Journal of

Medicinal Plant Research

Volume 11 Number 14, 10 April, 2017

ISSN 1996-0875

Academic Journals

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Journal of Medicinal Plants Research

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academicJournals

Vol. 11(14), pp. 264-274, 10 April, 2017
DOI: 10.5897/JMPR2017.6329
Article Number: 8F4996C63754
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Specialization in ethnomedicinal plant knowledge among herbalists in the forest region of Rivercess County, Liberia

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Received 3 January, 2017; Accepted 29 March, 2017

The ethnomedicinal uses of plants reported by 22 herbalists in the Rivercess County of Liberia show a rich knowledge of medicinal plant usage for both common and less common health problems. We set out with the objectives of documenting the ethnomedicinal plant knowledge and assessing gender differences in the use of the plants by male and female herbalists. Through semi-structured interviews conducted with herbalists, a total of 112 species belonging to 52 families in 93 genera were recorded to be in use. Seven plant families were known to account for 43.9% of the total number of species utilized including *Annonaceae, Apocynaceae, Costaceae, Rubiaceae, Euphorbiaceae, Fabaceae* and *Verbenaceae*. Traditional herbalists comprising male (64%) and female (36%) possessed extensive knowledge of the use of these plants, with male and female herbalists showing specializations in several categories of health problems based on their knowledge of medicinal plants. Female herbalists possessed a slightly higher knowledge of ethnomedicinal plants than male herbalists. Many ailments were reported with a large number of the plants utilized for malaria, snakebites, ulcer, spiritual/witchcraft, infection and diarrhoea. New and unrecorded ethnomedicinal uses for several plants were documented, including *Tetraberlinia tubmaniana* J. Léonard, *Delpydora gracilis* A.Chev., *Campylospermum subcordatum* (Stapf) Farron, *Cyathula prostrata* Blume, *Heisteria parvifolia* Sm., *Keetia rufivillosa* (Robyns ex Hutch. & Dalz.) Bridson, *Pavetta sonjae* W.D.Hawth., *Chrysophyllum pentagonocarpum* Engl. & K.Krause, *Placodiscus pseudostipularis* Radlk, and the naturalized plant, *Caladium bicolor* (Aiton) Vent. Further documentation of this knowledge is recommended, with the goal of assessing gender differences in the use of medicinal plants in Liberia.

Key words: Ethnomedicine, medicinal plants, herbalists, remedies, Liberia.

INTRODUCTION

Liberia is home to one of the richest flora in the Upper Guinean region of West Africa, with initial exploration dating back to the 1800s, beginning with collections made by European and American botanists like Bunting,

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Dinklage, Harley, Johnston, Leeuwenberg, Voorhoeve, etc. In recent times, additional documentations of the flora were carried out in three National Forest Reserves, with some 548 species recorded, including records of three new species to science (Jongkind, 2007). It has been estimated that some 2,300 species of vascular plants occur in Liberia, although given the apparent location of two centers of endemism in the country, the actual number of plant species occurring in the country might be higher (Poorter et al., 2004).

This rich diversity of plants also features extensively in the cultural heritage of the local inhabitants, and has been a source of herbal remedies for several health problems faced by the population. However, scientific documentations of the ethnomedicinal uses of the plants have been sporadic, and very little exist on the local uses to which these plants are put. Early documentations of this knowledge date back to the early 19th Century when a medical doctor stationed in the interior of the country documented the ethnomedicinal uses of over 200 medicinal plants among the Mano of Nimba County (Harley, 1941). Similar documentations of plant use among the Mano tribe were conducted by Adam and Adam (1970), and some four decades later, the local names and uses of over 250 important plant species in 7 communities was carried out by Marshall and Hawthorne (2013). In the Salala District of central Liberia, Orr (1968) conducted an anthropological examination of medical practices and belief systems of the Kpelle tribe, sometimes indicating the local names of plants and their ethnomedicinal uses. The actual scientific names of these plants were not reported.

Following this long hiatus, recent documentations of ethnobotanical knowledge have resumed, with assessments of Non-timber forest products (NTFP) in communities around the Sapo National Park in Liberia (Manvell, 2011). In the rainforest around the Putu Range in Grand Gedeh County, of 624 species of plants inventoried in five different habitats, 69% were of medicinal significance, with 55 species reported to include shade-bearing guild (Marshall and Hawthorne, 2012). In the Wonegizi region of Lofa County, the knowledge of local traditional medicinal practitioners was documented, producing a list of 101 species of plants in 48 families commonly used in treating 11 categories of ailments (Kpadehyea et al., 2015).

Liberia is currently going through a major transformation following the last civil war, and the need for rapid development is leading to increased exploitation of its natural resources, especially its vast resources of tropical rainforest (Blackett et al., 2009). Timber features highly in this drive, but the needs of local people are also centred on the use of important forest products such as poles, fruits, fibre and medicinal plants, among others. Like other developing countries around the world, poorly developed health care delivery systems have led to more reliance on the use of medicinal plants in most rural areas (Farnsworth and Soejarto, 1991). As local communities become speedily absorbed into the global economy, ethnomedicinal plant knowledge can quickly disappear and fears have been expressed in this regard (Lambert et al., 1997). Documenting the medicinal plant knowledge of forest dwelling communities is crucial before much of the forest disappears, and with it the extensive knowledge of traditional herbalists that interact with this rich plant biodiversity. In this regard, the main objectives of this study were to document the ethnomedicinal plant knowledge of herbalists in a forest region of Liberia, and to assess gender differences in the use of plants by male and female herbalists.

MATERIALS AND METHODS

Study area

Fieldwork for documenting the medicinal plant knowledge took place in central Rivercess County of Liberia, located some 320 km from the capital city of Monrovia. Garpue Town was the main study site (Figure 1), and its population like the rest of the county is dominated by the Bassa speaking tribe of Liberia, and comprised stakeholders drawn from 7 communities located within walking distances of each other. The 7 communities included Garpue Town, Beh Town, Gbetar, Saykpayah, Poewoh Town, Bodaza and Vondeh Town, all located in the central Morweh District. Rivercess County has an area of 5,263.4 km^2 and its geographic coordinates are N 5° 36' 23" and W 9° 39' 31" at an altitude of 84 m. The total population of the County is less than 120,000 people and 93% of them speak Bassa. It is largely a forest-dominated County and was home to three major logging activities during the past civil conflict (Blackett et al., 2009).

Data collection and analyses

We worked with traditional herbalists, identified through consultative meetings held in the chiefdom headquarter (Garpue Town), where the paramount resides. During a natural resource mapping exercise with community stakeholders, information was proffered on the heavy reliance on traditional herbalists given the lack of health facilities (Lebbie et al., 2009). These herbalists were reported to be members of the male and female secret societies, and are generally reputed for their knowledge in the use of plant remedies (Lebbie and Guries, 1995).

The number of herbalists in each community was not assessed and therefore unknown, but it was revealed through the consultative meetings that nearly every adult person possesses knowledge of the use of some plants in traditional medicine. Through further consultations with key stakeholders present at the resource mapping exercises conducted in Garpue Town, 22 practicing herbalists voluntarily consented to participate in the documentation of their ethnomedicinal plant knowledge. Using semi-structured questionnaires, we collected information on age, number of plants known and utilized, and ailments the herbalists specialized in treating. Following a brief practical guidance on how to collect specimens for taxonomic identification and herbarium vouchers, we asked herbalists to collect plant specimens known to each of them for proper scientific identification. For each plant collected and presented by herbalists, information on the ethnomedicinal uses and the parts utilized were obtained from the herbalists. No questions were asked about mode of preparation, manner of administration and local names, as herbalists had expressed concern that such information were proprietary, and we agreed not to extract such information from them.

Scientific identification and naming of medicinal plants were done on the spot, and confirmed with the aid of a field guide for woody plants known for this part of West Africa (Hawthorne and Jongkind, 2006), as well as the several volumes of the "Flora of West Tropical Africa" (Keay et al., 1954, 1958, 1963, 1968, 1972). While every effort was made to encourage herbalists to bring plant materials with complete of morphological features (leaves, fruits/seeds and flowers) to aid in identification, on a few occasion, some herbalists brought bark specimens without leaves, flowers and fruits, and the difficulties in identification of such specimens necessitated us to discard them. Some sterile materials were insufficient to identify beyond the genus level. All specimens were tagged and with voucher numbers, are housed in the National Herbarium of Sierra Leone (SL), given the lack of a formal herbarium in Liberia. Data obtained were compiled into tables, percentages and portrayed graphically.

RESULTS AND DISCUSSION

Gender differences and specialization in ethnomedicinal knowledge

Twenty-two (22) herbalists aged 27-77 years consented to participate in the documentation of their knowledge associated with ethnomedicine and the use of plants in treating various ailments. More of the herbalists were male (64%) than female (36%), with the majority of the herbalists over the age of 40 years (Table 1). The number of plants known and reportedly used by herbalists ranged between 2-40, with female herbalists knowing on average 18.1 plants more, while male herbalists knew 16.3 plants. Herbalists between the ages of 41-50 years reported knowing on average 19.1 medicinal plants, which was higher than other age categories.

Gender and age relationships have been observed in various studies involving medicinal plant knowledge. In Brazil, generally older people are reported knowing more medicinal plants than the younger people (Silva et al., 2011; Voeks and Leony 2004). For male herbalists in Nasarawa State of Nigeria, it was reported that they held more knowledge of medicinal plants than female herbalists (Ibrahim et al., 2016), although another study from Nigeria found men and women holding similar knowledge (Ayantunde et al., 2008). In three rural communities in Niger, Guimbo et al. (2011) utilizing participant observation and key-informant interviews found ethnobotanical knowledge to increase with age, but noted differences in ethnobotanical knowledge for both men and women as it related to medicinal plants. However, our study found female herbalists to know more medicinal plants than their counterpart male herbalists. Such differences can arise as a result of "limited experience", little interaction with other members of the community or due to problems with memory and forgetfulness on the part of the elderly as age sets in. The increased role often played by older women in child rearing is known to play a role in the acquisition of medicinal plant knowledge (especially over younger women).

In a study done around the Sapo National Park to document the use of non-timber forest products in adjacent communities, Manvell (2011) found women to be able to name more plants than men for the most common categories of illnesses. He attributed this to the unwillingness of the participants to share their knowledge. This is not uncommon among herbalists, and the differences in the number of medicinal plants reportedly known by each herbalist could also reflect under-reporting for fear of sharing their knowledge. We found a few of the men unwilling to share their knowledge in comparison to the women. A similar situation was observed in Sierra Leone, although in the same country, women of the sande/bondo secret societies willingly shared their knowledge of medicinal plants (Lebbie and Guries, 1995). In a review and meta-analysis of the effect of gender on medicinal plant knowledge conducted at various scales comprising national, regional and global (Torres-Avilez et al., 2016), no significant differences were apparent in the medicinal plant knowledge of men and women. Significant differences in medicinal plant knowledge were however observed at the national and continental level for both men and women. In Niger, Müller et al. (2015) found older people to be more knowledgeable about medicinal plants, with women knowing more about food plants. Given the small number of herbalists in the various age and gender categories we sampled, observed differences in reported knowledge of medicinal plants might be due purely to sample size, and possibly the unwillingness of herbalists to sometimes share their proprietary knowledge. In the Himalayas, Gupta et al. (2014) noted that herbalists (*Amchis*) are reluctant to disclose knowledge of medicinal plants given that this might reduce the effectiveness of the remedy. But more importantly, the knowledge of the remedies to the herbalists is what brings them "recognition", and sharing this knowledge might be seen as reducing their control over such proprietary knowledge.

Gender differentiation in ethnomedicinal knowledge and use of plants to treat certain categories of diseases was apparent, with female herbalists possessing specialized knowledge in treating children's health problems, snakebite, cholera and other medical problems related to women (Table 2). Male herbalists possessed diverse knowledge on various health problems, including broken bones, epilepsy, evil spirit, heart disease, high blood pressure, erectile dysfunction and infection of the testes, among others. Kpadehyea et al. (2015) noted also that female herbalists in the Lofa County of Liberia reported more traditional medicines that focused on children's health issues and menstrual problems than their male counterparts. Given the limited access to health facilities and drugs by these communities (Lebbie et al., 2009), women's traditional role of taking care of

Table 1. Gender and age distribution of herbalists and knowledge of medicinal plants.

Categories	Frequency	Percent	Mean number of plants known by herbalist
Gender			
Male	14	64.0	16.3
Female	8	36.0	18.1
Age (years)			
≤40	1	4.5	3.0
41 – 50	7	31.8	19.1
51 - 60	8	36.4	15.5
≥60	6	27.3	18.8

Table 2. Gender differentiation in ethnomedicinal knowledge in treating diseases.

Gender	Specialized strength of herbalists
Male	Broken bones, epilepsy, diarrhoea, evil spirit, heart disease, high blood pressure, erectile dysfunction, infection of the testes, back pain, bodily pain, asthma, insanity, worms, rheumatism, “African sign”, infection
Female	Children’s medical problems, treating pain associated with teenage rape, snakebites, stomach complaints, abortion, hookworms, infections, cholera, headache, “African sign”

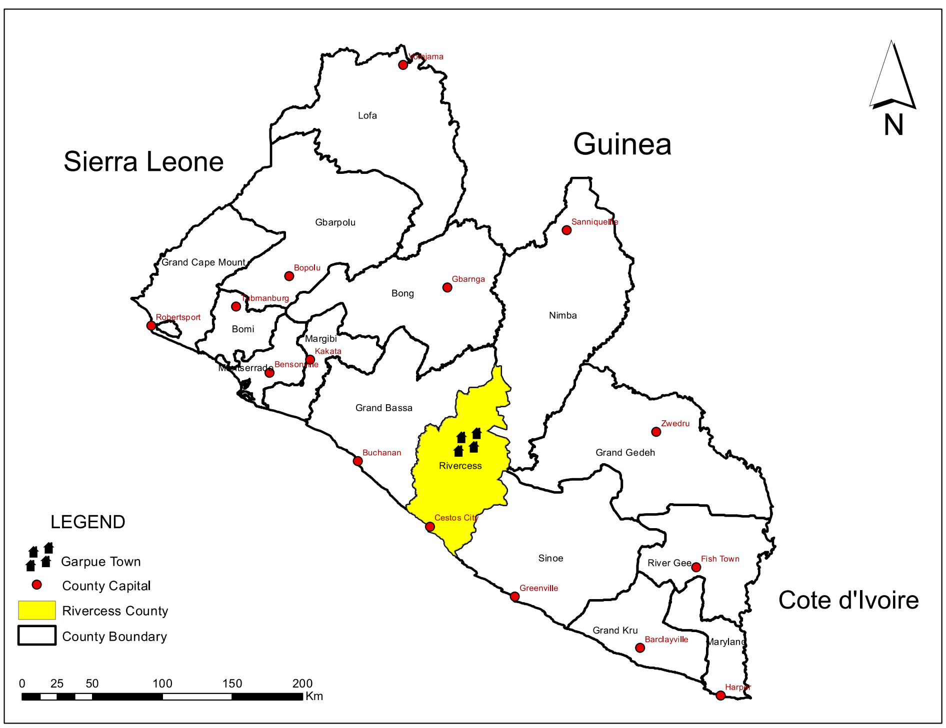

Figure 1. Map of Liberia showing the study area, Garpue Town, in Rivercess County. Source: http://d-maps.com/m/africa/liberia/liberia51.pdf Downloaded 2017

children could potentially help to explain their knowledge of medicinal plants associated with children’s health.

One common area of specialization among some male and female herbalists is an ethnomedical problem

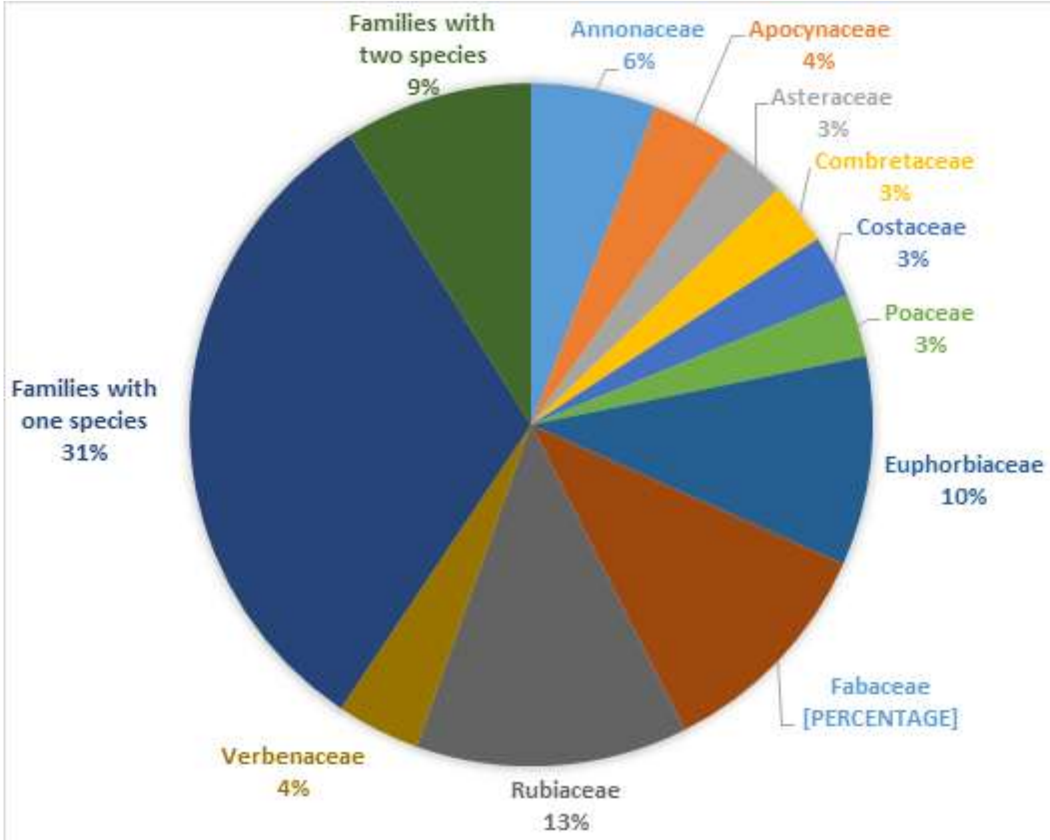

Figure 2. Distribution of medicinal plant families reported by herbalists.

referred to as "African sign"; a condition of ill health reported to be associated with unforeseen forces perpetuated by evil persons, "witchcraft" and or supernatural forces, for which western medicine is believed to be unable to address. It is apt to say that ill health, as perceived by the Bassa herbalists of Rivercess County is not seen entirely in the context of witchcraft or supernatural forces. Most of the ailments/diseases for which the herbalists were specialists in were not pegged on witchcraft or sorcery *per se*, as is often painted by ethnocentric western medical and social anthropologists (Konadu, 2008). Herbalists were quick to affirm their ability to distinguish between disease conditions in which malevolence was involved, either through witchcraft or supernatural forces, or generally as a result of some underlying etiologic or physiological condition. "African sign" and "dragon spirit" or "evil spirit" were not situated in the context of etiologic agents, but rather ailments for which evil persons have caused to happen to an otherwise perfectly healthy person. Such a sickness can be cured only by herbalists skilled in traditional Bassa ethnomedicine, with western medical interventions believed to be powerless in treating it, as has been noted in some parts of Kenya (Good, 1987). In South Africa, Petrus (2011) noted the increasing role of witchcraft in the lives of the people, and cited instances where allegations of ill health resulted from the interplay of witchcraft.

Medicinal plant species and ethnomedicinal uses

A total of 112 medicinal plants, belonging to 98 plant genera and 52 families, were indicated by the herbalists as forming part of their local ethno-pharmacopoeia (Table 3). The most species rich families with at least 3 species were Rubiaceae (13 species), Euphorbiaceae (10 species), Fabaceae (11 species), Annonaceae (6 species), Apocynaceae (4 species), Asteraceae (3 species), Combretaceae (3 species), Poaceae (3 species), Costaceae (3 species) and Verbenaceae (4 species) (Figure 2). They constitute 53.4% of all medicinal plants, with the remaining 46.6% distributed among 42 families comprising of 1 or 2 species per family. In a study conducted in the Nasarawa State of Nigeria utilizing the knowledge of traditional herbalists, it was found that medicinal plants belonging to the families Euphorbiaceae, Fabaceae and Rubiaceae comprised the most species rich (Ibrahim et al., 2016). While we recorded slightly different species composition and

Table 3. Medicinal plants and ethnomedicinal uses reported by herbalists in the study area.

Scientific name of plant	Voucher specimen	Family	Ethnomedicinal uses reported by herbalists	Part used
Cyathula prostrata (L.) Blume	LKK 23	*Acanthaceae*	Sanitary pad for women	Leaf
Annickia polycarpa (DC.) Setten & Maas	LKK 36	*Annonaceae*	Malaria	Bark
Cleistopholis patens Engl. & Diels	LKK 5	*Annonaceae*	Miscarriage caused by worms, bloody diarrhoea, rheumatism, stomach pain	Bark
Greenwayodendron oliveri (Engl.) Verdc.	LKK 45	*Annonaceae*	Male infertility	Leaf
Isolona campanulata Engl. & Diels	LKK 16	*Annonaceae*	Thrush (fungal infection in new born)	Leaf
Xylopia aethiopica A.Rich.	LKK 35	*Annonaceae*	Pneumonia, protrusion of uterus, snake bite, weakness in limbs of children, miscarriage caused by worms, jaundice, typhoid, pain in the ribs	Leaf, bark
Xylopia sp.	LKK 50	*Annonaceae*	Snake bite	Leaf
Baissea sp.	LKK 58	*Apocynaceae*	Chicken pox	Leaf
Funtumia elastica (P. Preuss) Stapf	LKK 6	*Apocynaceae*	Sore, diarrhoea	Leaf
Landolphia dulcis (Sabine) Pichon	LKK 65	*Apocynaceae*	Side and rib pains, chronic sore, dysentery	Leaf, bark
Rauvolfia vomitoria Wennberg	LKK 24	*Apocynaceae*	Vomiting, prolonged menstruation, hookworm, malaria, jaundice, snake bite, diarrhoea	Leaf, root
Anchomanes difformis Engl.	LKK 49	*Araceae*	Broken bone	Leaf
Caladium bicolor (Aiton) Went.	LKK 71	*Araceae*	To constrict the vagina	Petiole
Elaeis guineensis Jacq.	LKK 4	*Arecaceae*	Maturity (in children)	Leaf
Chromolaena odorata (L.) R.M.King & H.Rob.	LKK 57	*Asteraceae*	Malaria	Leaf
Vernonia cinerea (L.) Less.	LKK 88	*Asteraceae*	Side and rib pains	Leaf
Vernonia conferta Benth.	LKK 46	*Asteraceae*	Gonorrhea, African sign, food poison, rheumatism, broken bone	Leaf
Newbouldia laevis Seem.	LKK 103	*Bignoniaceae*	Snake bite	Leaf
Canna indica L.	LKK 15	*Cannaceae*	Malaria	Leaf
Myrianthus libericus Rendle	LKK 110	*Cecropiaceae*	Snake bite	Leaf
Salacia camerunensis Loes.	LKK 37	*Celastraceae*	Malnutrition	Leaf
Combretum comosum G.Don	LKK 111	*Combretaceae*	Infertility, sore in stomach	Leaf
Combretum racemosum P.Beauv.	LKK 1	*Combretaceae*	Cataract, African sign	Leaf
Terminalia superba Engl. & Diels	LKK 56	*Combretaceae*	Ulcer	Bark
Palisota hirsuta Schumann	LKK 70	*Commelinaceae*	Measles, joint pain, thrush, sore in the stomach of new born baby	Leaf
Manotes expansa Sol. ex Planch.	LKK 87	*Connaraceae*	Difficulty in a woman becoming pregnant	Leaf
Costus afer Ker Gawl.	LKK 102	*Costaceae*	Open sore in babies, poison, joint pain	Leaf
Costus dubius (Afzel) Schumann	LKK 2	*Costaceae*	Pain in the eye	Leaf
Costus sp.	LKK 92	*Costaceae*	Miscarriage	Fruit
Bryophyllum pinnatum (Lam.) Oken	LKK 66	*Crassulaceae*	Childhood cough	Leaf
Cyathea sp.	LKK 108	*Cyatheaceae*	African sign	Leaf
Scleria naumanniana Boeck.	LKK 25	*Cyperaceae*	Difficulty breathing, pain in the sides, cataract	Leaf
Scleria sp.	LKK 77	*Cyperaceae*	Bodily pain	Leaf
Nephrolepis biserrata (Sw.) Scott	LKK 47	*Davalliaceae*	Difficulty in breathing, heartburn, retention of urine, bleeding in pregnant women, epilepsy, sore, rash, joint pain, painful and swollen female breast, gonorrhea	Frond
Dichapetalum dictyospermum Bret.	LKK 3	*Dichapetalaceae*	Elephantiasis	Leaf

Table 3. Cont'd.

Tetracera potatoria (Afzel.) G.Don	LKK 34	*Dilleniaceae*	Oedema, retained placenta, impotence, open sore, pile	Leaf, bark
Triphyophyllum peltatum (Hutch. & Dalz.) Airy Shaw	LKK 89	*Dioncophyllaceae*	Open sore	Bark
Diospyros kamerunensis Gürke	LKK 7	*Ebenaceae*	Diarrhoea, abnormal heart beats, sore in the stomach of newborn child, dysentery	Leaf, bark
Diospyros mannii Hiern	LKK 40	*Ebenaceae*	Dysentery	Leaf
Alchornea cordifolia (Schumach.) Müll.Arg.	LKK 27	*Euphorbiaceae*	Toothache, gonorrhea, high blood pressure, post-delivery pain, sore in the stomach (ulcer), irregular menstruation pain, heartburn, wound, high fever	Leaf, bark, fruit, root
Croton hirtus L'Hér.	LKK 17	*Euphorbiaceae*	Jaundice	Leaf
Euphorbia hirta L.	LKK 61	*Euphorbiaceae*	White growth on the eye (cataract)	Leaf
Macaranga heterophylla Müll.Arg.	LKK 90	*Euphorbiaceae*	Diarrhoea, sore in stomach, malaria	Leaf
Maesobotrya barteri Hutch.	LKK 79	*Euphorbiaceae*	Snake bite	Leaf
Manihot esculenta Crantz	LKK 107	*Euphorbiaceae*	Heartburn	Bark
Manniophyton fulvum Müll.Arg.	LKK 8	*Euphorbiaceae*	Wound, sore, rheumatism, African sign	Leaf
Mareya micrantha Müll.Arg.	LKK 69	*Euphorbiaceae*	Food poison, snake bite, infection in pregnant women	Leaf
Phyllanthus muellerianus (Kuntze) Exell	LKK 86	*Euphorbiaceae*	Rib and chest pains, jaundice	Leaf
Tetrorchidium didymostemon (Baill.) Pax & K.Hoffm.	LKK 101	*Euphorbiaceae*	"Dragon spirit"	Bark
Albizia adianthifolia W.Wight	LKK 95	*Fabaceae*	Open sore	Leaf
Amphimas pterocarpoides Harms	LKK 39	*Fabaceae*	Prolonged labor pains	Leaf
Anthonota macrophylla P.Beauv.	LKK 26	*Fabaceae*	Headache, African sign, oedema in children, malnutrition	Leaf
Dalbergia saxatilis Hook.f.	LKK 91	*Fabaceae*	Miscarriage	Leaf
Dalbergiella welwitschii Baker f.	LKK 18	*Fabaceae*	Stomach pain due to pregnancy, side/rib pains, oedema, toothache	Leaf, stem
Desmodium adscendens (Sw.) DC	LKK 60	*Fabaceae*	Convulsion	Leaf
Desmodium sp.	LKK 68	*Fabaceae*	Pile, excessive bleeding during menstruation, African sign, oedema, low blood, hernia	Leaf, Bark
Leptoderris trifoliolata Hepper	LKK 85	*Fabaceae*	Erectile dysfunction	Bark
Tetraberlinia tubmaniana J.Léonard	LKK 106	*Fabaceae*	Sore in stomach	Bark
Entada sp.	LKK 9	*Fabaceae*	Pain in the leg	Leaf
Mimosa pudica L.	LKK 112	*Fabaceae*	Irregular heartbeats, bloody diarrhoea, thrush	Stem, leaf
Schrankia leptocarpa DC.	LKK 92	*Fabaceae*	Heartburn	bark
Harungana madagascariensis Poir.	LKK 32	*Hypericaceae*	Sore in the stomach of newborn child, rheumatism, pain in the ribs, sore, thrush, snake bite	Leaf
Vismia guineensis Druce	LKK 67	*Hypericaceae*	Oedema (of arms, legs and stomach)	Leaf
Beilschmiedia mannii Benth. & Hook.f.	LKK 78	*Lauraceae*	African sign	Leaf
Napoleonaea heudelotii A.Juss.	LKK 19	*Lecythidaceae*	Heart failure, erectile dysfunction, painful redness of the eye, sore in stomach, sore in the stomach of newborn child/baby	Leaf, bark, root
Napoleonaea sp.	LKK 38	*Lecythidaceae*	Snake bite	Leaf
Anthocleista nobilis G.Don	LKK 48	*Loganiaceae*	Bodily pain, skin rash, rheumatism, sore, snake bike	Bark, leaf
Lycopodiella cernua (L.) Pic.Serm.	LKK 100	*Lycopodiaceae*	Rheumatism	Leaf
Tristemma sp	LKK 59	*Melastomataceae*	Incontinence	Leaf
Carapa procera DC	LKK 84	*Meliaceae*	Weak penis	Root
Ficus capensis Hort.Berol. ex Kunth & C.D.Bouché	LKK 10	*Moraceae*	Low blood, snake bite, malaria	Leaf

Table 3. Cont'd.

Psidium guajava L.	LKK 93	*Myrtaceae*	Dysentery	Leaf
Nymphaea lotus L.	LKK 80	*Nymphaeaceae*	Typhoid	Leaf
Campylospermum subcordatum (Stapf) Farron	LKK 28	*Ochnaceae*	Dysentry, chest pain in children caused by worms	Leaf
Heisteria parvifolia Sm.	LKK 33	*Oleaceae*	Asthma	Leaf
Microdesmis keayana J.Léonard	LKK 97	*Pandaceae*	Severe stomach pain, gonorrhea, pneumonia, pain in the heart, abscess, tuberculosis, snake bite, jaundice, whooping cough, convulsion, toothache, painful and swollen testicles, severe side/rib pains	Leaf, bark
Smeathmannia laevigata Sol. ex R.Br.	LKK 11	*Passifloraceae*	Dysentery, stomach pain in pregnant women, abnormal heart beats	Leaf
Smeathmannia pubescens Sol. ex R.Br.	LKK 31	*Passifloraceae*	Diarrhoea, thrush	Leaf
Eleusine indica (L.) Gaertn.	LKK 42	*Poaceae*	Miscarriage caused by worms, joint pain	Leaf
Paspalum scrobiculatum L.	LKK 20	*Poaceae*	Worms, toothache, severe rib pain	Leaf, stem
Setaria chevalieri Stapf	LKK 54	*Poaceae*	Sore, diarrhoea	Leaf
Craterispermum laurinum Benth.	LKK 73	*Rubiaceae*	Malaria, cough	Bark
Geophila obvallata Didr.	LKK 72	*Rubiaceae*	Neck pain, food poison, severe heart pain	Leaf
Hallea ledermannii (K.Krause) Verdc.	LKK 62	*Rubiaceae*	Pile, rheumatism, used in divination to tell the fate of a sick person, sore, severe headache	Leaf, bark
Hallea sp.	LKK 98	*Rubiaceae*	Poison	Leaf
Keetia rufivillosa (Robyns ex Hutch. & Dalz.) Bridson	LKK 12	*Rubiaceae*	Malaria	Leaf
Massularia acuminata (G.Don) Bullock ex Hoyle	LKK 53	*Rubiaceae*	Used in divination to tell the fate of a sick person, typhoid, gonorrhea	
Morinda longiflora G.Don	LKK 83	*Rubiaceae*	Bodily pain	Leaf
Morinda lucida Benth.	LKK 104	*Rubiaceae*	Jaundice, malaria, dysentery	Leaf, stem
Morinda morindoides (Baker) Milne-Redh.	LKK 43	*Rubiaceae*	Malaria, measles, worms	Leaf, stem
Pavetta sonjae W.D.Hawth.	LKK 99	*Rubiaceae*	Cataract	Bark
Pavetta sp.	LKK 74	*Rubiaceae*	Diarrhoea	Bark
Psychotria peduncularis (Salisb.) Steyerm.	LKK21	*Rubiaceae*	Sore in stomach	Leaf
Psychotria sp.	LKK 105	*Rubiaceae*	Hernia	Bark
Zanthoxylum gilletii (De Wild.) P.G.Waterman	LKK 82	*Rutaceae*	Weak babies, rheumatism	Bark, leaf
Placodiscus pseudostipularis Radlk	LKK 13	*Sapindaceae*	Difficulty breathing	Stem
Placodiscus sp.	LKK 55	*Sapindaceae*	Ringworm	Leaf
Chrysophyllum pentagonocarpum Engl. & K.Krause	LKK 75	*Sapotaceae*	Pile	Leaf
Delpydora gracilis A.Chev.	LKK 63	*Sapotaceae*	Failure of the testes to descend (cryptochidism)	Leaf
Selaginella myosorus (Sw.) Alston	LKK 29	*Selaginellaceae*	Rheumatism, bloody diarrhoea	Leaf
Capsicum frutescens L.	LKK 41	*Solanaceae*	Severe headache (migraine)	Stem
Clappertonia sp.	LKK 109	*Tiliaceae*	Malaria	Leaf
Triumfetta sp.	LKK 81	*Tiliaceae*	Inflammation of breast	Leaf
Urera sp	LKK 96	*Urticaceae*	Malaria	Leaf
Clerodendrum splendens G.Don	LKK 30	*Verbenaceae*	Burning sensation in the foot, pain in the leg	Leaf
Premna hispida Benth.	LKK 76	*Verbenaceae*	Typhoid	Leaf
Vitex micrantha Gürke	LKK 22	*Verbenaceae*	African sign associated with pain on the sole of one's foot	Leaf
Vitex rivularis Gürke	LKK 51	*Verbenaceae*	Jaundice, malaria, abnormal bowel movement	Leaf

Table 3. Cont'd.

Rinorea sp.	LKK 64	*Violaceae*	Bloody diarrhoea, nightmare	Leaf, bark
Cissus aralioides Planch.	LKK 14	*Vitaceae*	Ulcer	Bark
Aframomum sceptrum Schumann	LKK 52	*Zingiberaceae*	Rashes on skin	Rhizome
Aframomum sp.	LKK 44	*Zingiberaceae*	Impotence, rheumatism	Rhizome

numbers for these families in our study area, all three species also comprised the most important families in terms of medicinal plants recorded among the herbalists.

Most medicinal plants had single or double uses, but we recorded twelve plants with at least five uses including *Xylopia aethiopica* A.Rich., *Rauvolfia vomitoria* Wennberg, *Vernonia conferta* Benth., *Nephrolepis biserrata* (Sw.) Schott, *Tetracera potatoria* Afzel. ex G.Don, *Alchornea cordifolia* (Schumach.) Müll. Arg., *Harungana madagascariensis* Poir., *Napoleonaea heudelotii* A.Juss., *Desmodium adscendens* (Sw.) DC., *Anthocleista nobilis* G. Don, *Microdesmis keayana* J.Léonard and *Hallea ledermannii* (K.Krause) Verdc. (Table 2). Among these, *M. keayana* J. Léonard had over 13 different medicinal uses, while *N. biserrata* (Sw.) Schott was utilized for 11 different medical problems. A large number of the medicinal uses indicated for *M. keayana* J. Léonard by herbalists appear new in the ethno-pharmacopoeia of Liberia, although its utilization in treating gonorrhoea is also corroborated by the folk medicine of the Edo of Nigeria (Burkill, 1998), and none by Kpadehyea et al. (2015) for Lofa County of Liberia. An unspecified species of *Nephrolepis* sp*.* is reportedly utilized in Cameroon for the management of gonorrhoea (Kuete, 2013). Burkill (1998) noted the use of the plant as a haemostatic, and in the prevention of abortion, uses which mirror utilization by female herbalists in stopping bleeding in pregnant women in the Rivercess County.

Herbalists in Central Rivercess District are utilizing medicinal plants to treat an ethnomedical problem referred to as "African Sign". This is a condition of ill health that may result from allegedly the evil workings of bad persons through witchcraft, supernatural forces and "bad medicine" (Good, 1987). Seven (7) medicinal plants are used to treat persons with such afflictions and include *Vitex micrantha* Gürke, *Desmodium sp., Anthonota macrophylla* P. Beauv., *Manniophyton fulvum* Müll.Arg., *Cyathea sp., Combretum racemosum* P. Beauv. and *Vernonia conferta* Benth. In Sierra Leone, the use of fetish like *hale nyamu* (bad medicine) can result in chronic illness or death (Wallis, 1905), which is similar to the "African sign" reported by herbalists in the study area.

Remedies for snakebite were common knowledge among all the female herbalists, with 11 medicinal plants commonly employed, including *X. aethiopica* A.Rich., *R. vomitoria* Wennberg, *Newbouldia laevis* Seem., *Myrianthus libericus* Rendle, *Maesobotrya barteri* Hutch., *Mareya micrantha* Müll. Arg., *Harungana madagascariensis* Poir., *Napoleonaeae sp.*, *Anthocleista nobilis* G.Don*, Ficus capensis* Hort. Berol. ex Kunth & C.D. Bouché and *M. keayana* J.Léonard*.* In the ethnomedicine of Africa, the anti-venom uses of *R. vomitoria* Wennberg and *M. keayana* J. Léonard have been noted (Burkill, 1985, 1994; Chifundera, 1987; Neuwinger, 1996; Kutalek and Prinz, 2007). Harley (1941), in an elaboration of the ethnomedicine of snakebite among the Mano of Liberia, reports the use of *M. spicata* Baill., which is a synonym of *M. micrantha* Müll. Arg as a snakebite remedy. Traditional herbalists in Lofa County have also confirmed the use of *M. micrantha* Müll. Arg and *R. vomitoria* Wennberg as anti-venom plants (Kpadehyea et al., 2015). It is not yet known to what extent the other plants reported by the herbalists as anti-venom plants are effective, as the current ethno-pharmacopoeia of Liberian plants does not mention their anti-venom uses among other cultures. However, this does not preclude the fact that herbalists could come up with new uses for such plants in their communities, given that we are yet to fully document ethnomedicinal uses of plants across the different cultures in Liberia.

Various health problems were reported by herbalists for which medicinal plants are used including malaria, ulcer and stomach sores, impotence and erectile dysfunction. Ten plants were indicated as remedies for malaria (Table 3), 9 for ulcer (and sore in the stomach of babies/children), and 6 plants for impotence (infertility/erectile dysfunction). Some of these plants are also known in the folk medicine of other countries (Lebbie and Guries, 1995; Neuwinger, 1997) as well as Liberia (Harley, 1941; Kpadehyea et al., 2015). However, some uses reported for certain medicinal plants by the herbalists might represent new and unrecorded uses. *Tetraberlinia tubmaniana* J. Léonard, *Delpydora gracilis* A. Chev., *Campylospermum subcordatum* (Stapf) Farron, *Cyathula prostrata* (L.) Blume, *Heisteria parvifolia* Sm., *Keetia rufivillosa* (Robyns ex Hutch. & Dalz.) Bridson, *Pavetta sonjae* W.D.Hawth., *Chrysophyllum pentagonocarpum* Engl. & K.Krause, *Placodiscus pseudostipularis* Radlk and *Caladium bicolor* (Aiton) Went., all represent plants which have new uses in the ethno-pharmacopoeia of Liberia. *Tetraberlinia tubmanniana* J. Léonard, a tree endemic to the Upper Guinean forest, is used by herbalists to treat stomach ulcer, while the naturalized plant, *C. bicolor* (Aiton) Went., has found a new use hitherto unreported. Its use to constrict the female vagina is reported by herbalists as a measure to increase sexual satisfaction in both men and women, as a dilated vagina is reported to reduce sexual satisfaction. In the ethnomedicine of the Lofa County, *C. bicolor* (Aiton) Went., is used in combination with *Musanga cecropioides* R. Br., to treat skin cancer (Kpadehyea et al., 2015).

Conclusion

There is a rich medicinal plant knowledge base among male and female herbalists in the forest region of Liberia, with clear gender differentiation and specialization in the use of medicinal plants for the treatment of illnesses. A total of 112 species of medicinal plants in 52 families were recorded through interviews conducted with traditional herbalists, with the most commonly utilized medicinal plants reported by them belonging to plants in the following families: *Annonaceae, Apocynaceae, Costaceae, Rubiaceae, Euphorbiaceae, Fabaceae* and *Verbenaceae*. Species in these families were utilized in the treatment of ailments such as malaria, snakebites, ulcer, evil spirit, witchcraft, infection and diarrhoea. New and unrecorded ethnomedicinal uses for several medicinal plants were reported by the traditional herbalists, including *Tetraberlinia tubmaniana* J. Léonard*, Delpydora gracilis* A.Chev., *C. subcordatum* (Stapf) Farron, *C. prostrata* (L.) Blume, *H. parvifolia* Sm., *K. rufivillosa* (Robyns ex Hutch. & Dalz.), *P. sonjae* W.D.Hawth., *C. pentagonocarpum* Engl. & K.Krause, *P. pseudostipularis* Radlk and *C. bicolor* (Aiton) Went. Further documentations are required to fully comprehend the wealth of knowledge possessed by herbalists, as well as unravel the gender differentiation and specialization in the use of medicinal plants in the ethnomedicine of Liberia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors' sincere thanks go to all the herbalists and community members and leaders who participated in the inventory work and in documenting the ethno-pharmacopoeia of their region. We thank the following who provided support in the field: Francis Odoom, David Mulbah Jr, Joe Flomo, Georgia Richards, Tenema Coleman, Nancy Gbakoyah, Morris Kamara, Wollorma Hurlowulu, Garvoie Kardoh and Daniel Karr. We also appreciate the funding provided by the GEF Small Grants of Liberia and IUCN-Liberia Office for the fieldwork. Many thanks to Alfred Bockarie of Njala University for developing the study area map.

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academicJournals

Vol. 11(14), pp. 275-283, 10 April, 2017
DOI: 10.5897/JMPR2017.6343
Article Number: A4C830663757
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition, antiacetylcholinesterase inhibition and toxicity activities of essential oil from *Hyptis dilatata* (Lamiaceae) flowers

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Received 26 January, 2017; Accepted 28 March, 2017

The content and chemical composition of the essential oil may vary in certain species according to the climatic period. The aim of this study was to evaluate the influence of seasonality on the chemical composition of the essential oil from *Hyptis dilatata* flowers, to perform biological activities such as antimicrobial, inhibition of acetylcholinesterase enzyme and to evaluate the toxicity of the essential oil using for the test as indicator *Artemia salina*. *H. dilatata* flowers were collected in rainy and dry periods and extracted by hydro distillation using extractor of Clevenger condenser double Spell model. Analysis of essential oil resulted in 22 chemical components. The major constituents for dry and rainy periods were α-pinene (26.2 and 10.9%), 3-Carene (12.2 and 3.7%), fenchone (17% and 14.8%) and β-cariophyllene (16.36 and 30.9%), respectively. The essential oil inhibited the acetylcholinesterase enzyme in 93.4% (rainy period) and 92.4% (dry period). Between the dry and rainy periods, the best LC_{50} in microbial activity *in vitro* was obtained in the rainy period tested in *Staphylococcus aureus* bacterium (LC_{50} 49.8 mg ml^{-1}). The cytotoxic activity of *A. salina* in *H. dilatata* essential oil proved LC_{50} results below of 100 µg ml^{-1}. Therefore, the chemical characterization and testing of biological activities of essential oils showed promising results in the search for new active substances and development of bioproducts of vegetable origin.

Key words: *Hyptis dilatata*, α-pinene, fenchone, β-cariofilene, 3-carene.

INTRODUCTION

The genus *Hyptis* belongs to Lamiaceae family, consisting of approximately 580 species, many of them with great ethnopharmacological importance. They are distributed mainly in tropical America, southern United States, Caribe and Argentina. In Brazil, it is mainly found in Minas Gerais, Bahia, Goiás and Amazonas states (Lima, 2010). In this family of plants, the use of essential oil is common in the population of folk medicine as coding and remedy and they have been used as antioxidant, anti-inflammatory, antihypertensive, antitumoral, gastroprotective, insecticide, antibacterial, antifungal and antiherpetic (Barros et al., 2010; Hussain et al., 2011). Some biological activities have been associated to the content of phenolic compounds, present in the plant (Zgórka and Glowniak, 2001; Valant-Vetschera et al., 2003). In addition, owing to the diversity of volatile constituents found in essential oils of various species of the Lamiaceae family, these essential oils have aroused the interest of the perfume, cosmetics, food and pharmaceutical industries (Fernandez-Alonso and Rivera-Diaz, 2002). Species of genus *Hyptis* are mostly aromatic with great economic potential, due to its essential oil and presents important pharmacological action, such as anesthetic, anti-inflammatory (Botrel et al., 2010). Defense mechanisms of plants can vary in distinct environmental conditions, leading, consequently, to the production of different secondary metabolites. Factors such as cultivation conditions, soil type and parts of the plant analyzed, may influence the content and chemical composition of essential oils (Botrel et al., 2010). Botrel et al. (2009) studied the chemical composition and content of the essential oils of *Hyptis marrubioides* Epling flowers of two genotypes, white and purple. In this research, they identified that the content of some of the major principal compounds of the white genotype, such as β-caryophyllene, γ-muurolene and caryophyllene oxide, was higher compared to the purple genotype, and also observed that the essential oil content was the highest in the inflorescence of the white genotype. Researchers like Santos et al. (2008) studied the essential oils of *Hyptis pectinata* leaves and identified calamusenona as the major compound, which possess antimicrobial activity. Niero and Malheiros (2009) studied the essential oils of *Hyptis suaveolens* leaves and identified the antimicrobial compound sabinene as major constituent (antimicrobial activity) while from *Hyptis crenata,* α-pinene was the majoritary one (antioxidant activity). Urones et al. (1998), isolated, from a *Hyptis dilatata* species collected in Veraguas (Panama), the well-known compounds carnosol, rosmanol and methyl-rosmanol. Tafurt-Garcia et al. (2014) studying the flowers of *H. dilatata* from Arauca (Colombia) identifield δ-3-carene, camphor, bornyl acetate, E-caryophyllene and palustrol as the principal constituents. According to Lang and Buchbauer (2012), the δ-3-carene, camphor and bornyl acetate compounds have antimicrobial activity (Lang and Buchbauer, 2012; Misharina et al., 2009), and α-humuulene, caryophyllene and fenchone have antimicrobial and antioxidant activities. *H. dilatata* species is a perennial sub-bush known as field mint, in Tepequem saw, where it was collected in the municipality of Amajari, Roraima State, Brazilian Amazon rainforest. This species is usually used by the population for medicinal purposes, such as intestinal problems, influenza, and as insecticide. The aim of this work was to evaluate concentration of the chemical compounds present in the essential oil of *H. dilatata* flowers in two seasonal periods and to realize the biological tests for antimicrobial activity, fungicide, activity with acetylcholinesterase enzyme, as well as the cytotoxic activity using *Artemia salina* with essential oils.

MATERIALS AND METHODS

Plant and essential oil extraction

The flowers of *H. dilatata* were collected during the dry period (January to March) and in the rainy period (June to August) in 2015 in Paiva village at 634 m (meters above sea level), in Tepequem saw (RR 203), municipality of Amajari in Roraima State, Brazil. A voucher specimen was deposited in the INPA herbarium with registration number 263670 and another voucher specimen was deposited in the integrated museum of Roraima State (MIR 12754). The essential oil was extracted by hydrodistillation method, drag the water vapor for 2 h using extractor of Clevenger condenser double Spell model. The percentage of essential oil collected in the dry and rainy periods was measured in triplicate. The essential oil was placed in amber flask, weighed, and stored under nitrogen in a freezer until further analysis (Castro, 2006).

GC-FID analysis

The composition of the chemical constituents present in the essential oils was determined by gas chromatography after preparation of the methyl esters. The analyses were performed on a Gas Chromatograph HP7820A (Agilent). Column: HP5 30 m × 0.32 mm × 0.25 µm (Agilent). Temperature: Column: 50°C (0 min), 0°C min^{-1}, up to 230°C. Injector: 250°C Split (1:30). Detector FID: 250°C. Vector gas: H_2 at 3.0 ml min^{-1}. Injection volume: 1 µl. Data acquisition software: EZChrom Elite Compact (Agilent). Samples diluted at 1% in chloroform.

GC-MS analysis

Identification of the chemical constituents was performed on

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GCMS-QP2010 ULTRA (Shimadzu) apparatus. Column: Rxi-1MS 30 m × 0.25 mm × 0.25 µm (Restek). Temperature Column: 50°C (3 min), 3°C min^{-1}, up to 230°C. Injector: 250°C Split (1:10). Interface CG-MS a 250°C. Detector MS (electronic Impact at 70 eV) up to 250°C. Vector gas: He at 2.0 ml min^{-1}. Injection volume: 1 µl. Samples diluted at 1% in chloroform. Data acquisition software: GCMS Solution (Shimadzu). Spectral library: NIST11.

Acetylcholinesterase (AChE) inhibition assay

Quantitative evaluation of acetylcholinesterase (AChE) inhibition activity was performed according to the methodology of Ellman (1961), modified by Rhee et al. (2001). This bioassay was performed on microplates of 96 wells. Eserine and galantamine (10 mg ml^{-1}) were used as positive controls while the negative control was performed without inhibitor. In each well were added 25 µl of acetylcholine iodide (15 mM); 125 µl of 5.5'-dithiobis (2-nitro benzoic acid) (DTNB); 50 µl of tris-HCl pH 8 0.1% w/v buffer of bovine serum albumin and 25 µl of extract (10 mg ml^{-1}) solubilized in Tween/DMSO (30:70). The tests were performed in triplicate. The plates were read nine times at 405 nm over a period of 10 min. Immediately after the first reading, 25 µl of acetylcholinesterase enzyme (*Electrophorus electricus,* Sigma Aldrich) (0.222 U mL^{-1}) was added and nine readings were performed over a period of 10 min at 405 nm. The interference of spontaneous hydrolysis of the substrate was corrected from the subtraction of the average of the absorbance measured before the addition of the enzyme. The percentage inhibition of the enzyme was calculated from the following mathematical formula:

% inhibition = [(C- A) / C × 100]

where C = control containing enzyme and substrate; A = assay containing the extract, enzyme and substrate.

Toxicity on *A. salina*

The toxicity on *A. salina* was carried out through the methodology adapted from Meyer et al. (1982). Artificial saline solution (40 g of coarse salt in 1 L of distilled water) was added in an aquarium that was used as an incubator. The pH of this solution was adjusted between 8 and 9 with sodium carbonate (Na_2CO_3 at 10%). The incubator was divided into two environments: an uncovered environment was artificially illuminated with a fluorescent lamp with aeration and the other environment was placed, approximately 100 mg of *A. salina* eggs, and covered with aluminum foil so that organisms at born remained isolated due to the difference in illumination, during an incubation period of 48 h. After hatching, the *A. saline* eggs were added in each test tube, 10 nauplii, containing serial concentrations (1000, 500, 250, 125, 62.5, 31.25 and 15.625 µl ml^{-1}). After a period of 24 h, the number of live and dead nauplii in each test tube was counted, using macroscopic visualization. The tests were done, in triplicate, for each concentration. As a positive control, DMSO and also saline water were used without the essential oil. The probability of mortality was calculated using the Abbot formula:

M (%) = Amount of dead organisms / Total number of organisms in the tube × 100

A calibration curve was used to obtain the LC_{50} through the toxicity assessment test. Low toxicity was considered when the LC_{50} is greater than 500 µg ml^{-1}; moderate when the LC_{50} was between 100 and 500 µg ml^{-1} and very toxic when the LC_{50} was less than 100 µg ml^{-1} (Amarante, 2010).

Antibacterial and yeast assay

The microorganisms used in the tests were two Gram-negative bacteria: *Salmonella typhimurium* (ATCC 13311) and *Citrobacter freundii* (ATCC8090), two gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778) and a fungus (yeast) *Candida albicans* (ATCC 18804). The concentrations of essential oils in the tests were: 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95 µg ml^{-1} (Zacchino and Gupta, 2007). The samples were weighed, dissolved in DMSO (500 mg ml^{-1}) and added to Brain Heart Infusion broth (BHI) for bacteria and Sabouraud for yeasts to produce a solution with final concentration of 20 mg ml^{-1}. A pre-inoculum was prepared in which the bacteria and yeast were transferred with a platinum ring to test tubes containing 3 ml of BHI broth. The tubes were incubated at 37°C for 24 h. The pre-inoculum (500 µl) was transferred to tubes containing 4.5 ml of distilled water. The tubes were homogenized and the concentration adjusted to 0.5 McFarland turbidity standard (10^8 UFC ml^{-1}), thus obtaining the inocula used in the bioassays. The tests were performed on 96 microwell plates, in duplicate. Two controls were performed, one to monitor the growth of the microorganism, in which there was no addition of the working solution (to verify cell viability) and another one, in which the microbial inoculum was not added (in order to eliminate the effect of extracts color of the working solution). A control plate containing 100 µl of BHI culture medium and 100 µl of sterile distilled water was added to the experiment to control the sterility of BHI culture medium. Another control was prepared, containing the standard antibiotics: ampicillin (antibacterial), miconazole and nystatin (antifungals) to observe the activity of these antibiotics on the microorganisms. The microplates were incubated in an oven at 37°C and after 24 h the Elisa plates (492 nm) were read. The results were calculated as percent inhibition using the formula:

% Inhibition = 100 - AC1 - AC2 × 100AH - AM

where AC1 = absorbance of the sample; AC2 = absorbance of control sample; AH = absorbance in the control of microorganism and AM = absorbance of the control of the culture medium.

RESULTS AND DISCUSSION

Analyses of GC-FID for quantification and GC-MS for identification of the essential oil components of *H. dilatata* flowers showed the presence of 22 constituents (Table 1).

The main compounds and their respective concentrations in the essential oil of dried flowers harvested during the dry period (DP) and rainy period (RP) were α-pinene (26.2%), fenchone (17%), β-caryophyllene (16.3%), 3-carene (12.2%) for DP and α-pinene (10.9%), 3-Carene (3.7%), fenchone (14.8%), and β-caryophyllene (30.9%) for RP (Figure 1).

Figure 2A and B shows the chromatographic profile for *H. dilatata* essential oil. Twenty-two components corresponding to 89.1 and 76.8% of essential oil composition of the flower collected in the dry and rainy period, respectively, were identified. Four major compounds gave 71.7% of the essential oil collected in the dry period and 60.3% of the essential oil collected in the rainy period.

The chemical composition of the essential oils of *H.*

Table 1. Chemical constituents in the essential oils of *H. dilatata* flower.

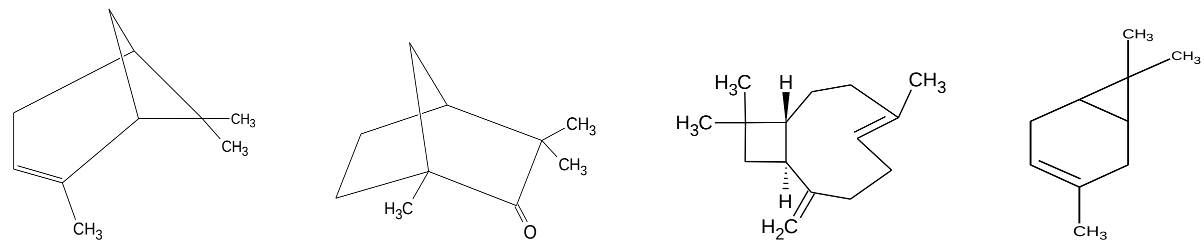
Peak	*KI	*RT	* FDP	* FRP	Compounds
		min	%	%	
1	973	3.75	26.2	10.9	α-pinene
2	980	4.03	1.5	0.7	Camphene
3	993	4.53	0.2	0.0	Sabinene
4	996	4.65	2.6	1.5	β-pinene
5	1008	5.07	0.4	0.2	Myrcene
6	1015	5.37	0.3	0.2	α-felandrene
7	1019	5.52	12.2	3.7	3-carene
8	1024	5.70	0.3	0.2	α-terpinene
9	1031	5.94	0.4	0.2	p-cymene
10	1033	6.04	2.4	0.7	Limonene
11	1058	6.96	0.4	0.3	g-terpinene
12	1083	7.90	17.0	14.8	Fenchone
13	1110	8.95	0.3	0.2	Menthenol
14	1133	9.80	2.0	2.4	Camphor
15	1170	11.20	0.8	0.9	Fenchol
16	1190	11.96	0.6	0.4	terpinen-4-ol
17	1212	12.77	1.4	2.2	α-terpineol
18	1413	20.39	16.3	30.9	β-caryophyllene
19	1432	21.10	1.2	1.9	Aromadendrene
20	1447	21.66	1.2	2.2	Humulene
21	1491	23.31	0.7	1.2	D-germacrene
22	1574	26.47	0.7	1.1	Caryophyllene oxide
-	-	-	10.9	23.2	Others
-	-	-	89.1	76.8	Identified
-	-	-	100.0	100.0	Total

*FDP: Essential oil from dry period; FRP: *Essential oil from rainy period; * KI: Kovats index calculated by GC using an *n*-alkane series under the same conditions as for samples; *TR: retention time.

dilatata was mainly constituted by monoterpenes, being fenchone, a bicyclic monoterpene compatible with literature data for volatile constituents of species of genus *Hyptis* (Martins et al., 2006). The values of α-pinene (0.07%) in the flowers of this species collected in Colombia (Tafurt-García et al., 2014) were much lower than those collected in the Tepequem saw in Roraima State, Brazil, according to the Table 1. In the work of Melo (2013), the 3-carene (9.5%) and camphor (16%) levels were above the levels found in the present work results of our research 3-carene (3.7%) and camphor (2.4%) ,respectively, but lower than the concentration of 3-carene (12.2%) obtained from *Hyptis* essential oil collected in dry period in this work. Brant et al. (2008) report that in different environments, there are differences in chemical composition of plant species due to different efficiencies in the production of active principles. They also emphasizes that the periods where there is greater production of oil usually do not match the largest production of chemical constituents.

In this research, the content of monoterpenes in essential oil of *Hyptis* flower presented in the dry and rainy periods were 69 and 39.5%, respectively. According to Passos et al. (2009), monoterpenes present in volatile oils have potential activities on the GABAergic neurotransmitters (gamma-aminobutyric acid), which are a good tool for the development of anxiolytic and anticonvulsant drugs.

The essential oils of *H. dilatata* showed high inhibition of acetylcholinesterase enzyme in the rainy (93.4%) and dry (92.4%) periods when compared with the standard drug used eserine (91.72%) and galatamine (94.36%) inhibitions. Extracts above 50% enzyme inhibition are indicated for isolation as potentially inhibitory substances of the enzyme; extracts ranging from 30 to 50% are considered moderate inhibitors and below 30% are weak inhibitors (Trevisan and Macedo, 2003; Vinutha, 2007). Inhibition of the acetylcholinesterase enzyme has demonstrated efficiency in the clinical treatment of Alzheimer's disease, which is associated with deficits of various brain neurotransmitters, such as acetylcholine, noradrenaline and serotonine. Symptomatic treatment of the disease primarily involves restoration of cholinergic function. It is suggested, therefore, that an increase in the



α-pinene fenchone β-caryophyllene 3-carene

Figure 1. Chemical structures of the major constituents of the essential oils of *H. dilatata* flowers.

Table 2. Antimicrobial activity of the flower essential oil of *H. dilatata* harvested in the rainy period.

Microorganisms	% inhibition of growth ($\mu g\ ml^{-1}$)							
	250	125	62.5	31.25	15.62	7.81	3.90	1.95
C. albicans	43.3	45.7	41.3	20.5	10.1	17.7	6.1	23.4
B. cereus	75.4	50.2	49.3	33.8	24.2	17.4	18.9	12.1
S. aureus	52.6	61.2	54.1	41.6	41.7	29.2	31.4	15.8
S. typhimurium	75.0	61.2	70.1	76.6	76.5	76.3	84.1	74.1
C. freundii	62.6	47.0	30.0	11.6	6.4	17.1	11.0	17.4

Table 3. Antimicrobial activity of the flower essential oil of *H. dilatata* harvested in the dry period.

Microorganisms	% inhibition of growth ($\mu g\ ml^{-1}$)							
	250	125	62.5	31.25	15.62	7.81	3.90	1.95
C. albicans	51.9	59.7	38.9	42.9	26.4	24.7	26.5	37.1
B. cereus	62.1	55.2	31.9	39.9	13.4	12.7	15.0	13.0
S. aureus	71.8	61.3	53.1	39.9	46.7	30.9	40.2	27.6
S. typhimurium	75.9	61.2	62.0	70.6	78.7	80.6	81.3	66.5
C. freundii	88.2	56.5	37.7	23.3	45.8	39.4	35.3	27.5

level of acetylcholine could be useful to improve patients health (Trevisan and Macedo, 2003; Vinutha, 2007). Monoterpenes and sesquiterpenes, such as elemol, linalool and α-pinene have been reported to inhibit acetylcholinesterase enzyme (Miyazama et al., 1998; Perry et al., 2000). In the study by Miyazama and Yamafugi (2005), some bicyclic monoterpenes such as α-pinene and 3-carene showed inhibitory effect on acetylcholinesterase. The results obtained for essential oil of *H. dilatata* flowers in the present work, show that this oil is a potent acetylcholinesterase inhibition.

In the toxicity assay on *A. salina,* the samples were tested at concentrations of 1000, 500, 250, 125, 62.5, 31.25 and 15.625 $\mu g\ ml^{-1}$. Survivors larval were counted after 24 h. The found for mortality percentages FRP (LC_{50}=5.25) and FDP (LC_{50}=6.91) are as shown in Figure 3.

According to the percentage of dead nauplii, in the lethality assay against *A. salina*, samples can be considered toxic when $LC_{50} < 100\ \mu g\ ml^{-1}$ (Amarante, 2010). In this assay, the control with DMSO (solvent) did not influence the results, because no larvae died in the presence of this solvent, in the same way as the control performed with salt water.

In the antimicrobial activity assay, essential oil of the *H. dilatata* flowers collected in both rainy and dry periods, were active against the pathogenic microorganisms. The controls used in the yeast test, showed LC_{50} of 7.73 $\mu g\ ml^{-1}$ and the miconazole with LC_{50} <1.95 $\mu g\ ml^{-1}$. The essential oil of the flowers collected in the dry period showed, greater effectiveness in relation to the inhibition of this microorganism by the essential oil harvested in the dry period, in the concentrations of 250 and 125 $\mu g\ ml^{-1}$, with respective inhibitions of 51.9 and 59.7% as shown in Tables 2 and 3.

It was observed that they inhibited more than 50% at concentrations of 250 and 125 $\mu g\ ml^{-1}$ both essential oils of all bacteria (Table 2). These results are very important

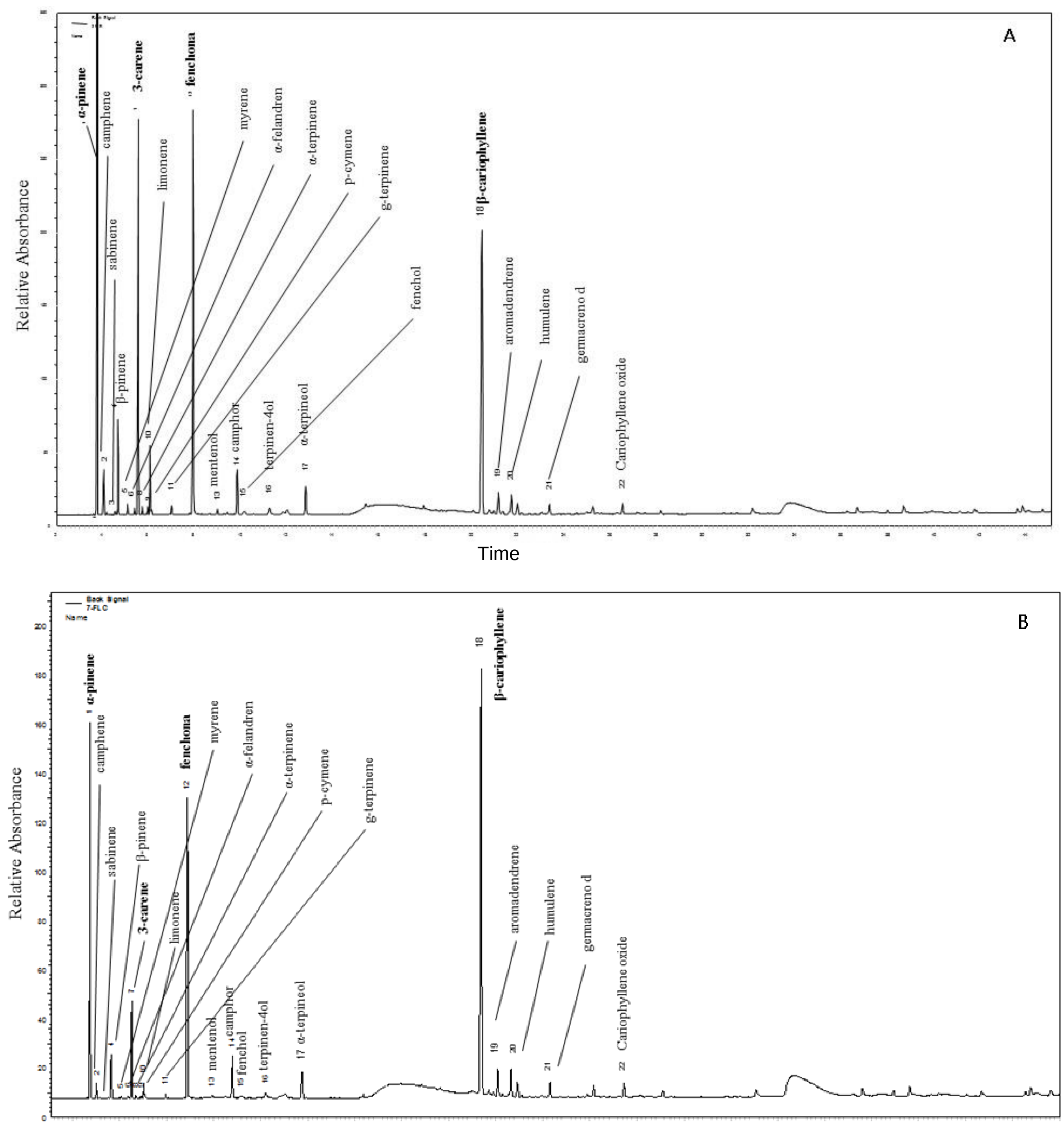


Figure 2. Chromatogram of the essential oil from *H. dilatata* flowers, harvested in the rainy period (A) and dry period (B).

and suggest future studies *in vivo*, which will contribute to a better knowledge of the potential of this species for producing bioproducts of medicinal interest. Tables 2 and 3 present the percentage of bacterial and yeast inhibition by essential oils of *H. dilatata* harvested in the rainy and dry period.

The LC_{50} values of the essential oils were calculated using software origin 8.0 (Figure 4). The control used in the bacterial assays was ampicillin that showed a LC_{50} value < 1.95 μg ml^{-1}. Although, the inhibition of *S. aureus*

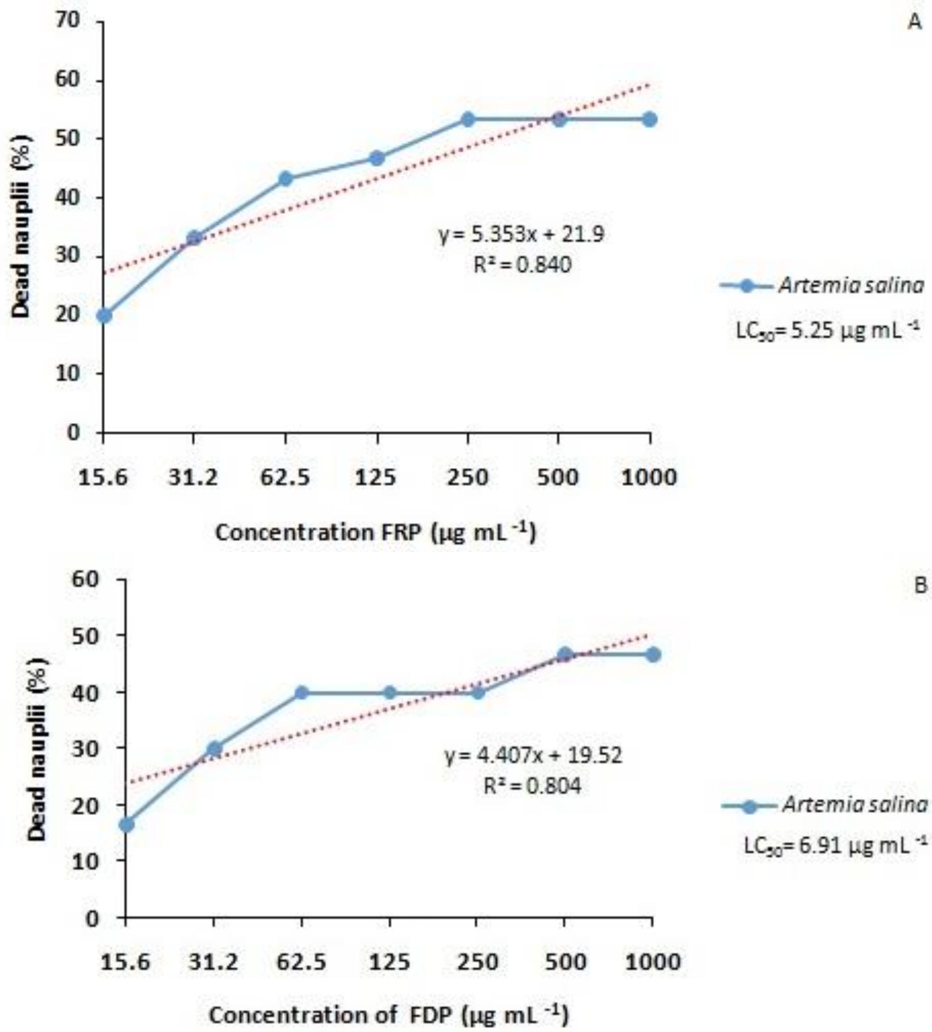


Figure 3. Curve of essential oil activity of *Hyptis dilatata* essential oil against *Artemia salina*. A: Essential oil obtained in rainy period; B: Essential oil obtained in dry period.

bacterium was higher when the oil was collected during the dry period, the values of 250 µg ml^{-1} of LC_{50} were better for the essential oil collected in the rainy period with LC_{50} = 49.8 µg ml^{-1} while when harvested in the dry period furnished a LC_{50} = 121.1 µg ml^{-1}.

According to Alvarez et al. (2015), a *H. dilatata* species, collected in San Jose Del Graviane (Colombia), presented monoterpenoid hydrocarbons represented by 2-β-pinene (12.29%), camphor (5.67%) and 1-β-pinene (4.21%) and between sesquiterpenoids and aromadendrene (6.48%), the main components. However, working with complex extracts, it is not possible to attribute the antimicrobial effects to a single component, since the major components can make a significant contribution to the biological activity. In this research, some of the chemical constituents of essential oil of *H. dilatata* collected in Tepequem saw, Amajari, Roraima, Brazil, in the dry and rainy periods were the same than those detected by Alvarez et al. (2015), but with different concentrations, such as β-pinene (2.6 and 1.5%), camphor (2.0 and 2.4%) and aromadendrene (1.2 and 1.9%). On the other side, there are some differences in the percentages of some major constituents such as α-pinene, 3-carene, fenchone and β-caryophillene.

Conclusion

Differences were observed on the components of the essential oil of *H. dilatata* flowers, harvested in rainy and dry periods, as well as in the biological activities towards aceticolinesterase enzyme, bacteria and yeast inhibitions in addition, the bioassays results showed that the species under study has potential to be used in the development of alternative medicine for treatment of neurodegenerative diseases and against pathogenic

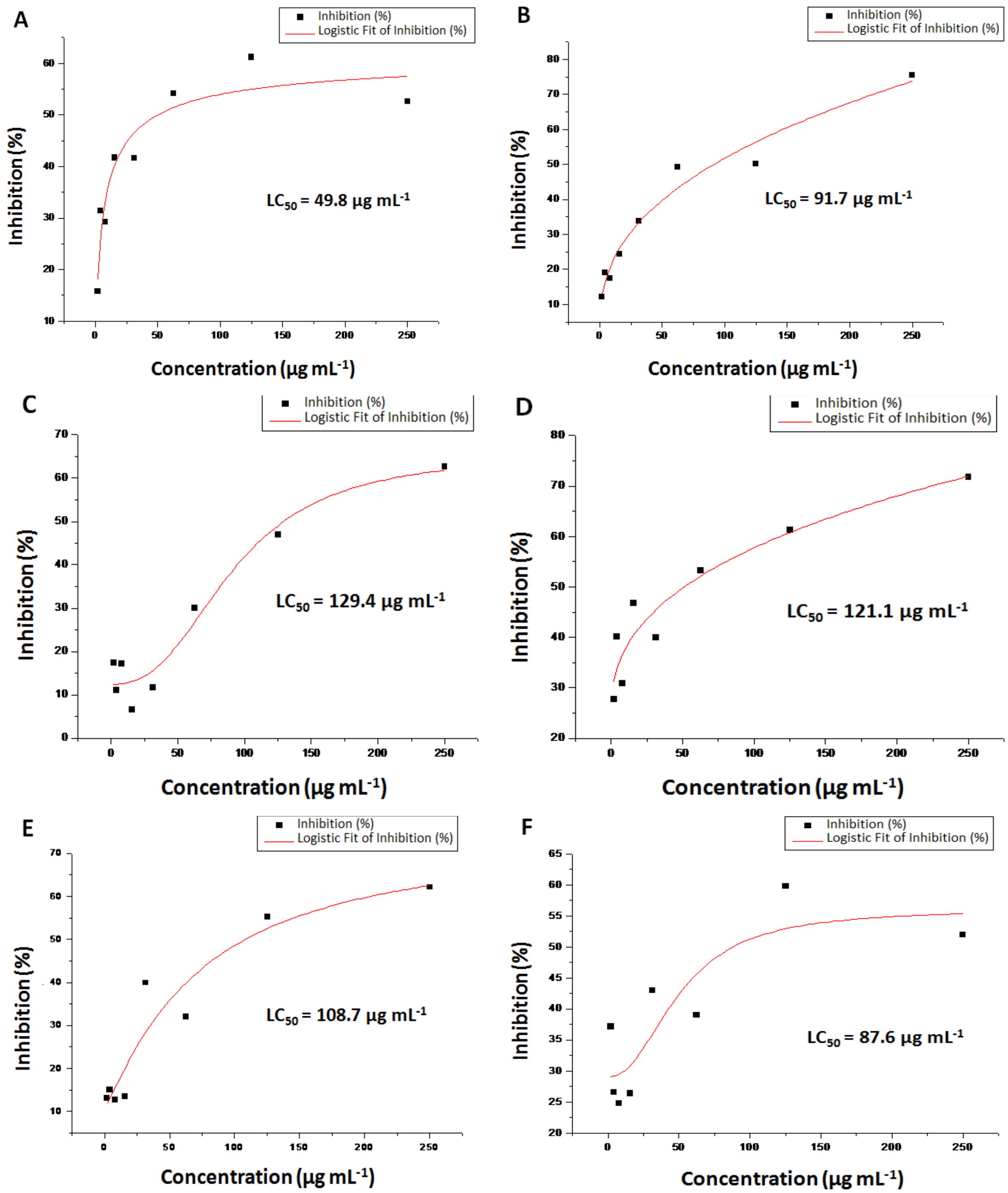


Figure 4. Curve of LC_{50} for essential oil of *H. dilatata*: Rainy Period: A - *Staphylococcus aureus;* B - *Bacillus cereus;* C - *Citrobacter freundii*. Dry period: D - *Staphylococcus aureus;* E - *Bacillus cereus;* F - *Candida albicans*.

microorganisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to FAPEAM for a fellowship and to the staff of the Herbarium of the INPA, especially Mr. José Ramos for identification and EMBRAPA-RR for the collection assistance.

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