures

The SJW dried plant material and capsules were extracted with 99% ethanol (and distilled water for dried plant material) by gentle oscillation for 3 hours at room temperature. The ethanolic extracts of the dried material and capsules, and tincture were spun in Labconco Centrivap Concentrator with an attached Centrivap Cold Trap overnight to remove alcohol. All samples were lyophilized in an EC Modulyo Freeze Drier (Thermo Electron, Ottawa, ON, Canada) to remove water. Dried extracts were weighed and stored in darkness at -20 °C until use.

For the CES1 assay, 10 mg of each dried extract was dissolved in 20% acetone to obtain 10 mg/mL stock solutions. Each stock solution was vortexed and sonicated immediately before use to ensure homogeneity.

Table 7-1 Product label information of commercial *Hypericum perforatum* (St. John's wort) natural health products (NHPs) examined in this study.

NHP	Medicinal Ingredient	Standardization	Formulation	Product Claim	Suggested Dose
SJW-1	1350 mg ¹ SJW ² flowering tops and buds (<i>Hypericum perforatum</i>)	2.7 mg ¹ hypericins per 2250mg dry herb	Capsules	Supports positive emotional health and promotes a healthy mood	1 capsule 3 times daily
SJW-2	250 mg SJW tops (<i>Hypericum perforatum</i>)	-	Tincture	Relieves nervousness and restlessness	Adults: 2 mL, 3 times per day
SJW-3/4 ³	SJW dried herb (<i>Hypericum perforatum</i>)	-	Dried plant material	N/a	Tea: 1 teaspoon dried herb over 237-355 mL boiling water

¹ per 3 capsules

² SJW-St. John's wort

³ SJW7-alcohol extract, SJW8-water extract.

7.3.3 Carboxylesterase-1 Assay procedure

A microtiter plate assay was used to evaluate the inhibitory potential of commercial SJW products and marker phytochemicals towards CES1 mediated metabolism of 4-NA. The CES1 inhibition assay procedure outlined in Chapter 5 (5.3.3) was used.

Single concentration and concentration-dependent inhibition of CES1 by SJW extracts

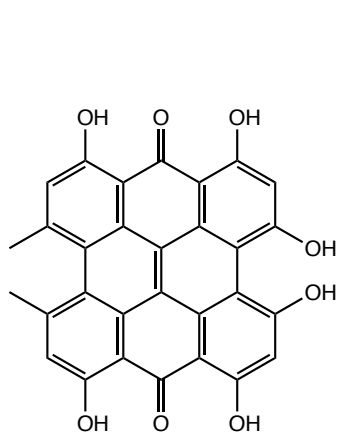
SJW extracts were first evaluated at a single supraphysiological concentration (200 µg/mL). Subsequently, serial dilutions of stock solutions were performed to yield six concentrations ranging from 800 µg/mL-6.25 µg/mL to evaluate a concentration-dependent response, and determine half maximal inhibitory concentrations (IC₅₀) using methods described in Chapter 5 (5.3.3).

Time-dependent (irreversible) inhibition activity of extracts

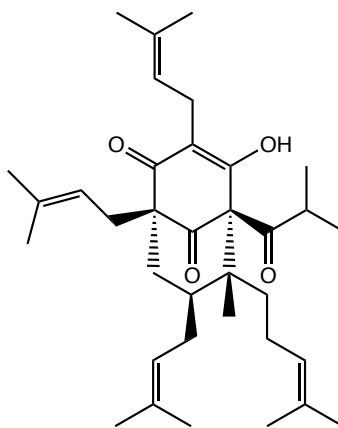
SJW extracts were assessed for potential time-dependent inhibition of CES1. The time-dependent response of SJW extracts (0.04 mg/mL) was compared to an irreversible inhibitor, JZL184 (0.5 µg/mL), and a reversible competitive inhibitor, oseltamivir (1 mg/mL) (Crow, Bittles, Borazjani, Potter, & Ross, 2012; Shi et al., 2006) using the pre-incubation method described in Chapter 5 (5.4.3).

Inhibitory effect of marker compounds on CES1

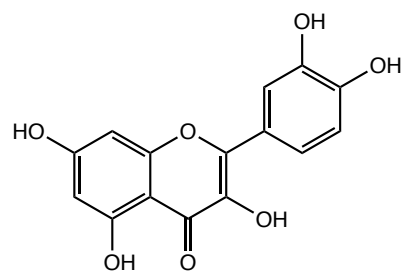
Marker phytochemical constituents of SJW (hyperforin, hypericin, quercetin-**Figure 7-1**), were tested at five to six concentration (19 µM- 199 µM) to determine the concentration-dependent inhibitory effects and IC₅₀, for CES1 mediated metabolism. This was determined using the CES1 inhibition assay methods described in Chapter 5 (5.3.3).



Hypericin



Hyperforin



Quercetin

Figure 7-1 Chemical structures of marker phytochemicals of St. John's wort.

7.3.4 Phytochemical quantification of marker compounds in SJW extracts

HPLC-DAD analysis was performed using previously published methods (Zeliou et al. 2017). In brief, SJW extracts were reconstituted in methanol at 5 mg/mL, sonicated, and filtered using 0.2 µm PTFE filters. Agilent 1100 HPLC system with Chemstation software (Version B 3.02) was used for phytochemical analysis. The system consisted of an autosampler (G1313A with 100 µL loop), Quaternary pump (G1311A), a solvent degasser (G1322A), a column oven (G1316A), and a photodiode array detector (G1315A).

Separation of marker compounds was performed on Luna C18 (4.6 mm x 150 mm, 5 µm particle size, Phenomenex Inc., Mississauga, Ontario). Mobile phase A consisted of 0.5% TFA in water, mobile phase B was acetonitrile, and mobile phase C was methanol. The separation gradient elution was performed with 85:15:0 for 10 minutes, then 85:15:0 in 10 minutes, 70:20:10 in 20 minutes, 10:75:15 in 10 minutes, and 5:80:15 in 15 minutes. The mobile phase was brought to initial conditions in 1 minute, then kept constant for 6 minutes. The temperature was set at 30°C with a flow rate of 1 mL/min. The injection volume was 10 µL and DAD was set to the range of 250 nm to 590 nm. Phytochemical markers were identified in the extracts based on comparison to retention time of pure standard compounds.

Quantification was performed using area under the peak and calculated based on the linear calibration curves of the pure compounds.

7.3.5 Inhibition of HLM mediated metabolism of MPH

Two time points (0, and 90-minutes) were used to determine effects of SJW-3 on pooled HLM mediated metabolism of MPH. For the 0- and 90-minute time points, the reaction mixture (total volume: 200 µL), consisted of 50 mM Tris (pH: 7.5), MPH (final concentration: 1 µM), and SJW-3 extract (100 µg/mL). The reaction was initiated by adding HLM (final concentration:

1 mg/mL) to the mixture. The reactions were vortexed and incubated for 90 minutes at 37°C in a shaking incubator at 200 rpm. Ice cold methanol (200 µL) was added to the time 0 reactions immediately after the addition of microsomes and were vortexed. After 90 minutes ice cold methanol (200 µL) was added to the 90-minute reaction mixtures and were vortexed. The reactions were centrifuged at 10,000 x g, and the supernatant was filtered through 0.2 µ PTFE filters (Chromatographic Specialities Inc., Brockville, ON, Canada). JZL184 (1 µM) was used as a positive control, and 20% acetone was used as a vehicle control. Two negative controls were conducted, one without HLM and one without MPH. The supernatant fractions were stored at -20°C until HPLC analysis. HPLC methods are outlined in Chapter 6 (**section 6.3**).

7.3.6 Statistical Analysis

IC₅₀ values were obtained by plotting percent inhibition against log-transformed concentration (µg/mL) using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0). A one-way ANOVA with Bonferroni post hoc test was used to compare percent inhibition, IC₅₀ values, and phytochemical quantification of commercial SJW products. A one-way ANOVA with Dunnett's post hoc was used to compare inhibition of HLM mediated metabolism of MPH, relative to vehicle control. An ANOVA with Dunnett's post hoc was used to compare the differences in residual activity of time 0-minutes vs. 5-, 10- and 15-minute pre-incubation time for each control and extract. A Pearson Correlation was performed to explore potential relationships between inhibition of extracts and the concentration of marker phytochemicals in extracts. A $P \leq .05$ was considered significant for all comparison.

7.4 RESULTS

Single concentration and concentration-dependent inhibition of CES1 by SJW extracts

Extracts of commercially available SJW products were evaluated for their potential to inhibit recombinant CES1 mediated metabolism of 4-NA. Preliminary evaluation at a single supraphysiological concentration (200 $\mu\text{g/mL}$) revealed variable inhibition of CES1 ranging from 9.8-74.8% across the four tested extracts (**Figure 7-2**).

Serial dilutions of the extracts were tested to determine their concentration-dependent effects and IC_{50} for CES1. **Figure 7-3** depicts the non-linear regression curves and the IC_{50} values (with 95% confidence intervals). SJW-3 (ethanolic extract of dried SJW) had the strongest potency ($\text{IC}_{50} = 99.1 \mu\text{g/mL}$) with the remaining 3 products exhibiting $\text{IC}_{50} > 238.1 \mu\text{g/mL}$.

Time-dependent effects on inhibition of CES1 of SJW extracts

In the time-dependent study, the known irreversible inhibitor, JZL184, showed significant ($P < .05$) decreases in CES1 residual activity across all pre-incubation times, as expected (**Figure 7-4**). CES1 residual activity remained consistent with the known competitive inhibitor, oseltamivir. None of the 4 commercial SJW products showed significant time-dependent inhibition regardless of pre-incubation time, with CES1 activities remaining relatively consistent.

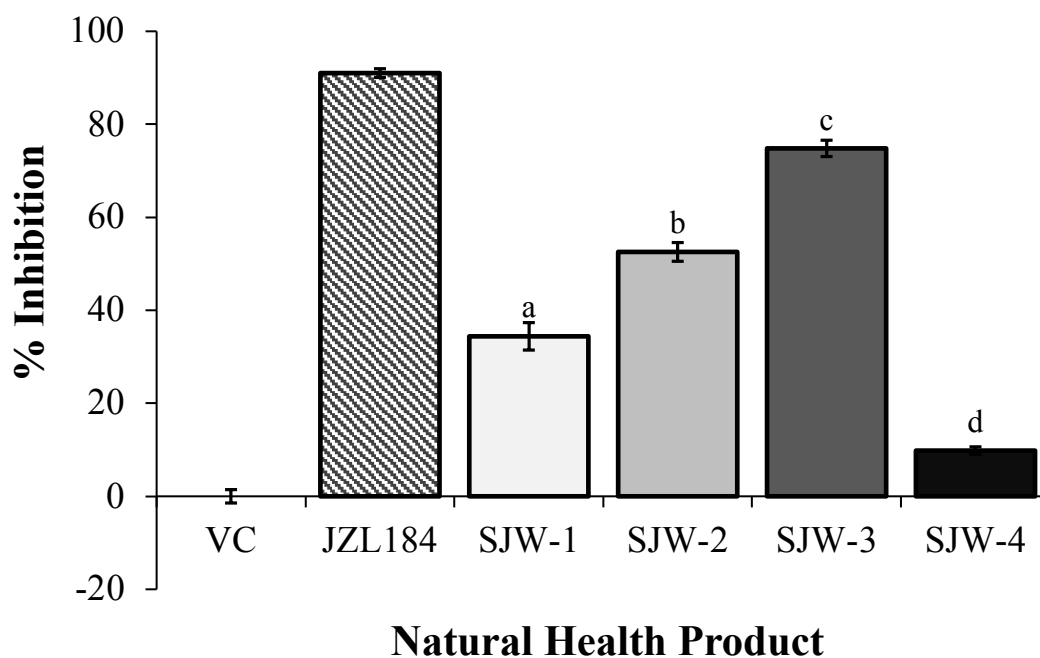


Figure 7-2 Mean percent inhibition of carboxylesterase-1 mediated metabolism of marker substrate by commercial St. John's wort natural health products (NHPs) (200 µg/mL) and positive control (JZL184 2 µM or 1 µg/mL). Data presented as means ± SEM of three independent experiments, relative to vehicle control (VC-2% acetone final concentration). Significant differences ($P < .05$) are denoted by letters, as determined by one-way ANOVA with Bonferroni post hoc test.

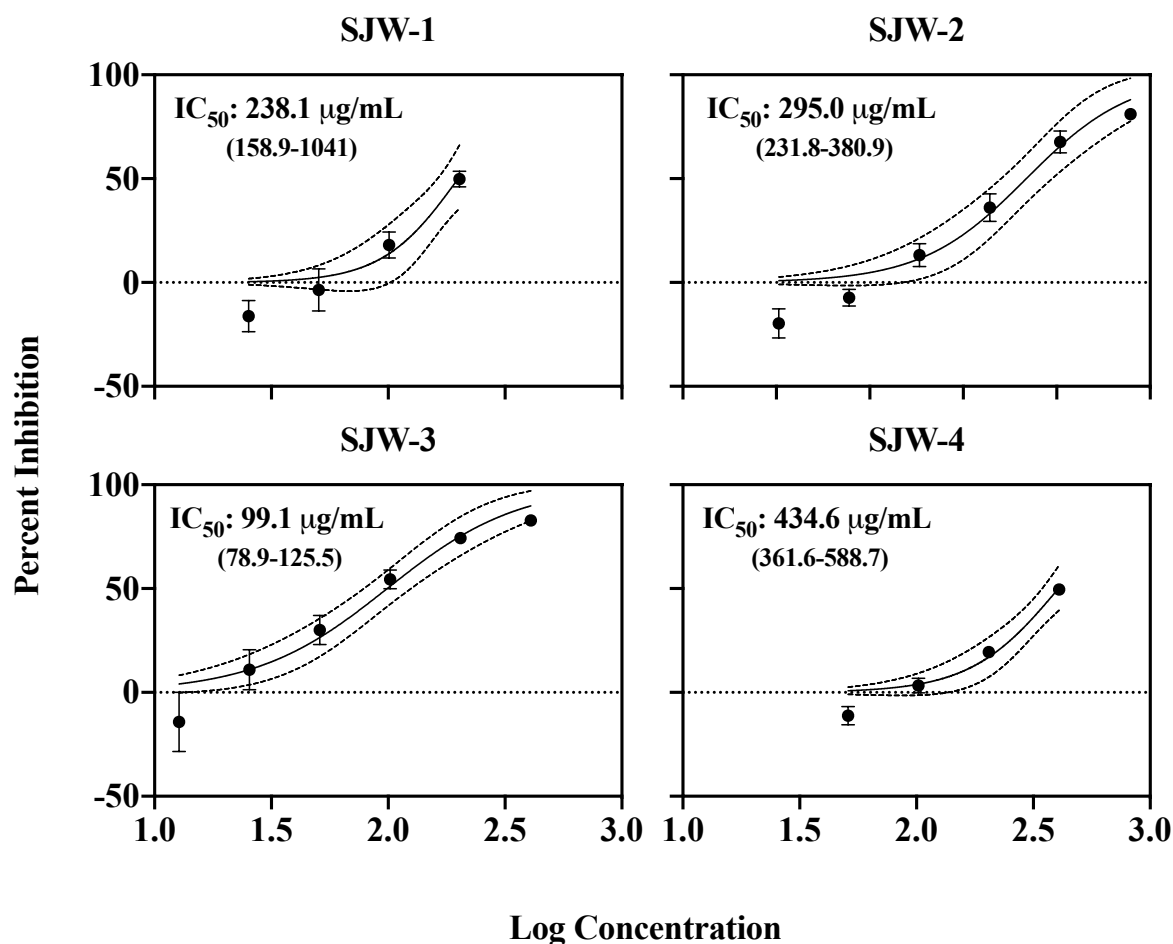


Figure 7-3 Relative potency and ranking of St. John's wort commercial natural health products (NHPs) towards carboxylesterase-1 as determined by IC_{50} values. Mean of $n = 3$ are presented with 95% confidence intervals. The concentrations were transformed into log form and percent inhibition was calculated relative to vehicle control (2% acetone final concentration), ($n = 3$). IC_{50} values were obtained using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0).

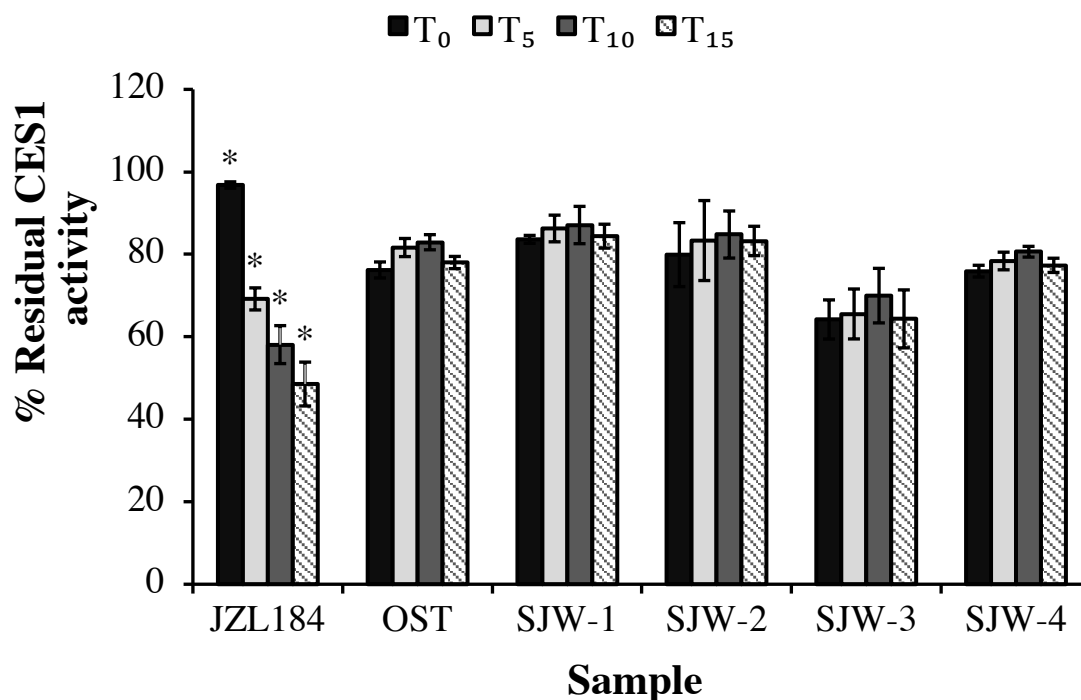


Figure 7-4 Percent residual activity of CES1 after pre-incubation with each commercial St. John's wort commercial product extracts (40 µg/mL) after 0, 5, 10, and 15 minutes. The results shown are means of 3 independent experiments \pm SEM and were relative to vehicle control (2% acetone final concentration). JZL184 (0.05 µg/mL) and OST (oseltamivir) (1 mg/mL) were used as positive and negative control respectively. An ANOVA with Dunnett's post hoc was used to compare the differences in residual activity of time 0- vs. 5-, 10- and 15-minute pre-incubation time for each control and extract. Significance is marked by* ($P < .05$).

Inhibition of CES1 by marker phytochemicals of SJW

The marker phytochemicals hypericin, hyperforin, and quercetin were serially diluted to determine their concentration-dependent effects, and IC_{50} for recombinant CES1. The non-linear regression curves and IC_{50} (with 95% confidence intervals) are shown in **(Figure 7-5)**.

Hyperforin was the most potent (65 μ M) ($P < .05$), relative to other phytochemical markers tested. Hypericin and quercetin had IC_{50} of >100 μ M for CES1.

7.4.1 Phytochemical quantification using HPLC-DAD

Phytochemical analyses of SJW extracts was conducted on HPLC-DAD with quantitation of hyperforin, hypericin and quercetin. **Table 7-2** displays the concentration of each marker phytochemical in the SJW extracts. The highest variation in concentration was observed for hypericin (34.73-106.87 μ g/g of extract). SJW-3 had the highest hypericin concentration, followed by SJW-1, SJW-4, and SJW-2. The concentration of hyperforin (16.92-19.11 μ g/g of extract) was similar across the different samples. Quercetin concentration varied amongst the SJW products, with the highest found in SJW-2 and SJW-3, and lowest in SJW-4. The concentration of marker compounds in the extracts was not significantly related to their observed potencies.

7.4.2 Inhibition of HLM-mediated metabolism of MPH by SJW NHP7

SJW-3 (100 μ g/mL) was tested for inhibition of HLM mediated metabolism of MPH. The extract showed almost 100% inhibition **(Figure 7-6)**. The positive control, JZL184, showed 53% inhibition. However, the results were not statistically significant relative to the vehicle control.

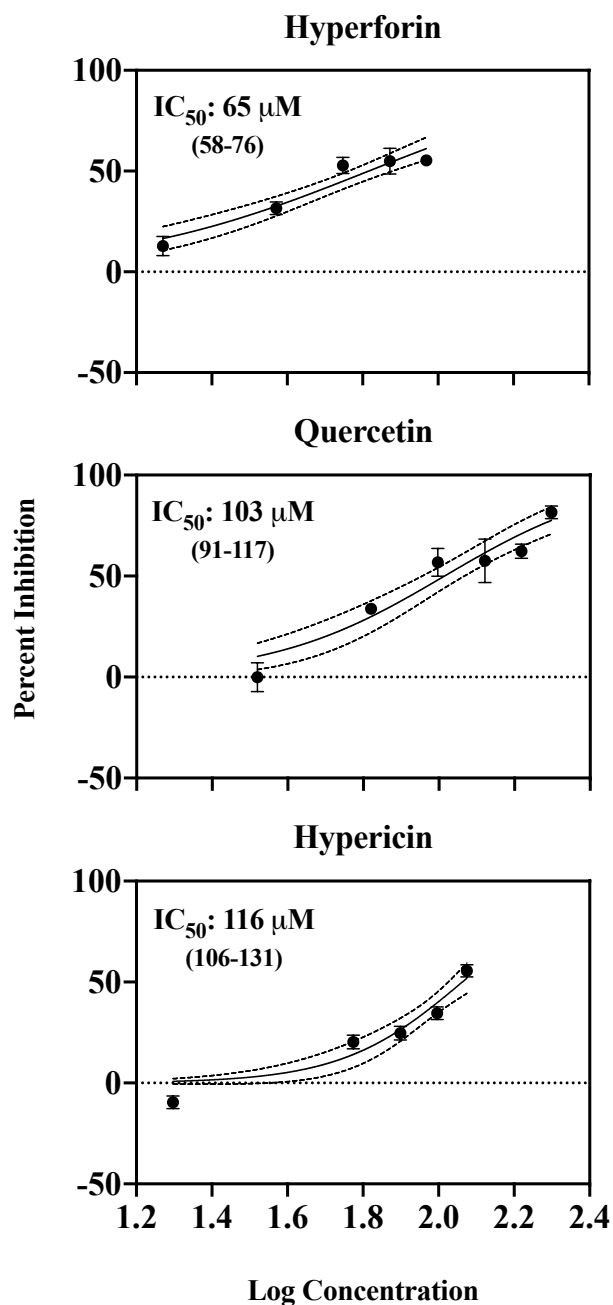


Figure 7-5 Relative potency and ranking of marker phytochemicals of St. John's wort on carboxylesterase-1 as determined by IC₅₀ values. Mean of $n = 3$ are presented with 95% confidence intervals. The concentrations were transformed into log form and percent inhibition was calculated relative to vehicle control (2% acetone final concentration), ($n = 3$). IC₅₀ values were obtained using the log[inhibitor] vs. normalized response- variable slope function on Prism GraphPad (version 8.1.0).

Table 7-2 Concentration of phytochemical marker compounds in commercial St. John's wort natural health products (NHPs) using high performance liquid chromatography-diode array detection. The concentration of each compound in individual extracts is presented in $\mu\text{g/g}$ of extract. Means of three injections are presented \pm SEM. Significant differences within each marker compound ($P < .05$) are denoted by letters, as determined by one-way ANOVA with Bonferroni post hoc test.

	Hypericin	Hyperforin	Quercetin
SJW-1	79.25 \pm 25.49 ^{ab}	16.92 \pm 1.76 ^a	5.64 \pm 1.59 ^a
SJW-2	34.73 \pm 5.20 ^a	18.20 \pm 4.53 ^a	23.07 \pm 3.21 ^b
SJW-3	106.87 \pm 6.21 ^b	19.11 \pm 3.36 ^a	24.42 \pm 0.91 ^b
SJW-4	44.50 \pm 12.17 ^{ab}	16.80 \pm 0.84 ^a	0.51 \pm 0.27 ^a

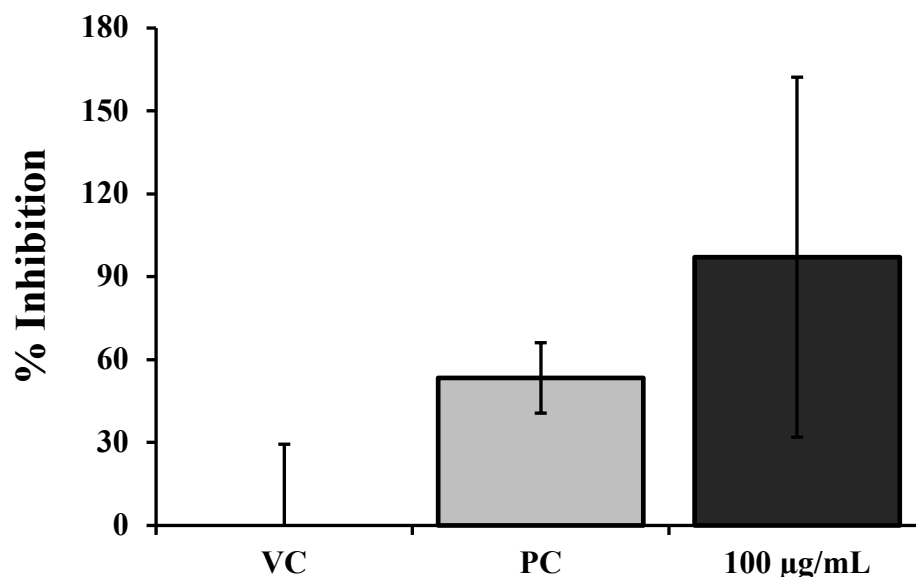


Figure 7-6 Mean percent inhibition of human liver microsome mediated metabolism of methylphenidate by SJW-3, an ethanolic extract (100 µg/mL) of a commercially available St. John's wort product. Data presented as means \pm SEM of three independent experiments of difference in peak area of parent compound after 90 minutes of incubation, relative vehicle control (VC- final concentration 1% acetone). Significant differences ($P < .05$) are denoted by letters, as determined by a one-way ANOVA with Dunnett's post hoc test. PC (positive control) was JZL184 (1 µM or 0.5 µg/mL).

7.5 DISCUSSION

One of the top selling herbals in North America, SJW is commonly used for treating depression in adults and children, but is also one of the top herbal remedies used by children with ADHD in the United States (Cala et al. 2003). The goal of this study was to evaluate the potential of SJW and its marker compounds for inhibiting the biotransformation of CES1 substrates, specifically MPH, and to gauge variability and mechanism of inhibition among different commercial SJW products.

There was varying inhibitory activity towards recombinant CES1 from the extracts of SJW products *in vitro*. The strongest inhibitory potency towards recombinant CES1 in this study was exhibited by SJW-3 (ethanolic extract). In our preliminary evaluation of 21 herbal remedies used in ADHD management, an extract of a SJW tincture (200 µg/mL) inhibited recombinant CES1 by 68% (Mazhar et al. 2019- unpublished work Chapter 4). At the same supraphysiological concentration in the current study, various commercial products of SJW showed 9-74.8% inhibition of CES1, with the extract of a tincture of SJW (SJW-2) showing comparable inhibition to Mazhar et al. 2019 (unpublished work Chapter 4). We observed varying inhibition potency of the SJW extracts in recombinant CES1 in this study (IC₅₀ range= 99-435 µg/mL). This suggests product-dependent inhibition of CES1 by SJW.

Subsequently, we evaluated hyperforin, hypericin, and quercetin for their potency to inhibit recombinant CES1. Amongst the three phytochemicals, hyperforin exhibited the strongest potency at 65 µM, however, consensus shows that this is considered weak inhibition (White 2000). Phytochemical quantification of the commercial SJW products was completed using HPLC-DAD. All products contained variable concentrations of hypericin and quercetin, but the concentration of hyperforin was not significantly different between products. Although SJW-3

had the highest concentrations of marker phytochemicals, and exhibited the strongest potency compared to other products in this study, inhibition and phytochemical concentrations were not correlated. The constituents could exhibit additive effects on inhibition, or it is possible that other constituents not identified/quantified in this study are responsible for the effect. It is plausible that the marker compounds of SJW evaluated in this study are not responsible for the pharmacokinetic activity *in vitro*. As it stands, different SJW products exhibit varying safety risks *in vitro*, making it difficult for patients to choose the safest products, and for healthcare professionals to advise patients on the safe use. This highlights the importance of stringent industry standardization on herbal remedies.

We further evaluated SJW-3 for HLM-mediated metabolism of MPH, a first-line psychostimulant for ADHD management. SJW-3 was further tested as it showed the strongest inhibition compared to other products in this study ($P < .05$). HLM contain a complex mixture of enzymes, making them a more biologically relevant model for studying herb-drug interactions (Dudda et al. 2013; Bradshaw et al. 2018). SJW-3 almost completely inhibited HLM-mediated metabolism of MPH in this study. Our results suggest that SJW can alter the pharmacokinetics of MPH *in vitro*. As no cofactors (e.g., NADPH) were used in our incubations, oxidation of MPH by the cytochrome P450 pathways as an alternative mechanism can be excluded. Gorman et al. (2013) reported that an extract of a commercial SJW product displayed weak non-competitive inhibition ($IC_{50} = 49.4$ mg/mL) of HLM-mediated metabolism of irinotecan, a chemotherapeutic agent metabolized through CES1 to its active metabolite, SN-38. Though the inhibition of irinotecan metabolism was weak, Gorman et al. (2013) reported there was statistically significant reduction of the biotransformation of irinotecan, a prodrug of SN-38. The differences in potency between studies could be possible due assay specific conditions and/or Gorman et al. (2013) not

detecting hyperforin or hypericin in their SJW extract. The discrepancy could also be due to the differences in the HLM (number of donors in the pool, mixed vs. single gender) and the substrate used.

Mathijssen et al. (2002) conducted a pharmacokinetic unblinded, randomized crossover study in a group of cancer patients treated with irinotecan, with and without SJW administration for 18 days. They reported a 42% decrease in plasma levels of the SN-38 in patients treated with irinotecan and SJW coadministration. As irinotecan is also a substrate for CYP 3A4, Mathijssen et al. (2002) concluded the decrease in plasma levels of SN-38 was due to the induction of CYP 3A4. However, they did not consider the inhibitory effects of SJW on CES1. The decrease in plasma levels of SN-38 was more prominent for patients who received the second dose of irinotecan, than for those who received it during the first dose (Mathijssen et al. 2002). Such time-dependent inhibition profiles can be the result of the formation of a reactive intermediate from the constituents of SJW (Burstein et al. 2000). Herb-drug interactions from time-dependent inhibition can have a delayed onset, and prolong after the herbal remedy is stopped, as enzyme activity is restored by *de novo* enzyme synthesis. Such inhibition can be characterized by a decrease in enzyme activity when the inhibitor is pre-incubated alongside the enzyme without any substrate (Orr et al. 2012; Grimm et al. 2009). However, in the current study, none of the commercial SJW products showed time-dependent inhibition. On the other hand, Hu et al. (2007) found significant decreases in the plasma concentration (39%) of SN-38 from long-term SJW coadministration with irinotecan *in vivo* in rats. A time-dependent effect by SJW pre-treatment was observed on irinotecan hydrolysis, and postulated, in part, due to reduced CES1 activity (Hu et al. 2005).

As a follow-up from the adverse events described as herb-drug interactions between SJW

and MPH by Mazhar et al. (2019), and Niederhofer (2007), it is possible that the adverse events are a result of both pharmacodynamic and pharmacokinetic interactions. Whereas Mazhar et al. (2019) suggested herb-drug interactions that increased bioavailability or activity of MPH and/or SJW, the adverse event described by Niederhofer (2007) had diminishing effects of MPH with SJW coadministration. Psychotic episodes, hallucinations, agitation, and excessive weeping are side-effects of MPH (Novartis Pharmaceuticals Canada Inc. 2007). Hence, pharmacokinetically, it is possible that SJW increases the side-effects of MPH by inhibition of CES1 with the corresponding reduction in MPH metabolism. Furthermore, these side-effects are listed for cases of MPH toxicity due to overdose in the product monograph. MPH is a weak substrate for P-glycoprotein transporter (P-gP), which plays a role in the disposition of MPH and its entry into the brain (Zhu, Wang, et al. 2008; Zhu et al. 2006). As an inducer of P-gP (Dürr et al. 2000), SJW can decrease the availability of MPH, leading to diminishing efficacy. The pregnane-X-receptor is also involved in the expression of CES1 (Shan et al. 2017). The activation of the pregnane-X-receptor by SJW (Moore et al. 2000; Wentworth et al. 2000) could result in increased expression of CES1, leading to decreased efficacy of MPH through faster clearance. Conversely, it is plausible that SJW has additive, or synergistic pharmacodynamic effects to MPH, as both inhibit the reuptake of dopamine, noradrenaline, and serotonin. Serotonin syndrome, characterized by agitation, restlessness, and hallucination (Volpi-Abadie et al. 2013), is possible with the administration of SJW and MPH concurrently. The mechanisms for the adverse events observed between MPH and SJW interactions are complex as there is significant overlap between side-effects, mode of action, and toxicity of both substances (**Figure 7-7**).

The risk of adverse events can increase for those with genetic polymorphisms of CES1. Polymorphisms of CES1 have been identified, with significant decreases in the metabolism and

disposition of methylphenidate. These variants of CES1 yield up to 7-fold increases in the serum concentration of MPH (Zhu, Patrick, et al. 2008; Patrick et al. 2007), which can lead to adverse events such as psychotic episodes, hallucinations, agitation, and excessive weeping, described in Mazhar et al. (2019). On the other hand, the ineffectiveness of MPH described by Niederhofer (2007) can be explained by rapid drug metabolism in Asian individuals with highly expressed CES1 genes (Rasmussen et al. 2017).

To our knowledge, this is the first study evaluating the mechanisms of interaction between extracts of selected SJW products, and marker compounds on CES1-mediated metabolism in recombinant enzymes, and HLM (with methylphenidate). Overall, our results indicate that some commercial SJW products may pose a risk of herb-drug interaction with conventional drugs metabolized by CES1. As there are similarities in the mode of action, side-effects and toxicity profile, interactions between SJW and MPH, there may be a risk of both pharmacokinetic and pharmacodynamic interactions. More clinical trials for NHPs are needed in pediatric populations. Moreover, any adverse events in clinical trials and case reports need to be described in detail to capture necessary information for safety signal detection. Herb-drug adverse reaction case studies need to be more thoroughly studied, analyzed and reported. Improved education on natural health product safety is necessary for patients (families) and healthcare professionals.

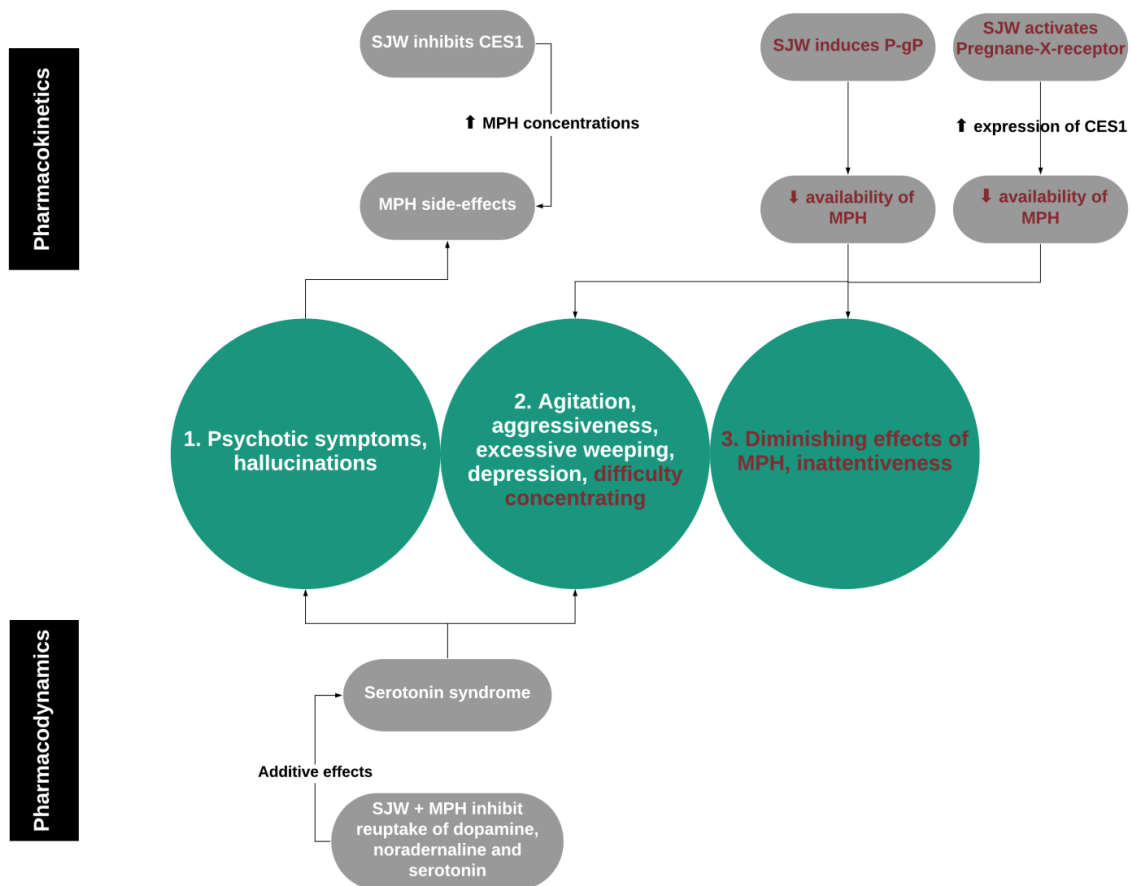


Figure 7-7 Graphical schematic of the overlap of the possible mechanisms for the adverse events observed between methylphenidate (MPH) and St. John's wort (SJW) in Mazhar et al. (2020) and Niederhofer (2007).

7.6 ACKNOWLEDGEMENTS

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Chapter 8
General Discussion

8.1.1 Novel Contributions

This thesis examined, for the first time, the use, and *in vitro* factors that may impact the safety of NHPs for management and improved pharmacovigilance in pediatric ADHD. I used a novel interdisciplinary approach to identifying the risk of herb-drug interactions achieved through using clinical questionnaires, pre-clinical probe-based assessment of CES1, and more specific pre-clinical assessment evaluating HLM mediated metabolism of MPH. In doing so, I was able to observe product variability and its effects on CES1 inhibition. Moreover, I assessed the risk of interactions using post-market surveillance tools by systematically searching for AERs. My thesis synthesized the level evidence of risk for pediatric ADHD patients using NHPs. Specifically, potential risks for those using herbal remedies concurrently with methylphenidate were highlighted through inhibition studies with recombinant CES1 and HLM. These interactions may not be obvious to regulators and healthcare professionals as most reported herb-drug interactions are caused by CYP P450 inhibition or induction.

This was the first thesis to:

- Develop and administer a detailed cross-sectional questionnaire regarding CIM use, specifically in ADHD patients in Canada, which was conducted at the CHEO ADHD outpatient clinic.
- Systematically determine the frequency, and assess the potential causality of clinical AERs related to the concurrent use of ADHD pharmacotherapy and herbal medicines by pediatric patients.
- Use multiple scoring tools to determine the quality of submitted AERs.

- Determine the effects of several major herbal remedies (and selected marker compounds) used by ADHD patients on recombinant CES1 activity *in vitro*, and evaluated time-dependent inhibition by extracts.
- Examine the effects of various *R. rosea* products and its major constituents (rosarin, rosavin, rosin, and salidroside) on the *in vitro* activity of recombinant CES1.
- Evaluate extracts of various *R. rosea* products for time-dependent inhibition of recombinant CES1.
- Evaluate the mechanisms of inhibition of recombinant CES1 in the context of ADHD of SJW products, its marker constituents (hyperforin, hypericin), and another abundant constituent, quercetin).
- Determine the direct effects of extracts of SJW and *R. rosea* products on the inhibition of HLM mediated metabolism of MPH.

This interdisciplinary approach can be used for evidence of initial assessment of the potential for risk of interactions with conventional drugs, and highlight herbal remedies needing *in vivo* characterization.

8.1.2 Overview and comparisons to literature

Chapter 2 synthesized available data on the efficacy and safety of herbal remedies commonly used by pediatric patients with ADHD, and other neurological disorders. A literature review identified 22 herbal remedies and determined possible interactions via the CYP 3A4 and CYP 2D6 pathways responsible for metabolism of some ADHD drugs (amphetamines, atomoxetine, and guanfacine) (Mazhar et al. 2016). However, little evidence of *in vitro* interactions and associated potential risks was found for CES1, the metabolizing enzyme for

MPH. The evidence of efficacy of the herbal remedies was weak or lacking. Nonetheless, many patients and families found them to be beneficial at alleviating symptoms (Cala et al. 2003; Soo et al. 2005; Galicia-Connolly et al. 2014). However, it was unclear if the herbal remedies were used and/or helpful for managing specific ADHD symptoms, comorbid conditions, and/or general or physical health. Determining more specifically how and for what reasons ADHD patients use herbal medicines, and other forms of CIM, would better aid in understanding CIM use within this population to improve communication and mitigate potential risks.

As a step to filling in the knowledge gaps identified in Chapter 2, Chapter 3 evaluated the use, knowledge, and determinants of CIM use in pediatric ADHD. A cross-sectional questionnaire regarding CIM was implemented specifically in ADHD patients in Canada and was conducted at the Children's Hospital of Eastern Ontario ADHD outpatient clinic. CIM use was common in the patients surveyed, and more than half of those using CIM used herbal remedies. Our results were comparable to other studies conducted in ADHD patients (Bussing et al. 2002; Huang et al. 2013; Chan et al. 2003), and as expected, CIM use in our study was more common than those captured in a general pediatric setting (Loman 2003; Ottolini et al. 2001; Barnes et al. 2008; Spigelblatt et al. 1994; Sawni-Sikand et al. 2002). CIM use in ADHD was likely higher compared to general pediatric setting, due to the chronic nature of ADHD, occurrence with comorbid conditions, concerns about adverse events from allopathic medicine, and the desire for a holistic approach to disease management and prevention (Kemper et al. 2013; Wang et al. 2018; Chan et al. 2003; Huang et al. 2013; Sinha et al. 2005; Stubberfield et al. 1999). Variation in the rates of use is possibly due to heterogeneity in the definition of CIM (e.g., some studies exclude vitamins), study population, sample size, and prevalence measurements (e.g., CIM use in the past year vs. ever used CIM), cultural and ethnic differences, and

differences in the regulation of NHPs internationally. Although previous surveys found CIM to be helpful in general, they did not capture the purposes of use (i.e., ADHD symptoms versus other health reasons) or the perceived helpfulness of CIM for these purposes (Sinha et al. 2005; Huang et al. 2013; Soo et al. 2005; Galicia-Connolly et al. 2014). In the current study, the incidence of CIM use was almost equal for ADHD, and general health maintenance. However, families found CIM more helpful for general health and relatively less helpful for ADHD, providing information for the knowledge gaps identified in Chapter 2. Families clearly want to use CIM to treat their child's ADHD symptoms, but in a lot of cases, CIMs do not seem to provide the desired benefits. More clinical trials in pediatric populations are needed on CIMs as, currently, pediatric patients may use CIM for off-label indications, which could explain the low levels of perceived helpfulness.

There were eight mild adverse events that were self-reported. It is noteworthy to highlight that half of the reported adverse events involved an NHP as compared to other CIM. This validates the importance of conducting a systematic search of adverse events in Chapter 4, and the *in vitro* assessments of NHPs in Chapter 5. Adverse events, especially those involving NHPs, are generally under-reported with passive surveillance methods (i.e., voluntary spontaneous reporting) such as those employed by national and international drug surveillance programs (Waller et al. 2004). Active surveillance methods, such as the current one, and in studies such as Necyk et al. (2014) and Vohra et al. (2012), all achieve increased reporting rates (ICH Steering Committee 2011).

The concurrent use of ADHD pharmacotherapy and CIM was common. This also confirmed the need to perform *in vitro* analysis (in Chapter 5, 6, and 7) of popular herbal remedies to identify potential risk factors. Comparing this data to *in vitro* results, displaying

varying inhibition across different herbal remedies and products, shows the need to monitor any adverse events, and for improved communication between families and healthcare professionals. Related to communication about CIM, interestingly, most families felt comfortable discussing CIM and consulted physicians about the use. However, physicians were often not perceived as a helpful resource for CIM information. Most physicians agree they need more knowledge in CIM to properly counsel patients (Patel et al. 2017; Kemper et al. 2004). This shows discomfort on the physician's part due to lack of knowledge on CIM, and it is possible that information provided by physicians about CIM often may not be satisfactory to the families. Hence, families often consult other informal resources such as health food stores, social media, and non-official websites with potentially anecdotal or misleading evidence for efficacy and safety. This scenario calls for improved medical education programs to ensure that physicians and other healthcare professionals feel comfortable providing reliable and evidence-based advice on CIM. Future studies should incorporate in-depth interviews of families, and healthcare professionals (including CIM practitioners, physicians, nurses, and pharmacist) to determine effective methods of overcoming communication barriers on CIM use.

Concomitant administration of herbal remedies and conventional drugs is common practice in ADHD, as validated by survey results in Chapter 3 and Galicia-Connolly et al. (2014). As a first step to the safety evaluation, Chapter 4 systematically determined the frequency, and assessed the potential causality, of clinical AER related to the concurrent use of ADHD pharmacotherapy and herbal medicines by pediatric patients. The systematic search identified 23 AERs of which, most involved use of 3 or more substances. Similar to other studies, the overall quality of the AERs in this study was poor (Awortwe et al. 2018; Gardiner et al. 2013). High quality AERs are important to understand and detect safety signals, and clinical

relevance of interactions. Safety signals aid pharmacovigilance centres to inform clinicians, and patients on risks. In addition to using active surveillance, the quality of adverse event reports can be improved by providing educational interventions, and providing detailed and specific feedback to reporters (Wallerstedt et al. 2007; McGettigan et al. 1997). This can facilitate regulatory framework and standards to facilitate more accurate procedures in AE reporting and surveillance programs. The systematic search identified pediatric adverse event reports involving concurrent use of ADHD drugs and herbal remedies including echinacea, garlic, ginkgo, ginseng, green tea, St. John's wort, and valerian. This guided the choice of herbal remedies tested *in vitro* in Chapter 5. Two potential herb-drug interactions were identified in Chapter 4, both from the use of MPH and SJW concurrently. A similar adverse event was reported by Niederhofer (2007), however, the potential mechanisms of interactions were not determined.

Other NHPs commonly reported in Chapter 3, such as melatonin and fatty acids, should be examined using the methods outlined in Mazhar et al. (2019) (Chapter 4) to determine their safety signals. For example, an NHP-drug interaction was observed by active surveillance by Necyk et al. (2014) and Vohra et al. (2012). Foster et al. (2015) investigated this interaction *in vitro* and reported that melatonin inhibits various recombinant CYP P450 isoenzymes. There is a possibility of both pharmacokinetic and pharmacodynamic interactions as melatonin is a serotonin-derived neurohormone (Haduch et al. 2016). Fatty acid supplements showed varying inhibition of recombinant CYP 3A4 and CYP 2D6 with IC₅₀ values ranging from 7-40 µg/mL (Strandell et al. 2004). Both melatonin and fatty acid supplements warrant further investigation for inhibition of CES1-mediated metabolism.

As a follow-up to the potential herb-drug interactions identified in Chapter 4, Chapter 7 examined the mechanisms of potential pharmacokinetic interaction between MPH and

commercial SJW products, as well as major SJW phytochemical marker compounds. Hyperforin showed the highest potency but was still a weak inhibitor of CES1. Extracts of commercial SJW products displayed varying inhibition of recombinant CES1. The ethanolic extract of dried product (SJW-3) completely inhibited HLM mediated metabolism of MPH, suggesting potential risk from the concurrent use. These results support my hypothesis that interactions between MPH and SJW can be explained, at least in part, by the ability of SJW to modulate CES1 activity *in vitro*. Contrary to my predictions, the concentrations of hypericin, hyperforin, and quercetin in the commercial products were not related to the degree of inhibition observed. Other oncological herb-drug interaction studies also show significant inhibition of the CES1-mediated biotransformation of the chemotherapeutic agent irinotecan in HLM (Gorman et al. 2013) and in humans (Mathijssen et al. 2002), suggesting pharmacokinetic herb-drug interactions. However, as the mode of action, side-effect profile, and toxicity profile of SJW and MPH are similar, it is possible the interactions are both pharmacokinetic and pharmacodynamic in nature. Herb-drug interactions observed between SJW and MPH are complex and can be difficult to delineate, rendering SJW a high-risk herbal remedy that should be used with caution with MPH. Patients using SJW and MPH concurrently should monitor their responses (efficacy, and side-effects), short and long term, and disclose the use to their healthcare professional. *In vivo* studies are needed to further characterize the risk and mechanism of interaction.

Chapter 5 evaluated the inhibitory effects of the herbal remedies identified in Chapter 2 on the activity of recombinant CES1. Most herbal remedies tested showed some inhibition of CES1-mediated metabolism of the probe substrate, 4-NA, providing evidence for my hypothesis that herbal remedies used in ADHD management would modulate CES1 activity. *R. rosea* extract showed strong inhibitory activity *in vitro*, suggesting potential risk of interactions with

MPH. However, the potency of the *R. rosea* extract could not be explained by rosavin, rosin, salidroside and tyrosol, as they did not show inhibition of CES1-mediation metabolism. The marker constituents of ginger [6]-gingerol, [8]-gingerol, [10]-gingerol showed strong inhibitory activity *in vitro* and could potentially explain the potency of the ginger extract. With only a few studies having examined herb-drug interactions via CES1 enzyme, this was the first study to determine the effects of several major herbal remedies and their marker compounds on CES1 activity *in vitro*. Psoralea (and its constituents including the flavonoids and their derivatives neobavaisoflavone, corylifolinin, coryfolin, and corylin) (Sun et al. 2016), oleanolic acid, urosolic acid (Zou et al. 2017), black cohosh, ginger (Gorman et al. 2013), and goldenseal (Liu et al. 2010) were all identified as moderate to potent inhibitors of CES1. Since CES1 is involved in the metabolism of numerous drugs and is increasingly targeted as an activation mechanism for prodrugs, studies such as the current one are needed for evaluation of other herbal remedies in CES1 models to identify potential inhibitors to support patient safety and greater pharmacovigilance.

Building on results from Chapter 5, Chapter 6 focused on *R. rosea*, identified as the most potent inhibitor of CES1 among the tested herbal extracts. Consistent with my predictions, commercial *R. rosea* products showed varying inhibitory effects on CES1. However, in contrast to my predictions, the concentration of phytochemicals (as determined by HPLC-DAD), were not correlated to potency. Two commercial *R. rosea* products showed time-dependent (irreversible) effects on CES1 inhibition, rendering them high-risk for clinically relevant pharmacokinetic interactions in patients using them. Since some *R. rosea* products did not exhibit time-dependant inhibitory activity, as described in Chapter 5 and Chapter 6, this indicates variability between extracts can affect the mode and magnitude of inhibition as well. This

emphasizes the value of testing different products, and further presents an opportunity for industry to optimize formulations that reduce risk without impacting product quality. Moreover, it can guide regulators to place greater attention on currently non-standardized compounds in products, and consider requiring them (and their quantities) to be listed on product labels.

RR-2 showed concentration-dependent inhibitory effects on HLM mediated metabolism of MPH *in vitro*. As this was the first study to examine the effects of commercial *R. rosea* products and their major constituents *in vitro* on CES1 mediated metabolism, direct comparisons were not possible. However, other studies found *R. rosea* products to be moderate to potent inhibitors of AChE, a related enzyme in the serine hydrolase family. Specifically, hydroquinone (Wang et al. 2007), gossypetin-7-*O*-L-rhamnopyranoside and rhodioflavonoside (Hillhouse et al. 2004) were found to inhibit AChE. Moreover, rhodiosin and rhodionin were potent inhibitors of CYP 2D6 (Xu et al. 2013). Similarly, in this study, rosavin, rosarin, rosin and salidroside showed weak inhibition, indicating that other phytochemicals present in the extract used in this study are likely to be responsible for the inhibition. Overall, *R. rosea* products have a high potential to interact with MPH, and other CES1 drug substrates. Bioassay-guided fractionation of *R. rosea* is needed to isolate and determine the pharmacologically active constituents responsible for effects on CES1, for improved safety of the product. Moreover, *in vivo* assessment of potent inhibitors of CES1 identified in this study require *in vivo* validation. A better understanding of the mechanisms of herb-drug interactions *in vitro* and *in vivo* can minimize and/or avoid therapeutic failures and toxicity.

Overall, this study adds to the previous limited evidence that interference by medicinal plants with CES1 could alter the metabolism of methylphenidate or other CES1 drug substrates, resulting in changes in efficacy and/or safety associated with this interaction with CES1. This

provides evidence to support my overall hypothesis that popular herbal remedies could pose a risk to patients using them concomitantly with drugs used for ADHD.

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APPENDIX A

Research Ethics Board Approval from Children's Hospital of Eastern Ontario for the survey (Chapter 3).



CHEO Research Ethics Board Approval - Delegated Review

Principal Investigator: Dr. Philippe Robaey

REB Protocol No: 15/104X

Romeo File No: 20150287

Project Title: CHEOREB# 15/104X - Evaluating the use and knowledge of Complementary and Alternative Medicines (CAM), specifically herbal medicines in ADHD (Attention-Deficit Hyperactivity Disorder) patients and families.

Primary Affiliation: Mental Health\Psychiatry

Protocol Status: Active

Approval Date*: May 18, 2016

Valid Until:** July 15, 2016

Annual Renewal Submission Deadline: June 15, 2016

Documents Reviewed & Approved:

Document Name	Comments	Version Date
Protocol	Protocol-CAM use and knowledge in ADHD ,Amendment1 - Version1_Clean copy.docx	2015/10/01
Consent Form	English_Consent Form - CAM use and knowledge in ADHD ,Amendment1 - Version1_clean copy .docx	2015/10/01
Recruitment Materials	Email invitation-CAM use and knowledge in ADHD ,Amendment1 - Version1.docx	2015/11/02
Recruitment Materials	Consent Form for Disclosure and Use of Email Address Amendment 1 version 1.docx	2015/10/01
Questionnaire/Survey	Survey CAM in ADHD_amendment1, version1.pdf	2016/05/08

This is to notify you that the Children's Hospital of Eastern Ontario Research Ethics Board has granted approval to the above named research study on the date noted above. Your project was reviewed under the delegated review stream, which is reserved for projects that involve no more than minimal risk to human subjects.

Final approval is granted for the above noted study, with the understanding that the investigator agrees to comply with the following requirements:

1. Please be advised that any translated documents must be submitted to the REB via the **'translated study documents'** event in ROMEO and including the signature page for this event.
2. The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
3. Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated above.
4. The investigator must not implement any deviation from, or changes to, the protocol, consents or assents without the approval of the REB.
5. The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials.
6. Investigators must provide the Board with French versions of the consent form, unless a waiver has been granted. An interpreter should be offered to participants as required or at the request of the participant throughout the course of research.
7. The investigator must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
8. Investigators must notify the REB of any study closures (closed to accrual, temporary, premature or permanent).
9. Investigators must submit a final report at the conclusion of the study.

Should you have any questions or concerns, please do not hesitate to contact the Research Ethics Board Office at 613-737-7600 ext. 3350 or 2128.

Regards,

[Redacted Signature]

Dr. Carole Gentile
Chair, Research Ethics Board
Présidente, Comité d'éthique de la recherche

[Redacted Contact Information]

*The final approval date for initial delegated study applications approved with or without modifications will be the date the REB has determined that the conditions of approval have been satisfied.

**The expiry date of REB approval for initial study application that required no modifications will be as follows:

- If the date of review and approval was **on or before** the 15th of the month, the expiry date will be the 15th of the month prior to the date of review and approval by the Chair and/or delegate *in the following year*;
- If the date of review and approval was **after** the 15th the expiry date will be the 15th of the month in which the date of review and approval by the REB *in the following year*.

The expiry date of REB approval for initial study applications that **require modifications** will be as follows:

- If the initial feedback was sent **on or before** the 15th of the month, the expiry date will be the 15th of the month prior to the date the letter of REB feedback is issued to the investigator(s) *in the following year*;
- If the initial feedback was sent **after** the 15th the expiry date will be the 15th of the month in which the feedback was sent *in the following year*.

Research Ethics Board approval from the University of Ottawa for the Survey (**Chapter 3**).



Université d'Ottawa University of Ottawa

Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

June 29, 2016

Philippe Robaey
Mental Health/Psychiatry
CHEO Research Institute
[Redacted]

Co-investigators: Hajra Mazhar, University of Ottawa
Cory Harris, University of Ottawa

Re: U of O Ethics file no. A06-16-07 – “Evaluating the use and knowledge of Complementary and Alternative Medicines (CAM), specifically herbal medicines in ADHD (Attention-Deficit Hyperactivity Disorder) patients and families”

Dear Dr. Robaey, Dr. Harris and Ms. Mazhar,

Thank you for the approval documents from the CHEO-REB (REB Protocol # 13/89X) for your project named above.

This is to confirm that, in accordance with the agreement between the University of Ottawa and The CHEO-REB, the University of Ottawa has authorized this board to act as Board of Record for the review and oversight of research involving human subjects conducted at or through the hospital.

We remind you of your obligation to:

- Follow all procedures of the CHEO-REB including reporting and renewal procedures;
- Submit to the authority of the CHEO-REB and that you are subject to the CHEO-REB requirements, including, without limitation, the requirement to modify or stop the research on demand of the CHEO-REB.

If you have any questions, please contact our ethics office at 562-5387.

Sincerely yours,

[Redacted Signature]

Catherine Paquet
Director, Office of Research Ethics and Integrity

550, rue Cumberland 550 Cumberland Street
Ottawa (Ontario) K1N 6N5 Canada Ottawa, Ontario K1N 6N5 Canada
(613) 562-5387 • Téléc/Fax (613) 562-5338
<http://www.recherche.uottawa.ca/deontologie/>
<http://www.research.uottawa.ca/ethics/>

APPENDIX B

Inactive survey link: <https://redcap.cheori.org/surveys/?s=H7YAM3APLT>

APPENDIX C

Table C-1 Other CIM reported by respondents in an open-ended question without additional information on use.

Complementary and Integrative Medicine	Frequency of mention
Aloe	1
Deep Immune (St. Francis Herb Farm)	1
Epsom salt bath	1
Essential oils and aromatherapy	4
Iron	1
L-theanine	1
Marijuana	1
Massage	1
Meditation	1
Melatonin	8
Mindfulness	1
Neurofeedback	3
Omega fish oils	4
Vitamin B12	1
Vitamin D	1
Yoga	1
Zinc	1

APPENDIX D

Copy of adverse event case reports from U.S. FDA FOIA.

Case I

FDA - Adverse Event Reporting System (FAERS)	
FOIA Case Report Information	
Case ID: 7378242	
Case Information:	
Case Type: EXPEDITED (15-DAY)	eSub: Y HP: Y Country: ROM Event Date: 01-Sep-2009 Outcomes: HO Application Type: NDA
FDA Rcvd Date: 05-May-2010 Mfr Rcvd Date: 23-Apr-2010 Mfr Control #: RO-ELI_LILLY_AND_COMPANY-RO201005000579	Application #: 021411
Patient Information:	
Age: 8 YR	Sex: Male Weight: 34 KG
Suspect Products:	
# Product Name	Compounded Drug ? Dose/Frequency Route Dosage Text Indications(s) Start Date End Date
1 STRATTERA	25 MG/ Unknown 25 mg, UNK ATTENTION DEFICIT/HYPERACTIVITY DISORDER Oct-2008 Sep-2009
# Product Name	Interval 1st Dose to Event DeC ReC Lot# Exp Date NDC # MFR/Labeler
1 STRATTERA	NA Unk ELI LILLY AND CO
Event Information:	
Preferred Term (MedDRA Version: 17.0)	ReC
Glaucoma	Unk
Event/Problem Narrative:	
<p>This spontaneous case reported by a child psychiatrist, concerns a 9 year old male patient of unknown origin. The patient's medical history included astigmatism, conduct disorder and asthma. The concomitant medications included ginkgo biloba for learning disabilities. The patient received atomoxetine hydrochloride (Strattera) 25mg daily, at an unknown route of administration, for the treatment of attention deficit / hyperactivity disorder (ADHD), beginning on an unknown date in Oct-2008. On an unknown date in (b) (6) at an unknown time after starting atomoxetine, the patient experienced glaucoma with subjective symptoms of headaches and eyes pain. The event of glaucoma was considered serious by the reporter for hospitalisation reasons. Relevant investigations on an unknown date included an ophthalmologic examination that raised the suspicion of glaucoma. Corrective treatment was not reported. On an unknown date in Sep-2009, atomoxetine treatment was discontinued. The patient had not recovered from the event of glaucoma. It was reported that the subjective symptoms of headaches and eyes pain did not disappear after discontinuation of atomoxetine. It was also reported that it was possible that atomoxetine increased the semiology. The hospitalisation dates were not reported. The reporting child psychiatrist stated that</p>	



FDA - Adverse Event Reporting System (FAERS)

FOIA Case Report Information

Case ID: 7378242

the event of glaucoma was related to atomoxetine.

Relevant Medical History:

Disease/Surgical Procedure	Start Date	End Date	Continuing?	
ASTHMA			UNKNOWN	
ASTIGMATISM			UNKNOWN	
CONDUCT DISORDER			UNKNOWN	
Medical History Product(s)	Start Date	End Date	Indications	Events

Relevant Laboratory Data:

Test Name	Result	Unit	Normal Low Range	Normal High Range	Info Avail
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Concomitant Products:

#	Product Name	Dose/ Frequency	Route	Dosage Text	Indications(s)	Start Date	End Date	Interval 1st Dose to Event
1	GINKGO BILOBA	80 MG/	Unknown	80 mg, daily (1/D)	LEARNING DISABILITY	May-2001	01-Nov-2010	



FDA - Adverse Event Reporting System (FAERS)

FOIA Case Report Information

Case ID: 7378242

Reporter Source:

Study Report?: No

Sender Organization: ELI LILLY AND CO

**503B Compounding
Outsourcing Facility?:**

Literature Text:

Case II

CaseID: 3786087



3911156-9-00-01

MEDWATCH

THE FDA MEDICAL PRODUCTS REPORTING PROGRAM

For use by user-facilities,
distributors and manufacturers for
MANDATORY reporting
Novartis Pharmaceuticals

Page 1 of 2

RECEIVED

MAY 01 2002

CDW/

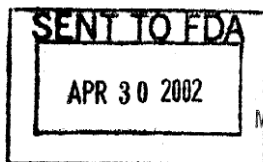
Relays International, Inc.
FDA Facsimile Approval: 30-JUN-1999

MR-report #	PHBS2002AU04398
UF/Dial report #	
FDA Use Only	

A. Patient information		C. Suspect medication(s)	
1. Patient identifier (b) (6) In confidence	2. Age at time of event: 7 Years or Date of birth: UNK	3. Sex <input checked="" type="checkbox"/> female <input type="checkbox"/> male	4. Weight UNK lbs or UNK kgs
B. Adverse event or product problem			
1. <input checked="" type="checkbox"/> Adverse event and/or <input type="checkbox"/> Product problem (e.g., defects/malfunctions)			
2. Outcomes attributed to adverse event (check all that apply)			
<input type="checkbox"/> death <input type="checkbox"/> disability			
<input type="checkbox"/> life-threatening <input type="checkbox"/> congenital anomaly			
<input type="checkbox"/> hospitalization - initial or prolonged <input checked="" type="checkbox"/> other: Medically Significant			
3. Date of event: 03/07/2002			
4. Date of this report: 04/29/2002			
5. Describe event or problem Movement disorder[Movement disorder NOS] Tic[Tic] Nightmares[Nightmare] Skin rash[Rash NOS] ([Dry skin]) Case Description: This is a consumer report: This patient started treatment with Ritalin (methylphenidate) in Oct 2001. On 07 Mar 2002, she developed a tic. Initially it involved both arms, then became more complex with hands, fingers and wrists, then neck, head and hips. Ritalin was discontinued on 09 Mar 2002. There had been a slight improvement since cessation, with movements of head, arm, neck and legs subsiding but the movements were still present. The patient's doctor will review the symptoms at the end of Apr 2002. continued in additional info section...			
6. Relevant tests/laboratory data, including dates NI			
7. Other relevant history, including preexisting medical conditions (e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.) NI			
1. Name (give labeled strength & mfr/labeler, if known) # 1. RITALINE(METHYLPHENIDAT (continued) # 2.			
2. Dose, frequency & route used # 1. 10 mg/day, Oral # 2.		3. Therapy dates (if unknown, give duration) # 1- 10/---/2001 to 03/09/2002 # 2.	
4. Diagnosis for use (indication) # 1. Attention (continued) # 2.		5. Event abated after use stopped or dose reduced # 1. <input checked="" type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply # 2. <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply	
6. Lot # (if known) # 1. UNK # 2.		7. Exp. date (if known) # 1. UNK # 2.	
8. Event reappeared after reintroduction # 1. <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply # 2. <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply			
9. NDC # - for product problems only (if known) # 1. # 2.			
10. Concomitant medical products and therapy dates (exclude treatment of event) EPALEX (EVENING PRIMROSE OIL, OMEGA-3 MARINE TRIGLYCERIDES, THYME OIL) UNK to UNK			
G. All Manufacturers			
1. Contact office - name/address (& mailing site for devices) Novartis Pharmaceuticals Corp. Clinical Safety and Epidemiology 		2. Phone number 	
3. Report source (check all that apply) <input checked="" type="checkbox"/> foreign AUS <input type="checkbox"/> study <input type="checkbox"/> literature <input checked="" type="checkbox"/> consumer <input type="checkbox"/> health professional <input type="checkbox"/> user facility <input type="checkbox"/> company representative <input type="checkbox"/> distributor <input type="checkbox"/> other:			
4. Date received by manufacturer (m/d/yyyy) 04/22/2002		5. (A)NDA # 10-187 IND # PLA # pre-1938 <input type="checkbox"/> yes OTC product <input type="checkbox"/> yes	
6. If IND, protocol #		7. Type of report (check all that apply) <input type="checkbox"/> 5-day <input checked="" type="checkbox"/> 15-day <input type="checkbox"/> 10-day <input type="checkbox"/> periodic <input type="checkbox"/> initial <input checked="" type="checkbox"/> follow-up # 1	
8. Adverse event term(s) Movement disorder NOS, Tic, Nightmare, Rash NOS, Dry skin		9. Mfr. report number PHBS2002AU04398	
E. Initial reporter			
1. Name & address Name and address withheld.		phone # Withheld	
2. Health professional? <input type="checkbox"/> yes <input checked="" type="checkbox"/> no		3. Occupation Consumer	
4. Initial reporter also sent report to FDA <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> unk			



3506A - Facsimile



DSS

MAY 02 2002

MAY 01 2002

30-Apr-2002 12:01:24



Experience Report
(continued)

mission of a report does not constitute
admission that medical personnel, user,
y, distributor, manufacturer or product
caused or contributed to the event.

Page 2 of 2

Novartis Pharmaceuticals

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service - Food and Drug Administration	
MR Report #	PHBS2002AU04398
URDR# Report #	
FDA Use Only	

Additional Information

B5. EVENT DESCRIPTION (cont.)

Follow-up information was received on 11 Apr 2002:

The mother of this patient reported that the symptoms could be described as a movement disorder as well as a tic. The patient did not have a history of tics or movement disorders and there was no family history of tics. Prior to the movement disorder, the patient had experienced night terrors (nightmares) for one week. She had also had a skin rash and dry skin around her mouth. The patient had been receiving concomitant Efalex since Dec 2001, as a nutritional supplement to replace essential fatty acids. The patient was improving.

Follow-up information was received on 22 Apr 2002:

The mother of this patient reported, that the patient continued to experience abnormal movements in her legs and arms. The movements were not as frequent but flare-ups occurred. The patient was seeing a pediatric psychiatrist for counseling regarding the nightmares she had whilst receiving Ritalin.

Novartis Comment:

The available information was considered inadequate to fully assess the case.

Serious spontaneous report (medically significant hazard) assessed as listed for tic and rash and as unlisted for nightmares and movement disorders NOS according to the Basic Prescribing Information. The information provided in this individual case does not warrant a change to the Basic Prescribing Information text. The topic will be monitored closely and will be re-evaluated on an ongoing basis based on cumulative experience. All spontaneous reports are considered suspected for reporting purposes.

C1. Name (cont.)

Suspect Medication #1: RITALINE(METHYLPHENIDATE HYDROCHLORIDE) Tablet

C4. Diagnosis for use (cont.)

#1: Attention deficit/hyperactivity disorder

DSS

MAY 02 2002

MAY 01 2002

Individual Safety Report		ALZA Corporation For use by user-facilities, butors and manufacturers for MANDATORY reporting		Approved by FDA on 09/25/95
 4049822-X-00-01		Page <u>1</u> of <u>2</u>		Mfr report # NSADSS2003000409 User/Inst report # _____ FDA Use Only
A. Patient information				
1. Patient identifier (b) (6)	2. Age at time of event or Date of birth: <u>11 yr</u> <u>??/??/??</u>	3. Sex <input type="checkbox"/> female <input checked="" type="checkbox"/> male	4. Weight <u>QNK</u> lbs or <u>QNK</u> kgs	
B. Adverse event or product problem				
1. <input checked="" type="checkbox"/> Adverse event and/or <input type="checkbox"/> Product problem (e.g., defects/malfunctions)				
2. Outcomes attributed to adverse event (check all that apply)				
<input type="checkbox"/> death (mortality) <input type="checkbox"/> life-threatening <input checked="" type="checkbox"/> hospitalization - initial or prolonged <input type="checkbox"/> disability <input type="checkbox"/> congenital anomaly <input type="checkbox"/> required intervention to prevent permanent impairment/damage <input type="checkbox"/> other: _____				
3. Date of event (m/d/y) <u>12/??/02</u>		4. Date of this report (m/d/y) <u>01/24/03</u>		
5. Describe event or problem				
Spontaneous report received from a physician: An 11-year-old boy experienced torticollis, rolling arm movements, lip chewing movements, slight pharyngitis, slurred speech, and not eating or drinking during the use of OROS methylphenidate. He initiated OROS methylphenidate 36 mg qd a few years earlier for treatment of attention-deficit hyperactivity disorder (ADHD). The physician reported that the boy ran out of medication 30-Dec-2002, and on (b) (6) the boy was taken to the emergency room with severe torticollis, rolling arm movements, lip chewing movements, and slight pharyngitis. He was treated with oral diazepam and ibuprofen, and discharged home. On 02-Jan-2003, the boy was taken to his pediatrician with continued symptoms. In addition, the boy's speech was slurred and he was not eating or drinking. He was treated with IV cetirizine hydrochloride 25 mg and admitted to the hospital. Torticollis somewhat improved with cetirizine hydrochloride, and the boy's pediatrician was planning to administer (Cont.)				
6. Relevant tests/laboratory data, including dates				
7. Other relevant history, including preexisting medical conditions (e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.)				
Moderate to moderate-severe developmental delays; Slow learner; Adopted (birth mother may have abused drugs while pregnant); Allergies - unknown				
C. Suspect medication(s)				
1. Name (give labeled strength & mfr/labeler, if known)				
#1 CONCERTA (36 mg sustained release tablet) (METHYLPHENIDATE)				
#2 _____ (Cont.)				
2. Dose, frequency & route used		3. Therapy dates (if unknown, give duration)		
#1 36 mg, 1 in 1 day(s), oral		#1 ??/??/?? - 12/31/02		
#2 _____		#2 _____		
4. Diagnosis for use (indication)		5. Event abated after use stopped or dose reduced		
#1 ATTENTION DEFICIT DISORDER OF CHILDHOOD		#1 <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> doesn't apply		
#2 _____		#2 <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply		
6. Lot # (if known)		7. Exp. date (if known)		
#1 _____		#1 _____		
#2 _____		#2 _____		
8. Event reappeared after reintroduction				
#1 <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> doesn't apply				
#2 <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply				
9. NDC # - for product problems only (if known)				
10. Concomitant medical products and therapy dates (exclude treatment of event)				
1) EVENING PRIMROSE ??/??/?? - Ongoing				
OIL (EVENING PRIMROSE OIL)				
2) FISH OIL (FISH OIL) ??/??/?? - Ongoing				
G. All manufacturers				
1. Contact office - name/address (& mailing site for devices)		2. Phone number		
ALZA Corporation		3. Report source (check all that apply)		
		<input type="checkbox"/> foreign <input type="checkbox"/> study <input type="checkbox"/> literature <input type="checkbox"/> consumer <input checked="" type="checkbox"/> health professional <input type="checkbox"/> user facility <input type="checkbox"/> company representative <input type="checkbox"/> distributor <input type="checkbox"/> other: _____		
4. Date received by manufacturer (m/d/y) <u>01/23/03</u>		5. (A) NDA # <u>21-121</u>		
6. If IND, protocol #		IND # _____		
7. Type of report (check all that apply)		PLA # _____		
<input type="checkbox"/> 5-day <input checked="" type="checkbox"/> 15-day <input type="checkbox"/> 10-day <input type="checkbox"/> periodic <input type="checkbox"/> Initial <input checked="" type="checkbox"/> follow-up # <u>1</u>		pre-1938 <input type="checkbox"/> yes OTC product <input type="checkbox"/> yes		
9. Mfr. report number NSADSS2003000409		8. Adverse event term(s)		
		1) ABSCESS 2) TORTICOLLIS 3) CHOREOATHETOSIS 4) PHARYNGITIS 5) SPEECH DISORDER 6) ANOREXIA		
E. Initial reporter				
1. Name, address & phone # (b) (6)		DSS JAN 29 2003		
				
2. Health professional?		3. Occupation		4. Initial reporter also sent report to FDA
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		Physician		<input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> unk

3500A Facsimile

Submission of a report does not constitute an admission that medical personnel, user facility, distributor, manufacturer or product caused or contributed to the event.

Individual Safety Report



4049822-X-00-02

Continuation Sheet for FDA-3500A Form

Mfr. report #: NSAIDSS2003000409

2 of 2

Date of this report: 01/24/03

B. Adverse event or product problem

B.5 Describe event or problem (C)

another dose. As of 3-Jan-2003 the status of OROS methylphenidate therapy and the outcome of the other events was unknown.

Additional information received 23-Jan-2003: The physician reported that the boy was diagnosed with lateral pharyngeal abscess. No further information was provided.

C. Suspect medication (Cont...)

Seq No.

: 1

C.1 Suspect medication

: CONCERTA(36 mg sustained release tablet) (METHYLPHENIDATE HYDROCHLORIDE)

C.4 Diagnosis for use(indication)

: 1) ATTENTION DEFICIT DISORDER OF CHILDHOOD WITH HYPERACTIVITY

DSS

JAN 29 2003

JAN 28 2003

Case IV



FDA - Adverse Event Reporting System (FAERS)

FOIA Case Report Information

Case ID: 6621671

Case Information:

Case Type: EXPEDITED (15-DAY) eSub: Y HP: Y Country: DEU Event Date: Outcomes: OT Application Type: NDA

FDA Rcvd Date: 23-Apr-2008 Mfr Rcvd Date: 15-Apr-2008 Mfr Control #: DE-JNJFOC-20080402794 Application #: 021121

Patient Information:

Age: 17 YR Sex: Female Weight:

Suspect Products:

#	Product Name	Compounded Drug ?	Dose/Frequency	Route	Dosage Text	Indications(s)	Start Date	End Date
1	CONCERTA			Oral		ATTENTION DEFICIT/HYPERACTIVITY DISORDER		
#	Product Name	Interval 1st Dose to Event	DeC	ReC	Lot#	Exp Date	NDC #	MFR/Labeler
1	CONCERTA		NA	Unk				

Event Information:

Preferred Term (MedDRA ® Version: 17.0) ReC

Psychotic disorder Unk

Schizophrenia Unk

Event/Problem Narrative:

This spontaneous report received from a physician concerns a 17 year old girl from Germany: A. The patient's concurrent conditions included attention deficit hyperactivity disorder (ADHD) subtype unspecified, and depression. The patient's weight was not reported. The patient was treated with OROS methylphenidate hydrochloride (sustained release tablets, oral) initiated about 1 year ago, for ADHD. Concomitant medications included: St. John's wort. On an unknown date the patient experienced schizophrenia-like symptoms and psychotic symptoms. It was described that the patient heard and saw things in the night which were not there and was therefore disturbed. The patient outcome was unknown for psychotic symptoms and schizophrenia like symptoms. Action taken with OROS methylphenidate was unknown. This report was serious (medically significant).



FDA - Adverse Event Reporting System (FAERS)

FOIA Case Report Information

Case ID: 6621671

Relevant Medical History:

Disease/Surgical Procedure	Start Date	End Date	Continuing?	
ATTENTION DEFICIT/HYPERACTIVITY DISORDER			YES	
DEPRESSION			YES	
Medical History Product(s)	Start Date	End Date	Indications	Events

Relevant Laboratory Data:

Test Name	Result	Unit	Normal Low Range	Normal High Range	Info Avail
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Concomitant Products:

#	Product Name	Dose/ Frequency	Route	Dosage Text	Indications(s)	Start Date	End Date	Interval 1st Dose to Event
1	ST. JOHN'S WORT		Unknown					

Reporter Source:

Study Report?: No Sender Organization: ALZA 503B Compounding
Outsourcing Facility?:

Literature Text:

Case V

CaseID: 3749511

 3852041-0-00-01+		RECEIVED JAN 11 2002 CORCOR		Novartis Pharmaceuticals Corp. FDA Form 101 (Rev. 10-2001)
MEDVVA ICH THE FDA MEDICAL PRODUCTS REPORTING PROGRAM		For use by user facilities, distributors and manufacturers for MANDATORY reporting Novartis Pharmaceuticals		PHHS2002CH00502 FDA Use Only
Page 1 of 2				
A. Patient information 1. Patient identifier: UNK 2. Age at time of event: 15 Years Date of birth: --/--/1986 3. Sex: <input checked="" type="checkbox"/> male 4. Weight: UNK lbs or UNK kg		C. Suspect medication(s) 1. Name (give labeled strength & mfr/labeler, if known) #1 RITALINE (METHYLPHENIDATE) (continued) #2 ST. JOHN'S WORT/HYPERICUM (continued) 2. Dose, frequency & route used #1 20 mg/day, Oral #2 5 goats/day, Oral 3. Therapy dates (if unknown, give duration) #1 --/--/1998 to Ongoing #2 06/01/2001 to 06/06/2001 4. Indication for use (indication) #1 Attention (continued) #2 Depressive symptom 5. Event abated after use stopped or dose reduced #1 <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> doesn't apply #2 <input checked="" type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply 6. Lot # (if known) #1 UNK #2 UNK 7. Exp. date (if known) #1 UNK #2 UNK 8. Event reappeared after reintroduction #1 <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> doesn't apply #2 <input checked="" type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> doesn't apply 9. NDC # - for product problems only (if known)		
B. Adverse event or product problem 1. <input checked="" type="checkbox"/> Adverse event and/or <input type="checkbox"/> Product problem (e.g., defects/malfunctions) 2. Outcomes attributed to adverse event (check all that apply) <input type="checkbox"/> death <input type="checkbox"/> disability <input type="checkbox"/> life-threatening <input type="checkbox"/> congenital anomaly <input type="checkbox"/> hospitalization - initial or prolonged <input checked="" type="checkbox"/> required intervention to prevent permanent impairment/damage <input type="checkbox"/> other Medically Significant 3. Date of event: 06/01/2001 4. Date of this report: 01/08/2002		10. Concomitant medical products and therapy dates (exclude treatment of event) NI		
5. Describe event or problem: Depression [Depression] [Agitation], [Crying], [Aggression], [Disturbance in attention] Drug interaction with Hypericum (St. John's wort) [Drug interaction NOS] Case Description: This is a health authority report: This patient started treatment with Ritalin (methylphenidate) in 1998. Ritalin was well tolerated. The patient had a period of sadness and Hypericum Ceres (hypericum, St. John's wort) was started on 01 Jun 2001. A few hours later, he developed agitation, unexplained weeping, aggressiveness alternating with depression and difficulties to concentrate. On 06 Jun 2001, Hypericum was discontinued and the reactions abated. 3 weeks later Hypericum was reintroduced during 2 days and the same reactions occurred. After discontinuation of Hypericum the symptoms abated again. Ritalin was continued. The causal continued in additional info section...		G. All Manufacturers 1. Contact office - name/address (if mailing site for devices) Novartis Pharmaceuticals Corp. Clinical Safety and Epidemiology 2. Phone number 3. Report source (check all that apply) <input checked="" type="checkbox"/> foreign CHC <input type="checkbox"/> study <input type="checkbox"/> literature <input type="checkbox"/> consumer <input checked="" type="checkbox"/> health professional <input type="checkbox"/> peer review <input type="checkbox"/> company representative <input type="checkbox"/> distributor <input checked="" type="checkbox"/> other (health authority) 4. Date received by manufacturer: 01/07/2002 5. FDA # ID-157 6. If IND, protocol # IND # P.L.A. # pre-1938 <input type="checkbox"/> yes <input type="checkbox"/> no OTC product <input type="checkbox"/> yes <input type="checkbox"/> no 7. Type of report (check all that apply) <input type="checkbox"/> 5-day <input checked="" type="checkbox"/> 15-day <input type="checkbox"/> 10-day <input type="checkbox"/> periodic <input checked="" type="checkbox"/> initial <input type="checkbox"/> follow-up # 8. Adverse event term(s): Depression, Drug interaction NOS, Agitation, Crying, Aggression, Disturbance in attention 9. Mfr. report number: PHHS2002CH00502		
5. Relevant test/lab data, including dates: NI		E. Initial reporter 1. Name & address: Name and address withheld phone # Withheld 2. Health professional? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no 3. Occupation: UNK 4. Initial reporter also sent report to FDA: <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> with		
7. Other relevant history, including pre-existing medical conditions (e.g., allergies, race, pregnancy, smoking and alcohol use, neurological dysfunction, etc.): NI		2. Health professional? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no 3. Occupation: UNK 4. Initial reporter also sent report to FDA: <input type="checkbox"/> yes <input type="checkbox"/> no <input checked="" type="checkbox"/> with		



(continued)

ion of a report does not constitute
 ion that medical personnel, user,
 distributor, manufacturer or product
 ed or contributed to the event.

Novartis Pharmaceuticals

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
 Food and Drug Administration

Version 1.0

PHRS2002CH00502

OTC Drug Label

FDA/CDR

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Additional Information

B5. EVENT DESCRIPTION (cont.)

relationship was assessed as probable

Novartis Comment:

Serious spontaneous report [medically significant hazard] assessed as listed for transient depressed mood and unlisted for depression when interacting with hypericum according to the Basic Prescribing Information. The information provided in this individual case does not warrant a change to the Basic Prescribing Information text. The topic will be monitored closely and will be re-evaluated on an ongoing basis based on cumulative experience. All spontaneous reports are considered suspected for reporting purposes.

C1. Name (cont.)

Suspect Medication #1: RITALINE(METHYLPHENIDATE HYDROCHLORIDE) Unknown

Suspect Medication #2: ST. JOHN'S WORT(HYPERICUM PERFORATUM)

C4. Diagnosis for use (cont.)

#1: Attention deficit/hyperactivity disorder

DSS

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