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Ethnobotany and Ethnopharmacology of Q'Eqchi' Maya Medicinal Plants from Southern Belize Used for Ethnopsychiatric and Neurological Purposes

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ETHNOBOTANY AND ETHNOPHARMACOLOGY OF Q'EQCHI' MAYA MEDICINAL PLANTS FROM SOUTHERN BELIZE USED FOR ETHNOPSYCHIATRIC AND NEUROLOGICAL PURPOSES

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Thesis submitted to the
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ABSTRACT

This study investigated plant use by Q'eqchi' Maya healers (Southern Belize) in the context of ethno-psychiatric and neurological conditions. The healers recognize eight of these disorders, which they treat with 82 identified plant species. Moreover, the *Piperaceae* family is the plant family most selected by the healers for these purposes. In a second section, the etiological basis of *susto*, a folk illness known throughout Latin America, was investigated. Some plants used by most of the healers showed anxiolytic and/or fear suppression activity. In addition, rituals surrounding the treatment process may have calming effects, and many plants used for *susto* are often used to treat mental/neurological disorders as well. This strengthens the basis for the inclusion of psychological/neurological factors in *susto*. A third section gives insights into the domestication and conservation of key medicinal species, indicating that harvesting practices are sustainable in the current context.

RÉSUMÉ

Cette étude a traité de l'utilisation de plantes pour traiter des désordres ethno-psychiatriques et neurologiques par des guérisseurs Maya Q'eqchi' du Belize. Les résultats indiquent que ces derniers traitent huit conditions à l'aide de 82 espèces végétales et que les *Piperaceae* sont préférentiellement sélectionnées dans ce contexte. Les bases étiologiques du *susto*, une maladie folklorique reconnue à travers l'Amérique Latine, ont aussi été sondées. Certaines plantes utilisées par la majorité des guérisseurs ont démontré des propriétés anxiolytiques et/ou de supression de la peur. Les rituels entourant le traitement du *susto* ont probablement aussi des effets calmants et les plantes utilisées pour traiter le *susto* sont souvent utilisées pour traiter des maladies neurologiques/mentales également. Ces résultats appuient l'idée d'une base psychologique/neurologique du *susto*. Une troisième section a examiné la domestication et la conservation de plantes médicinales culturellement importantes. Tout indique que les techniques de récolte sont durables dans le contexte actuel.

ABSTRACT IN Q'EQCHI' [1]

Cholcho' ut chutchu' i ru, li nawook' chi rish li kolbal ib sa' raihilal xban li naba'l pay chi tiajel. Tzilb'al i rish tyok'eb li xcuankeb lix tzolomik chirish aj cui li xcuankil li che kaham nar oksimank chi ok' b'an. Li aj tzolonel ain, cuankeb sa li nim laj tzolebal aran sa nim laj choch Canada. Ain lix nawbal chirix li jarub pay chi k'ehen nar ocsimank xban li tenamit aj Q'eqchi arin sa li ch'och'il Belize. Jarub pay chi kehen nar oksimank chi ok' banbal li chib li jolom, Ulul, ut malak' li Kahnil. Laj aj Q'eqchi lloneleb, nakesh taw i ru cua'shak'ib pay chi tiajel nar ek'amamk ut nar cuank sa li jolom, heb nake nawok' re ochenta y dos pay chi jalanil k'ehen re kirtasinkil li tiajel ain. Li chib i Jolom, Ulul ut li Kanhnil (*susto*) li tiajeleb ain mak'a ajcui arin sa Belize cuan, cuank aj cui se jalanil poyonam chiru li ch'och'. Lix nimkeb ixcuanquil sa li nim laj tzoleb'al aran sa tenamit Canada eshtawmank i ru, li k'eheneb ain li nar oksimank chi o'k re chib li jolom, ut, li et laj tiajel, kahnil, cuan li chabil ban chi sa' li k'ehen ain jo'hkan nar tenk'ank chi k'irtasinkil li tiaj, cuan sut tentoo li k'ehb'al lix cuut aran nar oksimak li Mayejak re' lix tenk'ankil ut re xcotzb'al li tiajel chi jun ajcuank. Li aj lloneeleb tyiok'eb ajcui' chi xokbalchak lix kommooneb li k'eheneb ain, re raub'al sa jun chi na'jej, re lix puktasinkil li kehen ut re puktasinkil resilal li nawom chi rix li b'anok. Xb'an jalan chi nahjej ut jalan chi ch'och'il nar tawmank chak cui'. Aran jo kawil lix puktasinkil li kehen, nab'al sut ink'a nar tyiolah, b'an tento' lix puktasinkil li xe' toon n'aleb.

[1] Translation by Victor Cal

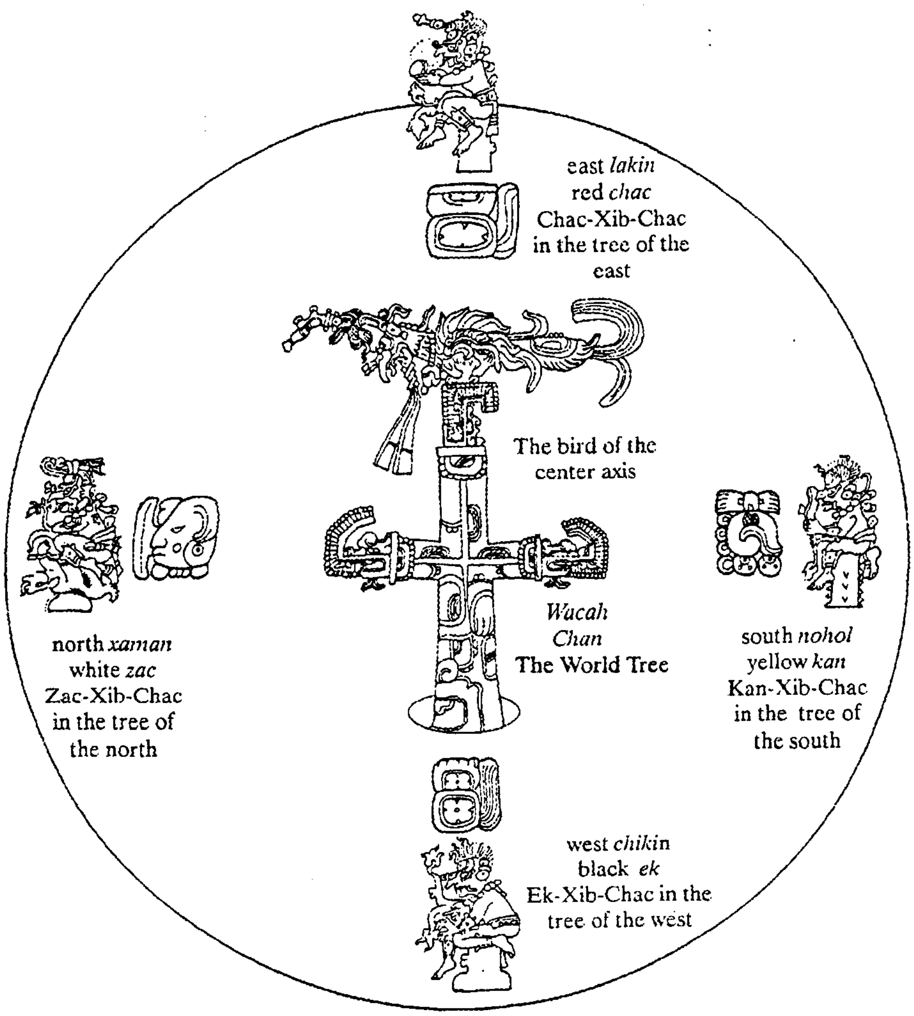

Figure 1.1: Cosmology of the Ancient Maya (Schele and Freidel; 1990) (Temple of the Cross, Palenque, Mexico); drawing by Linda Schele, © David Schele, courtesy Foundation for the Advancement of Mesoamerican Studies, Inc., www.famsi.org

CHAPTER 1

GENERAL INTRODUCTION

The Maya of the pre-colonial period (~ 1800 BC - 925 AD) envisioned a world where the World Tree (called *Wacah Chan* or "raised up sky", in the glyphs) (Schele and Freidel, 1990) was the central axis, and the four cardinal points (each represented by a tree) combined with this axis to constitute the fundamental community and world structure (Figure 1.1). According to their beliefs, the trunk of this sacred tree (ceiba tree; *Ceiba pentandra*) went through the Middleworld, while its crown was in the Heavens, and its roots reached the Underworld. This axis was not in a set place, but could be materialized through rituals during which the Kings and other shamans would be entranced and pass to the Otherworld, a parallel unseen world made up in part of the Heavens and the Underworld (Schele and Freidel, 1990).

Healing and shamanism undoubtedly played important roles in the lives of the Ancient Maya. Two main deities were associated with medicine: Itzamná (shaman, inventor of writing, patron of learning and the sciences, and one of the Creator Gods) and his wife, Ix-Chel (the old Goddess of weaving, medicine and childbirth, and old Moon Goddess) (Coe, 1999; Freidel et al., 1993) (Figure 1.2). They were believed to have given birth to all other deities in the Maya pantheon (Coe, 1999), attesting to their significance in the Maya belief system. This is also highlighted in the cult of the Goddess of Medicine (~ AD 1250), a pilgrimage to the island of Cozumel (Southern Mexico), and in the numerous shrines found of the representation of Itzamná (Coe, 1999).

Healers and spiritual leaders are important components of the contemporary Maya social structure. They use knowledge of astrology and the sacred Maya calendar to perform various rituals, much like their predecessors (Sharer, 1996). In fact, many aspects of Ancient Maya religion and culture have been conserved. Their culture did not disappear with the collapse of the Classic Maya civilization (800 AD – 925 AD), nor even under the sometimes violent pressures from European Conquerors. Rather, the Maya have displayed an incredible ability to adapt. At the time of Conquest, they mixed components of the Christian religion with components from their own, a characteristic that is obvious in many contemporary religious practices of the Maya. For example, the Cross of Christ was easily accepted as a religious symbol, as it was merged with the concept of the World Tree, traditionally represented in the shape of a cross (Freidel et al., 1993).

The study presented here stems from a desire to increase recognition of elders, healers, spiritual leaders, and traditional knowledge and practices of the contemporary Maya, and specifically of the Q'eqchi' Maya of Southern Belize. It focuses on traditional healing and the use of medicinal plants by members of this group. As such, this study is ethnomedical in nature, and belongs to the fields of ethnobotany and ethnopharmacology.

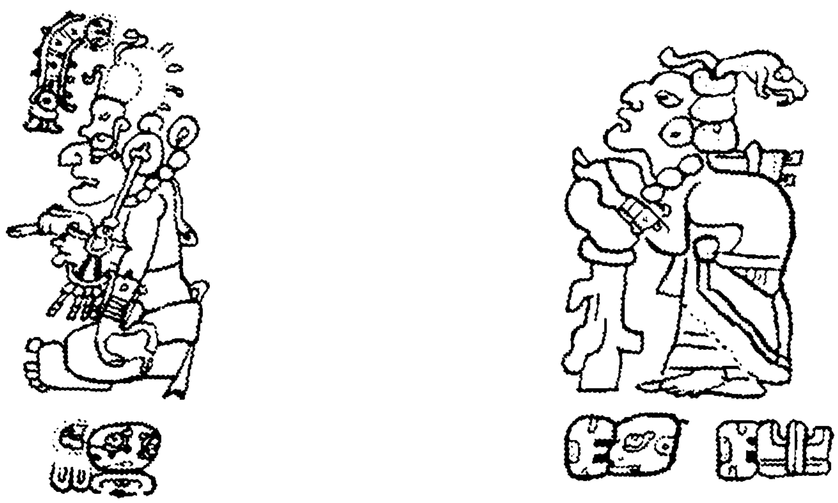

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Figure 1.2: Representations of Maya Gods with name glyphs. (A) Itzamná; (B) Ix-Chel; reproduced with permission from Coe (1999) (from the Dresden Codex)

1.1 The field of ethnobotany: past and present

Ethnobotany can be defined as the study of the interactions between people and the plants found in their environment (Ford, 1978). This discipline, which emerged at the turn of the twentieth century, has traditionally been concerned with recording the traditional knowledge of indigenous peoples from around the world by characterizing plant use and compiling lists of these plants. However, the 1970s presented innovative multidisciplinary contributions (e.g. Berlin et al., 1974; Schultes and Hoffman, 1973), and the scope of ethnobotanical research broadened (Balick, 1996). Since then, ethnobotany has become a truly multidisciplinary field, at the interface between anthropology, botany, nutrition, ecology, conservation, economics, phytochemistry, and pharmacology. Complementary fields of relevance to this study are ethnopharmacology, the study of the pharmacology of plants and other products used by indigenous and traditional peoples (Heinrich, 2001), and ethnomedicine, the study of traditional medical systems, practices and related beliefs (Genest, 1978).

In the past few decades, fascination and interest in the field of ethnobotany has greatly increased, both from the general public and the scientific community. With this attention, certain issues have become central in ethnobotany. These include the protection of intellectual rights, especially of indigenous peoples and their traditional knowledge, the application of more rigorous methodologies in ethnobotanical research, and the simultaneous conservation of rapidly eroding traditional knowledge and biodiversity.

1.1.1 Intellectual property rights

Intellectual Property Rights are certainly becoming a central issue in ethnobotanical and ethnopharmacological research. Both in commercial and academic endeavours, the respect of intellectual property rights is essential.

Commercial development

It is estimated that at least 50 pharmaceutical drugs have been discovered from screening based on ethnobotanical information (e.g. quinine, asperine) (Cox, 1994). In such situations, questions concerning the rights of indigenous and traditional people arise, and many examples exist where the interests of these people were overseen. One of the reasons for this is that intellectual property rights (IPR) laws, which are widely used legal recourses in the commercial use of traditional knowledge, were originally crafted to protect individuals and their inventions and are legally inadequate to protect the collective traditional knowledge of communities (Posey, 2002). It is important to mention, however, than some companies (e.g. Shaman Pharmaceuticals) are very aware of their moral debt towards indigenous collaborators and make an effort to provide equitable compensation (King and Tempesta, 1994). Clearly, as with bioprospecting in general, these issues need to be addressed by international organizations and serious legal procedures that take indigenous rights and customs into account should be put into place.

Academic research

Several issues pertaining to ethical concerns in research have been raised, such as the proper acknowledgement of indigenous sources, copyright property, the rights of indigenous peoples to be informed, and the respect of local customs (Battiste and Henderson, 2000; Posey and Dutfield, 1996). Furthermore, although recording and publishing ethnobotanical information is crucial to the conservation of disappearing traditional knowledge and to scientific studies where the free flow of information is very important, publishing renders the information public, thus available to individuals and organizations with commercial interests (Posey and Dutfield, 1996).

Fortunately, certain options are available to researchers. For example, most recent studies include indigenous authors and collaborators. Furthermore, prior informed consent regarding the reasons for the research, specific procedures, potential risks, and foreseeable implications of the study can and should be included in the research process (Posey and Dutfield, 1996). Moreover, many innovative means to protect the rights of indigenous and traditional peoples have been elaborated by communities, governments and individuals (see Posey and Dutfield (1996) for a discussion on "creative strategies and unique solutions"). However, the issue of public access to published information is still unresolved and few recourses are available for indigenous peoples to take action against companies in such situations. Posey and Dutfield (1996), for example, discuss the possibilities offered by defensive publication (publication of a thourough description of how the plant is used to prevent others from obtaining a patent), but warn that this practice is often unsuitable. An alternative is the publication of extrapolated information, such as information obtained using quantitative ethnobotanical methods for use in major publications (Phillips, 1996). Ethics in the practice of ethnobotany and its various implications are evolving and more measures to deal with such issues will most likely arise in the near future.

1.1.2 Quantitative ethnobotany

The general lack of methodological rigor in the field of ethnobotany was criticized in important contributions by Johns *et al.* (1990), Phillips and Gentry (1993a), and Trotter and Logan (1986), among other authors. Their dissatisfaction was mainly with the insufficiency of information given in research papers (e.g. number of informants interviewed, method used for data compilation) and with the qualitative nature of the research undertaken (e.g. absence of criteria to assess the relative importance of species cited, reliance on single informants, contradictions in the information received). Especially in ethnopharmacology, which provides a clear potential not only in the search for new drugs but also in the validation of traditional remedies (Croom, 1983), there was a need for identifying species from ethnobotanical studies that should be prioritized for pharmacological testing (Johns *et al.*, 1990). Following this call for more thorough research, several authors elaborated quantitative ethnobotanical methods. These not only provided criteria for the selection of plants with potential to be biologically active (e.g. Freidman *et al.*, Heinrich, 2000; Johns *et al.*, 1990; Leaman *et al.*, 1995; Moerman, 1991; Trotter and Logan, 1986), but offered the possibility to compare knowledge between geographical areas, cultural groups and villages (Begossi, 1996; Heinrich, 2000; Leonti *et al.*, 2003; Moerman *et al.*, 1999), to evaluate sampling effort (Begossi, 1996) and, broadly, to test falsifiable hypotheses (Phillips and Gentry, 1993a and 1993b) and identify patterns in ethnobotanical data (Johns *et al.*, 1994).

1.1.3 Ethnobotany and conservation

Ethnobotany is linked to conservation in many respects. In fact, the general field of ethnobotany could not exist without the plants and animals on which indigenous and traditional peoples depend. Moreover, a decrease in biodiversity, especially in tropical environments, entails a significant loss of cultural diversity because of the close association between traditional peoples and their environments. Accordingly, management strategies in conservation increasingly take into account the strong association between indigenous and traditional peoples and their environments, for example through the establishment of buffer zones (Martin, 1994). Additionally, ethnobotany provides strong justification for conservation: there have been a number of studies demonstrating the economic benefits of sustainable exploitation of non-timber forest products in tropical environments (e.g. Balick and Mendelsohn, 1992; Peters *et al.*, 1989). Therefore, providing healers with means to preserve their knowledge as well as the surrounding biodiversity could have considerable long-term impacts. Belize, with its high cultural and biological diversities, provides a good setting for such an undertaking.

1.2 Study site

Belize is located in Central America, sharing political borders with Mexico, Honduras and Guatemala. The general climate in Belize is sub-tropical, with rainfall increasing gradually with decreasing latitude (Hartshorn *et al.*, 1984). This country of only 22,963 km^2 sustains a wide diversity of ecosystems: the northern limestone plains support sub-tropical moist forests with various vegetation sub-types (e.g. savannas, inland marshes and forests comprising deciduous species); the coastline and off-shore islands are lined with mangrove forests; and the Maya Mountains of Southern Belize present a mountain pine ridge as well as semi-evergreen tropical forests (Balick *et al.*, 2000; Whitmore, 1998). Accordingly, over 3400 species of vascular plants have been recorded in Belize (1219 genera and 209 families), with 41 endemic species (1.2% of total flora) (Balick *et al.*, 2000).

1.2.1 Belize and conservation

Belize is becoming increasingly important from a conservation standpoint. Indeed, over 40% of the land is officially protected (Olivet and Asquith, 2004). Considering the diversity of ecosystems distributed throughout the country and the rapid deforestation and environmental degradation underway in many parts of Mesoamerica (FAO, 2003), the importance of such conservation measures is great. Furthermore, Mittermeier *et al.* (1998) have identified the Mesoamerican Forests (forests of Central America and of certain parts of Mexico) as one of the regions of the world with the highest plant endemism, recognizing it as a biodiversity hotspot. In this context, it is crucial to promote the sustainable use of forest resources in Belize (e.g. harvesting of medicinal plants) (Balick and Mendelsohn, 1992) and to emphasize the importance of natural resources and of their conservation to local populations.

1.2.2 The Q'eqchi'

The Maya comprise thirty-one distinct cultural groups with mutually unintelligible languages (Coe, 1999). They are dispersed mainly throughout Mexico, Guatemala and Belize (Figure 1.3). The Q'eqchi' language is thought to have derived from the Greater K'iche'an linguistic group in the last 2000 years, a group that also comprises Uspanteko, as well as the Poqom and K'iche'an linguistic groups (Coe, 1999; orthography according to the *Academia de Lenguas Mayas*). The Q'eqchi' are the fourth largest subgroup of Maya in population in Guatemala (Wilson, 1995). They are also found in Belize, where they are concentrated in small villages in the rural areas of the Toledo district, in the southernmost part of the country (Bolland, 1986). Also, because the Q'eqchi' live in relatively remote areas, less acculturation has occurred than with other indigenous groups of Belize (Treyvaud Amiguet *et al.*, 2004), which suggests a well preserved cultural tradition. In this thesis, the orthography promoted by the *Academia de las Lenguas Mayas* was used whenever possible. Thus "Q'eqchi'" refers to the same ethnic group as "Kekchi", from the previous orthography.

1.2.3 Ethnobotanical research among the Maya of Belize

Shamanism and traditional healing are still very important for the Q'eqchi' and in Belize generally. In fact, it is estimated that traditional practitioners provide 75% of primary health care to Belize's rural population (Balick and Mendelsohn, 1992). Since the healers rely chiefly on plants for traditional remedies (Arvigo and Balick, 1993), most studies interested in Belizean traditional medicine and associated remedies have focused on the use of medicinal plants. Such investigations include studies of the Yucatec by Arnason *et al.* (1980), Pendergast (1972), Roys (1931) and Thomson (1930). Also, *Rainforest Remedies* published by Arvigo and Balick (1993) and the *Checklist of Vascular Plants of Belize* by Balick *et al.* (2000) describe certain plants used by the Mopan, Yucatec, and Q'eqchi', among other ethnic groups. The most detailed study of Q'eqchi' use of plants is that of Treyvaud Amiguet *et al.* (2004), which provides an in depth description of Q'eqchi' medical ethnobotany. The present study builds on this previous research.

1.3 Hypotheses and objectives of the study

Generally, the study presented in this thesis seeks to better understand traditional Q'eqchi' medicine and the associated use of plants, focusing on neurological/mental disorders. Chapter 2 builds on the study by Treyvaud Amiguet *et al.* (2004) by giving a more in-depth analysis of ethno-psychiatric treatments used by the Q'eqchi', with an emphasis on the use of medicinal plants. Chapter 3 investigates the nature of *susto*, a folk illness recognized by the Q'eqchi', through the various treatments used. Chapter 4 is a preliminary study that aims to provide the healers with guidelines in conservation of selected medicinal plants used and insights into the domestication of important medicinal species. It is essential to note, however, that the objectives of this last chapter are practical. Nonetheless, it represents a significant first step in establishing research priorities for the conservation of medicinal plants in the area.

The main hypothesis examined in Chapter 2 is that some plant species and families are preferred for medicinal use in the context of neurological and/or mental disorders from the total flora available to the healers. It was evaluated with the following objectives:

1. to identify neurological and/or mental ailments recognized by the Q'eqchi';
2. to describe traditional healing techniques used by the Q'eqchi' for these disorders;
3. to identify consensus among healers to treat these ailments, in the favoured use of certain species (informant consensus);
4. to identify plant families preferentially selected by the healers for recognized neurological and/or mental disorders (regression analyses).

Chapter 3 examines the main hypothesis that underlying etiological neurological or psychological factors intervene in *susto*. From this general hypothesis, it is expected that treatments for *susto* should exhibit biological activity on the central nervous system. Specific hypotheses were that herbal remedies selected to treat *susto* have fear suppression and/or anxiolytic activities (quantitative assessment), that plants used to treat *susto* are often also used for neurological/mental disorders (quantitative assessment), and that concomitant forms of treatment consist of various healing techniques that help in relieving anxiety and fear (indirect qualitative assessment). These were evaluated by means of the following objectives:

1. to describe the perception of *susto* by the healers (e.g. symptomatology, folk etiology) and the various forms of treatment used in treating *susto*;
2. to identify plants used in common for *susto* and other ailments (ailment clusters);
3. to quantify the relative importance of plant species used for *susto* (informant consensus) and to evaluate the activity of preferred plants compared to plants selected at random in the same environment, in anxiolytic and fear suppression bioassays.

Finally, Chapter 4 has management and conservation objectives:

1. to compare edaphic and abiotic factors that affect plant growth between a natural site and a cultivation site;
2. to assess plant usage of selected species in relation to plant resilience or vulnerability to harvesting;
3. to provide the healers with guidelines for the conservation and domestication of important medicinal plants.

Figure 1.3: Distribution of colonial and contemporary languages in the Maya area; reproduced with permission from Coe (1999)

CHAPTER 2

PLANT USE BY THE Q'EQCHI' OF BELIZE IN ETHNOPSYCHIATRY AND NEUROLOGICAL PATHOLOGY

2.1 INTRODUCTION

Mental/neurological and behavioural disorders are very prominent worldwide, affecting 25% of individuals in their lifetime, and some of these debilitating conditions are more prevalent in developing countries (WHO, 2001). In 2002, the mental health Global Action Program (mhGAP) was created by the World Health Organisation (WHO) to provide improved monitoring, treatment/rehabilitation and preventative measures for individuals suffering from these disorders (WHO, 2002).

In Belize, as in most developing countries, traditional practitioners play an important role in general medical care. It is estimated that they supply 75% of the primary health care to rural inhabitants (Balick and Mendelsohn, 1992), relying chiefly on the surrounding semi-evergreen tropical forests (Whitmore, 1998) for their remedies. In this context, traditional medicine and the use of medicinal plants could provide valuable insights into financially and culturally viable ways of treating neurological and mental health disorders.

A study by Treyvaud Amiguet *et al.* (2004) found that the Q'eqchi' (Kekchi) healers of Southern Belize treat nervous system and mental health disorders with 55 plant species. They also reported the use of 169 plants to treat various ailments, attesting to the healers' extensive knowledge of herbal remedies. In the study presented here, the main objective was to provide a more detailed account of traditional healing. practices, in particular medicinal plant use, relating to neurological and mental health disorders among the Q'eqchi' of Southern Belize. Furthermore, the main hypothesis tested was that certain plant species and families are preferred for medicinal use in the context of neurological and/or mental disorders from the total flora available to the healers.

Quantitative methods have become increasingly important in ethnobotanical research, especially since they provide objective means of obtaining information comparable between studies and between geographical/cultural areas. Several such techniques provide measures to quantify consensus among healers relating to plants use (Phillips and Gentry, 1993a). Information gathered with these approaches is useful in identifying species that are culturally important and that could be interesting for pharmacological research (Heinrich *et al.*, 1998). In addition, considering the accelerating loss of traditional knowledge in countries like Belize, means to quantify this knowledge are extremely precious as a research tool.

Cultural and ethnobotanical context

The Mayan linguistic family comprises 31 mutually unintelligible languages (Coe, 1999). The Q'eqchi Maya are found mainly in Belize and Guatemala, whereas various Maya groups are distributed throughout the Yucatan Peninsula, the Chiapas and Tabasco states of Mexico, Belize, and Guatemala. Yucatec, Mopan and Q'eqchi' Maya are found in Belize, the Q'eqchi' being concentrated in small villages in the rural areas of the Toledo District, in the southernmost part of the country (Bolland, 1986).

The medicinal use of plants in Mesoamerica was prominent in prehispanic times (Ortiz de Montellano, 1990), and there is evidence of the use of phytotherapy as well as certain forms of psychotherapy among the Nahua (Aztec) before colonisation (Villaseñor-Bayardo, 1994). Furthermore, although similar information is not available for the Q'eqchi', the antiquity of ethnobotanical knowledge of two ethnic groups belonging to the Macro-Mayan linguistic family has been dated to Olmec times (pre-160 AD) (Leonti *et al.*, 2003), suggesting a long-standing tradition of ethnobotanical knowledge among these groups. The contemporary Maya have also attracted attention in their use of medicinal plants, with earlier ethnobotanies in Belize focusing mainly on the Yucatec Maya (Arnason *et al.*, 1980; Pendergast, 1972; Roys, 1931; Thomson, 1930). To our knowledge, the study by Treyvaud Amiguet *et al.* (2004) was the first to focus on the ethnobotanical knowledge of the Q'eqchi' of Belize. As well, although the anthropological literature has seen a number of studies in ethnopsychiatry, few studies have concentrated on the identification of medicinal plants used in indigenous cultures for disorders of the nervous system and of psychiatric nature.

2.2 METHODOLOGY

2.2.1 Ethical approval

The overall project was initiated by the Q'eqchi' healers, organized through the Q'eqchi' Healers' Association, in seeking to revive traditional knowledge and interest in the use of medicinal plants in the area. An agreement between the Q'eqchi' Healers' Association (through the Belize Indigenous Training Institute, a local NGO) and the University of Ottawa was made prior to fieldwork. In conformity with local conduct protocol, the researchers met with the village chairmen, who granted their permission for the study. An initial meeting with the healers was organized and the objectives of the project were explained and accepted. Furthermore, the healers granted their consent individually and their identity was kept confidential. The project was also reviewed and accepted by the University of Ottawa Ethics Committee (Approval # H-06-03-01) and the Ministry of Natural Resources of the Government of Belize issued a permit for plant collection in June 2003 (Ref # CD/6-/9/03 (18)). Close attention was paid to the respect of local customs, which was facilitated by working closely with two Q'eqchi' research collaborators, Pedro Maquin and Victor Cal.

2.2.2 Study area

Approximately 30 Q'eqchi' villages are present in Southern Belize. The research was conducted in three of these localities, in the Toledo District (Appendix 1; Jalacte, Indian Creek, Big Falls) and extended from May to June 2003. Healers were from these communities and plant collections were made in surrounding areas. Among the localities where the study took place Jalacte is the most remote, located on the Guatemala border, and is home to most spiritual leaders in the area.

2.2.3 Participating healers

Healers are locally defined as people consulted by patients from various communities who use herbal remedies as main source of treatment (often accompanied by prayers). Complementarily, spiritual leaders are experts in performing spiritual ceremonies in keeping with the sacred Maya calendar and ancient practices. As a healer/spiritual leader stated: "To become a healer, a person must complete the extensive training as an apprentice with a respected healer. To become a spiritual leader, the approach is the same, but the training is more complex, involving a very good understanding of the moon cycles, the sun and the stars, the heavens, the Earth's resources, etc."

Six healers from the Q'eqchi' Healers' Association participated in the study (out of 23 healers practising in the three localities), and one of the participating healers was also a spiritual leader. The healers were between the ages of 53 and 79 (average of 71 years), and had been practising for 35 to 59 years (average of 53 years). All practised traditional healing to various degrees, relying mostly on seasonal employment for income and family operated farms for subsistence. Although the initial aim was to collect information from both men and women healers, only men were interviewed. This was mainly because the Q'eqchi' Healers' Association was solely composed of men participants, although an effort is underway to integrate women healers in the group. To our knowledge, however, few Q'eqchi' women in the area perform traditional healing outside of their immediate families.

2.2.4 Interviews

An assessment of recognised neurological and mental ailments was first established through group discussions. We proceeded on the basis of the list of ailments identified by Treyvaud Amiguet *et al.* (2004), and expanded by describing symptoms of other disorders and asking the healers which of these they recognised. In the study by Treyvaud Amiguet *et* *al.* (2004), the disorders were classified according to the Kew Botanical Garden standard classification (Cook, 1995). Mental disorders and nervous system disorders are separate in this classification system. However, since mental disorders often have a neurological basis, which could have implications in the identification of plants with pharmacological potential and subsequent testing, they were grouped in the present study. Furthermore, headaches are usually analysed in a separate category ("pain"), but since they are culturally linked to the other disorders studied (especially epilepsy and madness) and since migraines are classified as nervous system disorders (Cook, 1995), this ailment was included in the analysis.

The healers were then questioned individually on the symptomatology, plant use, etiology and remedy preparation for the ailments identified with this method (Appendix 2). These structured interviews lasted from one to two hours and were generally translated from Q'eqchi' to English by Pedro Maquin who is an apprentice Q'eqchi' healer, BITI field officer and co-author on this study. Plant collections were made with the healers, each healer identifying plants he had mentioned during the interview. Subsequently, a specimen of each species collected was presented to the healers individually, and they were asked to confirm if they used the plant and for which ailments (neurological/mental and others).

2.2.5 Herbarium collections

Most plants listed by the healers were collected in triplicates, pressed (standard plant presses from Pacific Papers; Washington, USA), mounted (white buffered paper, neutral pH; Pacific Papers) and stored for identification and future reference. However, certain plants mentioned by the healers could not be found in the area surveyed due to the passage of hurricane Iris in October 2001 and subsequent forest fires (May 2003) that destroyed some of the trails used by the healers. Moreover, some of the plants could not be identified to the species level. However, according to the taxonomists, all the plants collected were from different species, even when partially identified specimens belonged to the same genus or family. Vegetation types were identified according to the *Checklist of the Vascular Plants of Belize* (Balick *et al.*, 2000) and/or personal observations.

Voucher specimens of each plant collected were distributed to the following herbaria: University of Ottawa (Canada); Q'eqchi' Healers Association (Belize); and *Universidad Nacional* (Costa Rica; *Herbario Juvenal Valerio Rodriguez*). Taxonomic identification was performed by Pablo Sanchez and Luis Poveda from the *Universidad Nacional de Costa Rica*.

2.2.6 Quantitative ethnobotanical methods

Agreement between healers was evaluated using the Informants' Consensus Factor (F_{ic}) (Heinrich *et al.*, 2000), which ranks ailments according to remedy consensus between healers and is calculated with the following equation for each ailment:

$$F_{ic} = (n_{\text{use reports}} - n_{\text{taxa}}) / (n_{\text{use reports}} - 1) \tag{2.1}$$

$n_{\text{use reports}}$: Number of reports of a healer using a plant to treat the ailment

n_{taxa}: Number of taxa used to treat the ailment, for all healers

(F_{ic} values range between 0 and 1, high values indicating strong consensus)

This consensus method gives an indication of the selection of certain species by the healers for their use in treating particular ailments. Indeed, the presence of consensus between healers implies that there is selection for the use of certain species.

To assess whether certain plant families were prefentially selected by the healers for neurological/mental disorders, a method developed by Moerman (1991) was employed. This method is also useful in identifying families with the most potential to yield interesting pharmacological properties. It consists of a regression of the number of species in families that are used medicinally on the total number of species available per family in the total flora. The *Checklist of the Vascular Plants of Belize* (Balick *et al.*, 2000) was used to assess the number of different plant species present in the area and respective plant families. The regression was calculated as follows:

$$\text{MPSPE} = \text{A} + (\text{B} * \text{FBSPE}) \qquad (2.2)$$

MPSPE: number of species used medicinally; A: intercept; B: slope; FBSPE: total number of species per family.

The constant and the coefficient were determined using a standard linear least-squares regression (SYSTAT®). Subsequently, residual values were calculated for each family by subtracting the predicted value from the actual value. Negative residuals indicate that the families are under-used, whereas positive values suggest over-use, or preferential selection (Moerman, 1991). The rationale is that plant families preferentially selected by the healers probably exhibit potent biological activity, hence their use by the healers. This approach was used because it is the most widely accepted to date. However, it should be mentioned that certain authors have objected to this method (Bennett and Husby, 2004; Caballero, 2004).

2.3 RESULTS AND DISCUSSION

2.3.1 Recognised neurological and mental ailments

Ten different neurological and mental health conditions recognised by the healers were identified. Eight of these are treated with herbal remedies or through spiritual ceremonies, and two are treated exclusively in spiritual ceremonies (Table 2.1).

Susto (fright) refers to a specific condition known throughout Latin America. According to popular belief, it is caused by a sudden frightening event that leads to the loss of an essence or "soul" and can create various general physiological symptoms (e.g. diarrhoea, loss of appetite, restlessness) (Klein, 1978). It was included in the description of recognised mental/neurological ailments since this condition is now formally part of the psychiatric diagnostic classification system as a "culture-bound syndrome", and has been linked to major depressive disorders, post-traumatic stress disorder, somatoform disorders (APA 1994: Appendix 1), undifferentiated somatoform disorder and other specified neurotic disorders (WHO, 1993). However, the etiology and classification of this disorder is an area of active research and some authors have identified social role stress and/or physiological causes (e.g. hypoglycaemia) as possible explanations for this syndrome (Bolton, 1981; Rubel, 1984).

According to the healers interviewed, headache, epilepsy, madness and *susto* (fright) are closely related. For instance, an untreated case of epilepsy can lead to severe headaches, and then to madness if left unattended. Febrile and non febrile seizures (tonic, clonic and tonic-clonic convulsions) are recognised, and many believe that non febrile seizures are caused by spirits from the mountains and must be treated with "special" plants, which are found in primary forests in surrounding mountainous areas. Although they were included as "ailments", fear (*xiu xiu*) and memory loss were considered as personality traits by most healers interviewed.

Interestingly, an ethnopsychiatric study found that the Tzeltal Maya of Mexico recognize 15 mental and neurological disorders, including headache, epilepsy, depression, anxiety, and *susto* (fright; referred to as *mal de espanto*) (Carod and Vázquez-Cabrera, 1996). Furthermore, the belief that spiritual elements intervene in the onset of certain mental/neurological disorders is present in both cultures, although this belief is certainly not unique to these two cultural groups. Nonetheless, the idea that herbal remedies can help in treating or alleviating most of these disorders (combined with various rituals) is present in both groups, and some elements of Tzeltal and Q'eqchi' treatments are similar (e.g. using herbal macerations to wet the head of headache patients). Unfortunately, information on plant use is very limited in this study and does not allow for a meaningful comparison.

Table 2.1: Traditionally ascribed symptoms, causes, epidemiology and treatments for neurological and mental ailments recognized by the six Q'eqchi' healers interviewed; ailment classification based on local descriptions, illness categories and symptomatology

Ailment	Translation (Q'eqchi')	Symptomatology	Treatment		Folk etiology	Age group most affected
			Herbal remedies	Spiritual ceremonies		
Epilepsy/ seizures	*Et ej tiajel*	Convulsions, teeth clenched, person falls to the ground; sometimes associated with fever	X	X	Disturbing something without permission (and disturbing certain spirits); also referred to as "spirit loss"	Children (febrile seizures)
Headache	*Chibl jolom*	Pain in the head	X	X	High blood pressure, being worried and tired, an abrupt change in temperature, fright (*susto*), diarrhea, fever, epilepsy, malaria, madness, evil spirits, witchcraft	Middle-aged people and elders
Madness	*Quaxiru*	Abrupt change of tone (normal to aggressive behaviour): running away, walking outside naked or imagining people approaching (e.g. soldiers)	X	X	Jealousy, obsessive thoughts, witchcraft	18 years old to middle-aged
Susto (fright)	*Kaanil*	Diarrhea, dehydration, weakness, vomiting, headache, fever, mild pain in stomach or heart, burning sensation	X	X	Fright which can lead to loss of the soul and sickness	Children or infants
Depression	*Rahil chol*	Feeling sad	X	X	Witchcraft	Not specified
Numbness	*Siiq*	Persistent lack of feeling in a specific part of the body	X		Witchcraft	Not specified
Insomnia	*Aj okil*	Trouble sleeping	X	X	Not specified	Not specified
Stress	*Ch'i chi il*	Not specified	X	X	Stressful situations	Not specified
Memory loss	*Sachal ch'ol*	Having trouble remembering things		X	Personality trait, witchcraft, being worried	Not specified
Fear	*Xiu xiu*	Nervousness, shyness, fearful, lack of energy		X	Personality trait, being afraid of something	Not specified

2.3.2 Plant use

A total of 82 species (266 use-reports) belonging to 36 plant families were recorded as being used for the treatment of mental and neurological conditions by the healers interviewed (Appendix 3). The healers mentioned the use of another 19 potential species, which could not be collected due to fieldwork constraints mentioned earlier (i.e. hurricane and forest fires). Since their uniqueness could not be verified, they were excluded from subsequent analyses. Species without common names were omitted from Appendix 3.

The leaves (and/or stems) are used most often in the preparations, although the bark and whole plants are sometimes used as well. These are macerated in cold water or boiled (decoction) to prepare the remedy, and they are either given orally or the water is used as a bath or to wet the patient's head. In treating *susto* (fright), the healers often perform a ritual of "smoking" the patient (burning certain plants with various other items and letting the smoke rise around the patient). For headaches, plants are sometimes tied directly around the patient's head and wetting the head with water from the preparation is often performed.

According to these results, the only species used by all the healers interviewed for conditions of the nervous system are *Justicia aurea* (*Acanthaceae*) and *Adiantum latifolium* (*Adiantaceae*) (Table 2.2). Unfortunately, the biological activity of these species has been understudied. Still, a compound from the *Justicia* genus, elenoside (found in *Justicia hyssopifolia*), has shown central depressant effects (i.e. general sedative properties) (Navarro *et al.*, 2001). Also, eight of the fifteen species used by more than half of the healers are from the *Pteridophyta* division. Consequently, this generally under-studied division warrants further investigation, especially for its effects on the CNS.

Headaches and epilepsy/seizures are the neurological disorders for which the healers use the most plant species, followed by madness, *susto* (fright), numbness, depression, insomnia, and stress, respectively (Figure 2.1). However, knowledge is unevenly distributed between healers (Figure 2.2). This tendency is highly skewed by one healer whose knowledge surpasses that of the others in the number of plants used for madness, epilepsy/seizures, and headache. According to this healer, it is important to know of many plants to treat these ailments, as each person responds differently to them. Therefore, in the treatment process, if the first plant does not seem adequate, a second one is used, and a third, and so on, until the plant shows the desired effect.

Certain elements stand out from a look at the etymology of Q'eqchi' names for plants used for neurological/mental disorders. Many names refer to morphological aspects of the plants (e.g. *Pu'chuch* = fat jointed) and others to uses (e.g. *Kan pom* = yellow incense) (Appendix 3). Interestingly, certain plants have names that refer to the Devil (e.g. Devil's finger), which indicate a conception of these conditions that is closely linked to the Spirit World.

Table 2.2: Description of medicinal plant use (vegetation type, medicinal use and proportion of healers using the plant) for plants used by more than half of the healers interviewed (more than three) in treating recognised neurological/mental conditions

Plant family	Botanical name	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Administration
Acanthaceae	*Justicia aurea* Schltdl.	39	Shrub	100	Headache, madness, epilepsy/seizures	Leaves [3]	Maceration	Oral, topical
Adiantaceae	*Adiantum latifolium* Lam.	68	Fern	100	Epilepsy/seizures, headache, *susto* (fright), depression, madness	Leaves	Maceration	Oral, topical
Acanthaceae	NA	60	Shrub	83,33	Headache, madness, epilepsy/seizures	Leaves	Maceration	Oral, topical
Fabaceae-Mimosoideae	*Mimosa pudica* L.	55	Herb	83,33	Insomnia, depression, madness, relaxing	Whole plant, Leaves	Decoction, maceration, burning	Oral, topical
Gesneriaceae	*Columnea* sp.	87	Shrub	83,33	Headache	Leaves	Maceration	Oral, topical
Piperaceae	*Piper* sp.	80	Shrub	83,33	*Susto* (fright), madness, epilepsy/seizures	Leaves	Decoction, maceration	Oral, topical
Adiantaceae	*Adiantum princeps* T. Moore	22	Fern	83,33	Epilepsy/seizures, *susto* (fright)	Leaves	Maceration, burning	Oral, topical
Adiantaceae	*Adiantum tetraphyllum* Humb. & Bonpl. ex Willd.	54	Fern	83,33	Epilepsy/seizures, *susto* (fright)	Leaves	Maceration	Oral, topical
Adiantaceae	*Adiantum wilsonii* Hook.	7	Herb	83,33	Epilepsy/seizures, headache, madness, insomnia	Leaves	Maceration	Oral, topical
Schizaeaceae	*Lygodium heterodoxum* Kunze	25	Fern	83,33	Epilepsy/seizures, *susto* (fright), headache	Leaves	Maceration, burning	Oral, topical

Plant family	Botanical name	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Administration
Schizaeaceae	*Lygodium venustum* Sw.	61	Fern	83,33	Epilepsy/seizures, *susto* (fright), headache, madness	Leaves	Maceration, burning	Oral, topical
Aristolochiaceae	*Aristolochia grandiflora* Sw.	20	Vine	66,67	Epilepsy/seizures, madness, headache	Leaves	Decoction, maceration, burning	Oral, topical
Aspleniaceae	*Bolbitis pergamentacea* (Maxon) Ching	53	Fern	66,67	Madness	Leaves	Decoction, maceration, burning	Oral, topical
Monimiaceae	*Siparuna* sp.	83	Shrub or sub-shrub	66,67	Headache	Leaves	Maceration	Topical
Selaginellaceae	*Selaginella aff. stellata Spring*	35	Herb	66,67	Epilepsy/seizures, *susto* (fright), madness	Leaves	Decoction, maceration, burning	Oral, topical

[1] Voucher number

[2] Relative frequency
RF = (No. healers using species for any neurological/mental ailment) / (Tot. no. healers interviewed) * 100

[3] When the leaves are said to be used, the stems are also often used in the preparation (although the healers did not distinguish between them in the interviews)

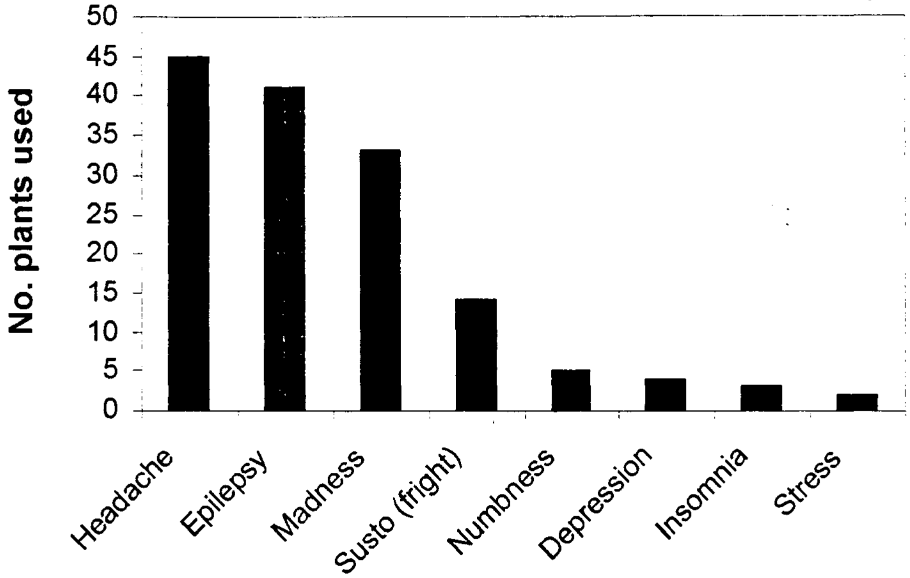

Figure 2.1: Number of plants used for each neurological/mental ailment recognized and treated with herbal remedies by participating Q'eqchi' healers; total number of plants used = 82 (the same plants can be used for more than one ailment; plants that could not be collected were excluded from this figure)

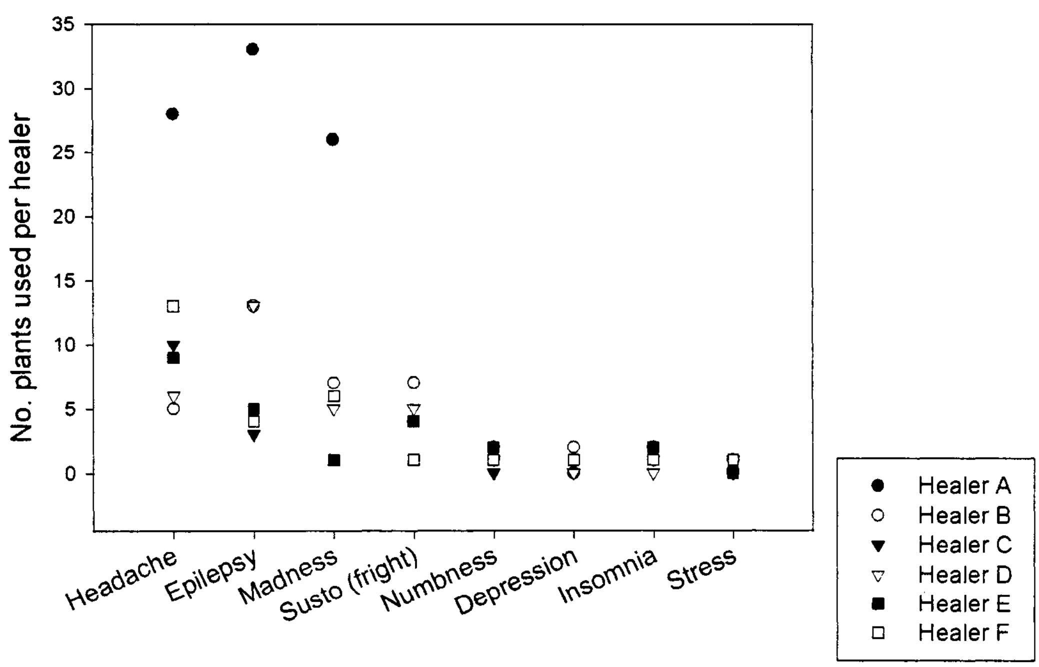

Figure 2.2: Distribution of the number of plants used per healer for each ailment recognized and treated with herbal remedies by participating Q'eqchi' healers (plants that could not be collected were excluded from this figure)

2.3.3 Consensus between healers

The disorders studied showed a wide range of consensus values (agreement among healers). For the ailments with highest plant use (i.e. headache, epilepsy, madness, *susto* (fright)), F_{IC} values ranged from 0.350 to 0.480 (Table 2.3). Considering that these healers are from the same ethnic group and are geographically close, these consensus values are lower than expected. This is probably due to the very large number of plants used by one of the healers for these conditions, as previously discussed. Insomnia and stress showed the highest F_{IC} values, whereas numbness had a low F_{IC} and depression had an F_{IC} of 0 since each plant was named only once. Few healers treat these four conditions, which probably explains the small numbers of plants used. Overall, it seems that there is preferential selection of certain species for treating neurological/mental ailments (except for depression), but with varying degrees. Ailments showing highest consensus and the plants used to treat these disorders merit further investigation, as their high usage is an indicator of potential biological activity.

Certain similarities emerged from the comparison of these results to those obtained by researchers using the same quantitative methods and having studied in the same general geographical area (i.e. Ankli *et al.*, 1999; Frei *et al.*, 1998; Heinrich *et* al., 1998; Leonti *et al.*, 2001; Weinmann and Heinrich, 1997). For instance, the Popoluca of Mexico have a consensus value for culture-bound syndromes (F_{ic}=0.470) (Leonti *et al.*, 2001) that is very close to the value calculated for *susto* (fright). However, studies from the Nahua and Zapotec of Mexico found that culture-bound syndromes had higher agreement between informants (Heinrich *et al.,* 1998). Furthermore, these studies reported higher F_{ic} values for categories that included headache remedies (in “fever and headache” or “pain/febrile diseases”) compared to the present study and the other neurological/mental ailments recognized by the Q’eqchi’ were either not mentioned or treated with very few plants. For instance, Weinman and Heinrich (1997) mention the use of plants for sleeplessness and nervousness (in "nervous disorders and illnesses associated with pain"), but the entire category is treated with only 13 plants. More significantly, the Popoluca treat epilepsy and madness with only four plants (Marco Leonti, personal communication). On the other hand, other ethnobotanical descriptions have shown greater usage of plants for neurological/mental conditions (e.g. El-Hilaly *et al.*, 2003; Hernandez Cano and Volpato, 2004), some having reported a large number of plants used for this purpose. For example, Tabuti *et al.* (2003) found patterns of use of plants for mental/neurological disorders in Uganda that are similar to those reported for the Q'eqchi'. These similarities and differences between groups could reflect cultural factors, for instance in believing that a certain ailment can or cannot be treated with herbal remedies, but they might also indicate methodological dissimilarities between studies and a possible under-representation of mental/neurological disorders in ethnobotanical studies.

Table 2.3: Information on plant use and remedy agreement between healers for each neurological/mental ailment recognized and treated by the six Q'eqchi' healers interviewed (consensus calculated according to the Informant Consensus Factor (F_{IC}))

Ailment	No. healers treating ailment	No. use-reports [1]	No. plants used [2]	F_{IC} [3]
Susto (fright)	6	26	14	0.480
Epilepsy	6	71	41	0.429
Headache	6	71	45	0.371
Madness	6	50	33	0.350
Insomnia	5	7	3	0.667
Stress	3	3	2	0.500
Numbness	5	7	5	0.333
Depression	3	4	4	0
Total	**6**	**266**	**82**	**---**

[1] No. use-reports: number of mentions of the use of a specific plant for a given ailment

[2] Some plants could not be collected and were not included in this table

[3] $F_{IC} = (n_{use\ reports} - n_{taxa}) / (n_{use\ reports} - 1)$ (values range between 0 and 1, high values indicating a strong consensus between informants) (Heinrich, 2000)

2.3.4 Regression analysis

Out of 211 native plant families present in Belize (with the *Fabaceae* divided in *Papilionoideae*, *Caesalpinioideae*, and *Mimosoideae*) (Balick *et al.*, 2000), 35 are used to treat disorders of the nervous system by the Q'eqchi' healers interviewed. One additional family (*Liliaceae*) was also included in the analysis since the healers mentioned the use of a species from that family. The following equation describes the regression of the number of plants per botanical family used by the Q'eqchi' for neurological/mental purposes on the number of plants per plant family in the Flora of Belize:

$$\text{MPSPE} = 0.212 + (0.0110 \times \text{FBSPE}) \qquad (2.3)$$

This regression analysis was highly significant (p-value<0.001), but the r^2 value was very low (r^2=0.0920), which indicates that only 9.20% of the variability can be explained with the proposed model (Figure 2.3). Furthermore, the r^2 value obtained in the overall ethnobotanical study was much higher (r^2=0.260; Treyvaud Amiguet *et al.,* submitted). This is probably due to the fact that the analysis was performed with a specific set of medicinal usages (i.e. neurological/mental) and further indicates the preferential selection of certain families for this category of use. Fourteen plant families were above the standard deviation of the residuals (S.D.=1.19), with the *Piperaceae* presenting the highest positive residual value (residual value=9.30). This tropical and sub-tropical family is mainly composed of the genera *Piper* and *Peperomia*, and certain species, especially from the *Piper* genus, exhibit potent activity on the nervous system (Parmar *et al.*, 1997), which could easily account for its wide use for neurological/mental disorders by the Q'eqchi' healers interviewed. For example, piperine and kavapyrones have been shown to depress central nervous system (CNS) activity, exhibiting sedative, anticonvulsant and relaxing effects (Kretzschmar and

Meyer, 1969; Lee *et al.*, 1984; Meyer, 1962). Four families displayed negative residual values greater than the standard deviation (e.g. *Euphorbiaceae*, *Cyperaceae*, *Poaceae*, and *Orchidaceae*), indicating that they were under-selected by the healers for neurological/mental disorders. The absence of these families in use for neurological/mental ailments in spite of the large number of species available to the healers could be due to the fact that they rely mainly on rainforest species and that the *Orchidaceae* are often rare and epiphytic, therefore not readily available to the healers.

These results differ from those obtained in other studies using the same methodology (i.e. Leonti *et al.*, 2003; Moerman, 1991; Moerman *et al.*, 1999). These comparative studies found that the *Asteraceae* was often the family most preferentially selected for medicinal use. Although this plant family is selected for use in neurological/mental disorders by the Q'eqchi', it ranks only 7th. Moreover, most other families preferentially selected by the Q'eqchi' are not top ranking families in ethnobotanies of Veracruz (Mexico), Chiapas (Mexico), Kashmir, North America, Korea or Ecuador (Leonti *et al.*, 2003; Moerman *et al.*, 1999). In addition, only the study in Veracruz found that the *Piperaceae* were preferentially selected for medicinal use (Leonti *et al.*, 2003). Again, these differences probably reflect the focus of this study on mental/neurological disorders as well as a preference of the Q'eqchi' for the *Piperaceae* (Treyvaud Amiguet *et al.*, submitted). They also reflect the fact that the flora of Southern Belize is neotropical rather than holarctic (see Leonti *et al.*, 2003 and Moerman *et al.*, 1999 for a discussion on this topic). Table 2.4 lists the sub-classes or classes to which they belong, along with the total number of plants, medicinal species and residual values from the regression analysis.

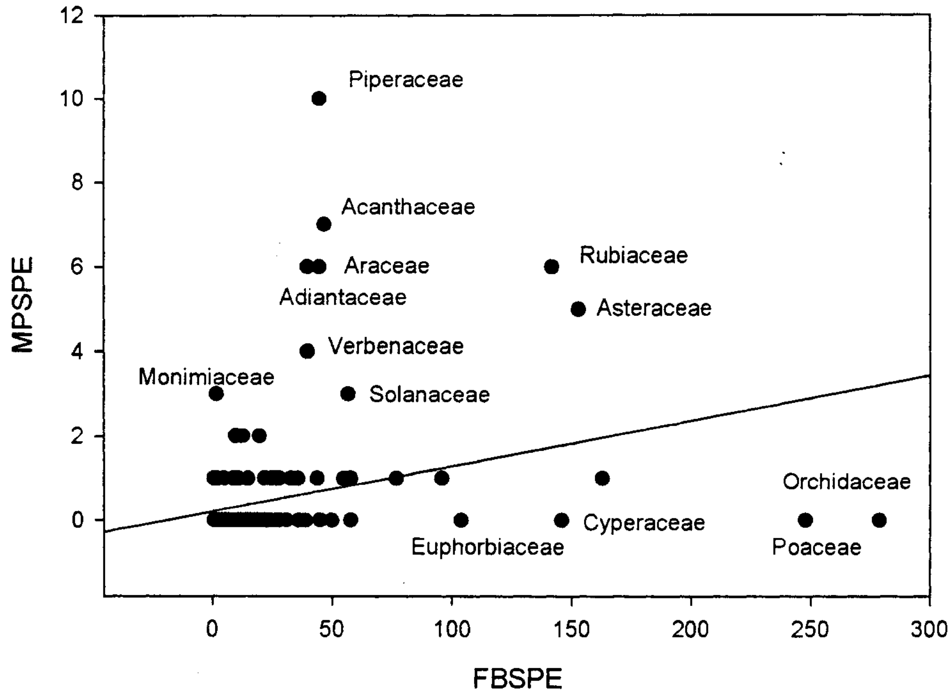

Figure 2.3: Regression plot of the number of species used for neurological/mental disorders per plant family (36 botanical families used for these purposes) (MPSPE) versus the number of species per botanical family present in the flora of Belize (FBSPE) (MPSPE = 0.212 + (0.0110 x FBSPE); N=212; p-value<0.001; r^2=0.0920; labelled points represent the nine families with residual values greater than 2.00 and the four families with residual values lower than -2.00 (S.D.=1.19))

Table 2.4: Number of species in the total flora of Belize and number of medicinal species for each plant family in the total flora of Belize, and residual values generated by the regression analysis of plants used for neurological/mental disorders by the Q'eqchi' healers interviewed on the 212 families present in Belize

Rank	Plant family	Division/Subclass	FBSPE[1]	MPSPE[2]	Residual[3]
1	*Piperaceae*	*Magnoliidae*	45	10	9.30
2	*Acanthaceae*	*Asteridae*	47	7	6.27
3	*Adiantaceae*	*Pteridophyta*	40	6	5.36
4	*Araceae*	*Arecidae*	45	6	5.30
5	*Rubiaceae*	*Asteridae*	142	6	4.25
6	*Verbenaceae*	*Asteridae*	40	4	3.36
7	*Asteraceae*	*Asteridae*	153	5	3.13
8	*Monimiaceae*	*Magnoliidae*	2	3	2.77
9	Solanaceae	*Asteridae*	57	3	2.17
10	Aristolochiaceae	*Magnoliidae*	10	2	1.68
11	*Gesnariaceae*	*Asteridae*	13	2	1.65
12	*Schizaeaceae*	*Pteridophyta*	13	2	1.65
13	*Selaginellaceae*	*Lycopodiophyta*	13	2	1.65
14	*Rutaceae*	*Rosidae*	20	2	1.57
15	*Buddlejaceae*	*Asteridae*	1	1	0.777
16	*Crassulaceae*	*Rosidae*	1	1	0.777
17	*Liliaceae*	*Lillidae*	1	1	0.777
18	*Bixaceae*	*Dilleniidae*	2	1	0.766
19	*Davalliaceae*	*Pteridophyta*	5	1	0.734
20	*Phytolaccaceae*	*Caryophyllidae*	5	1	0.734
21	*Combretaceae*	*Rosidae*	9	1	0.691
22	*Loranthaceae*	*Rosidae*	9	1	0.691
23	*Marantaceae*	*Commelinidae*	11	1	0.669
24	*Meliaceae*	*Rosidae*	15	1	0.626
25	*Passifloraceae*	*Dilleniidae*	22	1	0.550
26	*Cucurbitaceae*	*Dilleniidae*	25	1	0.518
27	*Lamiaceae*	*Asteridae*	27	1	0.496
28	*Scrophulariaceae*	*Asteridae*	28	1	0.485
29	*Boraginaceae*	*Asteridae*	33	1	0.431
30	*Apocynaceae*	*Asteridae*	36	1	0.399
31	*Malvaceae*	*Dilleniidae*	44	1	0.312
32	*Fabaceae-Caesalpinioideae*	*Rosidae*	55	1	0.193
33	*Aspleniaceae*	*Pteridophyta*	58	1	0.161
34	*Fabaceae-Mimosoideae*	*Rosidae*	77	1	-0.0445
35	*Actinidiaceae*	*Dilleniidae*	1	0	-0.223
36	*Alstroemeriaceae*	*Lillidae*	1	0	-0.223
37	*Balanophoraceae*	*Rosidae*	1	0	-0.223
38	*Bataceae*	*Dilleniidae*	1	0	-0.223
39	*Brunelliaceae*	*Rosidae*	1	0	-0.223
40	*Buxaceae*	*Rosidae*	1	0	-0.223
41	*Caryophyllaceae*	*Caryophyllidae*	1	0	-0.223
42	*Ceratophyllaceae*	*Magnoliidae*	1	0	-0.223
43	*Chloranthaceae*	*Magnoliidae*	1	0	-0.223
44	*Cistaceae*	*Dilleniidae*	1	0	-0.223

Rank	Plant family	Division/Subclass	FBSPE[1]	MPSPE[2]	Residual[3]
45	*Droseraceae*	*Dilleniidae*	1	0	-0.223
46	*Haemodoraceae*	*Commelinidae*	1	0	-0.223
47	*Hamamelidaceae*	*Hamamelidae*	1	0	-0.223
48	*Isoetaceae*	*Lycopodiophyta*	1	0	-0.223
49	*Juncaceae*	*Commelinidae*	1	0	-0.223
50	*Lacistemataceae*	*Dilleniidae*	1	0	-0.223
51	*Lecythidaceae*	*Dilleniidae*	1	0	-0.223
52	*Loasaceae*	*Dilleniidae*	1	0	-0.223
53	*Lophosoriaceae*	*Pteridophyta*	1	0	-0.223
54	*Magnoliaceae*	*Magnoliidae*	1	0	-0.223
55	*Martyniaceae*	*Asteridae*	1	0	-0.223
56	*Mayacaceae*	*Commelinidae*	1	0	-0.223
57	*Menyanthaceae*	*Asteridae*	1	0	-0.223
58	*Metaxyaceae*	*Pteridophyta*	1	0	-0.223
59	*Monotropaceae*	*Dilleniidae*	1	0	-0.223
60	*Musaceae*	*Commelinidae*	1	0	-0.223
61	*Myricaceae*	*Hamamelidae*	1	0	-0.223
62	*Najadaceae*	*Alismatidae*	1	0	-0.223
63	*Pedaliaceae*	*Asteridae*	1	0	-0.223
64	*Plantaginaceae*	*Asteridae*	1	0	-0.223
65	*Plumbaginaceae*	*Caryophyllidae*	1	0	-0.223
66	*Podocarpaceae*	*Pinophyta*	1	0	-0.223
67	*Polemoniaceae*	*Asteridae*	1	0	-0.223
68	*Proteaceae*	*Rosidae*	1	0	-0.223
69	*Psilotaceae*	*Psilotophyta*	1	0	-0.223
70	*Quiinaceae*	*Dilleniidae*	1	0	-0.223
71	*Refflesiaceae*	*Rosidae*	1	0	-0.223
72	*Salicaceae*	*Dilleniidae*	1	0	-0.223
73	*Sphenocleaceae*	*Asteridae*	1	0	-0.223
74	*Staphyleaceae*	*Rosidae*	1	0	-0.223
75	*Styracaceae*	*Dilleniidae*	1	0	-0.223
76	*Surianaceae*	*Dilleniidae*	1	0	-0.223
77	*Symplocaceae*	*Dilleniidae*	1	0	-0.223
78	*Tovariaceae*	*Dilleniidae*	1	0	-0.223
79	*Triuridaceae*	*Rosidae*	1	0	-0.223
80	*Trogoniaceae*	*Alismatidae*	1	0	-0.223
81	*Typhaceae*	*Commelinidae*	1	0	-0.223
82	*Vochysiaceae*	*Rosidae*	1	0	-0.223
83	*Zygophyllaceae*	*Rosidae*	1	0	-0.223
84	*Anthericaceae*	*Lillidae*	2	0	-0.234
85	*Basellaceae*	*Caryophyllidae*	2	0	-0.234
86	*Cabombaceae*	*Magnoliidae*	2	0	-0.234
87	*Cannaceae*	*Commelinidae*	2	0	-0.234
88	*Caricaceae*	*Dilleniidae*	2	0	-0.234
89	*Casuarinaceae*	*Hamamelidae*	2	0	-0.234
90	*Chenopodiaceae*	*Caryophyllidae*	2	0	-0.234
91	*Clethraceae*	*Dilleniidae*	2	0	-0.234
92	*Cuscutaceae*	*Asteridae*	2	0	-0.234
93	*Cyrillaceae*	*Dilleniidae*	2	0	-0.234

Rank	Plant family	Division/Subclass	FBSPE[1]	MPSPE[2]	Residual[3]
94	*Dichapetalaceae*	*Rosidae*	2	0	-0.234
95	*Hydrocharitaceae*	*Alismatidae*	2	0	-0.234
96	*Hydrophyllaceae*	*Asteridae*	2	0	-0.234
97	*Lemnaceae*	*Arecidae*	2	0	-0.234
98	*Marattiaceae*	*Pteridophyta*	2	0	-0.234
99	*Ophioglossaceae*	*Rosidae*	2	0	-0.234
100	*Papaveraceae*	*Magnoliidae*	2	0	-0.234
101	*Pinaceae*	*Pinophyta*	2	0	-0.234
102	*Portulacaceae*	*Caryophyllidae*	2	0	-0.234
103	*Primulaceae*	*Dilleniidae*	2	0	-0.234
104	*Ranunculaceae*	*Magnoliidae*	2	0	-0.234
105	*Rhizophoraceae*	*Rosidae*	2	0	-0.234
106	*Rosaceae*	*Rosidae*	2	0	-0.234
107	*Salviniaceae*	*Pteridophyta*	2	0	-0.234
108	*Valerianaceae*	*Asteridae*	2	0	-0.234
109	*Aizoaceae*	*Caryophyllidae*	3	0	-0.245
110	*Alismataceae*	*Alismatidae*	3	0	-0.245
111	*Aquifoliaceae*	*Rosidae*	3	0	-0.245
112	*Brassicaceae*	*Dilleniidae*	3	0	-0.245
113	*Cymodoceaceae*	*Alismatidae*	3	0	-0.245
114	*Dracaenaceae*	*Lillidae*	3	0	-0.245
115	*Gleicheniaceae*	*Pteridophyta*	3	0	-0.245
116	*Hypoxidaceae*	*Lillidae*	3	0	-0.245
117	*Icacinaceae*	*Rosidae*	3	0	-0.245
118	*Myristicaceae*	*Magnoliidae*	3	0	-0.245
119	*Nymphaeaceae*	*Magnoliidae*	3	0	-0.245
120	*Olacaceae*	*Rosidae*	3	0	-0.245
121	*Oleaceae*	*Asteridae*	3	0	-0.245
122	*Oxalidaceae*	*Rosidae*	3	0	-0.245
123	*Pontederiaceae*	*Lillidae*	3	0	-0.245
124	*Theaceae*	*Dilleniidae*	3	0	-0.245
125	*Melastomataceae*	*Rosidae*	96	1	-0.250
126	*Begoniaceae*	*Dilleniidae*	4	0	-0.255
127	*Campanulaceae*	*Asteridae*	4	0	-0.255
128	*Cecropiaceae*	*Hamamelidae*	4	0	-0.255
129	*Connaraceae*	*Rosidae*	4	0	-0.255
130	*Costaceae*	*Commelinidae*	4	0	-0.255
131	*Ebenaceae*	*Dilleniidae*	4	0	-0.255
132	*Elaeocarpaceae*	*Dilleniidae*	4	0	-0.255
133	*Erythroxylaceae*	*Rosidae*	4	0	-0.255
134	*Potamogetonaceae*	*Alismatidae*	4	0	-0.255
135	*Simaroubaceae*	*Rosidae*	4	0	-0.255
136	*Zamiaceae*	*Cycadophyta*	4	0	-0.255
137	*Zingiberaceae*	*Commelinidae*	4	0	-0.255
138	*Amaryllidaceae*	*Lillidae*	5	0	-0.266
139	*Apiaceae*	*Rosidae*	5	0	-0.266
140	*Araliaceae*	*Rosidae*	5	0	-0.266
141	*Cyclanthaceae*	*Arecidae*	5	0	-0.266
142	*Hippocrateaceae*	*Rosidae*	5	0	-0.266

Rank	Plant family	Division/Subclass	FBSPE[1]	MPSPE[2]	Residual[3]
143	*Iridaceae*	*Lillidae*	5	0	-0.266
144	*Ochnaceae*	*Dilleniidae*	5	0	-0.266
145	*Theophrastaceae*	*Dilleniidae*	5	0	-0.266
146	*Ulmaceae*	*Hamamelidae*	5	0	-0.266
147	*Agavaceae*	*Lillidae*	6	0	-0.277
148	*Burmanniaceae*	*Lillidae*	6	0	-0.277
149	*Podostemaceae*	*Rosidae*	6	0	-0.277
150	*Rhamnaceae*	*Rosidae*	6	0	-0.277
151	*Blechnaceae*	*Pteridophyta*	7	0	-0.288
152	*Burseraceae*	*Rosidae*	7	0	-0.288
153	*Ericaceae*	*Dilleniidae*	7	0	-0.288
154	*Lycopodiaceae*	*Lycopodiophyta*	7	0	-0.288
155	*Marcgraviaceae*	*Dilleniidae*	7	0	-0.288
156	*Vitaceae*	*Rosidae*	7	0	-0.288
157	*Xyridaceae*	*Commelinidae*	7	0	-0.288
158	*Fagaceae*	*Hamamelidae*	8	0	-0.299
159	*Grammitidaceae*	*Pteridophyta*	8	0	-0.299
160	*Loganiaceae*	*Asteridae*	8	0	-0.299
161	*Menispermaceae*	*Magnoliidae*	8	0	-0.299
162	*Smilacaceae*	*Lillidae*	8	0	-0.299
163	*Turneraceae*	*Dilleniidae*	8	0	-0.299
164	*Chrysobalanaceae*	*Rosidae*	9	0	-0.309
165	*Cyatheaceae*	Pteridophyta	9	0	-0.309
166	*Dilleniaceae*	*Dilleniidae*	9	0	-0.309
167	*Lythraceae*	*Rosidae*	9	0	-0.309
168	*Anacardiaceae*	*Rosidae*	10	0	-0.320
169	*Bombacaceae*	*Dilleniidae*	10	0	-0.320
170	*Cactaceae*	*Caryophyllidae*	10	0	-0.320
171	*Heliconiaceae*	*Dilleniidae*	10	0	-0.320
172	*Nyctaginaceae*	*Caryophyllidae*	10	0	-0.320
173	*Onagraceae*	*Pteridophyta*	10	0	-0.320
174	*Viscaceae*	*Rosidae*	10	0	-0.320
175	*Celastraceae*	*Rosidae*	11	0	-0.331
176	*Dennstaedtiaceae*	*Pteridophyta*	12	0	-0.342
177	*Dioscoriaceae*	*Lillidae*	12	0	-0.342
178	*Violaceae*	*Dilleniidae*	12	0	-0.342
179	*Capparaceae*	*Dilleniidae*	13	0	-0.353
180	*Eriocaulaceae*	*Commelinidae*	13	0	-0.353
181	*Tiliaceae*	*Dilleniidae*	14	0	-0.363
182	*Gentianaceae*	*Asteridae*	15	0	-0.374
183	*Sterculiaceae*	*Dilleniidae*	15	0	-0.374
184	*Lentibulariaceae*	*Asteridae*	16	0	-0.385
185	*Polygalaceae*	*Rosidae*	16	0	-0.385
186	*Annonaceae*	*Magnoliidae*	18	0	-0.407
187	*Commelinaceae*	*Commelinidae*	19	0	-0.417
188	*Polygonaceae*	*Caryophyllidae*	19	0	-0.417
189	*Thelypteridaceae*	*Pteridophyta*	21	0	-0.439
190	*Urticaceae*	*Hamamelidae*	21	0	-0.439
191	*Clusiaceae*	*Dilleniidae*	22	0	-0.450

Rank	Plant family	Division/Subclass	FBSPE[1]	MPSPE[2]	Residual[3]
192	*Hymenophyllaceae*	*Pteridophyta*	22	0	-0.450
193	*Myrsinaceae*	*Dilleniidae*	22	0	-0.450
194	*Flacourtiaceae*	*Dilleniidae*	23	0	-0.461
195	*Sapotaceae*	*Dilleniidae*	23	0	-0.461
196	*Amaranthaceae*	*Caryophyllidae*	24	0	-0.472
197	*Asclepiadaceae*	*Asteridae*	27	0	-0.504
198	*Polypodiaceae*	*Pteridophyta*	28	0	-0.515
199	*Lauraceae*	*Magnoliidae*	31	0	-0.547
200	*Malpighiaceae*	*Rosidae*	36	0	-0.601
201	*Moraceae*	*Hamamelidae*	36	0	-0.601
202	*Sapindaceae*	*Rosidae*	37	0	-0.612
203	*Arecaceae*	*Arecidae*	39	0	-0.634
204	*Bignoniaceae*	*Asteridae*	45	0	-0.699
205	*Bromeliaceae*	*Commelinidae*	50	0	-0.753
206	Convolvulaceae	*Asteridae*	50	0	-0.753
207	*Myrtaceae*	*Rosidae*	58	0	-0.839
208	*Fabaceae-Papilionoideae*	*Rosidae*	163	1	-0.974
209	*Euphorbiaceae*	*Rosidae*	104	0	-1.336
210	*Cyperaceae*	*Commelinidae*	146	0	-1.791
211	*Poaceae*	*Commelinidae*	248	0	-2.893
212	*Orchidaceae*	*Lillidae*	279	0	-3.228

[1] FBSPE: Total number of species per family
[2] MPSPE: Number of species used medicinally
[3] Standard deviation = 1.19

2.4 SUMMARY AND CONCLUSIONS

This study demonstrates selectivity in the choice of plants by Q'eqchi' healers of Southern Belize for the treatment of neurological and mental conditions, both for plant species and botanical families. A large number of plants are used for headache, epilepsy and madness, the last two ailments being under-reported in other ethnobotanical descriptions from the same geographical area. Means to treat these disorders are not limited to herbal remedies, but also include spiritual ceremonies, and these forms of treatment should not be overlooked, as they could certainly have a calming effect on the patient as well as being a central cultural component of the healing process.

Considering the results from the comparison of species present in the area versus species used to treat mental/neurological disorders, the *Piperaceae* species used warrant further investigation. In addition, many healers use *Pterydophyta* species to treat the recognized/mental neurological disorders, and their high consensus makes them interesting to study pharmacologically in the context of their possible activities on the nervous system. Indeed, consensus analyses provide insight into trial and error selection of indigenous pharmacopoeias, attesting to their pharmacological potential. Furthermore, validation of this plant material could provide valuable information on safe and efficacious treatment for the Q'eqchi', along with a reconnaissance of their healing abilities.

CHAPTER 3

INVESTIGATION OF TREATMENTS USED FOR *SUSTO* AND IMPLICATIONS FOR UNDERLYING ETIOLOGICAL FACTORS

3.1 INTRODUCTION

Susto is a traditional ethnomedical concept and is generally translated as "fright" or "shock" (synonyms include *espanto*, *pérdida de la sombra* and *pasmo*) (Mysyk, 1998). This traditional illness has attracted a great deal of attention from researchers since the publication of Gillin's "Magical Fright" in 1948. Medical anthropologists have been especially intrigued by this ailment in itself and as a model of the complex interplay between culture and illness. In this chapter, we provide an overview of the work from various authors on the subject and propose a look at treatment modalities to provide information on underlying factors of *susto*.

3.1.1 Background

Susto has been reported throughout Latin America, cutting across diverse linguistic and cultural borders. Studies include Latin groups from the Southern United States (Rubel, 1964; Rubel *et al.*, 1984; Trotter, 1982), indigenous and non-indigenous groups from Mexico (Castro and Eroza, 1998; Collado-Ardon *et al.*, 1983; O'Nell, 1975; Signorini, 1982; Tousignant, 1979; Uzell, 1974), Guatemala (Gillin, 1948; Logan, 1979; O'Nell and Selby, 1968), Belize (Lee and Balick, 2003), Colombia (Seijas, 1972), Bolivia (Crandon, 1983), Argentina (Palma, 1978), Peru (Gobeil, 1973; Sal y Rosas, 1958), and from the Venezuela/Brazil border (Carib-speaking groups) (Colson and de Armellada, 1983). The origin of *susto* is thought to be pre-Columbian and some authors believe that it is linked to the Nahua (Aztec) concept of the "loss of *tonalli*" (Ortiz de Montellano, 1990; Ortiz de Montellano, 1987; Villaseñor-Bayardo, 1994). However, *susto* might not be restricted to Latin America: accounts of similar conditions associated with fright have been reported in South-East Asia (Hart, 1969; WHO, 1993).

Although the use of the label "*susto*" is widespread (Logan, 1993), there are highly variable features in its symptomatology (Crandon, 1983; Tousignant, 1979), epidemiology (Rubel *et al.*, 1984), treatment (Trotter, 1982), and local perception (Crandon, 1983; Logan, 1979; Signorini, 1982). This syndrome affects people of different economic status, age, gender, and level of acculturation (Signorini, 1982; Trotter, 1991). In the indigenous conception, *susto* stems from a frightening event involving startle, which causes the loss of the soul, or vital force. This can then lead to sickness involving various general symptoms (Gillin, 1948; Gobeil, 1973, Rubel, 1964). It is also widely believed that *susto* can be fatal if uncured (i.e. if the patient's soul is not returned to its body) (Crandon, 1983; Gillin, 1948; Gobeil, 1973; Rubel *et al.*, 1984). The Ladino (acculturated indigenous people; Mestizo) conception is very similar, but soul-loss as a cause is less predominant, depending on the level of acculturation (Rubel, 1964; Signorini, 1982). The frightening events range from physical (e.g. falling into a river, an encounter with a dangerous animal) to social events (e.g. public humiliation, losing something valuable) (Klein, 1978). For both groups, the basic symptoms include restlessness during sleep, listlessness, loss of appetite, irritability, inability to urinate, loss of interest in personal appearance, weakness, depression, and introversion, and can occur days, months, or years later. Additional symptoms such as fever, muscular pain, nausea, vomiting, diarrhea, and vertigo are also often present, although with some variation between groups (Crandon, 1983; Klein, 1978; Rubel, 1964; Sal y Rosas, 1958; Signorini, 1982).

An initial diagnostic session to identify the cause of the fright is usually followed by rituals to bring back the lost soul (Gillin, 1948; Gobeil, 1973; Rubel, 1964). The use of herbal remedies is also widespread in treating *susto* and its diverse symptoms (Signorini, 1982; Trotter, 1982). Massages, sweat baths, "shock treatments", and ritual sweeping or rubbing the patient with various objects are often performed, with certain variations: guinea pigs are used for rubbing in Peru (Gobeil, 1973), while the use of hen's eggs is widespread in Guatemala (Gillin, 1948), and medicinal brushes ("sweeping") are used similarly in Southern Texas and Mexico (Rubel, 1964).

3.1.2 *Susto* as a culture-bound syndrome

In its latest version of the reference manual on mental disorders, the American Psychiatric Association (1994) dedicated an annex to "culture-bound syndromes", giving the DSM-IV (Diagnostic and Statistical Manual) more cultural depth than its predecessors (Maser *et al.*, 1991). Similarly, culture-bound syndromes (termed "culture-specific disorders") were included in the classification of mental and behavioral disorders from the World Health Organization (WHO, 1993), again as an annexed list of syndromes. Generally, these institutions define culture-bound syndromes as afflictions that are not easily categorized into existing classifications and are specific to a cultural area or locality. Of relevance to this study, both of the lists of syndromes provided include *susto* as a culture-bound syndrome.

This recognition certainly provides grounds for increased attention and research on these complex syndromes (Guarnaccia and Rogler, 1999). However, culture-bound syndromes as part of the Western nosological system represent a significant challenge for researchers, as they must either be linked to the established set of diagnostic categories and concepts (Hughes *et al.*, 1997), or be viewed as separate entities that cannot be directly associated to existing classifications, as has been suggested of *susto* by some authors (Rubel *et al.*, 1984; Tousignant, 1979). To date, research on *susto* has not resolved this issue, and information on factors intervening in *susto* might provide valuable indications on this matter. The general question remains whether *susto* is a cultural construct and must be analysed from an emic perspective (from within the culture), or if underlying organic causes are overshadowed by cultural beliefs and rituals (Crandon, 1983).

Nonetheless, many researchers disagree with the idea that *susto* is a culture-bound syndrome (e.g. Logan, 1993; Rubel, *et al.*, 1984). As Logan (1993) clearly stated, "[...] *susto* is so widely spread, and transects so many different regional, linguistic, demographic and class lines, it seems unreasonable to suggest [...] that this disorder is culture-bound. To the contrary, it is truly multi-cultural in its occurrence." There is more widespread acceptance of *susto* as a "folk illness", which can be defined as an illness that is not recognized as a genuine condition by Western medicine, in which symptoms are identifiable in the sufferer's culture as a form of illness, the cause is considered supernatural or as resulting from causes believed to be unique to that culture, and the patient's relations with others is different because of his or her condition (O'Nell, 1975). The term "folk illness" is used in this thesis because it is the usual term used in the litterature. However, the author recognizes its possible pejorative connotations.

Although *susto* is intrinsically linked to culture, it would be false and potentially hazardous to dismiss it as folkloric curiosity or superstition devoid of physiological consequences. Indeed, Collado-Ardon *et al.* (1983) showed that *susto* sufferers generally have a higher disease load and a higher likelihood of death over a seven-year period than non-sufferers. This disorder is therefore very serious, and can minimally be useful in screening for patients with high disease loads (Trotter, 1985). Much debate has surrounded its etiology, and elucidation of underlying factors in *susto* is still an area of active research. Insight into its causes and potential links with Western diseases is much needed.

3.1.3 Etiology of susto

Explanations proposed for the etiology of *susto* generally fall into one of three categories: physiological, psychological and social. Of course, all three categories are interrelated. Psychological disorders have neuronal, and therefore physiological, bases. Moreover, physiological and psychological processes are undoubtedly involved in social stress. However, since previous authors have classified proposed etiologies of *susto* according to those categories, they were accepted as such in the present study. Therefore, in this chapter, "physiological" refers to non-neurological physiological processes and "psychological" includes neuronal processes.

Proponents of physiological causes of *susto* include Bolton (1981) who linked *susto* to hypoglycemia on the basis of hostility, a symptom seen in victims of both ailments. As well, Signorini (1982) proposed intestinal parasitic infections as the underlying cause of *susto* because of the use of anthelmintic and antispastic herbal remedies, although acknowledging that other factors might be involved. Also based on treatments used, Trotter (1982) proposed that *susto* might be an array of closely related physiological ailments. Anxiety-related conditions have also been indicated to explain *susto* (Gillin, 1948; Gobeil, 1973; Kiev, 1968; Lopez-Ibor, 2003). For example, Gobeil (1973) linked *susto* to depression and obsession with soul loss and death leading to anxiety, and according to Lopez-Ibor (2003) *susto* is a clear panic disorder. Furthermore, in the DSM-IV *susto* is linked to post-traumatic stress disorder, anxiety disorder, major depressive disorders and somatoform disorders (APA, 1994), and the WHO associated *susto* to undifferentiated somatoform disorder and other specified neurotic disorders (WHO, 1993). Another hypothesis is that *susto* is caused by social role stress, which relates to the individual's inability to live up to social role expectations. This idea was proposed by Rubel (1964), and further developed by O'Nell and Selby (1968), O'Nell (1975) and Uzell (1974). However, as Rubel *et al.* (1984) pointed out, *susto* might not be the result of a single etiological factor, and all the factors mentioned above might be involved. Furthermore, an interesting account of the etiology of *susto* is given by Klein (1978), in which he proposes that *susto* is a disease of adaptation to stress and that it is the result of physiological, cultural and psychological factors.

In an attempt to provide novel information on possibilities relating to the causality of *susto*, we investigated treatments provided to *susto* patients, and in particular herbal treatments. Most previous studies of the etiology of *susto* have focused on the symptoms of patients and very few authors have investigated the effects of various treatments provided to infer possible underlying factors (Klein, 1978; Signorini, 1982; Trotter, 1982). In addition, we were interested in identifying potential neurological activity of plants used for treating *susto*, which has not been attempted in previous studies.

3.1.4 Objectives and hypotheses

Through this research, we sought to (1) describe symptoms and the folk etiology of *susto* for the Q'eqchi' of Belize; (2) identify treatment modalities for *susto* in the traditional Q'eqchi' healing system; (3) characterize plant use for treating *susto*; and (4) assess the anxiolytic activity of plants used as herbal remedies for *susto*. Our main hypothesis was that psychological or neurological factors are involved in the etiology of *susto*. Therefore, we would expect treatments used in *susto* to exhibit activity on the central nervous system (CNS). Accordingly, specific hypotheses were that plants used by the healers to treat *susto* have anxiolytic and/or fear suppression activity, plants used to treat *susto* are often used to treat for neurological/mental disorders as well, and concomitant forms of treatment also provide fear or anxiety relief for the patient (indirect qualitative assessment).

3.2 METHODOLOGY

3.2.1 Study area

The fieldwork for this study was conducted as part of the general ethnobotanical survey on neurological and mental health disorders (see Chapter 2). It therefore included the knowledge of the same group of healers from the same three localities in Southern Belize (Appendix 1), and extended from May to June 2003. Clarifications on treatments and the perception of *susto* were made in a second set of interviews held in March 2004. Ethical approval and herbarium collections are described in the second chapter of this thesis.

3.2.2 Interviews

The healers were interviewed individually on the symptomatology of *susto*, its folk etiology, treatment modalities and related plant use. These structured interviews were performed along with the more general interviews on neurological and mental health disorders (Appendix 2), which are described in Chapter 2.

In the second set of interviews (Appendix 4), the healers were questioned individually on the importance of herbal remedies in the treatment of *susto*, and clarifications were made in regards to concomitant forms of treatment (e.g. rituals). These semi-structured interviews lasted approximately 30 minutes. Translations were performed in the same manner as for the previous interviews.

3.2.3 Quantitative ethnobotanical methods

Several studies have demonstrated the relevance of using informant consensus to predict the biological efficacy of plants used by indigenous peoples for therapeutic purposes (Leaman *et al.*, 1995; Trotter and Logan, 1986; Johns *et al.*, 1990). Therefore, in this section, rather than quantifying traditional knowledge, we used consensus methods to select plants with high potential for showing biological activity, allowing focused efforts on a few plants for the *in vivo* assessment. Relative frequencies of mentions of plant use were selected as indicators of informant consensus, as proposed by Trotter and Logan (1986):

$$RF = N_A/N_t \ (*100) \tag{3.1}$$

RF: Relative frequency of mentions of plant A for a specific ailment

N_A: Number of mentions of use of plant A for an ailment (oral administration)

N_t: Total number of mentions of use of plants for the ailment (oral administration)

In addition, specificity of use was assessed using fidelity levels (FL), a method developed by Friedman *et al.* (1986):

$$FL = (I_p/I_u) * 100 \tag{3.2}$$

I_p: number of healers using a specific plant to treat a specific ailment (oral administration)

I_u: number of healers using the same plant for any purpose (oral administration)

3.2.4 Plant selection for testing

Plants were selected according to their relative frequencies of use (for oral administration only) (see Table 3.2 in the Results section), which was calculated with the methods described above. *Adiantum latifolium* (*Adiantaceae*), *Selaginella aff. Stellata* (*Selaginellaceae*), *Adiantum tetraphyllum* (*Adiantaceae*) and *Lygodium heterodoxum* (*Schizaeaceae*) were selected with this method. The selected plants were given orally to the test animals (rats) in the behavioral tests performed, attempting to remain consistent with the traditional administration of the remedies. Furthermore, given that all the healers use the leaves (and/or stems) of these plants, they were the only plant parts tested in the assays (exception: the whole plant of *Selaginella aff. stellata* is used and was tested in the assays).

One plant taken randomly from the same environment (control plant; *Commelina diffusa* Burm. (*Commelinaceae*)) was also included for testing on the basis that it was not used by the healers for treating *susto* or neurological/mental disorders. This plant was included to compare its biological activity to plants preferentially selected by the healers. The objective was to strenghten the hypothesis that neurological/psychological factors intervene in the etiology of *susto*. Indeed, considering that consensus methods have been shown to be good predictors of the biological activity of plants, if *susto* is caused by neurological or psychological factors (at least in part), plants used by the majority of healers should show greater activity on the CNS than plants taken randomly from the same environment. However, having tested only four high usage plants and one plant not used for these purposes, this comparison is merely used to provide an indication of different efficacies.

3.2.5 Plant extractions

The leaves (and/or stems) of selected plants were gathered in opaque white polypropylene Nalgene® bottles containing 70% isopropyl alcohol (rubbing alcohol) to sterilize and preserve the plant material for the duration of fieldwork. Once in the laboratory at the University of Ottawa, 95% EtOH was added to the bottles to ensure preservation of the plant material until extraction.

For the extractions, the solvent was first separated from the solid plant material using a Buchner filter system (Whatman #1 filter paper). The plant material was blended in

95% EtOH using a standard blender, with a biomass: solvent ratio of approximately 1 g fresh material: 10 ml EtOH. This plant-solvent mixture was put on a mechanic shaker overnight (~12 hours) and filtered to remove solid plant material. A second extraction was made using the same general procedure, but with a ratio of 1 g fresh material: 5 ml EtOH. The solid residues were dried and weighed after the final filtration. The filtrates were then roto-evaporated (Yamato rotary-evaporator), freeze-dried (E-C Supermodulyo freeze-dryer) and stored at 4°C until preparation for testing.

3.2.6 *In vivo* testing

3.2.6.1 Subjects

The experiments were conducted using male Sprague Dawley (SD) rats (250-275 g body weight; Charles River Laboratories Inc.), following the guidelines proposed by the Animal Care Committee at the University of Ottawa and the Canadian Council on Animal Care. The rats were individually housed in standard cages (45 x 24 x 20 cm) and familiarized with the researchers one week after their arrival. Testing started the following week. The animals were fed Purina™ Rat Chow and tap water *ad libitum*. They were maintained on a 12-hour light-dark cycle (7am-7pm) and room temperature was kept at 21°C. Efforts were made to avoid any situation potentially stressful to the animals (e.g. wet bedding material). Testing was done in sound-attenuated rooms and a mild detergent (Quatsyl®-D Plus; 8 ml/L water) was used for cleaning the equipment between all experiments.

3.2.6.2 Drug preparation, dosage, and administration

The plant extracts were dissolved one to four days prior to testing in 2% evaporated milk (Nestle Carnation) and kept at 4°C. To do so, the mixture was sonicated for 20 to 60 minutes and vortexed until the visible particles were dissolved. The animals were administered their respective drug orally by gavage (1 ml latex free sterile Becton Dickinson syringes with gavage tubes; gage 18, 5 cm in length).

The first series of tests took place in December 2003, in which plant extracts ([200 mg/ml]) from the five selected plants (four high usage plants and one plant not used by the healers for *susto* or mental/neurological conditions) were administered randomly to the rats at a dose of 200 mg/kg body weight (treatments: n=10; vehicle control: n=11). The behavior of treated rats was compared to that of control rats in the elevated plus maze (EPM), open field and acoustic startle response paradigms. Prior to the drug test, animals were gavaged on two consecutive days with water for habituation. The rats were tested in the acoustic startle response paradigm the week following EPM and open field testing. Animals that fell off the equipment during the test and those that failed to receive the full drug dose were excluded from data analyses.

The second set of tests was done in May 2004 with two of the plants previously tested. In this dose-response study. The first plant (*Adiantum tetraphyllum*) was administered at doses of 50 mg/kg, 150 mg/kg and 300 mg/kg body weight, whereas the second plant (*Lygodium heterodoxum*) was administered at a single 300 mg/kg dose. The dissolution and administration procedures were essentially the same as described above. Fifteen animals were tested per treatment group in the EPM, open field, and conditioned emotional response (CER) tests. The CER tests were performed one week after the EPM and open field tests. Again, depending on experimental circumstances, certain rats were excluded from the analyses.

The doses tested tended to be slightly higher in the assays performed with the rats, but were in the general range of doses administered by the healers (on a mg/kg basis). It is important to mention, however, that considerations such as dose variation between healers, differences in preparation methods (decoction and maceration used by the healers versus ethanol extractions in the laboratory due to methodological constraints), as well as the use of different species (rats versus humans) make direct comparisons somewhat difficult.

3.2.6.3 Apparatus and Procedure

Open Field Test

The open field test uses the animal's aversion of the central zone (open space: anxiogenic) to quantify anxiety behavior. It is also useful in assessing changes in motor activity after drug administration (Crawley *et al.*, 2003) by evaluating locomotive activity.

The arena was a light grey Plexiglas box (57 cm x 57 cm with 39 cm high walls) in which the floor was sub-divided into 36 equal-sized squares with marked lines. In this arena, the outermost series of squares (adjacent to the wall) is designated as the peripheral zone (perimeter) and the inner squares are designated as the central zone. In this paradigm, the time spent in the "safe" peripheral zone is thought to be proportionate to the level of anxiety in the rat. Conversely, the less anxious the animal is, the more time it is expected to spend in the central or more "vulnerable" zone of the arena. A black curtain surrounded the apparatus to minimise distractions. Video recording equipment was positioned above the centre of the arena and was connected to a remote monitor.

Animals were administered the drug and returned to their home cages for 45 minutes. They were then transferred to the testing room for fifteen minutes (total post-treatment time of 60 minutes). The rats were placed individually in the centre of the open field apparatus to initiate testing. Their behavior was recorded for five minutes using computer assisted real-time data logging. The session was videotaped concurrently for back-up. Parameters scored included: (1) the number of squares crossed in the central zone, (2) the number of squares crossed in the peripheral zone, (3) the time spent in the centre, and (4) the time spent in the periphery.

Elevated Plus Maze (EPM) Test

This behavioral test is among the most commonly used anxiolytic tests (Crawley *et al.*, 2003) and is based on the conflict between the instinct of the animal to explore novel environments and its inherent fear of heights and open (vulnerable) spaces. This test has been validated to identify anxiogenic and/or anxiolytic drug effects in rats by Pellow *et al.* (1985).

The maze consisted of an elevated wooden cross (or plus (+)-shaped structure), in which two opposing arms were closed on three sides (alley; 40 cm walls, but open on top), and the two other arms were open planks. The maze was elevated at 50 cm from the floor, and each arm was 50 cm in length and 10 cm in width. An open area at the centre (10 cm x 10 cm) separated each arm. The flooring was a black rubberised runway and the interior walls were of Plexiglas®. The "open arms" make up the anxiogenic portion of the maze, and the "closed arms" the safer or relatively less anxiogenic portion (Pellow *et al.*, 1985). In order to minimize extraneous distractions, a black curtain surrounded the apparatus.

One hour after drug administration, the rats underwent testing on the open field for five minutes, and were then directly transferred to the EPM. At the beginning of the test, they were placed facing the wall of a closed arm and were monitored via a video camera system positioned above the apparatus for five minutes, using computer assisted data analysis. They were also videotaped for back-up. The following behavioral parameters were quantified: (1) number of entries in the open arms, (2) number of entries in the closed arms, (3) time spent in the open arms, (4) time spent in the closed arms, (5) time spent in the centre, and (6) number of unprotected head dips. Operationally, only when all four paws of the animal entered an arm was it considered as an entry into the specific arm. Unprotected head dips were defined as the protrusion of the head over the edge of an open arm.

Acoustic Startle Response Test

This test is useful in measuring behavioral effects of drugs on the CNS, and uses amplitude of the startle reflex as a measure of anxiety (Davis and Gallager, 1988).

Because of individual differences, a brief startle test was done prior to the actual experiments to obtain individual baseline values for each animal. Rats with similar startle amplitude were distributed into treatment groups to obtain groups with equivalent startle amplitudes. In terms of the test procedure, the rats were placed in the Acoustic Startle Box, on pre-calibrated platforms. These were connected to a computer program to record startle amplitude. Testing started after a five-minute habitation period. The rats were then exposed to a set of 30 randomized bursts of white noise (95 dB, 105 dB, and 115 dB) over the 15 minute test period. All animals were tested 60 minutes following drug or vehicle treatment (via gavage).

Conditioned Emotional Response (CER) Test

The CER test is thought to assess fear in the animals and uses conditioning procedures. Drugs that reduce fear or anxiety are expected to attenuate the animal's response in the CER test (Davis, 1990). Two parameters were differentiated in this experiment, contextual fear (responses generalized to the conditioning test chamber) and cued fear (to a specific tone, paired to the aversive stimulus), as proposed by Lee *et al.* (2001).

For training, the rats were placed in a conditioning chamber. After one minute of habituation, they were exposed to a 20-second tone (85 dB), which was paired with a foot shock (1.0 mA) delivered during the final second of the tone delivery. The rats underwent six such trials at randomized intertrial intervals over the 10 minute training session. Rats were tested one hour following drug treatment (randomized assignment to treatment groups). The animals were reintroduced into the conditioning chamber where they had received the training 24 hours earlier. Freezing was assessed every 5 seconds for a total period of 4 minutes. Freezing was defined as complete absence of movement except for respiration. Following this, rats were immediately transferred to a novel environment (a clean cage identical to their home cage) and allowed one-minute habituation period. They were then exposed to 20 tones (each lasted 20 seconds and occurred every minute) and freezing was assessed every five seconds. The same procedure was repeated the following day, however during this final test session, no drugs were administered. This session was included to assess whether drug administration affected the reconsolidation or recall processes.

3.2.7 Statistical analyses

Statistical analyses were performed with the program SYSTAT®. Data were reported as means ± SEM. To compare data from the EPM and open field tests, analyses of variance (one-way ANOVAs) were done with Fisher's Least Significant Difference Test as post-hoc test. A repeated measures ANOVA was used in the acoustic startle response test to analyze the effects and interactions of time (before/after drug administration) and treatment on startle amplitude. One-way ANOVAs were also performed to verify the Treatment effect alone. Similarly, a repeated measures ANOVA was used to analyze the effects and interactions of Time and Treatment on freezing in the CER test, and one-way ANOVAs allowed the identification of Treatment effects for individual time frames. The level of significance was set at $p < 0.05$.

3.3 RESULTS

3.3.1 Illness perception and treatment

According to the healers interviewed, the general cause of *susto* is being frightened by someone (or something), which can lead to loss of the soul, and sickness. Specific causes include startling events (e.g. dog barking, encounter with a snake, lightning), people, and mountains spirits. Various symptoms were identified by the healers and include dehydration, weakness, vomiting, diarrhea, loss of appetite, mild pain in the stomach or heart, and a burning sensation. However, *susto* is not perceived as an illness *per se*, but as a cause of various symptoms. It is also considered to afflict children more frequently than adults.

To treat *susto*, the healers generally first give herbal remedies and then call back the soul of the patient by prayers and rituals performed to re-balance the body and spirit. These ceremonies were considered crucial to the patient's recovery by all healers interviewed. Healers often perform a ritual of "smoking" the patient by burning certain items and letting the smoke rise around the patient. Plants, hair or finger/toe nails, as well as elements from the frightening event (e.g. branches) can be burned for this ritual, along with copal (*Protium copal*) incense. The patient's name is called out repetitively during the ceremony. Some of the healers also use massages as therapy in this context. In all cases, either an item from the frightening event is brought to the patient, or the ritual is performed at the site itself.

3.3.2 Plant use

Fourteen plant species belonging to six plant families are used as remedies for *susto* (Table 3.1) (another two species mentioned by the healers could not be collected and were therefore excluded from the analyses). These are prepared by decoction (boiled) or macerated in cold water. In most cases, the leaves and/or stems are used. The preparations are either given orally or the water is used for the patient to bathe or to wet his/her head in. Some of the plants listed are also used for ritual "smoking" (see previous section). The majority of healers interviewed said they used plants from primary forests and/or mountains to treat *susto* because of their spiritual value. Plant families most represented were the *Adiantaceae* (four species) and *Piperaceae* (three species). Some of the healers (three healers out of six) believed the plants administered are important in relieving the symptoms of *susto* but not the syndrome itself, while the others thought the plants used for *susto* are useful in treating *susto* and act differently than plants used for the symptoms of *susto* when they occur by themselves.

Among the plants tested in the bioassays, *A. tetraphyllum* exhibited the highest relative frequency values and was cited as a favourite plant to treat *susto* by all the healers that used it (Table 3.2). Furthermore, the use of this plant was highly specific to *susto*, as indicated by the FL values. The other plants selected also had high relative frequency values and their use by the healers was very specific to *susto* compared to most other plants used. However, they were not considered favourites to treat *susto* by the healers who used them, appart from *S. aff. stellata*.

Table 3.1: Plants used by six Q'eqchi' healers of Southern Belize to treat *susto* and description of remedy preparation and administration.

Botanical name	Plant family	Voucher #	Vernacular name (Q'eqchi')	Mode of preparation	Mode of administration	Plant parts used
Adiantum princeps T. Moore	*Pteridaceae*	22	*Colantra pim*	Maceration	Oral, topical [1]	Leaves and/or stems
Adiantum latifolium Lam.	*Pteridaceae*	68	No name	Maceration	Oral, topical	Leaves and/or stems
Adiantum tetraphyllum Humb. & Bonpl. Ex Willd.	*Pteridaceae*	54	*Roq chit cuan*	Maceration	Oral, topical	Leaves and/or stems
Combretum sp.	*Combretaceae*	79	*Qa'xa'an caham*	Decoction	Oral	Leaves and/or stems
Guarea grandifolia DC.	*Meliaceae*	89	*Bolb*	Decoction	Oral	Bark
Lygodium heterodoxum Kunze	*Schizaeaceae*	25	*Ruxb'i'kaak*	Maceration, ritual burning	Oral, topical	Leaves and/or stems
Lygodium venustum Sw.	*Schizaeaceae*	61	*Ruxb'i'kaak*	Maceration, ritual burning	Oral, topical	Leaves and/or stems
Piper sp.	*Piperaceae*	80	*Cux sawi*	Maceration, decoction	Oral	Leaves and/or stems
Piper sp.	*Piperaceae*	50	*Pu'chuch*	Maceration	Oral, topical	Leaves and/or stems
Piper sp.	*Piperaceae*	99	*Pu'chuch*	NA	Oral, topical	Leaves and/or stems
Pityrogramma calomelanos (L.) Link	*Pteridaceae*	66	*Roq chit cuan*	Maceration	Oral, topical	Leaves and/or stems
Pteris plumula Desv.	*Pteridaceae*	107	No name	Maceration	Oral, topical	Leaves and/or stems
Scoparia dulcis L.	*Scrophulariaceae*	73	*Colantra pim*	Maceration	Oral, topical	Leaves and/or stems
Selaginella aff. Stellata	*Selaginellaceae*	35	No name	Maceration, decoction, ritual burning	Oral, topical	Whole plant

[1] Remedies given as baths or to wet head of the patient, or used for ritualized burning (locally referred to as "smoking" the patient);

Table 3.2: Description and informant consensus information for identified plants used orally and/or topically to treat *susto*

Scientific name	Plant family	Voucher #	Vernacular name	Favourite [2]	RF [3]	FL [4]
Adiantum princeps T. Moore	*Pteridaceae*	22	*Colantra pim*	No	4.17	16.67
Adiantum latifolium Lam.	*Pteridaceae*	68	No name	No	12.50	50.00
Adiantum tetraphyllum Humb. & Bonpl. Ex Willd.	*Pteridaceae*	54	*Roq chit cuan*	Yes *	16.67	80.00
Combretum sp.	*Combretaceae*	79	*Qa'xa'an caham*	Yes *	4.17	25.00
Guarea grandifolia DC.	*Meliaceae*	89	*Bolb*	Yes *	4.17	33.33
Lygodium heterodoxum Kunze	*Schizaeaceae*	25	*Ruxb'i'kaak*	No	4.17	25.00
Lygodium venustum Sw.	*Schizaeaceae*	61	*Ruxb'i'kaak*	No	4.17	25.00
Piper tuerckeimii C.DC. ex Donn. Sm. [1]	*Piperaceae*	80	*Cux sawi*	Yes	8.33	33.33
Piper sp.	*Piperaceae*	50	*Pu'chuch*	No	4.17	20.00
Piper sp.	*Piperaceae*	99	*Pu'chuch*	No	4.17	25.00
Pityrogramma calomelanos (L.) Link	*Pteridaceae*	66	*Roq chit cuan*	Yes *	4.17	50.00
Pteris plumula Desv. [1]	Pteridaceae	107	No name	No	8.33	100
Scoparia dulcis L.	*Scrophulariaceae*	73	*Colantra pim*	No	4.17	25.00
Selaginella aff. stellata	*Selaginellaceae*	35	No name	Yes	8.33	50.00

[1] These species could not be collected in sufficient quantities for testing;

[2] Plants cited as favourites to treat *susto* by at least some of the healers interviewed (* favourite plant to treat *susto* according to all the healers interviewed who use the plant as treatment for *susto*);

[3] Relative frequency of mentions of use = (Number of mentions of use of plant A for a specific ailment / Total number of usage mentions for the ailment) *100 (Trotter and Logan, 1986) (for oral administration only);

[4] Fidelity Level = (Number of healers using plant A for a specific ailment / Number of healers using plant A for any purpose) *100 (Freidman, 1986) (for oral administration only).

Plants used to treat *susto* were also used by the healers in treating 15 other ailments (Table 3.3). Many such ailments are associated with symptoms of *susto* (i.e. fever, diarrhea, heartburn, muscle pain, ulcers and depression), according to information available in the literature and to symptoms described by the healers interviewed. Several plants were also used to treat both *susto* and neurological/mental ailments. The number of mentions of use in common was greater for neurological/mental ailments than for ailments associated with symptoms of *susto*, and the same number of species in common was used for both categories of treatment. In addition, most of the plants used for *susto* were also used in treating epilepsy.

Table 3.3: Ailments that have plant remedies in common with *susto*, number of mentions of use in common and number of plants in common (out of 14 plants used for *susto*)

Ailment category	Ailment	Number of plants	Number of mentions
Associated with symptoms of *susto*[1]	Fever	5	6
	Diarrhea	4	5
	Heartburn	4	4
	Pain	4	5
	Ulcers	1	1
	Depression[2]	2	2
	Total	**13**	**23**
Neurological/mental	Epilepsy/seizures	10	20
	Headache	5	7
	Madness	5	5
	Depression[2]	2	2
	Total	**11**	**34**
Others	Snakebite	5	5
	Heavy menstruation	1	1
	Repositioning uterus after accident	1	1
	Coughs	1	1
	Diabetes	1	1
	Rheumatism	1	1
	Total	**9**	**10**

[1] According to both symptoms mentioned in the literature and by the group of healers interviewed
[2] Depression is a neurological/mental ailment and an ailment associated with symptoms of *susto*

3.3.3 Anxiolytic and fear suppression activity of plants tested

3.3.3.1 Open Field test

In the open field test (dose: 200 mg/kg body weight), there were no significant differences between treatments across any of the parameters assessed (number of squares crossed in center: $F(5,55)=1.155$, $P=0.343$; time in central zone: $F(5,55)=0.812$, $P=0.546$; number of squares crossed in periphery: $F(5,55)=0.529$, $P=0.753$) (Figure 3.1, Appendix 5). However, there was a trend for the animals that were administered *L. heterodoxum* to cross more squares in the central zone, when compared to the control animals (p-value: 0.091) (Figure 3.2).

In the second set of experiments (*A. tetraphyllum* [50 mg/kg], [150 mg/kg] and [300 mg/kg], and *L. heterodoxum* [300 mg/kg]), no significant differences were detected between treatments (no. squares crossed in center: $F(4,70)=1.390$, $P=0.246$; time in central zone: $F(4,70)=0.934$, $P=0.449$; no. squares crossed in periphery: $F(4,70)=0.812$, $P=0.522$) (Figure 3.3, Appendix 6).

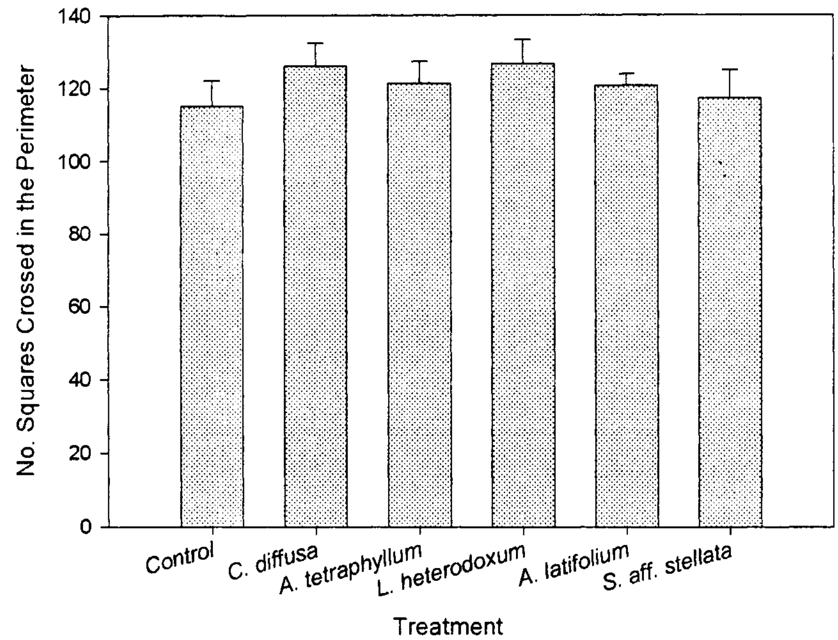

Figure 3.1: Number of squares crossed in the peripheral zone of the open field for different treatments (dose: 200 mg/kg), after a one hour post-drug interval (n=10-11). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

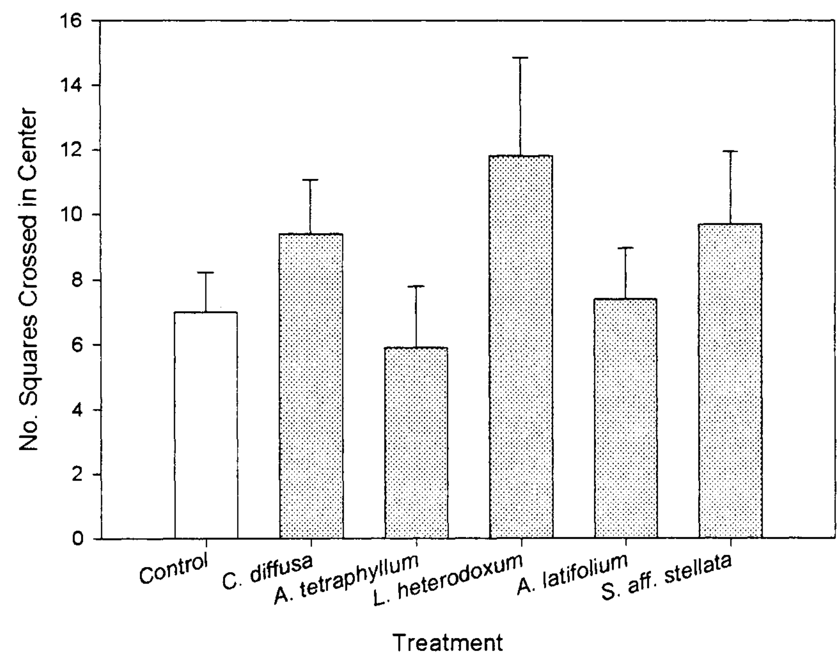

Figure 3.2: Number of squares crossed in the central zone of the open field for different treatments (dose: 200 mg/kg), after a one hour post-drug interval (n=10-11). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

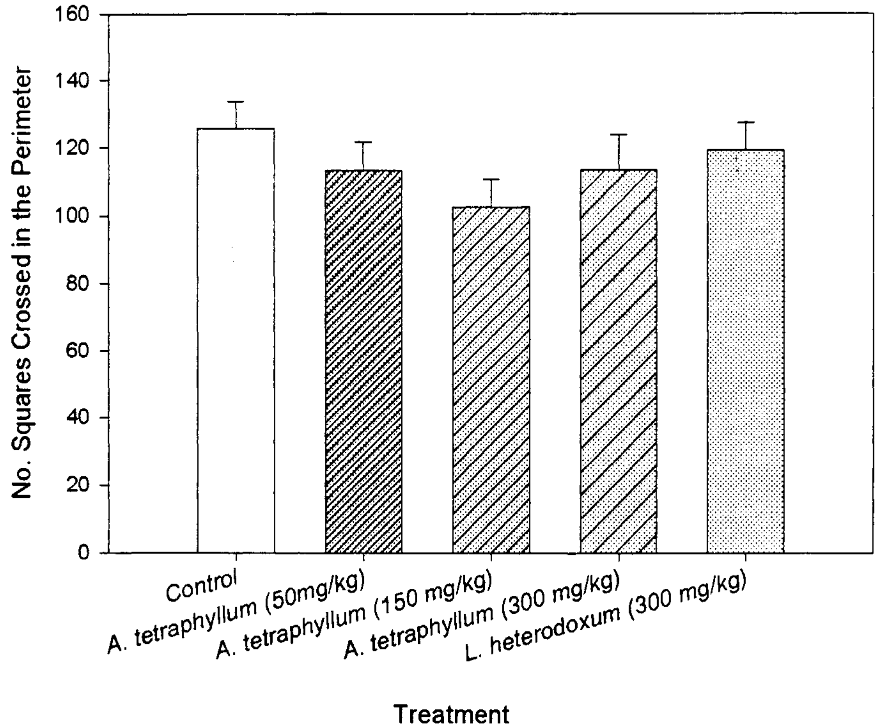

Figure 3.3: Number of squares crossed in the perimeter of the open field for different treatments (doses: 50 to 300 mg/kg), after a one hour post-drug interval (n=10-14). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

3.3.3.2 Elevated-Plus Maze test

At a dose of 200 mg/kg body weight, the treatments administered had a significant effect on certain parameters in the elevated plus maze test (i.e. number of open arm entries: $F(5,52)=2.480$, $P=0.040$; time spent in open arms: $F(5,52)=4.209$, $P=0$; number of unprotected head dips: $F(5,52)=3.552$, $P=0$; % open arm entries: $F(5,52)= 3.194$, $P=0$; % time spent in open arms: $F(5,52)=4.209$, $P=0$). When comparing control animals vs. drug-treated animals, *A. tetraphyllum* showed significant anxiolytic effects in the time spent in the open arms, the number of unprotected head dips performed and the percentage of time spent in the open arms (Figures 3.4, 3.5 and 3.6; Appendix 7), whereas the other treatments administered did not have a significant effect on the parameters evaluated when compared to the control animals.

In the second set of experiments (*A. tetraphyllum* [50 mg/kg], [150 mg/kg] and [300 mg/kg], and *L. heterodoxum* [300 mg/kg]) treatment had no significant effect on time spent in the open arms ($F(4, 61)=0.396$, $P=0.811$), the number of occurrences in the open arms ($F(4, 61)=0.684$, $P=0.606$), the number of unprotected head dips ($F(4, 61)=0.480$, $P=0.750$), the percentage of open arm entries ($F(4,61)= 0.441$, $P=0.778$), or the percentage of time spent in the open arms ($F(4,61)=0.396$, $P=0.811$) (Appendix 8).

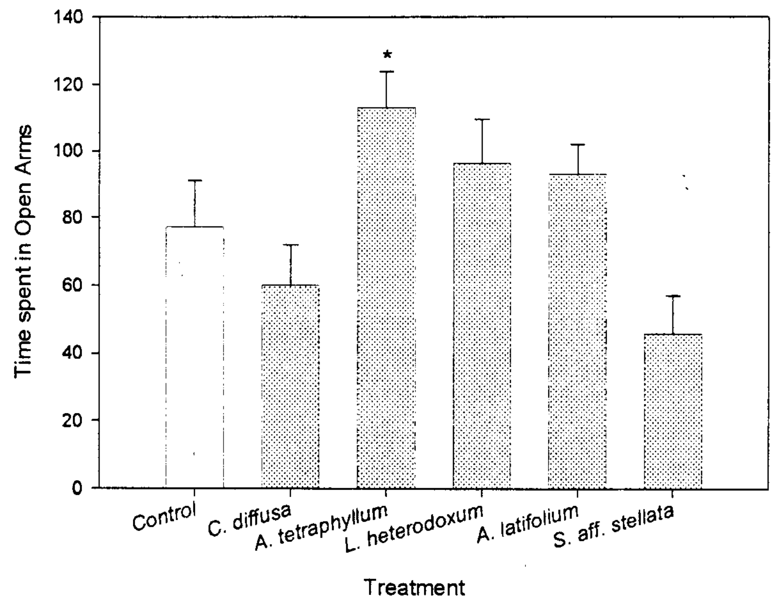

Figure 3.4: Time spent in the open arms of the elevated plus maze for different treatments (dose: 200 mg/kg), after a one hour post-drug interval (n=9-11). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

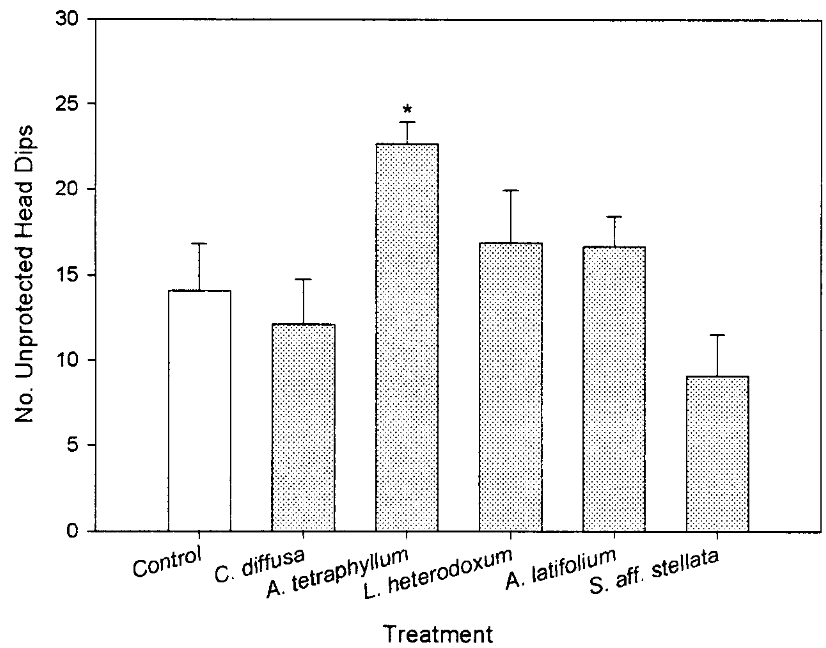

Figure 3.5: Number of unprotected head dips performed in the elevated plus maze for different treatments (dose: 200 mg/kg), after a one hour post-drug interval (n=9-11). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

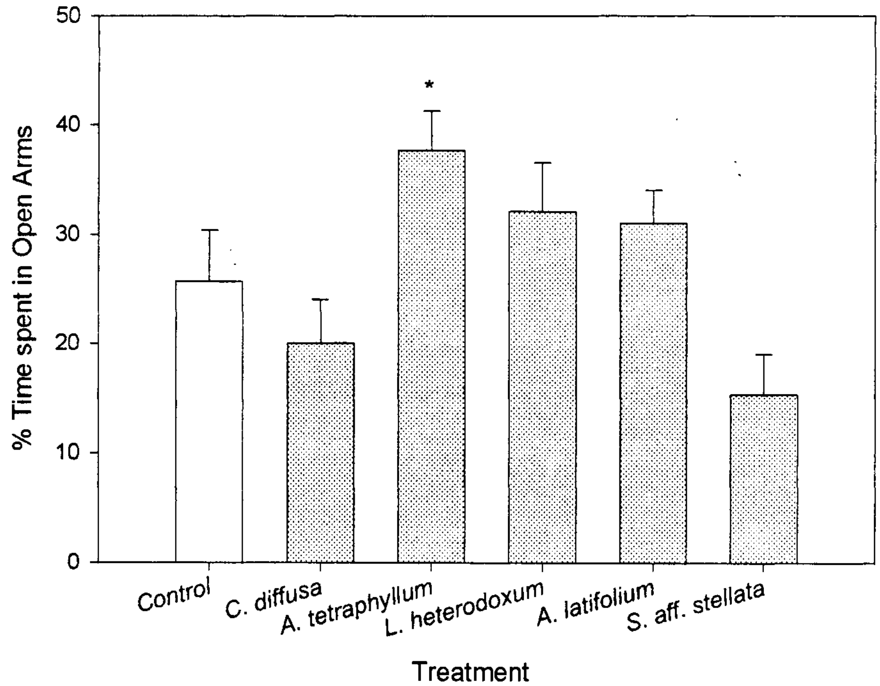

Figure 3.6: Percentage of the time spent in the open arms of the elevated plus maze for different treatments (dose: 200 mg/kg), after a one hour post-drug interval (n=9-11). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

3.3.3.3 Acoustic Startle Response test

Rats showed similar mean startle amplitudes (in arbitrary units) for all intensities of white noise combined (95 dB, 105 dB and 115 dB) regardless of the treatment administered ($F(5,54)=1.516$, $P=0.200$) (Figure 3.7). Furthermore, although there was a general increase in mean startle amplitude with increasing white noise intensity (Figure 3.8), there was no significant difference between treatments at different white noise intensities (dB) (95 dB: $F(5,54)=0.774$, $P=0.573$; 105 dB: $F(5,54)=0.455$, $P=0.808$; 115 dB: $F(5,54)=1.117$, $P=0.362$).

Comparisons of startle amplitudes before and after drug administration showed that there was no significant Treatment x Time interaction (95 dB: $F(5,54)=0.376$, $P=0.863$; 105 dB: $F(5,54)=0.076$, $P=0.996$; 115 dB: $F(5,54)=0.640$, $P=0.670$, all intensities combined: $F(5,54)=0.329$, $P=0.893$) and no Treatment effect (95 dB: $F(5,54)=0.289$, $P=0.917$; 105 dB: $F(5,54)=0.249$, $P=0.939$; 115 dB: $F(5,54)=0.304$, $P=0.909$, all intensities combined: $F(5,54)=0.561$, $P=0.730$). However, Time (before/after treatment) had a significant effect on startle amplitude (95 dB: $F(1,54)=21.01$, $P=0$; 105 dB: $F(1,54)=29.04$, $P=0$; 115 dB: $F(1,54)=48.001$, $P=0$, all intensities: $F(1,54)=12.2$, $P=0.001$) (Figure 3.9). Therefore, the rats treated with the plant preparations did not have significantly lower startle amplitudes than the animals in the control group, and all animals (including the control animals) decreased their startle amplitudes in the "after treatment" experiment.

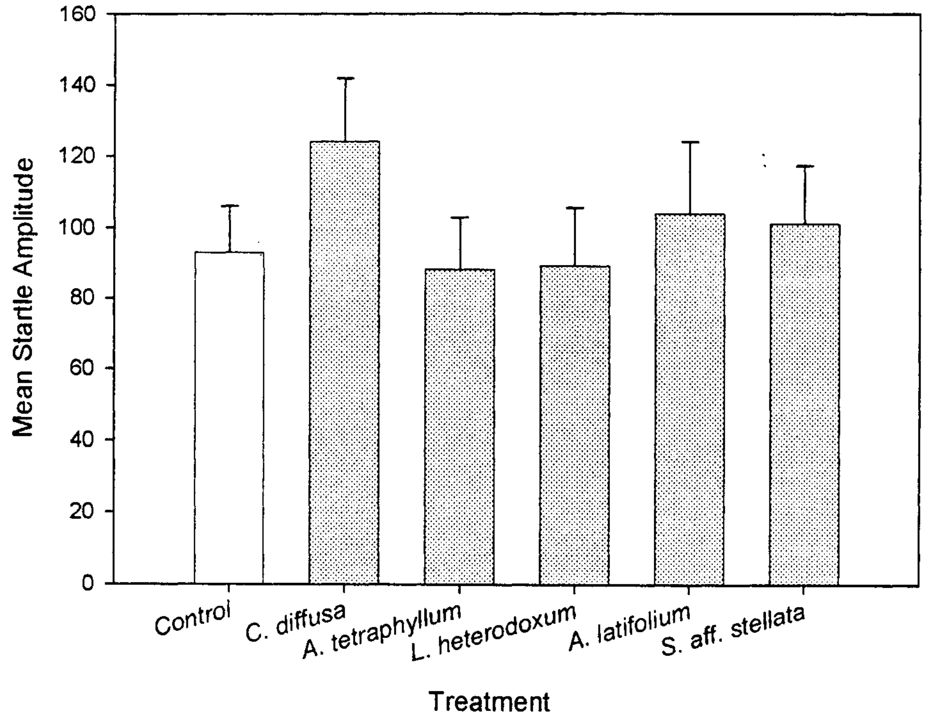

Figure 3.7: Mean startle amplitude in response to all intensities of white noise combined (95 dB, 105 dB and 115 dB) one hour after drug administration, for different treatments (n=9-11; dose: 200 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

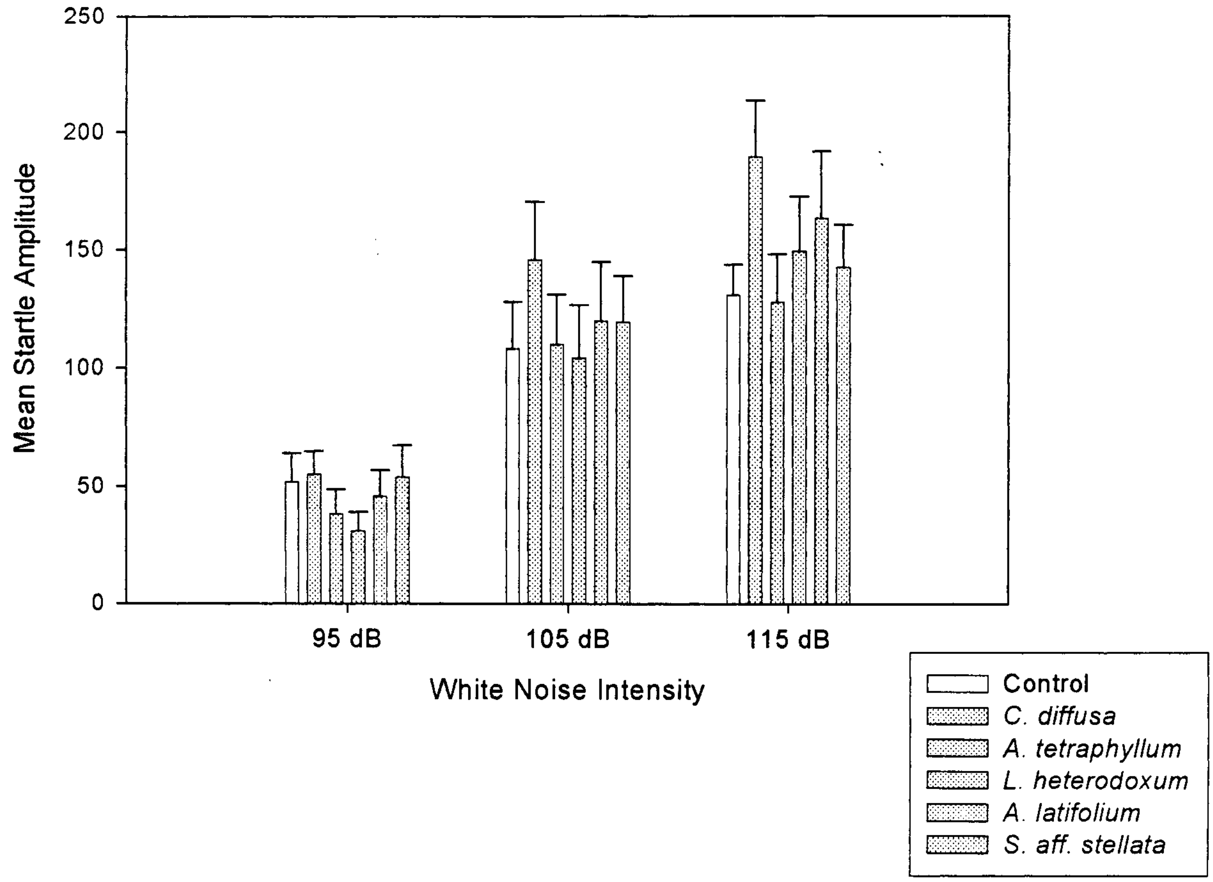

Figure 3.8: Mean startle amplitude in response to 95 dB, 105 dB and 115 dB bursts of white noise one hour after drug administration, for different treatments (n=9-11; dose: 200 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

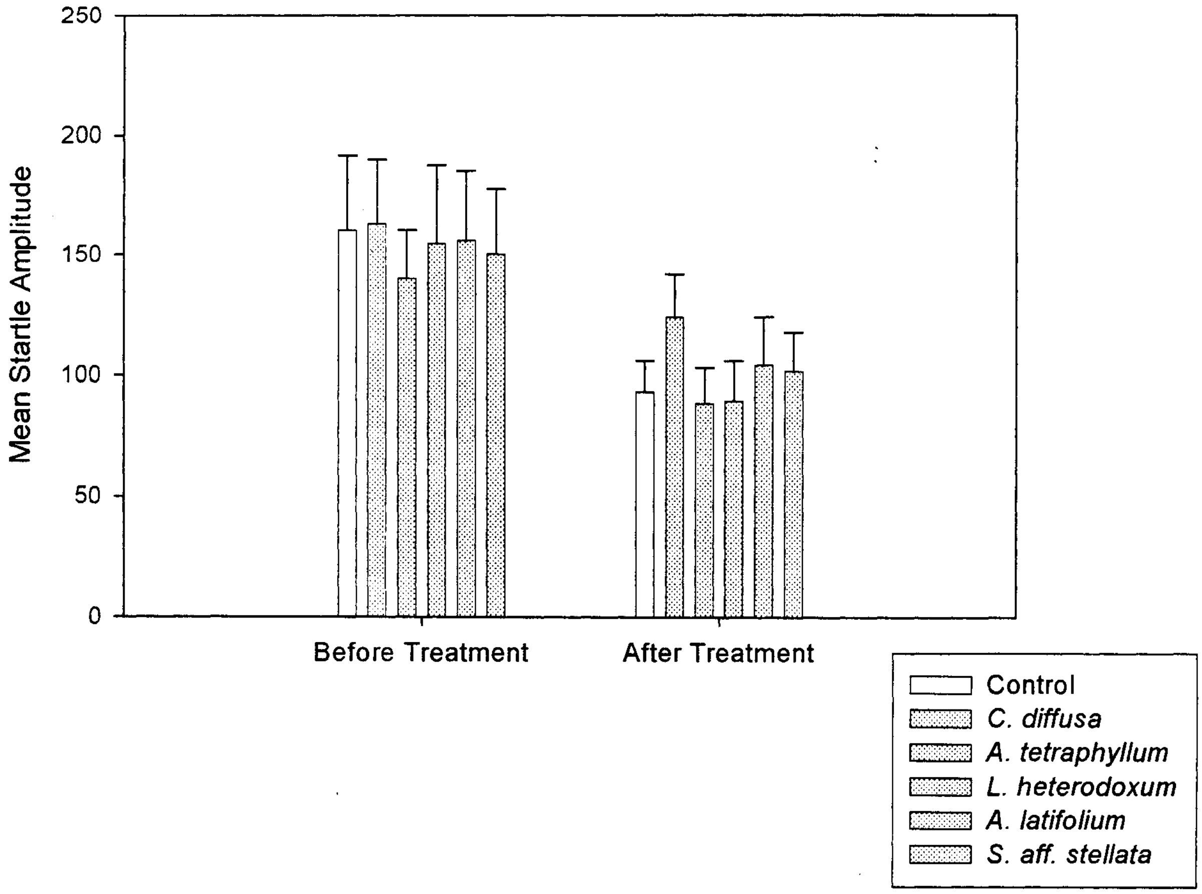

Figure 3.9: Mean startle amplitude before and after drug administration in response to all intensities of white noise (95 dB, 105 dB and 115 dB), for different treatments (n=9-11; dose: 200 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

3.3.3.4 Conditioned Emotional Response test

Contextual fear

Overall, analyses performed showed that when the rats were re-exposed to the contextual cues associated with the place where they received the training (aversive stimuli), there was a significant Time effect on freezing behavior ($F(3,207)=10.024$, $P=0$) (Figure 3.10). For this same period, no Time x Treatment interaction was found ($F(12,207)=0.384$, $P=0.384$), nor was there a significant Treatment effect ($F(4, 69)=0.581$, $P=0.677$). However, in the first minute of context, there was a significant effect of treatment on freezing ($F(4,69)=2.587$, $P=0.044$). Post-hoc analyses revealed that the rats treated with *A. tetraphyllum* [300 mg/kg] froze significantly less than the control animals ($P=0.032$). Furthermore, when the rats were tested 24 hours later in the same paradigm, the results were generally the same (Figure 3.11): Time had a significant effect on freezing ($F(3,198)=23.636$, $P=0$), but no Time x Treatment interaction was found ($F(12,198)=0.849$, $P=0.600$), and the Treatment effect was not significant ($F(4,66)=0.394$, $P=0.812$). Further analyses showed that there was a near-significant anxiolytic effect of *A. tetraphyllum* [300 mg/kg] compared to the control group in the first minute of testing ($P=0.055$).

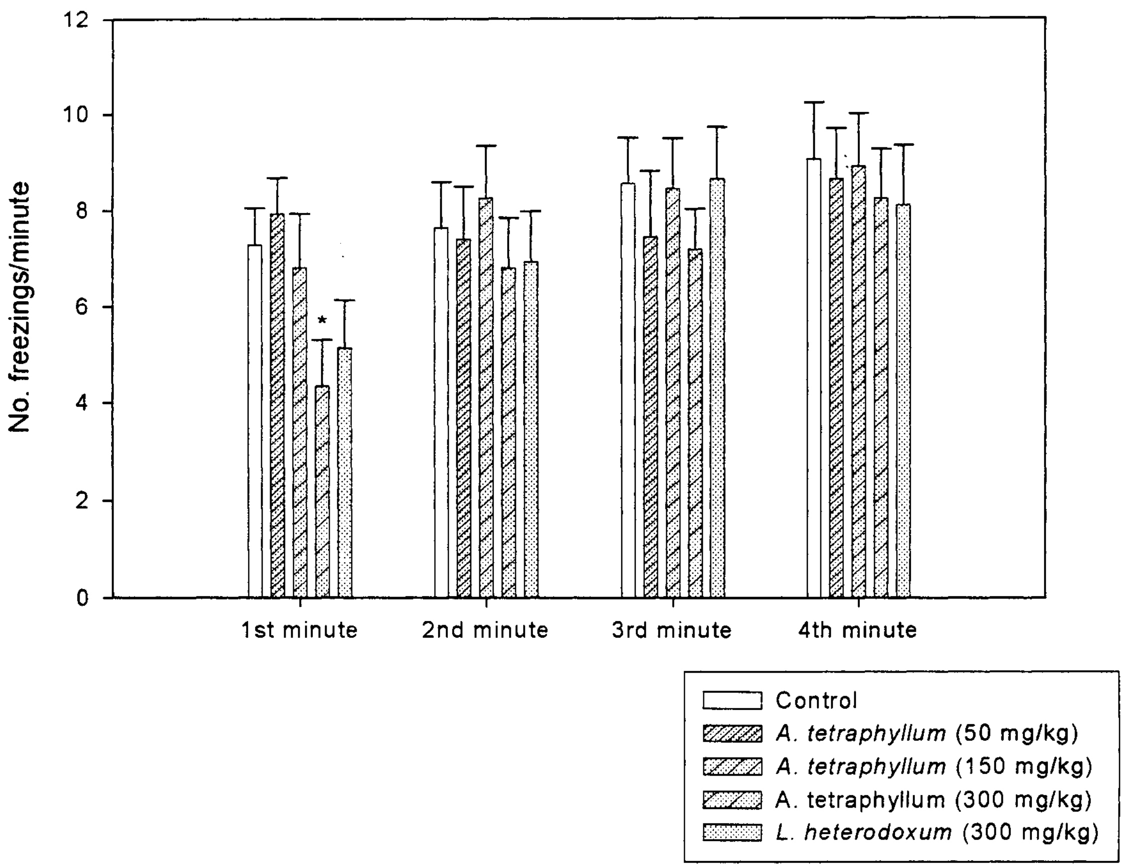

Figure 3.10: Number of times the animals froze each minute when put in the cage where they received the tone (context), one hour after drug administration (n=14-15; doses: 50 to 300 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

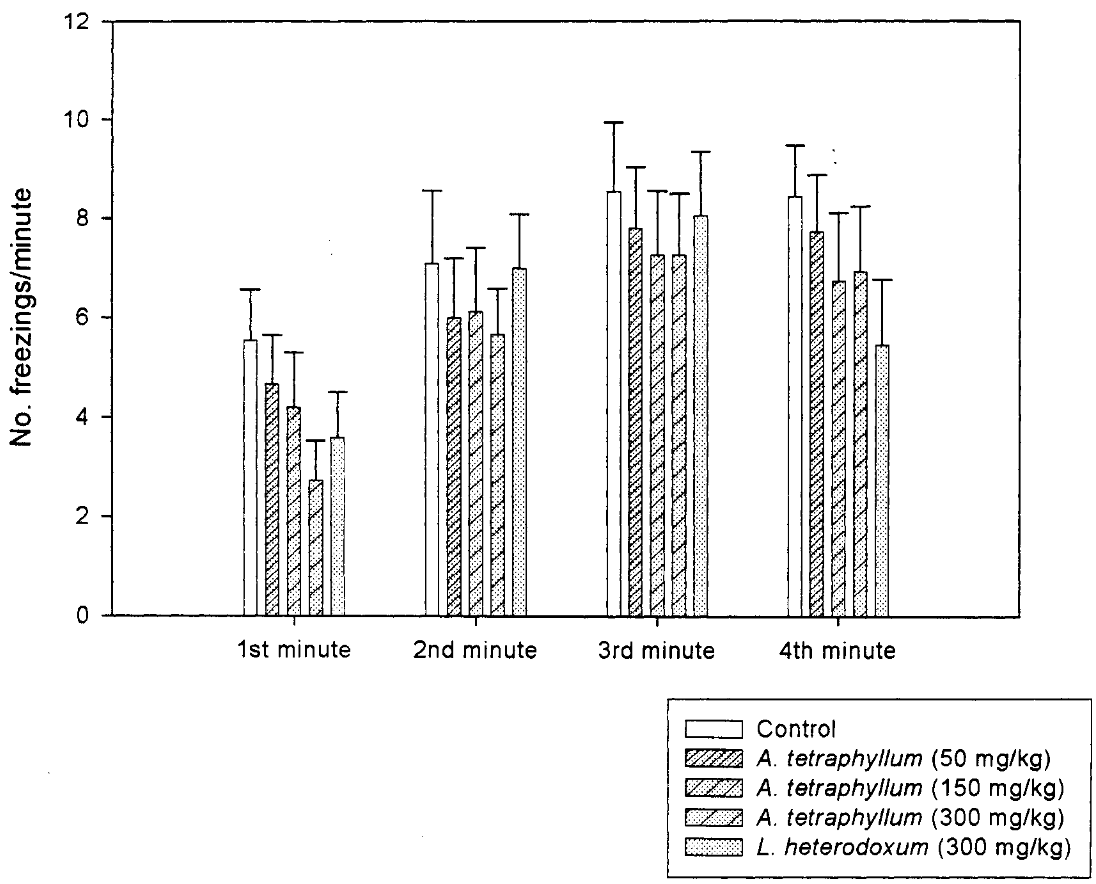

Figure 3.11: Number of times the animals froze each minute when put in the cage where they received the tone (context), 24 hrs after drug administration (n=14-15; doses: 50 to 300 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk).

Cued fear

When the rats were exposed to the tone (which was previously paired with foot shock), there was a significant Time effect on freezing ($F(19,1311)=23.990$, $P=0$), and the interaction between Time and Treatment was significant ($F(76,1311)=1.392$, $P=0.016$). However, the treatment effect was not significant in this analysis ($F(4,69)=0.882$, $P=0.479$). Figure 3.12 shows selected time frames (every five minutes) to illustrate cued freezing behavior over time. The interaction effect was that as time progressed, rats treated with *A. tetraphyllum* at a dose of 150 mg/kg became increasingly different from the control group in their freezing behavior. At the time frame 18:20-19:40, these animals froze significantly more than the control animals ($F(4,69)=4.313$, $P=0.004$; post-hoc analysis: $P=0.001$), while animals treated with 50 mg/kg and 300 mg/kg of the same plant displayed similar behavior as the control animals (post-hoc analyses: $P=0.334$ and 0.926, respectively), and this trend was constant until the end of the experiment (Figure 3.12). The same trend was seen 24 hours after drug administration (Figure 3.13): Time had a significant effect on freezing ($F(19,1254)=10.446$, $P=0$), a Time x Treatment interaction was found ($F(76,1254)=1.564$, $P=0.002$), and the treatment effect was not significant ($F(4,66)=1.413$, $P=0.239$). A more in depth look showed that there was a trend for animals treated with *A. tetraphyllum* [150 mg/kg] to freeze more than the control animals (p-value: 0.082) in the time frame from 11:40 to 13:00. Furthermore, animals treated with *A. tetraphyllum* [50 mg/kg] and *A. tetraphyllum* [300 mg/kg] displayed trends in freezing less than the control group (*A. tetraphyllum* [50 mg/kg] 2:20-3:40 $P=0.099$ and *A. tetraphyllum* [300 mg/kg] 2:20-3:40 $P=0.058$; 5:00-6:20 $P=0.094$; 6:20-7:40 $P=0.068$; 7:40-9:00 $P=0.055$).

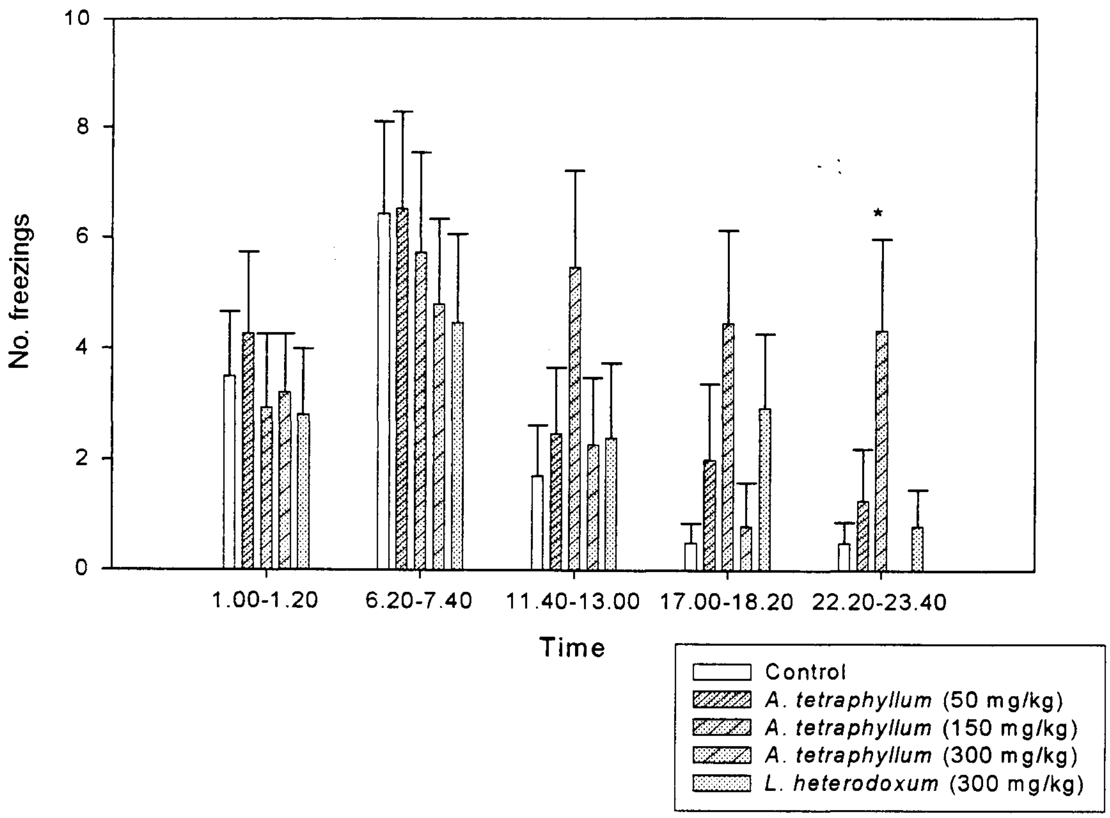

Figure 3.12: Number of times the animals froze for selected time frames, when subjected to the acoustic tone (previously paired with foot shock), one hour after drug administration (n=14-15; doses: 50 to 300 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

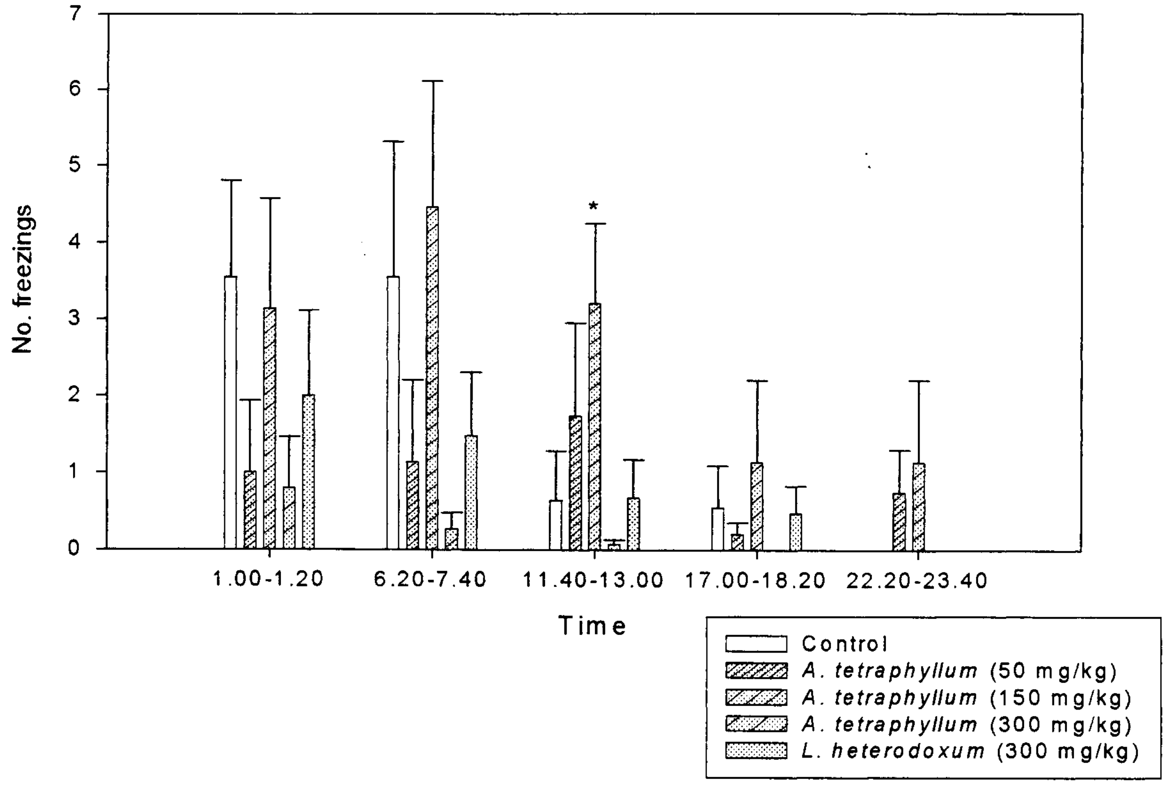

Figure 3.13: Number of times the animals froze for selected time frames, when subjected to the acoustic tone (previously paired with foot shock), 24 hours after drug administration (n=14-15; doses: 50 to 300 mg/kg). All values represent the group mean ± SEM; control: vehicle control (2% evaporated milk); significance of difference from control animals: * p-value < 0.05.

3.4 DISCUSSION

3.4.1 Rituals as treatment

All the healers interviewed stated that even in cases where herbal remedies were administered, the patient would eventually fall ill again from *susto* if the specific rituals were not performed. These rituals, and especially calling back the spirit of the patient, were in fact an essential part of the treatment according to the healers. Interestingly, three major components of the rituals described are seen throughout the literature. First, calling back the soul of the patient is a crucial component of treating *susto* (Gillin, 1948; Gobeil, 1973; Rubel, 1964). Secondly, the patient must be relaxed during the treatment, as well as the use of massages, prayers, and ritual sweeping with various objects (or in this case "smoking" the patient) (Gillin, 1948; Klein, 1978; Rubel, 1964; Weller *et al.*, 2002). Thirdly, the rituals are either performed at the site of the frightening event, or an element of the fright is brought to the patient for the ritual (Gillin, 1948; Rubel, 1964; Signorini, 1982). This step of the ritual is of significant interest, as it is similar to certain aspects of "desensitization to the trauma", a technique used to treat post-traumatic stress disorder (PTSD) (Scott and Stradling, 1992). Although *susto* cannot be directly equated to PTSD, the perception that one has lost his soul could certainly be traumatic for the patient (Klein (1978) addresses the issue of symbolic danger in his account of *susto* as a disease of adaptation) and desensitization could prove valuable in treating *susto*. Through desensitization in the presence of a respected healer, the patient can relive the frightening experience in total security. Furthermore, Weller *et al.* (2002) provide an interesting assessment of various forms of treatment for *susto*. They report that according to their informants, the use of sedatives, massages and prayers, staying relaxed, and consulting a psychiatrist or psychologist are helpful in treating *susto*, along with consulting herbalists and healers.

The elements described above combine to give a clearer picture of the use of rituals in *susto.* Specific strategies are used to help the patient relax, thus providing initial calmness. Then the trauma is re-experienced in the presence of a healer, helping the patient cope with the distress. And finally, the patient's soul is called back to its owner, providing resolution of the strongest cultural dimension of this syndrome (i.e. soul loss).

3.4.2 Herbal remedies in *susto*

In the literature, descriptions of treatments for *susto* concentrate mainly on magico-religious rituals, seldom providing detailed accounts of herbal remedies used. The most in depth description available is from Trotter (1982), in which he found that *yerba aniz* (*Pimpinella anisum* L.; anise) was widely used for *susto*. As well, his study revealed that preparations of *manzanilla* (*Matricaria chamomilla* L.; camomile), *yerba buena* (*Mentha spicata* L.; mint), *hoja de naranjo* (*Citrus aurantium* L.; bitter orange), *albacar* (*Ocimum basilicum* L.; sweet basil), and *toronjil* (*Melissa officinalis* L.; lemon balm) were administered as teas for *susto* in Southern Texas. He argued that these ethnopharmacological treatments probably have physiological effects, since many compounds from the plants could produce such effects, and that *susto* would therefore be mainly physical rather than related to social stress or psychological disorders. However, many of the species mentioned for treating *susto* exhibit activity on the nervous system, and could therefore support the idea that *susto* has a psychological and/or social stress component. For instance, anise has been shown to exhibit relaxant (Boskabady and Ramazani-Assari, 2001) and anticonvulsive proprieties (Pourgholami *et al.*, 1999), along with its recognised antimicrobial and antifungal effects. Moreover, camomile has anxiolytic and mild sedative proprieties (Viola *et al.*, 1995), and sour orange has shown anxiolytic, anticonvulsive and sedative activity (Carvalho-Freitas and Costa, 2002). Lemon balm has also exhibited efficacy in reducing agitation in patients suffering from dementia (Ballard *et al.*, 2002). Hence, neurological and relaxing effects have been demonstrated in most of the plants described by Trotter (1982) for use against *susto*. Consequently, Trotter's description of the use of plant species does not necessarily provide grounds for the exclusion of psychiatric or stress-related components in *susto*, even if some of these plants possess antimicrobial and other non-neurological activities.

The biological activity of the plants used by the healers and identified to species have been understudied, apart from *Pityrogramma calomelanos*, which has shown cytotoxic and anti-tumoral effects (Sukumaran and Kuttan, 1991), and *Scoparia dulcis* which has anti-diabetic (Latha and Pari, 2004) as well as antiviral activity (Hayashi *et al.*, 1988). Of the plants tested for anxiolytic and/or fear suppression activity, no substantial information was available in the literature. Furthermore, when comparing the use of plants for *susto*, none of the plants mentioned by the healers interviewed were found to be used against *susto* in studies from Mexico that include culture-bound syndromes (Frei *et al.*, 1998; Leonti *et al.*, 2001). However, this may be due to cultural variations, since these studies were done with the Popoluca and Zapotec of Mexico. Furthermore, the ethnobotany of the Yucatec Maya of Mexico was studied by the same group of researchers (Heinrich *et al.*, 1998), but the authors did not mention *susto* as a syndrome of the Yucatec (although a previous study with the Yucatec of Belize showed that they recognize *susto*) (Arnason *et al.*, 1980), and merged culture-bound syndromes and gastro-intestinal disorders (Ankli *et al.*, 1999). Therefore, a clear assessment of the use of the plants identified in the present study is arduous.

3.4.3 Ailment clusters

The assessment of herbal treatments common to *susto* and other ailments revealed that many plants are used for ailments associated with symptoms of *susto* as well as for *susto*, which was observed earlier by Trotter (1982). The direct implication of this finding is that plants used to treat *susto* might be selected on the basis that they relieve physiological symptoms of this syndrome. This is in accordance with the perception of some of the healers interviewed that the plants used for *susto* are administered mainly for alleviating its symptoms. Trotter also proposed that plants used might treat *susto* itself and that *susto* would have underlying physical causes, based on the association of treatments between ailments, the biochemical effects of some of the plants administered, and the fact that his study revealed a majority of ethnopharmacological treatments as opposed to magico-religious therapies.

However, the data from the present study also demonstrate a clear association between plants used to treat *susto* and those used for neurological/mental ailments. In fact, the number of plant species used in common between *susto* and neurological/mental disorders is equal to those in common between *susto* and its symptoms. Moreover, the number of mentions of use in common is higher for neurological/mental disorders and *susto*, denoting that more healers use the same plants for *susto* and mental/neurological ailments than for *susto* and ailments associated with its symptoms. This could indicate that these plants act through similar modes of action in neurological/mental disorders and *susto*, offering support for the hypothesis of a psychological (or neurological) basis of *susto*. This is in agreement with the view expressed by some of the healers that believe plants used in *susto* act differently than plants used strictly for its symptoms (e.g. diarrhoea, fever).

Along these lines, most of the plants used for *susto* are also used in treating epilepsy/seizures. Considering our hypothesis that plants used for *susto* have anxiolytic and/or fear suppression activity, the association between anxiety and epilepsy in drug use deserves elaboration. Many pharmaceutical drugs used for epilepsy are also used in treating anxiety (e.g. gabapentin, vigabatrin, tiagabine, lamotrigine, pregabalin), through their action on γ-aminobutyric acid (GABA) metabolism (Ashton and Young, 2003). In addition, investigation of the activity of plant products has revealed that certain plants (e.g. *Sesbania grandiflora*, *Citrus aurantium*, and *Zingiber officinale*) possess both anxiolytic and anticonvulsant properties (Carvalho-Freitas and Costa, 2002; Kasture, *et al.*, 2002; Vishwakarma *et al.*, 2002). Therefore, plants mentioned by the healers could certainly be active both in epilepsy and anxiety, and a plausible mechanism of action of the plant species used to treat *susto* would be through mediation of GABA metabolism, potentially explaining the strong association between *susto* and epilepsy in the treatments used. Nevertheless, other mechanisms could certainly be involved.

3.4.4 Anxiolytic and fear suppression properties of selected plants

Although there were no clear significant effects of treatment in the open field test, rats treated with *L. heterodoxum* at a dose of 200 mg/kg displayed marginal anxiolytic activity. Therefore, *L. heterodoxum* might have anxiolytic activity that was not fully detected in the behavioral assays undertaken. Furthermore, the plants administered did not impair locomotion, since the number of squares crossed in the perimeter was constant between treatments and did not differ significantly from the control animals.

In the elevated plus maze, *A. tetraphyllum* showed anxiolytic activity at 200 mg/kg when compared to the control animals. However, at 50, 150 and 300 mg/kg, this effect was not detected. This might be due to the sensitivity to dosage of this plant, as illustrated in the CER paradigm (see below). The conditions of the tests might also have been different, since they were performed at different times, even though all efforts were made to keep the experimental conditions constant.

In the acoustic startle response test, the animals' general reaction was standard, since startle amplitude increased with the intensity of white noise bursts (Marable and

Maurissen, 2004). In addition, startle amplitude was significantly different before and after drug administration for all treatments, probably due to habituation to the test. However, treatment did not have a significant effect on startle amplitude. Therefore, the plants tested did not influence the animals' responsiveness to the startle reflex.

Different components of anxiety were assessed in the elevated plus maze, open field and acoustic startle paradigms. Therefore, it was expected that plants would not necessarily display similar activity in all the tests. *A. tetraphyllum* showed potent activity in the EPM, but not in the acoustic startle or open field tests, and *L. heterodoxum* showed an anxiolytic trend only in the open field test. This outlines the importance of using different models when studying potential anxiolytic products.

In the assessment of contextual fear, no overall effect of treatment was detected. However, during the first minute of being placed in the conditioning chamber, rats treated with *A. tetraphyllum* (300 mg/kg) showed less freezing than control animals, indicating clear fear suppression to context. It is interesting to note that in the treatment of *susto*, the healers seem to incorporate some elements of the context surrounding the triggering event (by bringing an item from the frightening event to the patient or performing the ritual at the site of the event). Therefore, the fact that *A. tetraphyllum*, the plant most used for *susto* by the healers, showed attenuation of contextual fear might indicate that some treatments used help in supressing fear when the patients are placed in the context of the triggering event, thus aiding the therapy.

The cued fear test showed interesting complementary information. At lower (50 mg/kg) and higher (300 mg/kg) doses of *A. tetraphyllum*, rats behaved similarly to the control animals, but at an intermediate dose (150 mg/kg) they showed more fear of the cue than the control animals, and this trend became more significant with time. Although this trend was not observed in the conditioning chamber, similar effects might have been detected if more extended test periods had been used (20 min vs. 4 min). Hence, the dose is absolutely crucial in obtaining the desired effect with *A. tetraphyllum*, since it showed an inverted "U"-shaped dose-dependence in suppressing fear to the cue. It is of interest that many psychoactive compounds show a similar therapeutic window with doses above or below that window displaying almost opposite or no effects.

In both contextual and cued fear testing, when the animals were tested 24 hours after drug administration, the same pattern emerged as when the animals were tested one hour after gavage. This indicates either that the drugs were still in the animals' circulatory system 24 hours after drug administration, or that the process of reconsolidation was affected, the animals remembering their drug-modified level of fear rather than their initial fear of the tone. Moreover, the rats gavaged with *A. tetraphyllum* at 50 and 300 mg/kg displayed less responsitivity to the cue 24 hours after gavage than one hour after drug administration (at certain time frames the activity was not significant one hour after administration, but significant 24 hours after when compared to controls). Considering that the plants are used for treating *susto*, fear extinction and longer-term effects might be selected by the healers in order to help the patient deal with and forget the initial frightening event.

Among the four high usage plants tested, *A. tetraphyllum* not only had the highest consensus values (for both measures of relative frequency), but was also mentioned as a favourite plant to treat *susto* by all the healers who used it as such. This plant showed potency in two of the tests undertaken. In addition, the plant not used by the healers for *susto* or neurological/mental conditions (*C. diffusa*) did not show significantly different activity compared to the control treatment in any of the assays performed. This observation lends credence to the hypothesis that *susto* has a neurological/psychological component since the plant preferred by the healers showed more anxiolytic and fear suppression activity than a plant selected at random in the same environment. In addition, among the plants tested, *A. tetraphyllum* was the plant most specific to *susto* in its use, indicating that specificity might also be a good predictor of activity.

The data presented show that some plants used by the healers to treat *susto* seem to be anxiolytic and/or suppress fear. Specifically, the plant with highest consensus showed potency for suppressing anxiety and fear, and a second plant showed a trend for being anxiolytic. Although other physiological activities of these herbal remedies could also be present and associated with their use in treating *susto*, their neurological activity suggests an association between anxiety/fear and *susto*.

3.5 SUMMARY AND CONCLUSIONS

One of the plants traditionally used for *susto* (*A. tetraphyllum*) showed anxiolytic and fear suppression activity in the animal tests of anxiety and/or fear, while a second plant (*L. heterodoxum*) showed potential marginal anxiolytic activity. Furthermore, most plants used for *susto* are also used for mental/neurological disorders, and rituals performed involve steps to calm the patient as well as ritual processes focused on the patient facing his initial fright. These results indicate that treatments used for *susto* might have an effect on the nervous system, therefore providing support for the hypothesis that *susto* has a neurological (psychological) component related to anxiety and/or fear. However, the findings that many plants are also common between *susto* and its symptoms and that certain plants have been shown in the literature to have non neurological physiological effects reveal that other activities are probably also present in the plants used. Of note, the suggestion that psychological factors might be involved in *susto* does not necessarily imply the absence of other factors: anxiety/fear could be caused by general stressful situations, social role stress, or could co-occur with physiological illnesses. Moreover, plants used in treating *susto* might act both neurologically and to relieve physical symptoms of *susto*. In fact, many factors probably interact in *susto*, from physical, psychological and social dysfunctions to culture. Klein's (1978) interpretation of *susto* as a disease of adaptation to stress is extremely pertinent in this context, as he proposes a model that combines all three etiological components, and which might prove very useful in the elucidation of folk illnesses related to fright. Further research could concentrate on the possible mechanisms of action of the plants used and other potential biological activities of these plants (e.g. anti-microbial and anti-fungal). Moreover, it would be interesting to conduct interviews with patients to discuss the treatments used (including rituals) and their efficacy.

In investigating folk illnesses and culture-bound syndromes, looking at treatment modalities can be useful in identifying potential underlying factors, especially when symptoms and folk etiologies are difficult to interpret due to cultural discrepancies between researchers and sufferers/traditional practitioners. *Susto* has been studied extensively and still remains an excellent model for examining interactions between culture and illness. Clearly, cultural factors are central in *susto*, both in its etiology and treatment, and physiological causes might also intervene. This study nonetheless strengthens the basis for the inclusion of neurological/psychological factors in the etiology of *susto*.

CHAPTER 4

PRELIMINARY INVESTIGATION OF CULTURALLY IMPORTANT PLANTS: CONSERVATION AND DOMESTICATION

4.1 INTRODUCTION

The use of medicinal plants by rural people is widespread in Belize (Balick and Mendelsohn, 1992) and these plants are often harvested directly from natural environments. Generally, harvesting practices include destructive (e.g. total removal of the bark) and non-destructive (e.g. removal of small pieces of bark) techniques. However, even in instances where these practices are destructive in specific sites, the general practice can be sustainable when the sites are small and sufficient rotation time is allotted (Balick and Mendelsohn, 1992). In this context, it is crucial to assess the sustainability of such practices, both at specific sites and in larger areas where they take place. However, gathering information required to accurately predict population sustainability under pressure from harvesting (e.g. demographic information and direct harvesting impact measurements) is very time-consuming. In lieu of those data, the present study relied on a method developed by Cunningham (2001), which uses easily assessed ecological characteristics to predict the general vulnerability of specific species to harvesting (Cunningham, 2001).

Another important aspect of plant conservation in the context of harvesting by local people is providing measures to reduce harvesting pressure on natural populations, for instance through plant domestication. The idea of a medicinal plant garden was initiated by the Belize Indigenous Training Institute (BITI) and the Q'eqchi' Healers Association (QHA) to promote healing activities and traditional practices through education at the garden, and

50 acres of land were given to BITI and QHA by the Government of Belize in September 2000 for this purpose (Arnason *et al.*, 2004). A garden was established in part of the land given to the association. This garden also serves as an additional source of plants for the healers. However, the healers have faced obstacles with the domestication of plants at this garden: many transplanted species have not survived, and survival is especially difficult during the dry season. Therefore, this study also investigated plant domestication at the garden, by comparing the environment where the plants were collected for transplantation (collection site) and the garden itself.

4.2 METHODOLOGY

4.2.1 Study area

The research took place in the Toledo District (Appendix 1) in March 2004. Environmental measurements were taken in the Jalacte area, a hilly area bordering Guatemala (collection site), and at the medicinal plant garden (Itzama), in Indian Creek.

4.2.2 Plant selection and identification

Eight species were selected for this study. These had high degrees of consensus of use by the healers according to the general ethnobotanical description by Treyvaud Amiguet *et al.* (2004), and had been transplanted at the garden. Consensus (% of healers that use these plants for any ailment) was re-evaluated during interviews, and indicates the cultural importance of these plants (Heinrich, 2000). Information on the use and conservation of the selected species was recorded through interviews with the healers.

The voucher specimens were collected in triplicates, pressed (standard plant presses from Pacific Papers; Washington, USA), mounted (white buffered paper, neutral pH; Pacific Papers) and stored for identification and future reference. The Ministry of Natural Resources of the Government of Belize issued a permit for plant collection in June 2003 (Ref # CD/6-/9/03 (18)). Voucher specimens of each plant collected were distributed to the following herbaria: University of Ottawa (Canada); Q'eqchi' Healers Association (Belize); and *Universidad Nacional* (Costa Rica; *Herbario Juvenal Valerio Rodriguez*). Taxonomic identification was performed by Pablo Sanchez and Luis Poveda from the *Universidad Nacional de Costa Rica*.

4.2.3 Interviews

Interviews were conducted in March 2004 with healers having previously participated in collaborative research (see Chapters 2 and 3). A specimen of each species collected was presented to the healers and they were questioned individually on plant use, traditional ecological knowledge and conservation practices (Appendix 9). These structured interviews lasted from one to two hours and were translated from Q'eqchi' to English by trusted members of the healers' families that were fluent in English.

4.2.4 Vulnerability/resilience to harvesting

A matrix method developed by Cunningham (2001) was used to identify plants with greater vulnerability/resilience to harvesting. Included predictors of resilience or vulnerability were geographic distribution, habitat specificity, growth rate, general resource group (i.e. parts of the plants used), types of uses, reproductive biology (pollination and dispersion), habitat diversity and phylogenetic distinctiveness. For the plants included in the present study, these characteristics were established according to the interviews with the healers, as local knowledge is useful for rapid assessments of plant resources (Dalle and Potvin, 2004). Various literature sources were also used, primarily the *Flora of Guatemala* (Standley *et al.*, 1946-1977), the Missouri Botanical Garden ("Specimen Data Base") and the Montreal Botanical Garden ("The Green Pages"). Although local population size was included as a predictor in the original matrix (Cunningham, 2001), such information was unavailable and was therefore excluded.

4.2.5 Environmental measurements

Ten quadrats (10m x 10m) were set up randomly in the medicinal plant garden (Indian Creek), and five were set up at the collection site (Jalacte). Light measurements

(Sper Scientific 840006 light meter) were taken at each corner and at the centre of each quadrat (~1m from the ground). Relative humidity (Bacharach Sling Psychrometer) and temperature (°C) were taken once at each quadrat, and once in an open area close to the sampling sites (as a reference point). Because measurements were taken on two different days for the two sites, temperature, relative humidity (air) and light measurements were analysed in reference to measurements taken in the open area. Therefore, parameters analysed were Δ temperature, Δ relative humidity and Δ light intensity, which allowed comparisons between environments (Table 4.1). Surface soil samples (top soil, ~ 10 cm deep) were taken at each corner of the quadrats and soil moisture was evaluated by weighing the samples before and after drying (soil moisture (%) = wet weight – dry weight / soil wet weight * 100). The soil was dried on a *comal*, over fire. The time was recorded for each measurement taken. Percent organic matter, pH, as well as macronutrient (N, P, K, Ca, Mg) and micronutrient (B, Mn, Zn, Cu, Fe) content from five surface soil samples per site (from different quadrats, samples selected randomly) were evaluated at Accutest Laboratories (Ottawa, Canada) (Canadian Food Inspection Agency permit #: P-2004-01917). Litter fall was also estimated in both environments by setting four 1m x 1m nets in each site for seven days and weighing dry leaf litter for each net.

4.2.6 Statistical analyses

To test the hypothesis that the two environments were different according to the measurements taken, litter fall, temperature, humidity, and micro- and macronutrients were compared using two-tailed t-tests. A nested ANOVA was used for soil moisture, and a Scheirer-Ray-Hare test was performed for the light measurements. When soil nutrient content was at the detection limit, the limit was retained as the value for the analyses. All analyses were performed with SYSTAT© and statistical significance was set at $p < 0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Environment comparison (collection site vs. garden)

Certain edaphic and abiotic factors were significantly different between the collection site and the medicinal plant garden (Table 4.1). For instance, the difference in humidity between the open reference point and the sampling sites under canopy were greater at the collection site than at the garden, indicating that the collection site provided a more humid environment (in comparison to the reference point). Similarly, the differences in temperature and light intensity were greater at the collection site (negative value), therefore the collection site provided cooler and shadier conditions compared to the garden. Soil moisture (%) was also greater at the collection site. Importantly, humidity, temperature, soil moisture and light intensity were most probably affected by the density of the canopy, which was damaged by the passage of hurricane Iris in 2001. Nonetheless, it seems from earlier observations and discussions with the healers that the garden was more open than the collection site even before the hurricane. Furthermore, organic matter, litter fall and pH were all significantly greater at the collection site. The higher pH at the collection site is probably due to the greater amounts of Ca and Mg present at this site compared to the garden, and might affect the availability of certain soil nutrients, although the differences in acidity are not very marked. These results indicate that certain general conditions are probably more suitable for plant growth at the collection site compared to the garden, especially considering that many of the plants domesticated grow preferably in primary forests (see Table 4.3).

Many nutrient levels were present in significantly greater amounts at the collection site (N, P, Ca, Mg, B), although a few were found in significantly lesser amounts (Fe, Cu) (Table 4.1). However, when comparing these values to values obtained in previous studies in Belize (Arnason *et al.*, 1984), it becomes clear that certain nutrients are probably limiting in both environments. For instance, extractable phosphorus was found to be present at 19 ppm in the previous study, whereas it was found at levels of 1.00 ppm and 2.40 ppm at the garden and collection site, respectively. A similar observation can be made with boron, zinc, iron, manganese and copper. Therefore, plant survival is probably more affected by light intensity, humidity, temperature, and soil moisture than by nutrient levels in the soil.

Table 4.1: Comparison of edaphic and abiotic parameters (environmental parameters) between the collection site and medicinal plant garden (light intensity: n_{garden}=50, $n_{collection\ site}$=25; relative humidity and temperature: n_{garden}=10, $n_{collection\ site}$=5; soil moisture, organic matter, pH, micronutrient and micronutrient content: n_{garden}=5, $n_{collection\ site}$=5; litter fall: n_{garden}=4, $n_{collection\ site}$=4)

Environmental parameters		Garden	Collection Site
Δ Light intensity (Lux) [1]		-8236.7 ± 1940.5	-19061.2 ± 252.7 ***
Δ Relative humidity (air) (%) [2]		0.500 ± 1.00	19.0 ± 1.23 ***
Δ Temperature (°F) [3]		-2.80 ± 0.442	-6.20 ± 0.200 ***
Soil moisture (%) [4]		22.8 ± 1.69	49.4 ± 3.03 ***
Organic matter (%)		7.80 ± 1.30	28.4 ± 5.60 *
Litter fall (g)		4.48 ± 0.725	21.1 ± 2.08 **
pH		6.00 ± 0.20	7.00 ± 0.100 *
Macronutrient content (surface soil)	N (total Kjeldahl Nitrogen; %)	0.400 ± 0.100	1.40 ± 0.200 **
	P ($NaHCO_3$ extractable; ppm)	1.00 ± 0.00	2.40 ± 0.500 *
	K (NH4 acetate extractable; ppm)	256.0 ± 81.5	130.4 ± 9.80
	Ca (NH4 acetate extractable; ppm)	2660.0 ± 584.4	5280.0 ± 141.9 *
	Mg (NH4 acetate extractable; ppm)	700.8 ± 48.4	1226.0 ± 28.4 ***
Micronutrient content (surface soil)	Fe (DTPA extractable; ppm)	32.3 ± 5.7	10.3 ± 3.10 *
	B (ppm)	0.500 ± 0.000	4.80 ±- 1.00 *
	Mn (H_3PO_4 extractable; ppm)	69.8 ± 9.10	68.7 ± 27.6
	Cu (DTPA extractable; ppm)	1.50 ± 0.100	0.700 ± 0.100 *
	Zn (DTPA extractable; ppm)	2.00 ± 0.400	1.60 ± 0.300

All values represent the group mean ± SEM
* $p < 0.05$
** $p \leq 0.001$
*** $p \leq 0.0001$

[1] Δ Light intensity (Lux) = Light intensity at sampling site – Light intensity in open area; although mean values were very different, the non-parametric ANOVA did not detect significant differences; the differences in variances is probably due to greater irregularities in the canopy at the garden compared to the collecting site

[2] Δ Relative humidity (air) (%) = Relative humidity at sampling site – Relative humidity in open area

[3] Δ Temperature (°F) = Temperature in open area – Temperature at sampling site

[4] There was no quadrat effect (calculated with a nested ANOVA)

4.3.2 Vulnerability/resilience to harvesting

4.3.2.1 General harvesting practices

All the healers interviewed stated that they collect most of the medicinal plants from primary forests, mainly based on a perception that these plants have "more force" and are "more ancient" than plants from secondary forests or *guamils* (secondary forests regenerating from abandoned corn fields). This has important implications for conservation, as the harvesting pressure is then principally on primary forests.

The regular harvesting sites of the healers who collaborated in this study are generally distinct, although they sometimes collect specific plants at sites that are different from their regular collection site. Moreover, the individual harvesting sites are relatively small (under 2 km of trail per site; personal observation). Therefore, the general harvesting pressure is probably small, although certain temporary sites might overlap for specific species, as the data collected for the eight selected species seem to indicate (Table 4.3). However, with the participating healers, even when the collection locality is the same the specific locations are different. Furthermore, the healers pay close attention to the health of the plant patches at their collection site: they leave leaves on each individual plant and when they notice that patches of plants are getting smaller (i.e. the plants are collected at a faster rate than they can regenerate), they change patches. According to all the healers interviewed, most plants used are fairly resilient to harvesting, although some healers have identified specific plants that seem to show signs of having difficulties regenerating (e.g. *Philodendron* sp.). According to the healers, forest fragmentation (forest fires, agriculture) and flooding (around river beds) are the most likely threats to certain species.

This suggests that rotation time might be sufficient to allow regeneration. Furthermore, the healers mostly use the leaves of the plants (Treyvaud Amiguet *et al.*, 2004), which are parts of the plants that offer high opportunity for sustainable harvest (Cunningham, 2001). Overall, rotation time, site distribution and size of harvesting sites seem to indicate that the general harvesting practices are sustainable. However, a study of the dispersion and abundance of plant species in the area coupled with a thorough harvesting study would provide much needed information to give clear conclusions as to the sustainability of these harvesting practices.

4.3.2.2 Harvesting of selected culturally important species

Resilience to harvesting was characterized according to the matrix method developed by Cunningham (2001) (Table 4.2). Since the selected species (culturally important species for the participating Q'eqchi' healers) thrive in primary forests, their growth rate was deemed to be relatively slow (Whitmore, 1998) and habitat specificity was assessed with the help of the healers. General resource groups, in this case, were parts of the plants used medicinally by the healers.

The culturally important plants all have a wide distribution, being found in various areas of Central America, some in South America and Southern Mexico. Furthermore, since all of these species are found in the same general environment, the costs and complexity of monitoring and management are the same, and growth rates and habitat specificity are similar. There are nonetheless marked differences between the selected species in their vulnerability/resilience to harvesting according to the included predictors, and it seems that *Piper tuerckeimii* is the most vulnerable according to the available information (Table 4.3). However, a more specific survey with the healers should be undertaken to assess local population sizes, an important predictor of vulnerability/resilience to harvesting. Another factor that was not included in Table 4.3 is the amount of plant material collected. This was estimated through interviews and, although precise measures are not available, it provides a good general indicator of quantities collected. According to this survey, the healers collect between 1-5 handfuls per collection and, apart from *Justicia aurea*, the frequencies of collection are not very high (generally 1-50 times per healer per year).

Table 4.2: Predictors of resilience/vulnerability to harvesting of botanical species (modified from Cunningham, 2001)

Criteria	Resilience to harvesting		
	HIGH	MEDIUM	LOW
1-Geographic Distribution	Wide	Limited	Restricted
2-Habitat Specificity	Broad	Restricted	Highly specific
3-Growth Rate	Rapid	Fairly rapid	Slow
4-General Resource Group	Leaves, flowers, fruit	Exudates, sap, dead wood	Whole plant, bark, roots, apical meristems
5-Single vs. Multiple Use	Single or non-competing	Few, low conflict between uses	Multiple-use species
Reproductive Biology			
6-a) Pollination	Wind, abiotic, asexual	Common biotic (birds, insects)	Highly specific (e.g. specific beetles, bees, bats)
7-b) Dispersal	Wind, water	Common generalists (birds, small mammals)	Large mammals and large birds
8-Costs and Complexity of Monitoring and Management	Vegetation dominated by few species (<5)	Low diversity (e.g. savanna; <10 tree spp./ha)	High diversity systems
9-Phylogenetic Distinctiveness (worldwide)	Large genera	Medium to large genera	Monotypic families or genera

Table 4.3: Description and ranking according to predictors of vulnerability/resilience to harvesting, location of collection sites and consensus values (RF) of selected plants

Species	Criteria 1	2	3	4	5	6	7	8	9	Rank (score out of 27)	Collection site (number of healers) [1]	RF (%) [2]
Justicia aurea Schltdl.	Mexico, Central and South America (North)	Primary forests mainly (sometimes found in secondary forests)	Slow	Leaves and flowers	Non-competing use	Humming-birds, bees, ants	Animals	High diversity systems	420 spp	4 (14)	Big Falls(1), Hicattee(1), Indian Creek(3), Golden Stream(1), Silvercreek (1), San Felipe(1)	100
Anthurium wildenowii Kunth.	Caribbean and South America	Primary forests mainly (sometimes found in secondary forests)	Slow	Leaves	Single use	Insects, bees	Birds	High diversity systems	700-900 spp	4 (14)	Indian Creek(2), Punta Gorda(1), Boom Creek(1), Jalacte(1), Golden Stream(1), Silvercreek(1), Hicattee (1)	83
Begonia heraclifolia Schltdl. & Cham.	Mexico, Central America (North)	Primary forests mainly (sometimes found in secondary forests)	Slow	Leaves	Single use	Insects	Wind, water and/or animals	High diversity systems	1500 spp	5 (16)	Big Falls(1), Indian Creek(3), Laguna(1), Hicattee(1), Jalacte(1)	100
Mollinedia guatemal-ensis Perkins	Mexico (South), Central America (North)	Primary forests mainly (sometimes found in secondary forests)	Slow	Leaves	Single use	Bees	Animals	High diversity systems	95 spp	3 (13)	Big Falls(1), Golden Stream(1), Jalacte(2), Hicattee(1), Indian Creek(1), Otoxa(1), Dolores(1), Creek circle(1)	83

Species	Criteria 1	2	3	4	5	6	7	8	9	Rank (score out of 27)	Collection site (number of healers) [1]	RF (%) [2]
Piper tuerckeimii C.DC. ex Donn. Sm.	Mexico (South), Central America (North)	Primary and secondary forests	Slow	Leaves and/or whole plant	Low conflict	Bats	Bats	High diversity systems	700 spp	1(9)	Indian Creek(4), Hicattee(1), Punta Gorda(1)	100
Peperomia hispidula (Sw.) A. Dietr.	Mexico, Central and South America (North)	Primary forests only	Slow	Leaves	Single use	Bats	Bats	High diversity systems	600 spp	2 (12)	Indian Creek(2), Jalacte(1), Silvercreek (1), Golden Stream(1)	83
Justicia pectoralis Jacq.	Mexico, Central and South America	Secondary forests mainly (sometimes found in primary forests)	Slow	Leaves, flowers, stems	Non-competing use	Humming-birds, bees, ants	Animals	High diversity systems	420 spp	4 (14)	Big Falls(2), Golden Stream(2), Punta Gorda(1)	83
Epiphyllum phyllanthus (L.) Haw. var. strictum (Lem.) Kimnach	Mexico (South), Central and South America	Primary forests only	Slow	Leaves	Single use	Bats, moths	Resprouter, and seed dispersal by animals	High diversity systems	15 spp	3 (13)	Big Falls(1), Jalacte(1), Golden Stream(1), Silvercreek (1), Hicattee(1)	83

[1] Healers can collect a specific plant from one or more environments
[2] Relative frequency of use (Number of healers using the plant / Total number of healers interviewed *100)

4.4 CONCLUSIONS, RECOMMENDATIONS AND FUTURE RESEARCH

The comparison between the garden environment and the collection site shows that light intensity, humidity, temperature and soil moisture are significantly different and probably affect survival of the transplanted species. Certain soil nutrients might also have an effect, but are not likely to affect short term survival. Installing temporary shade cloths, irrigating and misting might improve the conditions at the garden until the canopy recovers from hurricane Iris. Focusing on non-epiphytic plants and plants that grow in secondary forests as well as in primary forests might also be a valuable option. Furthermore, considering the factors limiting growth at the garden and that healers value primary forest plants more than plants from secondary forest, another option to consider would be to set up a garden in the area of the collection site or in accessible areas of primary forest not destroyed by hurricane Iris. Either way, the garden, both as an education tool and to increase access to plants for the healers, is very important to the practice of traditional Q'eqchi' medicine. Furthermore, investing energy in this garden could prove to be very useful in the future, as it could eventually be a way to relieve harvesting pressure from natural populations.

This study also aimed to provide much needed information on the vulnerability/resilience to harvesting of certain culturally important species. As this study was based on set characteristics of selected plants, it should evidently be followed by an extensive study of plant abundance and distribution in the area. Nonetheless, it showed that the general harvesting practices of the healers seem sustainable (small collection sites, rotation, little or no depletion of plant material). However, it would be interesting to map out the specific trails used by the healers for specific species and see if they overlap (and to what extent). It would also be useful to assess the actual amount of plant material collected and frequencies of collection over a long-term period.

CHAPTER 5

GENERAL CONCLUSION

This thesis investigated various aspects of the medical ethnobotany of the Q'eqchi' of Southern Belize. The collaboration between the University of Ottawa and the Q'eqchi' Healers' Association stemmed from a general desire to increase the recognition of traditional Q'eqchi' medicine in Southern Belize. Indeed, the healers have had few apprentices and, considering that most of them are elders, there is a significant challenge for the transmission of their knowledge to the next generation. Therefore, a primary objective of this study was to record part of the extensive body of knowledge they possess.

To our knowledge, this study is the first to focus on Q'eqchi' plant use for neurological/mental disorders, an area of research that is seldom the focus of ethnobotanical and ethnopharmacological investigations (Etkin, 1986). Ethnobotanical survey results showed that the Q'eqchi' healers possess extensive knowledge of plant use for these purposes, far greater than that of culturally similar groups from the same geographical area (Chapter 2). This emphasizes the importance of ethno-psychatric and neurological traditional knowledge of the Q'eqchi' healers, but also indicates that neurological/mental disorders might be under-represented in certain general ethnobotanical studies. Although most of these conditions have strong cultural components in many societies, they should not be overlooked in ethnobotanical studies, since thorough research on these plants could provide new and culturally relevant ways to alleviate mental and neurological disorders in developing countries. Furthermore, this research emphasized the importance of certain plant species and families for such uses by the Q'eqchi', notably the *Piperaceae* and species of the *Pteridophyta*. It was therefore demonstrated that the healers preferentially select certain plant species and families for their use in neurological/mental disorders. As few studies have investigated plant use for neurological/mental disorders specifically, these ethno-directed leads could prove to be very useful in the search for new psychotherapeutic drugs.

The investigation of the etiology of *susto* (Chapter 3) provides an important contribution, using treatment as an indicator of underlying etiological factors. This differs from most previous investigations, which focused mainly on the symptomatology of *susto*, although a few authors have proposed using the effects of treatment procedures to infer the role of stress and/or physiological causes in *susto* (Klein, 1978; Signorini, 1982; Trotter, 1982). However, none have investigated the effects of plants used on the nervous system. This research indicates that some treatments used in *susto* have an effect on the nervous system (i.e. *A. tetraphyllum* showed fear suppression and anxiolytic activity, rituals used are similar to "desensitization to the trauma" and seem to help in claming the patient), and that plants used for *susto* are also frequently used to treat mental/neurological disorders. Therefore, this study suggests that there are underlying neurological factors in *susto* that might be related to anxiety and/or fear. This finding has important implications as the interest in "culture-bound syndromes" and cross-cultural psychology is increasing. Indeed, the decision to categorize folk illnesses into existing psychological classifications or to treat them as separate disorders poses a significant problem in psychology. Therefore, information on etiological factors might provide insights into means to respond to *susto* and *susto* sufferers, as well as refining the perception of folk illnesses.

Conservation is a key aspect of any ethnobotany, as medicinal plants are often extracted from natural environments. This preliminary investigation aimed to provide information on the vulnerability/resilience to harvesting of culturally important species (Chapter 4). Furthermore, domestication of wild species has potential in relieving pressure from natural population, and was the second aim of this chapter. This study showed that although there were differences in soil nutrients, the same nutrients were probably limiting in both environments (garden and collection site). Therefore, other factors probably affected survival to transplantation more significantly (e.g. soil moisture, humidity, temperature, light intensity). To counter the low survival rates, certain measures discussed in Chapter 4 could be undertaken (e.g. shade cloths, irrigation). Moreover, since the healers value plants from primary forests and mountainous areas more than from secondary forests (mainly for cultural reasons), it could be beneficial to set up another garden in the area of the collection site. Generally, medicinal plant gardens can help in relieving pressure from natural environments and are useful tools for education, which makes them an important component of conservation in the context of ethnobotany. Although the information provided is limited, it is a first step in the study of the domestication of these plants and of the potential risks associated with harvesting.

Overall, this research shows that the Q'eqchi' healers possess great ethnomedicinal knowledge for neurological/mental disorders. Traditional medicine is indubitably important for the physical, mental and cultural health of the Q'eqchi' and recognition of the value of traditional healers is crucial in this context. Generally, culturally important plants should be the focus of future research, in the study of their biological activity, status and conservation.

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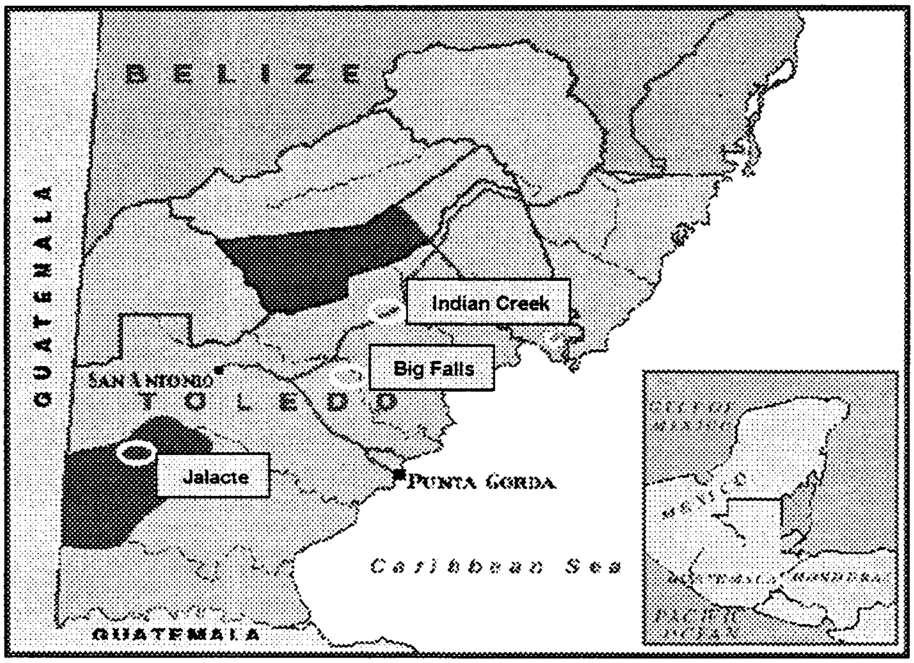
Appendix 1: Map outlining sampling sites in the Toledo District, Southern Belize (modified from www.inuitcircumpolar.com; produced by Strata360; Jalacte: 16°15'14"N/ 89°10'43"W; Big Falls: 16°15'14"N/88°52'85"W; Indian Creek: 16°20'13"N/88°47'69"W).



Appendix 2: General questionnaire on neurological and mental health disorders

Name:

Type of healer:

Age and sex:

Years of practice as a healer:

Village:

1. Do you treat [name of ailment]?
2. If yes, with plants, spiritual ceremonies, or both?
3. Which symptoms do you associate with this ailment?
4. What causes this ailment?
5. If treated with plants, which plants do you use for [name of ailment]?
6. Are they used in combination with other plants?
7. Which parts of the plant(s) do you use?
8. How do you prepare the remedy (ex: boiled in water)?
9. How much [plant part(s) used] do you use?
10. How is the remedy administered?
11. How much of the preparation does the patient have to take?
12. How many times a day and for how long?
13. Are there any restrictions for the patient during treatment?
14. Is this herbal remedy the one you prefer for treating [name of ailment]?

Appendix 3 (CONFIDENTIAL): Summary information on plants used by Q'eqchi' healers (n=6) to treat recognized neurological/mental disorders (vegetation types were identified according to the *Checklist of the Vascular Plants of Belize* (Balick *et al.*, 2000) and/or personal observations)

Plant family	Botanical name	Vernacular name	Translation	V #[1]	Vegetation type	RF (%)[2]	Ailments treated	Plant parts used	Preparation	Admistration
Acanthaceae	*Aphelandra* sp.	Roq sosol	Vulture's foot	48	Herb or sub-shrub	50	Madness, headache	Leaves	Decoction, maceration in cold water, burning	Oral, topical
Acanthaceae	*Aphelandra* sp.	No name	NA	90	Shrub	33.33	Epilepsy/seizures, headache	Leaves	Maceration in cold water	NA
Acanthaceae	*Justicia albobracteata* Leonard	Xna kejen	Mother's herb	13	Shrub	16.67	Epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Acanthaceae	*Justicia aurea* Schltdl.	Saxjolom chacmut	Feather of Curasow's head	39	Shrub	100	Headache, madness, epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Acanthaceae	*Justicia pectoralis* Jacq.	X'cuy i kok or Santa Maria kejen	Santa Maria's herb	14	Balsam vine	33.33	Epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Acanthaceae	NA	Saxjolom chacmut	Feather of Curasow's head	45	Shrub	50	Headache, madness, epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Acanthaceae	NA	Saxjolom chacmut	Feather of Curasow's head	60	Shrub	83.33	Headache, madness, epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Adiantaceae	*Adiantum latifolium* Lam.	No name	NA	68	Fern	100	Epilepsy/seizures, headache, *susto* (fright), depression, madness	Leaves	Maceration in cold water	Oral, topical
Adiantaceae	*Adiantum princeps* T. Moore	Colantra pim	Coriander plant	22	Fern	83.33	Epilepsy/seizures, *susto* (fright)	Leaves	Maceration in cold water, burning	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
Adiantaceae	*Adiantum tetraphyllum* Humb. & Bonpl. ex Willd.	Roq chit cuan	Black-bird's foot	54	Fern	83.33	Epilepsy/seizures, *susto* (fright)	Leaves	Maceration in cold water	Oral, topical
Adiantaceae	*Adiantum wilsonii* Hook.	Ruj i rak' aj tza	Devil's tongue	7	Fern	83.33	Epilepsy/seizures, headache, madness, insomnia	Leaves	Maceration in cold water	Oral, topical
Adiantaceae	*Pityrogramma calomelanos* (L.) Link	Roq chit cuan	Black-bird's foot	66	Fern	16.67	Epilepsy/seizures, *susto* (fright)	Leaves	Maceration in cold water	Oral, topical
Adiantaceae	*Pteris plumula* Desv.	No name	NA	107	Fern	33.33	*Susto* (fright), depression	Leaves	Maceration in cold water	Oral, topical
Apocynaceae	NA	No name	NA	101	Vine	33.33	Epilepsy/seizures	Leaves	Decoction, maceration in cold water	Oral, topical
Araceae	*Anthurium* sp.	Xtye pu'u	Quam's tail	58	Herb	16,67	Madness, headache	Leaves	Maceration in cold water	Oral, topical
Araceae	*Philodendron* sp.	Rubelsa' i' xul	A snake's stomach	11	Herb	16.67	Epilepsy/seizures	Leaves	Maceration	Oral
Araceae	*Philodendron* sp.	Par'i'pim	Skunk plant	47	Herb	16.67	Madness, headache	Leaves	Maceration in cold water	Oral, topical
Araceae	*Philodendron* sp.	Ma'raq'	NA	78	Vine	16.67	Numbness	Leaves	Decoction	Topical
Araceae	*Syngonium* sp	Ruj i ruk' aj tza or Ruk maus	Devil's finger	2	Vine	16.67	Epilepsy/seizures, madness	Leaves	Maceration in cold water	Oral, topical
Araceae	*Anthurium willdenowii* Kunth	Xchich maus	Devil's sword	94	Herb	33.33	Madness, headache	Leaves	Maceration in cold water, decoction	Oral, topical
Aristolochiaceae	*Aristolochia grandiflora* Sw.	Patz pim	Duck plant	20	Vine	66.67	Epilepsy/seizures, madness, headache	Leaves	Decoction, maceration in cold water, burning	Oral, topical
Aristolochiaceae	*Aristolochia* sp.	Xa'ab maus	Devil's feet	42	Vine	16.67	Madness	Leaves	Maceration in cold water	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
Aspleniaceae	*Bolbitis pergamentacea* (Maxon) Ching	Quax kay pim	Madness plant	53	Fern	66.67	Madness	Leaves	Decoction, maceration in cold water, burning	Oral, topical
Liliaceae	*Cordyline* sp.	Cuxtzi	Marco's dog	92	Shrub	33.33	Numbness	Leaves	Decoction	Topical
Asteraceae	*Liabum* sp.	Saq sa'ab	Whitish plant	86	Herb or shrub	16.67	Headache, madness	Leaves	Maceration in cold water	Oral, topical
Asteraceae	*Pluchea* sp.	Mai pim	Pain plant	88	Herb or sub-shrub	16.67	Headache	Leaves	Decoction, maceration in cold water	Oral, topical
Asteraceae	*Porophyllum ruderale* (Jacq.) Cass.	Sosol pim	Vulture's plant	67	Herb or sub-shrub	33.33	Headache, madness, epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Asteraceae	*Pseudobaccharis trinervis* (Lam.) V. M. Badillo	Tzelek za'ak	Grasshopper's legs	40	Herb or sub-shrub	16.67	Epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Asteraceae	NA	Juruch aj pak	NA	41	Vine	33.33	Epilepsy/seizures	Leaves	Decoction, maceration in cold water	Oral, topical
Bixaceae	*Bixa orellana* L.	Xaq xa yaw	Annato	84	Tree	16.67	Madness	Leaves	Decoction	Oral, topical
Boraginaceae	*Tournefortia* sp.	Kan'bolie kejen	NA	64	Vine	16.67	Headache	Leaves	Decoction, maceration in cold water	Oral, topical
Buddlejaceae	*Buddleja americana* L.	Saq rix pim	Plant with white under leaf	44	Shrub or tree	16.67	Headache, madness	Leaves	Maceration in cold water, decoction	Oral, topical
Combretaceae	*Combretum* sp.	Qa'xa'an caham	Boolywood vine	79	Herb	16.67	*Susto* (fright)	Leaves	Decoction	Oral
Crassulaceae	*Kalanchoe pinnata* (Lam.) Pers.	No name	NA	57	Herb	16.67	Headache	Leaves	Maceration in cold water	Topical
Cucurbitaceae	*Melothria pendula* L.	Sandia cho	Mice watermelon	63	Vine	16.67	Headache	Leaves	Maceration in cold water	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
Davalliaceae	*Nephrolepis biserrata* (Sw.) Schott	Xqu'uq mo'coch	Cohune palm vine	27	Fern	33.33	Headache	Leaves	Maceration in cold water	Oral, topical
Fabaceae-Mimosoideae	*Mimosa pudica* L.	Quare kix	Sleeping prickle	55	Herb or sub-shrub	83.33	Insomnia, depression, madness, stress	Whole plant, Leaves	Decoction, maceration in cold water, burning	Oral, topical
Fabaceae-Papilionaoideae	*Machaerium riparium* Brandegee	Pur caham	Snail vine	43	Tree, shrub or liana (spiny)	33.33	Madness	Leaves	Maceration in cold water	Oral, topical
Fabaceae-Caesalpinioideae	*Senna* sp.	Kara'bansi che	A bean's tree	104	Shrub	16.67	Madness	Leaves	Decoction, maceration in cold water	Oral, topical
Gesneriaceae	*Columnea* sp.	Kaki pim	Red plant	87	Shrub	83.33	Headache	Leaves	Maceration in cold water	Oral, topical
Gesneriaceae	NA	Baknel pim	Snake's plant	69	Herb	50	Headache, epilepsy	Leaves	Maceration in cold water	Oral, topical
Lamiaceae	*Hyptis* sp.	Xtye caway	Horse's tail	56	Herb	16.67	Insomnia, headache, stress	NA	NA	NA
Loranthaceae	*Oryctanthus* sp	Ne'ba pim	Poor plant	106	Shrub	16.67	Madness	Leaves	Decoction	Oral, topical
Malvaceae	*Hibiscus rosa-sinensis* L.	Hibiscus	NA	98	Shrub	16.67	Headache	Leaves	Maceration in cold water	Topical
Marantaceae	*Calathea* sp.	Koq mush	Small Waha leaf	46	Herb	16.67	Madness	Leaves	Maceration in cold water	Oral, topical
Melastomataceae	*Adelobotrys adscendens* (Sw.) Triana	Chunac kejen	Momi seed herb	52	Liana	33.33	Epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
Meliaceae	*Guarea grandifolia* DC.	Bolb	NA	89	Tree	16.67	Susto (fright)	Bark	Decoction	Oral
Monimiaceae	*Mollinedia* sp.	Saki pim	White plant	6	Herb	16.67	Epilepsy/seizures	Leaves	Maceration in cold water, Decoction	Oral, topical
Monimiaceae	*Siparuna* sp.	Co moj che	NA	77	Shrub or small tree	16.67	Headache	Leaves	Maceration in cold water	Topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
Monimiaceae	*Siparuna* sp.	Roq xa'an	Old lady's foot	83	Shrub or sub-shrub	16.67	Headache	Leaves	Maceration in cold water	Topical
Passifloraceae	*Passiflora* sp.	Tu' kej	Deer's breast	91	Vine	33.33	Headache	Leaves	Maceration in cold water	Oral, topical
Phytolaccaceae	*Petiveria alliacea* L.	Par'i'pim	Skunk plant	30	Herb	50	Epilepsy/seizures, madness, headache	Leaves	Decoction, maceration in cold water, burning	Oral, topical
Piperaceae	*Peperomia* sp.	Xcua'aj aw chan	Boa constrictor's food	93	Herb or sub-shrub	33.33	Epilepsy/seizures, headache	Leaves	Maceration in cold water	Oral, topical
Piperaceae	*Peperomia* sp.	No name	NA	97	Shrub	33.33	Epilepsy/seizures, headache, depression, madness	Leaves	Maceration in cold water	Oral
Piperaceae	*Piper amalago* L.	Kan pom or Pu'chuch	Yellow incense or fat jointed	100	Shrub or small tree	16.67	Epilepsy/seizures	NA	NA	NA
Piperaceae	*Piper auritum* Kunth	U'bel	NA	65	Herb or sub-shrub	16.67	Numbness	Leaves	Maceration in cold water, decoction	Topical
Piperaceae	*Piper* sp.	Kan pom	Yellow incense	26	Shrub	50	Epilepsy/seizures, headache, madness, insomnia	Leaves	Maceration in cold water	Oral, topical
Piperaceae	*Piper* sp.	Pu'chuch	Fat jointed	50	Shrub	50	Epilepsy/seizures, madness, headache, *susto* (fright)	Leaves	Maceration in cold water	Oral, topical
Piperaceae	*Piper* sp.	Cux sawi kejen	Marco Sawi's herb	80	Shrub	83.33	*Susto* (fright), madness, epilepsy/seizures	Leaves	Decoction, maceration in cold water	Oral, topical
Piperaceae	*Piper* sp.	Mai pim	Pain herb	95	Herb or sub-shrub	33.33	Epilepsy/seizures, headache	Leaves	Maceration in cold water	Oral, topical
Piperaceae	*Piper* sp.	Pu'chuch	Fat jointed	99	Shrub	50	Epilepsy/seizures, headache, *susto* (fright)	Leaves	Decoction, maceration in cold water, burning	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
Piperaceae	*Piper* sp.	Pu'chuch	Fat jointed	51	Shrub	33.33	Epilepsy/seizures, headache	NA	NA	NA
Rubiaceae	*Gonzalagunia rosea* Standl.	Tzu'ul che	Mountain tree	33	Shrub or small tree	50	Epilepsy/seizures	Leaves	Maceration in cold water, decoction	Oral, topical
Rubiaceae	*Hamelia patens* Jacq.	Chaj' mash	Monkey's ash	103	Shrub or small tree	16.67	Headache	Leaves	Decoction, maceration in cold water	Oral, topical
Rubiaceae	*Psychotria poeppigiana* Müll. Arg.	Saxjolom chilan	Chicken's comb (feather of head)	24	Shrub	16.67	Headache	Leaves	Maceration in cold water	Oral, topical
Rubiaceae	*Psychotria tenuifolia* Sw.	Colaras	Vine	12	Shrub	50	Epilepsy/seizures	Leaves	Decoction, maceration in cold water	Oral, topical
Rubiaceae	*Sabicea* sp.	Colaras	Vine	1	Vine	16.67	Headache	Leaves	Maceration in cold water	Oral, topical
Rubiaceae	NA	Xaq ka pe	Coffee	85	Shrub or small tree	16.67	Madness	Leaves	Decoction	Oral, topical
Rutaceae	*Citrus limetta* Risso	Xaq i'ch'in	Orange	105	Tree	16.67	Madness	Leaves	Decoction	Oral, topical
Rutaceae	*Citrus* sp.	Lamux	Lime	75	Tree	16.67	Madness	Leaves	Decoction	Oral, topical
Schizaeaceae	*Lygodium heterodoxum* Kunze	Ruxb'i'kaak	Thunder vine	25	Fern	83.33	Epilepsy/seizures, *susto* (fright), headache	Leaves	Maceration in cold water, burning	Oral, topical
Schizaeaceae	*Lygodium venustum* Sw.	Ruxb'i'kaak	Thunder vine	61	Fern	83.33	Epilepsy/seizures, *susto* (fright), headache, madness	Leaves	Maceration in cold water, burning	Oral, topical
Scrophulariaceae	*Scoparia dulcis* L.	Colantra pim	Coriander plant	73	Herb or sub-shrub	16.67	*Susto* (fright)	Leaves	Maceration in cold water	Oral, topical
Selaginellaceae	*Selaginella aff. stellata Spring*	No name	NA	35	Herb	66.67	Epilepsy/seizures, *susto* (fright), madness	Leaves	Decoction, maceration in cold water, burning	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V #[1]	Vegetation type	RF (%)[2]	Ailments treated	Plant parts used	Preparation	Admistration
Selaginellaceae	*Selaginella* sp.	No name	NA	34	Herb	50	Epilepsy/seizures, headache	Leaves	Maceration in cold water	Oral, topical
Solanaceae	*Solanum* sp.	Ik kejen	Pepper herb	49	Herb or sub-shrub	33.33	Epilepsy/seizures, numbness	Leaves	Decoction, maceration in cold water	Oral, topical
Solanaceae	*Solanum* sp.	No name	NA	76	Shrub	16.67	Headache, numbness, madness	Leaves	Decoction, maceration in cold water	Oral, topical
Solanaceae	*Solanum* sp.	Sajom te	NA	81	Herb	50	Epilepsy/seizures	Leaves	Maceration in cold water, decoction	Oral, topical
Verbenaceae	*Aegiphyla* sp.	No name	NA	36	Shrub	16.67	Epilepsy/seizures	NA	NA	NA
Verbenaceae	*Cornutia pyramidata* L.	Job lo te	Monkey cap tree	59	Shrub or small tree	16.67	Headache	Leaves	Maceration in cold water	Oral, topical
Verbenaceae	*Lippia* sp.	Tu' lush pim	Dragonfly plant	102	Herb or sub-shrub	33.33	Headache	Leaves	Maceration in cold water, decoction	Oral, topical
Verbenaceae	*Stachytarpheta frantzii* Pol.	Xtye aj pak	Lizard's tail	21	Herb	50	Epilepsy/seizures, headache	Leaves	Maceration in cold water	Oral, topical
NA	NA	Kaki pim	Red plant	NA	Shrub	33.33	Epilepsy/seizures, headache	Leaves	Decoction, maceration in cold water	Oral, topical
NA	NA	Kehal kejen	Cold herb	NA	NA	16.67	Epilepsy/seizures, headache	NA	NA	NA
NA	NA	Pepem pim	Butterfly plant	NA	Vine	16,67	Epilepsy/seizures, headache	Leaves	Maceration in cold water	Oral, topical
NA	NA	Pu'chuch	Fat jointed	NA	NA	16,67	Numbness	Leaves	Decoction	Oral, topical
NA	NA	Pu'chuch	Fat jointed	NA	Shrub	16,67	Epilepsy/seizures, *susto* (fright)	Leaves	Decoction	Oral, topical
NA	NA	Pu'chuch re'tz'ul	NA	NA	NA	16,67	Epilepsy/seizures	Leaves	Maceration in cold water	Oral, topical
NA	NA	Rax' ik che'	Green pepper tree	NA	NA	33.33	Headache, depression	Leaves	Maceration in cold water	Oral, topical

Plant family	Botanical name	Vernacular name	Translation	V # [1]	Vegetation type	RF (%) [2]	Ailments treated	Plant parts used	Preparation	Admistration
NA	NA	Rubelsa' i' xul	A snake's stomach	NA	NA	16,67	Epilepsy/seizures	Leaves	Decoction	Oral, topical
NA	NA	Sa jolom masa'an	NA	NA	NA	16,67	Headache	Leaves	Maceration in cold water	Oral, topical
NA	NA	Saki pim	White plant	NA	Vine	16,67	Headache	Leaves	Maceration in cold water	Topical
NA	NA	Xcua'i'xul	Snake's food	NA	NA	16,67	Headache	Leaves	Maceration in cold water	Oral, topical

[2] Voucher number
[1] Relative frequency:
RF = (No. healers using species for any neurological/mental ailment) / (Tot. no. healers interviewed) * 100

Appendix 4: Complementary questionnaire on *susto*

Name:

Type of healer:

Age and sex:

Years of practice as a healer:

Village:

1. Are there different types of *susto*?
2. Could you describe the general treatment procedure for *susto*?
3. Are the plant remedies and plant species different for *susto* than for treating its symptoms (e.g. diarrhea)?
4. Are the herbal treatments important in treating the patient? Are the other treatments provided also important?

Appendix 5: Comparison of four selected high usage plants for *susto* and the vehicle control (2% evaporated milk; Nestle Carnation) in the open field paradigm, after a one hour post-drug interval (n=10-11). *A. tetraphyllum*, *L. heterodoxum*, *A. latifolium*, and *S. aff. stellata* were tested at a concentration of 200 mg/kg body weight, in December 2003.

Treatment	# S.C.	T.C.	# S.P.	T.P.
Vehicle control (n = 11)	7.00 ± 1.22	7.61 ± 1.66	115.09 ± 7.07	292.24 ± 1.67
Commelina diffusa (plant control) (n = 10)	9.40 ± 1.67	9.90 ± 2.16	126.00 ± 6.25	289.98 ± 2.15
Adiantum tetraphyllum (n = 10)	5.90 ± 1.89	5.779 ± 2.12	121.20 ± 5.98	294.10 ± 2.13
Lygodium heterodoxum (n = 10)	11.08 ± 3.05	11.11 ± 2.46	126.50 ± 6.75	288.62 ± 2.46
Adiantum latifolium (n = 10)	7.40 ± 1.57	8.37 ± 2.21	120.70 ± 3.18	291.47 ± 2.22
Selaginella aff. stellata (n = 10)	9.70 ± 2.24	9.98 ± 2.22	117.10 ± 7.76	289.86 ± 2.21

All values represent the group mean ± SEM (n = 10-11)

S.C. – number of squares crossed in the central zone, T.C. – time spent in the central zone, C.E. – number of center entries, # S.P. – number of squares crossed in the peripheral zone, T.P. – time spent in the peripheral zone

Appendix 6: Activity of *A. tetraphyllum* and *L. heterodoxum compared to that of* the vehicle control (2% evaporated milk; Nestle Carnation) in the open field paradigm, after a one hour post-drug interval (n=11-14). *A. tetraphyllum* was tested at a concentration of 50, 150 and 300 mg/kg body weight, and *L. heterodoxum* at 300 mg/kg body weight, in April 2004.

Treatment	# S.C.	T.C.	# S.P.	T.P.
Vehicle control (n = 11)	10.36 ± 2.59	9.48 ± 2.56	125.64 ± 8.14	290.29 ± 2.56
Adiantum tetraphyllum ([50mg/kg]; n = 14)	5.29 ± 1.33	6.89 ± 1.74	113.36 ± 8.36	292.61 ± 1.91
Adiantum tetraphyllum ([150mg/kg]; n = 14)	6.93 ± 1.98	6.75 ± 1.58	102.57 ± 8.24	292.70 ± 1.57
Adiantum tetraphyllum ([300mg/kg]; n = 10)	5.00 ± 1.83	6.90 ± 2.32	113.50 ± 10.29	292.89 ± 2.31
Lygodium heterodoxum ([300mg/kg]; n = 12)	4.83 ± 1.48	3.96 ± 1.07	119.25 ± 8.26	295.74 ± 1.07

All values represent the group mean ± SEM (n = 10-14)

S.C. –number of squares crossed in the central zone, T.C. – time spent in the central zone, # S.P. – number of squares crossed in the peripheral zone, T.P. – time spent in the peripheral zone

Appendix 7: Comparison of four selected high usage plants for *susto* and the vehicle control (2% evaporated milk; Nestle Carnation) in the elevated plus maze paradigm, after a one hour post-drug interval (n=9-11). *A. tetraphyllum*, *L. heterodoxum*, *A. latifolium*, and *S. aff. stellata* were tested at a concentration of 200 mg/kg body weight, in December 2003.

Treatment	# O.A.E.	T.O.A.	# C.A.E.	T.C.A.	# U.P.H.D.	% O.A.E.	% T.O.A.
Vehicle control (n = 11)	5.091 ± 0.78	77.22 ± 13.95	7.91 ± 0.81	135.01 ± 20.02	14.09 ± 2.72	35.30 ± 4.88	25.74 ± 4.65
Commelina diffusa (plant control) (n = 10)	4.10 ± 0.81	60.12 ± 11.96	8.40 ± 0.56	140.88 ± 12.12	12.10 ± 2.64	30.40 ± 4.74	20.04 ± 3.99
Adiantum tetraphyllum (n = 9)	6.44 ± 0.71	112.97 ± 10.78 *	7.56 ± 0.29	115.82 ± 12.64	22.67 ± 1.28 *	45.15 ± 2.95	37.66 ± 3.59 *
Lygodium heterodoxum (n = 9)	6.00 ± 0.76	96.30 ± 13.22	7.89 ± 0.61	122.28 ± 9.84	16.89 ± 3.09	42.73 ± 3.91	32.10 ± 4.41
Adiantum latifolium (n = 9)	5.11 ± 0.56	93.11 ± 8.99	7.78 ± 0.47	124.51 ± 11.07	16.67 ± 1.78	39.17 ± 3.31	31.04 ± 3.00
Selaginella aff. stellata (n = 10)	3.20 ± 0.81	46.02 ± 11.22	8.30 ± 0.42	167.27 ± 17.21	9.10 ± 2.45	24.29 ± 5.18	15.34 ± 3.74

All values represent the group mean ± SEM (n = 9-11)
* p-value < 0.05 vs. control: one-way ANOVAs, Fisher's LSD (multiple comparison)

O.A.E – number of open arm entries, T.O.A. – time spent in the open arms, # C.A.E. – number of closed arm entries, T.C.A – time spent in the closed arms, # U.P.H.D. – number of unprotected head dips, % O.A.E. – percentage of open arm entries, % T.O. – percentage of time spent in the open arms

Appendix 8: Activity of *A. tetraphyllum* and *L. heterodoxum* compared to that of the vehicle control (2% evaporated milk; Nestle Carnation) in the elevated plus maze paradigm, after a one hour post-drug interval (n=12-14). *A. tetraphyllum* was tested at a concentration of 50, 150 and 300 mg/kg body weight, and *L. heterodoxum* at 300 mg/kg body weight, in April 2004.

Treatment	# O.A.E.	T.O.A.	# C.A.E.	T.C.A.	# U.P.H.D.	% O.A.E.	% T.O.A.
Vehicle control (n = 14)	3.36 ± 0.80	52.64 ± 13.95	8.00 ± 0.73	203.32 ± 17.28	3.57 ± 1.16	25.10 ± 5.26	17.55 ± 4.65
Adiantum tetraphyllum [50mg/kg] (n = 14)	3.71 ± 0.72	71.62 ± 14.15	8.07 ± 0.54	193.09 ± 15.14	5.21 ± 1.03	29.06 ± 4.72	23.87 ± 4.72
Adiantum tetraphyllum [150mg/kg] (n =14)	4.00 ± 0.67	67.94 ± 13.08	6.57 ± 0.72	185.61 ± 17.49	5.57 ± 1.05	31.91 ± 4.77	22.65 ± 4.36
Adiantum tetraphyllum [300mg/kg] (n = 12)	5.00 ± 0.95	74.26 ± 13.99	8.25 ± 0.52	178.53 ± 17.32	5.42 ± 1.25	33.37 ± 5.01	24.75 ± 4.66
Lygodium heterodoxum [300mg/kg] (n = 12)	4.58 ± 0.82	69.61 ± 12.91	8.83 ± 0.65	186.56 ± 14.90	5.17 ± 1.52	31.52 ± 4.69	23.20 ± 4.30

All values represent the group mean ± SEM (n = 12-14)

O.A.E – number of open arm entries, T.O.A. – time spent in the open arms, # C.A.E. – number of closed arm entries, T.C.A – time spent in the closed arms, # U.P.H.D. – number of unprotected head dips, % O.A.E. – percentage of open arm entries, % T.O. – percentage of time spent in the open arms

Appendix 9: Questionnaire on plant use, traditional ecological knowledge and conservation practices

Name:

Type of healer:

Age and sex:

Years of practice as a healer:

Village:

1. Where can you find this plant?
2. Is it (a) abundant, (b) fairly common, (c) scarce?
3. Does it grow in very specific habitats (e.g. *milpas*, roadside, primary forests)?
4. Could you find it more easily before the passage of hurricane Iris?
5. Which plant parts do you use?
6. Which ailments do you use this plant for?
7. How much plant material do you collect every time you collect this plant?
8. How many people have you treated with this plant in the past year (average)?
9. How much plant material do you use for each patient (average)?
10. Do you always collect the plants in the same areas or patches?
11. When you collect medicinal plants, do you practice certain collection methods that help to conserve the plants?
12. Do you generally collect medicinal plants from primary or secondary forests?
13. Do you think the survival of some of the plants discussed in the area could be endangered (short or long term)?

Appendix 10: Ethnobotanical investigation of the anxiolytic plant "*sin susto*" (by J.T. Arnason)

Following previous research at the University of Ottawa on the *Marcgraviaceae* species collected in Costa Rica which showed anxiolytic activity in animal trials, an ethnobotanical enquiry was made with the Kekchi Healers Association during a field trip to Belize by John Thor Arnason in January 2004. Nine of the healers were present at the meeting and were shown a herbarium specimen of *Souroubea sympetala*, and asked to describe the traditional use of this plant. The following responses were recorded:

1) Four of the healers were very familiar with this plant and treat patients with it;

2) Three healers used the leaf for witchcraft, and their name for the plant is *Holobob kahim* or "vine of the monkey top tree". The dose administered is 20-30 g/litre of water, boiled for 10 minutes (we weighed a traditionally used amount). Patients are advised to drink 1 cup, twice a day, for three days. There are no reported adverse effects or cautions. Patients affected by witchcraft were reported to be "sad". According to the healers, the patients do not talk a lot and become thin and withdrawn. This appears to be a traditional medical description of depression or anxiety. Response to the plant is reported to be very good and patients recover quickly. Withcraft is a delicate subject and it was clear that this information would not be volunteered without direct enquiry. Furthermore, it seems that the plant is frequent and commonly used;

3) One healer used the bark for treating ulcers and dysentery, with good results.