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Full Length Research Paper

Versatility and consensus of the use of medicinal plants in an area of cerrado in the Chapada do Araripe, Barbalha - CE- Brazil

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This study aimed to conduct an ethnobotanical survey of medicinal species in an área of cerrado vegetation in the chapada do Araripe, Ceara, as well as evaluating and selecting species with potential for bioprospecting, based on the relative importance of the species and the consensus among the informants. The ethnobotanical data were collected through semi-structured interviews and a free list, and by using the technique "snowball". It was analyzed the relative importance of the mentioned plants as well as species that stood out on the bodily systems, based on the factor:consensus among the informants. It was recorded a total of 38 species distributed into 35 genera and 25 families. Of the total eight species (21%) presented great versatility (IR≥1) regarding to its use. The therapeutic indications were grouped into 14 categories of bodily systems. Disorder of respiratory and genitourinary systems, injuries, poisoning and other consequences of external causes and diseases of the blood, organs and connective tissue, obtained a greater consensus among the informants. *Ximenia americana, Himathantus drasticus, Stryphnodndron rotundifolium, Hancornia speciosa* and *Cecropia pachysthachya* were the species subject to further study, based on versatility, and consensus of the use among the informants.

Key words: Ethnobotany, factor in consensus, local knowledge, potential therapeutic, regional species, relative importance.

INTRODUCTION

Studies on plants with medicinal potential have represented a major focus on the search for new drugs (Elisabetsky, 1991). In accordance with the data from the World Health Organization, a large part of the population of developing countries uses popular herbal medicine in primary healthcare. This is due to the fact that diseases

that affect the population in these countries are mainly related to the lack of sanitation, malnutrition and difficulty of access to medicines (Kumate, 1997). Due to this, these communities per finish choose medical species available in the environment, low cost.

In Brazil, the use of medicinal plants is a therapeutic resource extensively explored by the population, although often their use does not contain the necessary information to ensure the safety of these agents (Souza et al., 2011). Hence, the importance of encouraging the transmission of knowledge from one generation to another in a traditional scope (Ceolin et al., 2011) however, accompanied by scientific proof. An analysis that generates information about traditional therapeutic practices and of the species which deserve attention from these people is valid when the goal is to promote information about cultural subjects and the Brazilian ecosystem sustainability.

In this sense, ethnobotanical investigations have focused on studying biologically active species, as well as obtaining information about the possible forms of sustainable use of these plant resources (Albuquerque, 2002). Among the least studied Brazilian ecosystems there are those, which are related to dry forests (Caatinga and Cerrado) (Albuquerque and Andrade, 2002). Specifically in the northeastern cerrados there is a visible lack of conducted studies, mainly regarding to ethnobotanical and pharmacological surveys. Most of these studies are concentrated in the states of Mato Grosso, Goias and Minas Gerais (Souza and Felfili, 2006; Botrel et al., 2006; Alves et al., 2008; Silva and Proenca, 2008), and there is a gap regarding to the northeastern disjoined cerrados.

Considering the importance of information about plants with therapeutic purposes and the analysis of their representativeness within the cerrado, the goal of this study was to investigate the use of medicinal plants in a cerrado area of Chapada do Araripe, Barbalha, State of Ceara. The study focused on assessing the local diversity of medicinal plants by checking the versatility of species used and the consensus of use and/or knowledge among the informants. At the same time, the study aimed at pointing out which group of species should deserve further study due to be potentially active as herbal medicines.

MATERIALS AND METHODS

Study area

This study was conducted in the rural community of Betania (7°18' 18'' S and 39° 18' 07'' W). It is situated in the municipality of Barbalha, established in a cerrado area of Chapada do Araripe, south of the State of Ceara, bordering the State of Pernambuco (Figure 1). Chapada do Araripe, which has a tabular surface with an altitude ranging between 760 and 920 m, is one of the most striking elements of the landscape of the region (Toniolo and Kazmierzak, 1998), within the semi-arid region. It behaves as an island for certain types of vegetation, such as the cerrado, and it is like a disjunction of its core area in this location (Costa et al., 2004). The dystrophic red and yellow latosols predominate in the region (Brasil, 1973), with warm tropical climate, average annual rainfall of around 760 mm and temperature between 24 and 26°C. This region has two well-defined seasons, dry and rainy, being the rainy occurring between January and April (Costa et al., 2004).

Ethnobotanical and floristic survey

The research was carried from August to December 2014, out by means of semi- structured interviews based on standardized forms according to the method proposed by Martins (1995) and information was obtained after the participants signed a consent form. It is important to mention that this research was submitted to the Research Ethics Committee of the Regional University of Cariri and approved with Opinion No.251.829/2013. The informants was selected through the snowball technique (Bailey, 1984).

The free list technique was used in the interviews in order to stimulate the interviewed cite of all medicinal plants they know and/or use. A total of 30 informants were interviewed, 19 males and 11 females, with ages ranging from 35 to 91 years.

The botanical material was collected in the backyard of the residences and, when possible, in the forest, at the time and/or after the interviews. The species were incorporated into the Caririense Dardano de Andrade Lima Herbarium of the Regional University of Cariri (HCDAL-URCA).

Data analysis

The index of relative importance (RI) of the medicinal plants identified was calculated according to the methodology proposed by Bennett and Prance (2000). The relative importance is a quantitative method which demonstrates the importance of specie based on its versatility, with '2', being the highest possible value. The calculation is made according to the following formula: RI = NCS + NP; where, RI= relative importance; NCS = the number of body systems treated by a given species (NCSS), divided by the total number of body systems treated by the most versatile species (NSCSV); NP = number of properties attributed to a species (NPS), divided by the total number of properties attributed to the most versatile species (NPSV) (Silva et al., 2010 (a); Almeida et al., 2006). The calculation of this index was performed based on the distribution of the indications for use of ethno-species in body systems, related to 12 categories of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (OMS, 2000).

The informant consensus factor (ICF) was calculated according to the technique proposed by Trotter and Logan (1986) aiming to identify the body systems that had greater consensus of current knowledge and/or use, and which plant groups require deeper studies (Almeida et al., 2006). For the calculation of the ICF, the following formula was used to calculate the ICF = nur −nt/ nur −1, where: nur is the number of citations of usage in each category and nt is the number of species indicated in each category. ICF values

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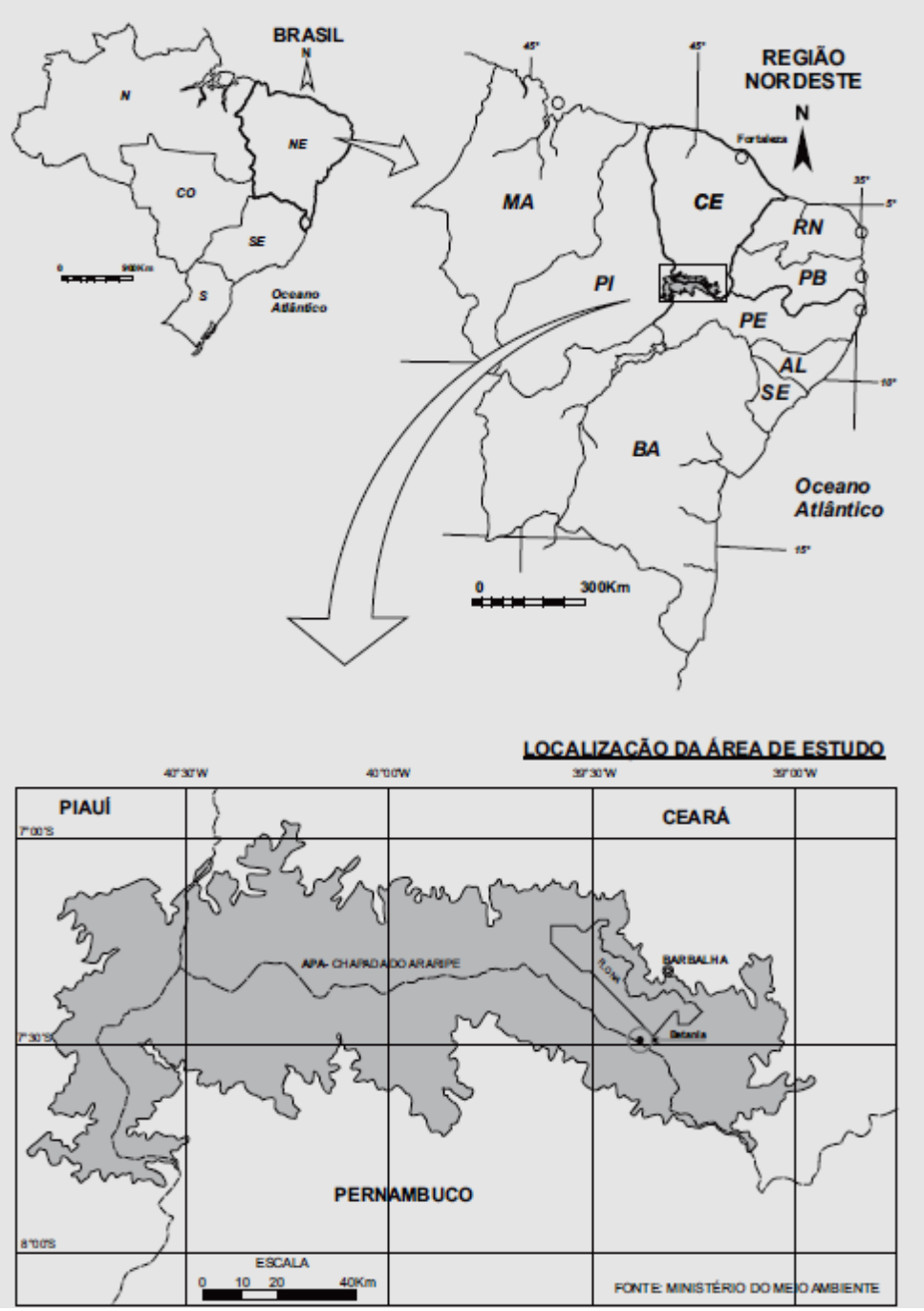

Figure 1. Localização geográfica da área de estudo na comunidade Betânia no município de Barbalha, Ceará, Brasil.

range from 0 to 1.

RESULTS AND DISCUSSION

Local diversity of medicinal plants

A total of 38 native medicinal plant species were reported, distributed in 25 botanical families and 35 genera (Table 1). Among these, seven were only identified at genus level and 31 at species level. Compared with similar studies related to the medicinal use of native species conducted in the cerrado (Souza and Felfili, 2006; Botrel et al., 2006; Souza, 2007; Cunha and Bortolotto, 2011), there is still a reduced number when compared to the medicinal flora diversity of this vegetation, which ranges from 24 to 143 species. The botanical families

Table 1. List of the medicinal species indicated by the residents of the Betânia community in the municipality of Barbalha, state of Ceara (NE Brazil).

Family/ Scientific name	Popular name	Part used	Preparation	Uses forms	Therapeutic indications	RI	Herbarium number
Anacardiaceae *Astronium fraxinifolium* Schott ex Spreng.	Gonçalo-Alves	Ib	of sauce	Drink	Cough, Influenza.	0.34	10.153
Anacardium microcarpum Ducke	Cajuí	Ib	of sauce	Bath, gargle	Sore throat, wound, toothache	0.80	Nc
Annonaceae *Annona coriacea* Mart.	Araticum	Sb	Decoction	Drink	Snakebite.	0.25	7973
Apocynaceae *Ditassa* R.Br.	Caninana	Ro	of sauce ou no álcool	Drink	Rheumatism, spine, Influenza.	0.60	7960
Himatanthus drasticus (Mart.) Plumel.	Janaguba	La	On the water	Drink	Cancer, ulcer, gastritis, stomach, varicose veins, hernia, rheumatism, wound, burning sensation.	1.65	8417
Hancornia speciosa Gomes.	Mangaba	La, Le	Decoction	Drink	Gastritis, infarction, thyroid, triage vein, varicose vein, stomach, cough, ulcer, heartburn, high blood pressure.	1.83	10.155
Aristolochiaceae *Aristolochia claussenii* Duch.	Jarrinha do Mato	Ro	Infusion	Drink	Bowel disease, heartburn.	0.35	Nc
Asteraceae *Acanthospermum hispidum* DC.	Espinho de cigano	Le, Ro	Decoction	Drink	Belly ache	0.25	Nc
Protium heptaphyllum March.	Amescla	Rs	-------	Inhalation	Headache	0.25	8426
Bromeliaceae *Tillandsia* sp.	Bananinha de raposa	Fr	-------	Eat the fruit	Worm	0.25	Nc
Caryocaraceae *Caryocar coriaceum* Wittm.	Piqui	Fr	extract the oil of the fruit and mix with water, licking	Drink	Influenza, sore throat, cough, fatigue, swelling.	0.80	8408
Euphorbiaceae *Croton heliotropiifolius* Kunth.	Velame branco	Le	Decoction	Drink	Thinning the blood, rheumatism, stomach pain, injury, internal inflammation, cicatrizant, spine pain.	1.46	7970
Croton sp.	Velame vermelho	Le	Infusion	Drink	Stomachache, thinning the blood, strepto.	0.80	8423
Fabaceae *Bowdichia virgiloides* Kunth.	Sucupira	Ib, Sb	of sauce or in the alcohol	Drink	Rheumatism, spine, stomach ache.	0.60	8425
Centrosema sp.	Alcançu	Ro	of sauce	Drink	Influenza, fever, cough, asthma, stomachache.	1.00	8407
Copaifera langsdorffi Desf.	Pau d'óleo	Le, Fr, Ib	Oil bast, Decoction	Drink	Spine, rheumatism, indigestion, epilepsy, thinning the blood, swelling in the belly, stomachache, general pain, injury.	1.81	7962
Dimorphandra gardneriana Tull.	Faveira	Se	Decoction	Drink	Heart	0.25	7941

Table 1. Cont'd.

Stryphnodendron rotundifolium Mart.	Barbatimão	Ib	of sauce	Drink	Cancer, ulcer, wound, inflammation of the uterus,blood infections, gastritis, sore throat, wound.	1.72	8406
Acosmium glasycarpa Benth.	Pau pra-tudo	Ib	Decoction	Drink	Hernia, chest pain.	0.51	Nc
Fabaceae (Cae) *Bauhinia cheilantha* (Bong.) Steud.	Mororó	Le, Ib, Ro	Decoction, Infusion	Drink	Stomachache, cramp of blood, heartburn.	0.60	8414
Hymenaea courbaril L.	Jatobá	Ib	Decoction	Drink	Cough, Influenza, bronchitis.	0.45	7957
Senna occidentalis (L.) Link	Manjirioba	Ro, Se	Infusion, seed toasted	Drink	Cough, Influenza, thinning the blood.	0.60	8411
Malvaceae *Sida galheirensis* Ulbr.	Malva-branca	Le, Ro	Decoction	Drink	Stomachache, Influenza, heartburn.	0.60	8409
Myrtaceae *Psidium* sp.	Araçá de veado	Le	Decoction	Drink	Swelling in the stomach.	0.25	8424
Psidium guineense Sw.	Araçá goiaba	Le	Decoction, Infusion	Drink	Stomachache, gastura.	0.25	Nc
Olacaceae *Ximenia americana* L.	Ameixa	Ib, Sb	Decoction	Drink	Rheumatism, bone pain, sore throat, wound, ulcer, spine, wound.	1.38	8419
Passifloraceae *Passiflora cincinnata* Mast.	Maracujá do mato	Le	Decoction	Drink	Nerves, depression, high pressure, insomnia.	1.00	7974
Polygonaceae *Bredemeyeria brevifolia* (Benth.) A.W. Benn.	Mal vizinho	Le	Decoction	Drink	Gastritis	0.25	7939
Phytolacaceae *Petiveria* sp.	Tipi	Ro	Of sauce or in the alcohol	Drink	Spine, Inflammation of kidneys.	0.51	Nc
Proteaceae *Roupala montana* L.	Congonha	Le	Decoction	Drink	Fever, spine	0.51	8420
Rubiaceae *Tocoyena formosa* (Cham. & Schltdl.) Schum.	Jenipapo	Ib	Infusion	Drink	Twist	0.25	7956
Solanaceae *Solanum lycocarpum* A. St. Hil.	Jurubeba	Se	seed toasted	Drink	blurry vision, gastritis.	0.55	7969
Rutaceae *Pilocarpus* sp.	Jaborandi	Le	Decoction	Drink	Fever, pain in general.	0.34	8413
Smilax japicanga Griseb.	Japecanga	Ro	Decoction	Drink	Headache, menstrual cramps, heartburn.	0.90	10.154
Urticaceae *Cecropia pachysthachya* Trec.	Toré	Le	of sauce, Decoction	Drink	Kidney pain, heartburn, sore throat.	0.80	Nc
Hybanthus arenarius Ule	Orelha de onça	Ro	Decoction	Drink	Swelling in the belly, liver, heartburn, indigestion.	0.70	Nc
Hybanthus ipecacuanha L.	Papaconha	Ro	Infusion	Drink	Influenza, intestine pain.	0.51	8412

LEGENDA: Le: leaves, Ro: root; Se: seed; Sb: stem bark; Ib: inner bark; Fr: fruit, La: latex, Rs: Resin; RI: Relative Importance; nc: number of collection in process by Herbaria.

that contributed with the greatest number of species were Fabaceae, with nine species, and Apocynaceae, with three. The fact that Fabaceae stood out over the others corroborates with the works conducted by Botrel et al. (2006); Moreira and Guarim Neto (2009); Oliveira et al. (2010); Cunha and Bortolotto (2011) and Souza et al. (2014), since the large medicinal use of this family can be explained by the great number and diversity of the species that compose it, being one of the largest in the cerrado. Most genera were represented by only one species, with the exception of *Croton*, *Psidium* and *Hybanthus*.

In this research, the leaves were the part of the plants most used in local home made medicines (31.61% of cases). The other plant parts used had a percentage distributed as follows: roots (24.49%); inner barks (20.43%); stem barks and fruits (6.12%) each, in addition to plant products such as resins, latex, and seeds (12.27%). Other studies have demonstrated that the leaves were also the part of the plant most used in medicinal preparations (Giraldi and Hanazaki, 2010; Pinto et al., 2006; Franco and Barros, 2006), followed by barks and roots. It is well known that plants have different concentrations of chemical compounds in their parts.

In the community under study, 07 different preparation methods were recorded, ranging from decoction (44,19%), soaking (18,60%), infusion (16,28%). The other forms, use of alcohol, in the water, licking and seed toasted totaled a percentage of 20.93%. (Intake, is the most cited by the interviewed) It is observed that decoction is the most common form staging also in other etnobotanicos surveys conducted in cerrado de Minas Gerais (Calábria et al., 2008) and Mato Grosso do Sul (Cunha and Bortolotto, 2011).

Among the species, 21% of medicinal plants had great versatility in relation to their uses, showing high relative importance (RI ≥ 1). The most versatile species were: *Hancornia speciosa*, *Copaifera langsdorffi*, *Stryphnodendron rotundifolium*, *Himatanthus drasticus*, *Croton heliotropiifolius*, *Ximenia americana*, *Centrosema* sp., and *Passiflora cincinnata*. The other species had RI <1, with variation from 0.25 to 0.90, ranging from one to three body systems per species. It is important to emphasize that none of the species mentioned in this survey reached the highest value of RI = 2.

The most versatile species had from three to six body systems, what comprehends from four to eleven healing properties. The species *S. rotundifolium* and *C. langsdorffi* stood out by offering larger number of body systems (6), having been related to eight and nine healing properties, respectively.

The species *H. drasticus*, *H. speciosa* and *S. rotundifolium* were highly recommended for the treatment of gastritis and ulcer. *C. heliotropiifolius*, *S. rotundifolium* and *X. americana* were mentioned for curing both internal and external inflammations. For hypertension problems, the informants made use of the species *P. cincinnata* and *H. speciosa*.

As much latex as for the leaves, however in Nova Xavantina-MT, the most used part of this species is the bark of the stems, and *C. langsdorffi* they use as much as the shells as the leaves and fruits, corroborating with the results achieved in our research (Silva et al., 2010b). Regarding *H. drasticus*, the informants' among versatile species: the leaves are the parts of the plants which are most used, followed by inner barks. This fact was also observed in studies conducted by Cartaxo et al. (2010), Medeiros et al. (2004) and Pinto et al. (2006). It is observed that the parts of the plants used will vary depending on the knowledge and practices of use that exists in each locale. Thus, regarding *C. heliotropiifolius* in the community studied and in a study conducted in the semi-arid region of the State of Piaui (Oliveira et al., 2010), the most common report was for the use of its leaves; however, in the carrasco area of the State of Ceara, besides the use of leaves, its roots had also been used by the informants (Souza et al., 2014).

Agreeing with this study, *X. americana* has also been reported in other studies with respect to its bark and inner bark (Roque et al., 2010; Cartaxo et al., 2010). For *H. speciosa*, informants mentioned the use of the latex of the plant to treat various ailments. Still for the same species in the surrounding communities of Serra das Almas Natural Reserve, states of Ceara and Piaui, the informants reported that the use of leaves, to make medicines is the most common (Magalhaes, 2006).

Among the species with relative importance in this research, some have pharmacological studies that demonstrate a variety of activities, for example, both bark extracts and leaves of *X. americana* have proven antifungal activity (Omer and Elnima, 2003). *H. drasticus* tests have presented its anti-inflammatory, gastro-protective and antinociceptive effects (Lucetti et al., 2010; Colares et al., 2008), and this research corroborates with the therapeutic indications mentioned by informants for treating ulcer, gastritis and wounds.

Despite informants not having cited *C. langsdorffii* for cancer, studies have demonstrated that kaurenoic acid isolated from the oil of *C. langsdorffii* presented anti-inflammatory and cytotoxic potential, inhibiting the growth of cancer cells (Costa Lofuto et al., 2002; Paiva et al., 2003). The ethanolic extract from stem bark of *S. rotundifolium* showed anti-microbial, anti-ulcer and gastro-protective activities in surveys conducted by Rodrigues et al. (2008) and Oliveira et al. (2011). This fact is confirmed in this study, in which the species were reported for the treatment of cancer, ulcer, gastritis, infections, and inflammations. Anti-hypertensive (Soares et al., 2006) and anti-inflammatory (Endringer et al., 2006) activities were verified through of ethanolic extract *H. speciosa* leaves.

For *Croton h.,* there were found pharmacological and

phytochemicals studies using the leaves of this species, only the root extract was observed antitumor activity (Torrance et al., 1977) and antimicrobial (Peres et al.,1997). For *Centrosema* sp and *P. cincinnata,* pharmacological studies were not found to report activities or medicinal use of these, given mainly to treat diseases of respiratory and nervous system.

Many of the healing properties of the species with the highest relative importance in this work were cited in other studies (Cunha and Bortolotto, 2011; Roque et al., 2010; Santos et al., 2012;), however, in other studies, the use of *X. americana* for the treatment of rheumatism had

not been reported yet.

Informant's consensus factor for therapeutic purpose

The medicinal species were used to treat 51 therapeutic purposes and were classified into 14 categories relating to body systems listed in Table 2. In general terms, there was an agreement regarding to the use of the species within the categories among informants, with consensus values ranging from 0.6 to 1.0. In only one category there was no consensus among the informants.

The categories, Certain infectious and parasitic diseases, Endocrine, nutritional and metabolic diseases, presented the highest indices of agreement, with the utilization of *Tillandsia* sp., To treat worm and *Hancornia speciosa* for thyroid, providing evidence of greater cultural importance of these species for the studied community. In another study in semi-arid region of northeastern Brazil, IPD category also obtained the greatest consensus among the informants (Almeida et al., 2006). It suggests that these diseases are related to the health habits, and to the economic conditions of the community.

The category RSD has received 114 citations of use, having being reported 12 species for therapeutic indications included in this body system, corresponding to 31% of species referred to in this work for the treatment of cough, sore throat, flu, asthma, bronchitis and tuberculosis, *C. coriaceum* (30), *H. courbaril* (28) and *H. ipecacuanha* (20) were the most reported species in this category, indicating a vast community knowledge about the medicinal plants which treat respiratory problems. Usually, this category is commonly found in other studies by the number of species or reports related to this body system, in Itacare, State of Bahia, and this system stands out by the high number of reports of use related to the treatment of flu and cough (Pinto et al., 2006). In the municipality of Caico, State of Rio Grande do Norte, RSD accounted for 31.6% of the reports and *Amburana cearensis* (Allemao) was the most common species indicated to treat flu and sinusitis (Roque et al., 2010). In a rural community of a caatinga region, in the municipality of Aiuaba, State of Ceara, this system obtained the largest number of reports of use (335), which corresponds to 28.44% of the total number of reports (1,178) and it also had a high number of species indicated (50), corresponding to 42.02% of the total. This fact demonstrates the broad knowledge that the community studied has about medicinal plants to treat respiratory problems

GSD had 23 citations of use grouping four species for the treatment of uterine inflammation, kidney disorders and menstrual cramps. *Cecropia pachysthachya* and *S. rotundifolium* were the most reported species to treat kidney disorders and internal inflammations, respectively. This category has been frequent in the studies conducted by Roque et al. (2010), Calabria et al. (2008), and Cunha and Bortolotto (2011), with kidney disorders as the most reported disease in this category.

The categorie IPOCEC, with 46 reports of use and nine species, covered the treatments indicated for injuries, healing and streptos, among others. *S. rotundifolium*, *C. langsdorffi* and *C. heliotropiifolius* were the most reported species. Similar results were observed in studies conducted by Roque et al. (2010), in which 'injury' was reported by 9% of the informants, and in studies conducted by Pinto et al. (2006), in which 'cut' received 30% of the reports within this system.

DSD and DMSCT received the same ICF values. DSD was the second most reported system (103 times), totaling 22 species. *H. drasticus*, *H. speciosa*, and *S. rotundifolium* were referred to treat ulcer and gastritis, which were the most frequent affections in this category. For DMSCT, nine species were reported to treat hernias, rheumatism and the vertebral spine pain.

It is common to find results which confirm the prevalence of DSD (Santos, 2009; Gazzaneo et al., 2005; Pilla et al., 2006), however, for diseases of the musculoskeletal system and connective tissue, ICF values are lower than those found in this study (Cartaxo et al., 2010).

The category CSD received 34 reports and comprised eight species. *P. cincinnata* and *H. speciosa* together had 15 reports for treating high blood pressure, having been the one most reported in this corporal system. In the semi-arid region of the State of Piaui, hypertension was also the most reported was the most reported disease (20), comprising 15 species for this purpose (Oliveira et al., 2010). Then the category of neoplasms, cancer was the illness of involve this system, cited by the informers the species *S. rotundifolium* and *H. drasticus* for their treatment. Similar results were found by Santos et al.(2012) in the semi-arid region of the State of Paraiba, where this category reached the same ICF value (0.75) of this study.

DBI, MBD and SSC obtained the lowest ICF values. *Hybanthus arenarius* stood out in the category diseases of the blood and blood-forming organs for treating of anemia. *P. cincinnata* and *X. americana* stood out in the treatment of insomnia and inflammations in general. Only

Table 2. Informant consensus factor (ICF) for body systems based on quotes from use of medicinal plants by the residents of the community of Betania, the town of Barbalha, Ceará (Northeast Brazil).

Code ICD-10	Categories: Therapeutic purposes	Number of uses reported in each category (nar)	Species reported in each category (na)	Percentual citation	ICF
A00- B99	**IPD**: worm	03	Bananinha de raposa	01	1.0
E00-E90	**DENM:** thyroid	03	Mangaba	01	1.0
J00-J99	**RSD:** Sore throat, cough, influenza, asthma, bronchitis, tuberculosis.	114	Barbatimão, mangaba, alcançu, caninana, ameixa, toré, jatobá, piqui, papaconha, manjuirioba, cajuí, malva branca.	12	0.90
N00-N99	**GSD**: Inflammation in the uterus, kidney pain, menstrual pain, clean the uterus, kidney inflammation.	23	Barbatimão, toré, japecanga, tipi.	04	0.86
S00-T98	**IPOCEC:** Cut, wound, snake bite, twist, strepto, healing.	46	Janaguba, barbatimão, pau d'óleo, ameixa, araticum, jenipapo, velame branco, cajuí, velame vermelho.	09	0.82
M00-M99	**DMSCT**: Hernia, rheumatism, spine pain.	40	Janaguba, caninana, pau d'óleo, sucupira, ameixa, congonha, tipi, velame branco, pau pra-tudo.	09	0.79
K00-K93	**DSD:** ulcer, gastritis, stomach pain, burning sensation, heartburn, abdominal pain, indigestion, gastura, birth tooth, toothache.	103	Janaguba, barbatimão, mangaba, alcançu, pau d'óleo, sucupira, ameixa, toré, mororó, jarrinha do mato, japecanga, papaconha, mal vizinho, Jitirana, jurubeba, cajuí, velame branco, araçá de veado, cajuí, velame vermelho, malva branca, espinho de cigano.	22	0.79
I00-I99	**CSD**: Varicose veins, vein triage, high blood pressure, heart attack, thinning the blood, body swelling, heart.	34	Janaguba, mangaba, pau d'óleo, piqui, maracujá do mato, manjuirioba, velame branco, velame vermelho.	08	0.78
C00-D48	**N**: câncer	05	Janaguba, barbatimão.	02	0.75
D50.0-D89	**DBI**: blood infection, anemia	07	Barbatimão, orelha de onça.	02	0.66
G00-G99	**NSD:** Nerves, migraine.	12	Maracujá do mato, japecanga, amescla.	03	0.27
F00- F99	**MBD**: Depression, insomnia, epilepsy**.**	06	Maracujá do mato, pau d'óleo.	02	0.8
R00-R99	**SSC**: Inflammation, fever, of blood cramp, pain in general.	21	Mangaba, alcançu, ameixa, jaborandin, mororó, congonha, pau pra-tudo, velame branco, pau d'óleo.	09	0.6
H00-H59	**DSSE**: blurry vision	01	Jurubeba	01	0

LEGENDA: RSD: Diseases of the respiratory system;IPOCEC: Injury, poisoning and certain other consequences of external causes; NSD: Diseases of the nervous system; DMSCT:Diseases of the musculoskeletal system and connective tissue; DSD: Diseases of the digestive system ; CSD: Diseases of the circulatory system ; N: Neoplasms ; GSD:; Diseases of the genitourinary system; DBI: Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism; MBD: Mental and behavioural disorders; SSC: Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified; IPD: Certain infectious and parasitic diseases;DENM: Endocrine, nutritional and metabolic diseases; DSSE: Diseases of the eye and adnexa.

the category of disorder sensory system has obtained FCI zero, with no agreement on the use of species in relation to this category in the locality. The bodily systems reported in this study, also stood out in other parts of the world, such as in China with the highest number of citations for the digestive system, injuries and other consequences of external causes and skin problems (Inta et al., 2008). In Serbia, the most frequently reported medicinal uses were for treating gastrointestinal, respiratory and cardiovascular (Jarić et al., 2007) diseases. In Ethiopia, the most cited systems were disorders of the digestive system, connective tissue diseases and skin lesions (Giday et al., 2003). In

the Latin America, other studies in the southern Ecuador showed 11% of the citations for the treatment of kidney problems (Tene et al., 2007) and in Peru were reported 34 species (39%) within the category of diseases and pains not set required to reduce inflammation in different parts of the body (De-La-Cruz et al., 2007). Based on these results, it is evident that many species stands out in some categories for their therapeutic applicability, where the most sought species are those which the population believes it has "healing power", confirming that the traditional herbal medicine remains being used by the local community.

Conclusion

According to the understanding of the versatility and use of plant species by informants, deeper studies are recommended for *H. drasticus species*, and *H. speciosa S. rotundifolium* that were highly reported for the treatment of gastrointestinal disorders, kidney disorders pachystachya to cecropia*, X. americana* and for the treatment of rheumatism.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Investigation of the inhibitory effects from the Brazilian medicinal plant *Pothomorphe umbellata* L. (Piperaceae) on the molecular pathways of cyclooxygenase-2 and nuclear factor kappa B

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Presently, medicinal plants, such as *Pothomorphe umbellata* L. (Piperaceae), have been assessed as sources of potential therapeutic medicines. The inhibitory effects of the crude extract, fractions and 4-nerolidylcathecol, a phenolic compound which has been assigned significant activity in several beneficial properties performed by the plant, on the molecular pathways of cyclooxygenase-2 (COX-2) and nuclear factor kappa B (NF-κB) were evaluated. The *in vitro* inhibition of COX-2 was performed by enzyme-linked immunosorbent assay (ELISA). Methylene chloride fraction demonstrated the preeminent inhibition of COX-2. In order to estimate the inhibition of NF-κB, an adaptation of the luciferase plasmid assay was developed. 4-Nerolidylcathecol presented the best inhibition NF-κB activity. The results obtained from the *in vitro* assays were promising, mainly on the molecular pathway of NF-κB, once 4-nerolidylcathecol demonstrated a remarkable inhibition activity.

Key words: Piperaceae, *Pothomorphe umbellata, Piper umbellatum*, 4-nerolidylcathecol, folk medicine, cyclooxygenase-2 (COX-2), nuclear factor-kappaB (NF-kappaB), inflammation.

INTRODUCTION

From thousand years until today, anti-inflammatory therapies have been applied with the use of natural resources. However, only recently, the cellular and molecular mechanisms of the inflammatory process have

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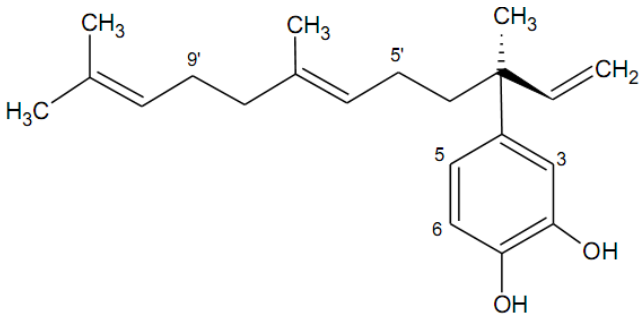

Figure 1. Chemical structure of 4-nerolydilcathecol.

been sufficiently detailed to promote anti-inflammatory strategies with reduced adverse effects (D'Acquisto et al., 2002).

In this context, medicinal plants have been receiving increasing attention. They are an important source of novel structures leading to the expansion of drugs as well as an alternative to the use of synthetic compounds in pharmaceutical technology (Kengne et al., 2016). Plants of the Piperaceae family are purported in the Ayurvedic system of medicine and in folk medicine of Latin America for their numerous curative actions (Parmar et al., 1997; Perazzo et al., 2005).

Innumerous folk preparations developed from Brazilian medicinal plants are generally used for the treatment of inflammation (Stasi et al., 1989). *Pothomorphe umbellata* sin. *Piper umbellatum* L. (Piperaceae), belonging to Brazilian biodiversity, known as "caapeba-do-norte" or "pariparoba", is a green shrubby plant which blooms naturally from the north to the south of Brazil, mainly in the states of Amazonas, Bahia, Espírito Santo, and São Paulo (Angely, 1969).

P. umbellata L. (Piperaceae) had been termed in the first edition of the Brazilian Pharmacopoeia (Silva, 1929). It has been relegated to several therapeutic properties, such as an antioxidant (Lopes et al., 2013), analgesic and anti-inflammatory (Perazzo et al., 2005), antibacterial (Isobe et al., 2002), antifungal (Rodrigues et al., 2012) and antimalarial (Bagatela et al., 2013).

As a part of our research on pharmacological activities of *P. umbellata* L., the inhibitory activities of its extract, fractions and 4-nerolidylcathecol, a phenolic compound which is assigned significant activity in several beneficial properties performed by the plant species, on the molecular pathways of cyclooxygenase-2 (COX-2) and nuclear factor kappa B (NF-κB) have been investigated.

MATERIALS AND METHODS

Plant material

Aerial parts of *P. umbellata* L. were collected in the campus of the University of São Paulo (Ribeirão Preto, SP, Brazil). Professor Pedro de Melillo de Magalhães made the botanical identification of the aerial parts. A sample was deposited in the herbarium of the Botany Department of the University of Campinas – UNICAMP, under register number #UEC 127123.

Preparation of crude extract and fractions

The crude extract and fractions from aerial parts of *P. umbellata* L. were prepared as previously described (Perazzo et al., 2005). Briefly, 500 g of dry material was used to obtain the ethanolic extract, yielding 112 g of dried extract (22.47%). This extract was suspended in methanol:water (9:1) mixture and extracted with methylene chloride (CH_2Cl_2) and ethyl acetate (EtOAc), in sequence, to furnish CH_2Cl_2 (7.45 g, 1.49%), EtOAc (12.4 g, 2.48%) and hydromethanol (residual) fractions.

Isolation and identification of 4-nerolidylcatechol

The isolation of 4-nerolidylcatechol was performed by column chromatography and identified by nuclear magnetic resonance (NMR) through ^{13}C NMR and ^{1}H NMR analyses (Kijjoa et al., 1980; Bagatela et al., 2013) (Figure 1).

In vitro inhibition of cyclooxygenase-2

Mouse macrophages were cultured in cell tubes (75 cm^2) in RPMI-1640 medium (Gibco™, Carlsbad, CA, USA) supplemented with 10% of bovine serum (HyClone Laboratories, Logan, UT, USA) and 60 mg/L of amikacin (Sigma-Aldrich, St. Louis, MO, USA). They were maintained in a temperature equal to 37°C in an atmosphere with 95% humidity and 5% carbon dioxide (CO_2). Then, cells were sprinkled in 96-well enzyme-linked immunosorbent assay (ELISA) microplates (Greiner Bio-One, São Paulo, SP, Brazil) and incubated for twenty-four hours at 37°C. Cells had been treated with 250 µM of aspirin for thirty minutes for complete inactivation of the activity of cyclooxyenase-1 (COX-1) enzyme. After washing with the culture medium described previously, cells were incubated with 5 mg/mL of lipopolysaccharide (LPS, Sigma-Aldrich Co., St. Louis, MO, USA) for sixteen hours to induce the production of cyclooxyenase-2 (COX-2) enzyme. The induced cells were washed again with culture medium for the completed removal of LPS and, then, treated with different concentrations (50, 10 and 2 µg/mL solubilized in DMSO) of the samples for two hours. It was added 300 mM of arachidonic acid (Sigma-Aldrich, St. Louis, MO, USA) and the cells were incubated for thirty minutes at 37°C. The supernatant was recovered for quantitation of prostaglandin E_2 (PGE_2) using the PGE_2 enzyme immunoassay reagent (Cayman Chemical Company, Ann Arbor, MI, USA). Preliminarily, the activity of COX-2 was determined by conversion of exogenous arachidonic acid into PGE_2 and expressed as percentage of control (DMSO, 0.5%). And, subsequently, the IC_{50} (inhibition concentration which decreases the investigated response by 50%) was calculated. N-[2-(Cyclohexyloxy)-4-nitrophenyl]methanesulfonamide (NS-398; Cayman Chemical Company, Ann Arbor, MI, USA), a specific inhibitor of COX-2, was used as control in the assay.

In vitro inhibition of nuclear factor kappa B

Chondrosarcoma cells SW1353 were cultured in a mixture of DMEM/F12 supplemented with 10% FBS (1:1), 100 U/mL of penicillin G sodium, and 100 mg/mL of streptomycin. The *in vitro*

Table 1. Inhibition of COX-2 by *P. umbellata* L. (Piperaceae) species.

Tested drug	Concentration (µg/ml)			IC_{50} (µg/ml)
	50	10	2	
	Inhibition percentage (%)			
Crude extract	20.27 ± 3.42	10.86 ± 3.06	6.95 ± 4.15	NA
CH_2Cl_2 fraction	57.38 ± 2.61	22.79 ± 2.40	10.86 ± 2.09	39.21 ± 2.22
EtOAc fractions	20.95 ± 2.37	13.87 ± 2.21	6.15 ± 3.10	NA
Residual fraction	12.71 ± 2.11	2.29 ± 2.05	0.00	NA
4-Nerolydilcathecol	11.24 ± 2.13	1.18 ± 2.10	0.00	NA
NS-398	100	100	98.73 ± 2.17	0.013 ± 0.02

NA, not active.

inhibition of nuclear factor kappa B (NF-κB) assay is an adaptation of a procedure previously described (Subbaramaiah et al., 2005). Preliminarily, the sensitivity of NF-κB to high dosages (30, 20 and 10 µg/mL solubilized in DMSO) of the drugs had been measured. The data is presented as percentage. And, thus, the IC_{50} was calculated. N-tosyl-L-phenylalanine chloromethyl ketone (TPCK; Sigma-Aldrich, St. Louis, MO, USA), a specific inhibitor of NF-κB, was used as control in the assay.

Statistical analysis

Analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison tests were applied (Sokal and Rohlf, 2012). The results with $P < 0.05$ were considered significant. Data were expressed as mean (M) ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical constituents of *P. umbellata* L.

Phytochemical studies on aerial parts of *P. umbellata* L. (Piperaceae) demonstrated the presence of several chemical constituents, such as β-stigmasterol, β-sitosterol, and campesterol (Perazzo et al., 2005; Pino et al., 2005; Baldoqui et al., 2009; Bagatela et al., 2013). Plants containing these bioactive molecules have previously demonstrated a significant anti-inflammatory activity (Navarro et al., 2001). *P. umbellata* L, among its relevant properties, evidenced a significant *in vivo* anti-inflammatory action (Perazzo et al., 2005).

In vitro inhibition of cyclooxygenase-2

The *in vitro* inhibition activity of *P. umbellata* L. (Piperaceae) on the molecular pathway of COX-2 is exhibited in Table 1. The obtained data are presented as inhibition percentage of COX-2 compared to untreated cultures. Thus, the tested material exhibited remarkable inhibition percentages in the primary assay. However, when the material were tested at lower concentrations in the secondary test, the preeminent result obtained was that of CH_2Cl_2 fraction, the only tested plant component that presented a considerable IC_{50} value and, consecutively, a substantial inhibition activity in the secondary assay.

Given the chemical complexity of medicinal plants, the use of crude extracts, and their fractions can offer significant advantages compared to isolated compounds. Although the reductionist path consists in a logical approach to drug development, crude extracts and fractions must be investigated in order to maintain the benefits of synergy (Orlando et al., 2010), as evidenced below.

In vitro inhibition of nuclear factor kappa B

The *in vitro* inhibition activity of *P. umbellata* L. (Piperaceae) on the molecular pathway of NF-κB is exhibited in Table 2.

This data presents the inhibition percentage of NF-κB compared to untreated cultures. Moreover, the tested drugs exhibited notable inhibition percentages in the primary assay. However, when the drugs were tested at lower concentrations in the secondary test, the preeminent result obtained was that of 4-nerolydilcathecol, the only tested plant component that presented an interesting IC_{50} value and, consecutively, a newsworthy inhibition activity in the secondary assay.

Notably, this assay demonstrated that complex plant extracts, fractions or isolated molecules can be investigated with the proposed experimental systems (Kim et al., 2004). Inflammation has been associated with several pathological disorders, especially several forms of cancers. NF-kB is one of the elements responsible for the link between inflammation and cancer. Several studies aim the search for new compounds that act on the molecular mechanisms involved in the process of inflammation and carcinogenesis. NF-kB is a transcription facto r present in the cytoplasm in an inactive form. When

Table 2. Inhibition of NF-κB by *P. umbellata* L. (Piperaceae) species.

Tested drug	Concentration (µg/ml)			IC_{50} (µg/ml)
	30	20	10	
	Inhibition percentage (%)			
Crude extract	0.00	0.00	0.00	NA
CH_2Cl_2 fraction	7.21 ± 2.11	4.13 ± 1.90	0.00	NA
EtOAc fractions	17.19 ± 2.29	0.00	0.00	NA
Residual fraction	8.62 ± 2.17	0.00	0.00	NA
4-Nerolydilcathecol	72.02 ± 2.68	47.93 ± 2.33	21.47 ± 3.34	22.73 ± 2.23
TPCK	100	72.38 ± 2.58	36.62 ± 2.42	15.07 ± 3.19

NA, not active.

not stimulated, NF-kB is bound to an inhibitory protein: IkB. This complex prevents the translocation of NF-kB to the nucleus, but when it is activated, either by free radicals, inflammatory stimuli, carcinogens, endotoxins or radiation, moves from the cytoplasm to the nucleus where it induces the expression of genes associated with inflammation, pro-angiogenic genes, pro-metastatic and anti-apoptotic genes, and others (Karin et al., 2002; Basak and Hoffmann, 2008; Wong and Tergaonkar, 2009).

Several isolated substances of aromatic plants demonstrated remarkable pharmacological actions through inhibition of NF-kB activity, as 4-nerolydilcathecol. Previous studies have shown that limonene, perillyl alcohol and menthol inhibited the activation of NF-κB (Berchtold et al., 2005; Salminen et al., 2008). α-Pinene, which is also a monoterpene, revealed a robust activity by inhibiting translocation of NF-κB to the cell nucleus, increasing the expression of the inhibitory protein IκB (Zhou et al., 2004). Genipin is one of the substances present in *Gardenia jasminoides* Ellis. This monoterpene exhibited anti-inflammatory activity by inhibiting the production of nitric oxide and blocked IκB degradation, which resulted in inhibition of NF-κB activation (Galvez et al., 2005; Koo et al., 2004; Salminen et al., 2008).

The strategy to use isolated compounds as precursors for synthesis was used for 4-nerolydilcathecol. This compound has been changed to (E)-4-(3,7-dimethylocta-2,6-dienylamino)phenol and showed *in* vivo antinociceptive and anti-inflammatory effects, related to the inhibition of COX-2 and phospholipase A2 (PLA2), demonstrated by *in vitro* assays (Lino et al., 2013). Besides 4-nerolydilcathecol, several natural products such as resveratrol, gingerol, capsaicin and ginsenosides, act towards NF-κB (Kim et al., 2006; Kim et al., 2004; Swales et al., 2006; Lee et al., 2004).

Thus, in these *in vitro* assays, it was possible to study the beneficial potential of *P. umbellata* L. (*Piperaceae*). In a reference survey, no other reports about the inhibition effects of this Brazilian medicinal plant on the molecular pathways of COX-2 and NF-κB. This is the first *in vitro* demonstration of the probable anti-inflammatory mechanism of this promising medicinal plant. However, further research is needed in *in vivo* and in clinical studies to confirm these findings.

Conclusion

The results obtained from the *in vitro* assays were promising, mainly on the molecular pathway of NF-κB, once 4-nerolidylcathecol demonstrated a remarkable inhibition activity. In consequence, this molecule should be considered a notable basis for the development of new beneficial medicines for alternative treatments.

Conflict of Interests

The authors have not declared any conflict of interests.

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Indigenous knowledge and antibacterial activity of selected herbs used locally to treat common cold in Central Uganda

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The study documented the medicinal plants used in the treatment of influenza and common cough and established efficacy of some plants locally used against bacteria causing upper respiratory tract infections in Uganda. It involved an ethnobotanical survey and laboratory experimental investigation to determine the bioactivity against selected bacteria that cause upper respiratory tract infections. Data on medicinal indigenous knowledge was collected with the aid of questionnaires, direct observations, key informant interviews and field excursions and voucher specimen collection. The plants were identified by a botanist at Makerere University Herbarium (MHU), Department of Biological Sciences and voucher specimen were deposited in the herbarium. Methanol and diethyl ether extracts of the commonly used plants were screened for antibacterial activity against *Streptococcus pneumonia* and *Klebsiella pneumonia* using agar well diffusion and agar well dilution methods. Ethno botanical survey showed that 43 plants were commonly used and the most commonly used plant was *Momordica feotida*. Three out of four extracts assayed had activity against *S. pneumonia* and *K. pneumonia,* while one showed activity against *K. pneumoniae*. Hence, plants extracts showed broad spectrum antimicrobial activity. There is need for further development and standardization of products to treat respiratory diseases at household level in the study area.

Key words: Indigenous knowledge, medicinal plants, bioactivity, minimum inhibitory concentration (MIC), upper respiratory infections.

INTRODUCTION

Upper respiratory tract infections contribute highly to the disease burden in Uganda, particularly, in children under5 years and the immune-compromised individuals (Mbonye, 2004; Uganda Ministry of Health, 2010). Allopathic medicine is the national mainstay in treatment of these infections. However, due to poor infrastructure and distribution of health care services, particularly in the rural communities'; majority of the population lack

accessibility of these medicines. In addition allopathic medicines are costly and sometimes cause severe side effects to patients; hence some people have resorted to the use of herbal medicine which is thought to be safe, cheaper, available and sometimes due to lack of alternatives.

The use of plants to treat various diseases includes use of plants' seeds, berries, roots leaves, bark and flowers (Izzo and Ernst, 2009). The World Health Organization (WHO) estimates that 4 billion people globally presently use herbal medicine for some aspect of primary health care (Uganda Ministry of Health, 2010). WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional medicinal uses by indigenous and local communities. It is estimated that about 80% of the population living in developing countries including Uganda use Traditional Medicine (TM), although, the % varies from country to country (Uganda Ministry of Health, 2010). For instance 90% in Ethiopia, 70% in Rwanda and 60% in Uganda and Tanzania use TM for their Primary Health Care (PHC) (Tabuti et al., 2012).

Majority of Uganda's population depends on herbal medicine because it is accessible, affordable and culturally familiar. With an estimated one health traditional practitioner for every 200 to 400 Ugandans compared to one western medicine trained doctor per 20,000 people, it is deduced that herbal medicine is more widely used compared to allopathic medicine. Herbal medicine has long been used to manage a range of common conditions including malaria, digestive and respiratory problems, tooth aches, skin diseases, reproductive health-related complications and upper respiratory infections (The Cross-Cultural Foundation of Uganda, 2008).

The use of allopathic medicine in Uganda, have led to various problems associated with the use of chemotherapeutic agents. Such problems include the pathogenic agents developing resistance to the drugs, for example the cough suppressants such as codeine or dextromethorphan are frequently prescribed but organisms have demonstrated resistance to the compounds, side effects of agents, for example expectorants have side effects of nausea and vomiting, inaccessibility due to high cost and limited treatment centers especially in rural areas.

Although the proportion of people living within 5 km of a health care facility in Uganda rose to 72% in 2010 from 49% in 2000 (Uganda Ministry of Health, 2010; The Uganda Ministry of Health, 2012) access to facilities is still limited by poor infrastructure, lack of medicine, lack of accommodation at facilities, shortage of medical human resource among other factors that constrain access to quality service delivery in rural areas (Kitula, 2007). Due to limited access to drugs, most people especially in rural areas in Uganda resort to use of herbal medicine to treat diseases such as respiratory infections. However, for most herbal medicine, their efficacy has not been validated (Ernst et al., 2006). This research documented the medicinal plants used in the treatment of respiratory tract infections and also established the efficacy of some plants used against the common bacteria causing upper respiratory infections in humans.

MATERIALS AND METHODS

Study design

This was a cross-sectional study involving both the ethno botanical survey to document the plants used in treatment of respiratory tract infections and experimental investigation to determine the bioactivity against selected bacteria that cause upper respiratory tract infections. The study was carried out between January and June, 2014.

Study area

The study was done in Luweero district in central Uganda (Figure 1). Luweero was chosen because it is accessible and also communication with local people was easy since majority of the people are Baganda. It is bordered by Nakasongola district to the north, Kayunga district to the east, Mukono district to the southeast, Wakiso district to the south and Nakaseke district to the west. The district headquarters at Luweero are located approximately 75 Km (47 miles), by road, North of Kampala, Uganda's capital and largest city. The coordinates of the district are: 00 50N, 32 30E (Latitude: 0.8333; Longitude: 32.500). The 2002 national census estimated the population of the district at about 336,600 with an annual population growth rate of 3.2%. It is estimated that the population of the district in 2010 was about 433,100 (City population.de, 2014).

The study was carried out in Katikamu Sub County in Musaale parish. This parish was chosen because it is far from Luwero town and hence the people do not easily access health facilities. Most people therefore resort to use of herbal medicine. In the parish, 4 villages were studied; Kakakala, Nnongo, Nsawo and Kakinga. Kakakala was the first village moved through, the first family was identified by the help of the local council chairperson, and then snowball method was used to identify other families and respondents that were interviewed. In each village, consent of the chairperson was first sought before interviewing the people. At least 25 people in each village were interviewed, making a total of 100 people.

Instruments for data collection

Data collection was aided by the use of questionnaires, direct observations, key informant interviews and field excursions which involved moving with the herbalist to the field to see the medicinal plants and also collect voucher specimen. Observation involved actual participation to learn how the local people process the

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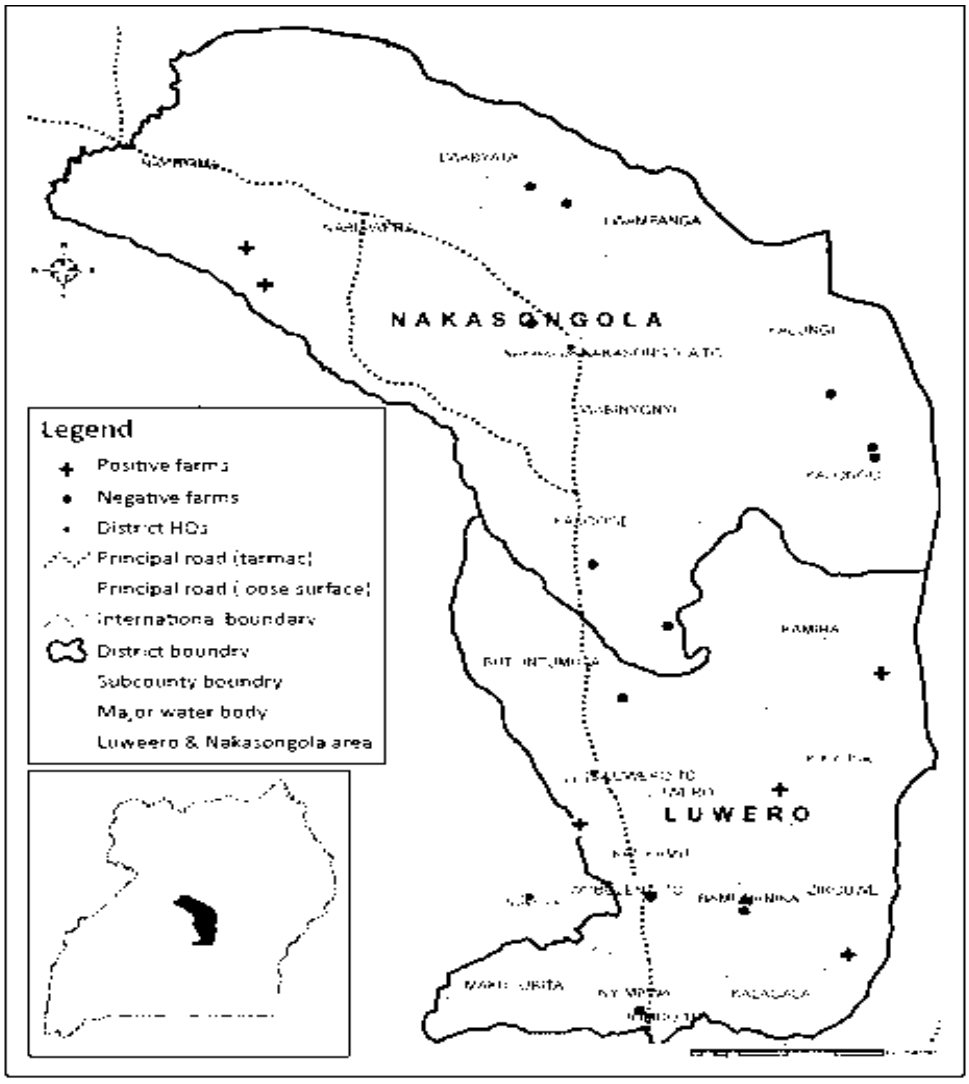

Figure 1. Map of Uganda showing location of Luwero district and the study villages.

medicinal plants and the actual plants/ plant parts used.

Identifying and documenting plants used in treating of upper respiratory tract infections

Sought information included; an inventory of plants used in the treatment of cough, part of used plant, preparation method, administration mode and dosage. A list of used plants was obtained, the most commonly used plants by the local people were determined based on the frequency of use and the priority plants which have not been worked on were chosen based on literature search. The plants were identified by a botanist at the Makerere University Herbarium (MHU), and a voucher specimen was deposited in the herbarium for future reference.

Collection and preparation of sample materials for laboratory analysis

The plants for laboratory analysis were collected from Luweero district in the early morning hours before sunrise, where plants/parts of plants were collected depended on the use information given by the respondents. Leaves were collected for *Momordica foetida,* and *Vernonia amygdalina,* stem bark was collected for *Acacia comphylacantha* and roots were collected for *Solanum macrocarpon.* These plant specimens were then cleaned by washing them and they were then air dried for a week at room temperature. They were then oven dried for 30 min, ground to powder with the grinding machine. The powder was weighed and then packed in air tight containers ready for extraction.

Laboratory analysis

This included extraction and microbiological testing carried out at Natural Chemotherapeutic Research Institute (NCRI) and Kyambogo University Microbiology laboratory/ Faculty of Science.

Extract preparation

Preparation of the plant crude extracts was carried out at NCRI. Extracts were obtained sequentially by maceration using non polar

diethyl ether followed by polar methanol. The extracts were then filtered and the filtrate were concentrated by evaporation using a rotary evaporator (BUCHI scientific equipments), weighed and reconstituted in Dimethyl sulfoxide (DMSO) to a concentration of 1 g/ml. These samples were then stored in a refrigerator at 4°C and later were used in the proceeding of antibacterial tests.

Determination of the antibacterial properties of the selected plants

The antibacterial assays were carried out using agar well diffusion method and agar dilution techniques. The antimicrobial activity of the plant extracts were tested on 2 standard bacteria species namely; *S. Pneumonia* a gram positive bacterium and *K. Pneumonia* a gram negative bacterium.

Inoculums' preparation

A colony of the organism was obtained using a wire loop; it was then emulsified in normal saline to make a suspension which is adjusted using 0.5 McFfarland standards so that its colour matches with that of the standard. This was to ensure that there is the right population cell per millimetre of normal saline. The adjusted cell suspension was inoculated on media by swabbing under aseptic conditions.

Antibacterial screening

The media of Mueller Hinton agar was prepared and treated according to manufacturer's guidelines, where 35 g of media was mixed with one litre of distilled water and enclosed in a container and autoclaved at 121°C for 15 min. The media was later dispensed into 90 mm sterile agar plates (Oxford, UK) and left to set. The agar plates were incubated for 24 h at 37°C to confirm their sterility.

Absence of growth after 24 h showed that the plates were sterile. The sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had wells of 6 mm diameter and 5 mm depth made into it using a sterile agar glass borer. Ceftriaxone was used as a positive control, while normal saline was used as a negative control. Approximately 0.2 ml of the bioactive test plant crude extracts of concentration 1 g/ml was suspended in the wells and thereafter inoculated, plates/culture were incubated for 24 h at 37°C. The plates/cultures were examined for the presence of bacterial inhibition zones around each well. Antibacterial activity was determined from the zone of inhibition around the wells. Single readings were carried out. Non-active compounds did not show any inhibition zone. The zones of inhibition were measured using a ruler and a pair of divider (Picfare) and results were reported in millimeter. All zone diameters were considered important since the extracts from the plants were still crude.

The agar well diffusion method as described by Esimone et al. (1998) was adopted for antibacterial screening of plant extracts. In this method, 15 ml of Mueller Hinton agar was seeded with 1.0 ml of a broth culture of the test organism. This was done by introducing the culture into sterile petri dishes, followed by adding molten agar to incorporate the broth culture and swirling gently to ensure uniform distribution of the microorganisms in the gel and then finally allowing the agar to solidify on a flat surface. Wells were made in the agar plates (about 5.0 mm diameter using a sterile agar borer) into which equal volumes of the extract were transferred using micropipettes. The plates were allowed to stand for one hour for pre-diffusion of the extract to occur and then incubated at 20 to 25°C for 1 to 2 days. Dimethyl Sulfoxide (DMSO) was set in a different well alongside the test to serve as a negative control and Ceftriaxone was used as a positive.

Minimum inhibitory concentration (MIC)

The MIC was determined by agar dilution technique. A set of 5 test tubes were dispensed with 1 ml of sterile normal saline. An equal volume (1 ml) of the test plant extract was added to the first test tube and the 2 were mixed thoroughly well. Then I ml of the solution in the first tube was transferred to the second tube and the 2 mixed well. This was repeated until the last tube and the last aliquot 1 ml was discarded so as to have uniform volume. This constituted a twofold dilution whereby each step moved to the right reduced the concentration by a factor of 2. This procedure was done for all extracts individually. A bacterial culture adjusted using 0.5 McFarland solution was inoculated on sterile media. Agar wells were bored into the inoculated plates and the samples from the extracts were impregnated into the wells and incubated for 24 h. The different dilutions for a particular extract were impregnated on the same plate. Control plates inoculated with standard drug and DMSO (Dimethylsulfoxide) were set as positive and negative controls, respectively. After 24 h incubation, the plates were examined for antibacterial activity and the results were recorded.

Data analysis and presentation

The data was analyzed by Microsoft excel and the results presented as tables, pie chart and bar graphs, while qualitative interpretations were made for qualitative data.

Ethical considerations

The proposal was presented to the Department of Biological Sciences, Kyambogo University for approval. Before entry into any village, permission to interview the people was sought from the chairperson of the village. Also, consent of the individual was sought before beginning the interviews. For children under the consenting age, a guardian or parent signed on their behalf. For laboratory investigations, standard strains of bacteria whose sensitivity to antibacterial agents is known were used in the study. Also, the experiments were conducted in standard laboratories.

RESULTS AND DISCUSSION

Demographic data

From the survey, demographic information characterizing human population (age, sex, marital status among others) was obtained. The information is summarized in Table 1.

According to the obtained results, majority of the respondents were peasant farmers (80%), while only 20% had a formal employment. Almost all the local people were Baganda (97%); this made it easy to get as much information from them as possible since the interviewer was fluent in the spoken language. Most of the people were not well educated, more than half (62%) of the respondents had not made it to secondary school.

Table 1. A summary of social demographic characteristics of the study participants.

Characteristic	Category	Frequency
Gender	Male	56
	Female	44
Age	Below 35	46
	36-65	31
	66-75	18
	Above 75	5
Marital status	Single married	20
	Divorced	69
	Widow	10
	Divorced	1
Education	No formal	8
	Primary	54
	Secondary	35
	Tertiary	3
Occupation	Peasant	80
	Teacher	1
	Business	9
	Others	10

N=122.

Majority of the respondents were below 35 years of age, the proportion of the aged (above 75 years) was only 5%. Thirty five years and below is the reproductive age in Uganda, since the study was targeting children below five years of age, probably that is why most respondents were falling into this age bracket. However this may be an indication that the African traditional extended family structure is giving way to the nucleated family structure. The elderly people have been known to be the custodians of indigenous knowledge including traditional medicine (United Nations Environment Programme, 2008). Hence the small proportion of aged people in this study is an indication that the older people who have knowledge on medicinal plant use and preparations are dying off; hence, necessitating documentation of medicinal plants so that the knowledge is not lost with the people. It is important that a comprehensive study on indigenous systems and knowledge in Uganda done in order to transfer this knowledge to modern innovations and technologies to match the current trends amongst the younger generations.

Medicinal plant use

The gathered information from the local people about these plants included parts of the used plants in the treatments, preparation methods, route of administration and dosage. Forty three used plants by local people to treat influenza and common cough in Luweero districts were documented (Table 2). These were mostly given personally by the respondents or in consultation with other community members.

The most commonly used plants were *Momordica foetida, Callistemon Citrinus (Curtis), Psidium guajava* and *Mangifera indica,* respectively. Others that were used by many households included; *Azadirachta Indica, Syzygium cumminii, Persea Americana, Euclyptus globules, Albizia coriaria, Physalis peruviana, Tetradenia riparia* and *Acacia Compylacantha.* The family that contained most of the plants mentioned by respondents was Myrtaceae, while the most commonly used plant part was the leaves. Majority of the respondents used the oral mode of administration and boiling was the most preferred form of preparation of the medicine. The mode of administration for all the plants was oral.

Results from laboratory analysis

The dry plant crude extracts obtained at Natural Chemotherapeutic Research Institute (NCRI) and the antibacterial analyses were done at Kyambogo University

Table 2. Species scientific name, family, local name, local medicinal plant use and dosage of the reviewed plants.

Species scientific name, (Family), Local name	FREQ	Part	Preparation method	Dosage	
				Child	Adult
Momordica foetida Schumach (Cucurbitaceae), Bombo[D]	53	L	Chew the leaves or squeeze, take juice	NA	NA
Mangifera indica, L. (Anacardiaceae), muyembe[D]	50	L and S	Boil, leave to cool filter	2spoons×3 daily	1 cup
Callistemon citrinus, Curtis (Myrtaceace) Nyambalabutonya[D]	19	L and S	Boil, cool, filter	2spoons×3 daily	1/4 cup×2 daily
Psidium guajava L(Myrtaceae), Mapeera[D]	18	L	Boil with mango leaves, filter	2spoons×3 daily	1 cup
Vernonia amygdalina Delile (Asteraceae), Mululuuza[D]	10	L	Boil with 8, leave to cool	1spoons×3 daily	1/8cup×3 daily
Azadirachta indica, A. Juss (Meliaceae), Niimu[D]	8	F and L	Boil leaves and fruit	1spoon×3 daily	2spoons×3 daily
Synzigium cuminii L. (Myrtaceae),.Jjambula[D]	8	S	Boil them with 9 and 10, cool, filter	2spoons×3 daily	1/4cup×3 daily
Persea americana Mill. (Lauraceae), Ovakedo[D]	7	L	Boil with 9, leave to cool	2spoons×3 daily	1/4cup×3 daily
Eucalyptus globules Labill (Myrtaceae), Kalitunsi[D]	5	L	Boil, leave to cool	2spoons×3 daily	1/2 cup×3 daily
Albizia coriaria Welw (Mimosoideae, Omugavu[D]	5	S	Boil with 2, 4, 8, 9 and 15, cool	1spoon×3 daily	1/8cup×3 daily
Physalis peruviana L. (Solanaceae), Ntuntunu[D]	4	L	Squeeze, drink fluid	NA	NA
Tetradenia riparia (Hochst.) Codd. (Lamiaceae) Kyewamala[D]	4	L	Smoke, crush and lick or squeeze fluid for child or mix with 8 and 20 and squeeze	NA	NA
Acacia comphylacantha Hochst.ex.(Fabaceae), Kibeere[D]	4	S	Boil with 8,9 and 18 cool	2spoons×3 daily	1/2cup×3 daily
Solanum marcrocarpon L. (Solanaceae), Akatengotengo[L]	3	R	Clean, get bark crush dry add H_2O and salt	NA	NA
Ocimum basilicum L. (Lamiaceae) Kakubansiri[D]	3	L	Boil with 9,10 and 6, allow to cool	1spoon×3 daily	4spoons×3daily
Canarium schweinfurthii, Engl (Burseraceae) Miwafu[D]	3	S	Boil with 8,10 and 13, cool	1spoon×3 daily	2spoons×3 daily
Combretum molle R. Br. G. Don (Combetaceae) Endagi[D]	3	L	Boil, leave to cool	NA	NA
Dracaena steudneri, Schweinf (Dracaenaceae), Kajjolyenjovu[L]	2	L	Dry, burn add ash and salt, mix and lick	NA	NA
Rubia cordifolia, L. (Rubiaceae), Kasalabakesi	2	L		NA	NA
Markhamia lutea Denth K Schum (Bignoniaceae), Musambya[D]	2	S	Boil, leave to cool	2spoons×3 daily	1/4cup×3 daily
Spathodea campanulata P Beauv (Bignoniaceae), Kifabakazi[D]	2	S	Boil, leave to cool	2spoons×3 daily	1/4cup×3 daily
Ocimum suave Willid (Lamiaceae), mujaaja[D]	2	L	Boil with 9,19and24, cool	1/8 cup×3 daily	1/2cup×3 daily
Artocarpus heterophylus, Lam (Moraceae), ffene[D]	2	L	Dry leaves with 9 and17, sieve and lick	NA	NA
Lantana camara L. (Verbenaceae), Kayukiyuki[D]	2	L	Squeeze with 8and28 and take liquid	NA	NA
Piliostigma thonningii, Schum (Caesalpiniaceae), Kigaali[D]	2	S	Boil with 4,6,9 and13 cool	2spoons×3 daily	1/2cup×3 daily
Symphonia globulifera, L. f (Clusiaceae), Omusaali[D]	2	S	Boil with 6,8and9 leave to cool and cool	1spoon×3 daily	2spoons×3 daily
Zingiber officinale, Roscoe (Zingiberaceae) Ntangawuzi[C]	1	R	Crush and add warm H_2O	2spoons×3 daily	1/2cup×3 daily
Imperata cylindrical, P. Beauv (Poaceae) Olusenke[D]	1	R	Boil the roots	2spoons×3 daily	1/4 cup×2 daily
Asparagus africanus Lam (Asparagoideae) Kadaali[D]	1	S and L	Boil, cool, filter	1/4cup×3 daily	1/2cup×3 daily
Hoslundia opposite Vahl (Lamiaceae) Kamunye[D]	1	W	Boil, leave to cool	1/8 cup×3 daily	1/2 cup×3 daily
Brideria micrantha (Hochst.) Baill. (Euphorbiaceae) Katazamiti[D]	1	R	Boil with 18 and 8, leave to cool	2spoons×3 daily	1/4cup×3 daily
Sapium ellypticum (Euphorbiaceae) Musasa[D]	1	S	Boil with 8, 18, 21 and 22 cool	2spoons×3 daily	1/4cup×3 daily
Albizia coriaria (Fabaceae) Nongo[D]	1	S	Boil with 4, 9 and 22 leave to cool	2spoons×3 daily	3spoons×3 daily
Vangueria apiculata K. Schun (Rubiaceae) Matugunda[D]	1	L and S	Boil with 6, 9 and10, leave to cool	1spoon×3 daily	2spoons×3 daily

Table 2. Cont'd.

Ipomoea hildebrandtii, Vatke (Convolvulaceae) Nalongo[D]	1	L	Boil with 18, leave to cool	1/8 cup×3 daily	1 cup
Ficus exasperate Vahl. (Moraceae) Luwawu[D]	1	L	Dry, crush add cassava flour, salt and lick	NA	NA
Ageratum conyzoides, L. (Asteraceae) Namirembe[D]	1	W	Boil with 8, 18, 39, leave to cool	1spoon×3 daily	2spoons×3 daily
Galinsoga parviflora, Cav. (Asteraceae) Kafumbe[D]	1	W	Boil with 8,18 38, leave to cool	1spoon×3 daily	2spoons×3 daily
Aristolochia elegans, Mast (Aristolochiaceae) Akasero[D]	1	L	Boil them with 8, 18 and 43, leave to cool	2spoon×3 daily	1/2cup×3 daily
Aloe vera, Burm.f (Xanthorrhoeaceae) Kigaji[D]	1	L	Boil them with 8, 18 and 42, leave to cool	1/2 spoon×3 daily	2spoons×3 daily
Loranthus sp. (Loranthaceae) Nzirugaze[D]	1	L	Boil, leave to cool	1spoon×3 daily	2spoons×3 daily

Plant part: L- Leaves, S- stem bark, R- roots, W- whole plant, F- fruit; Mode of administration: [D]- drinking; [L]- licking; [C]- chewing.

Microbiology Laboratory in Uganda. All the 4 methanol plant extracts had activity against *Klebsiella pneumoniae*, while all except the extract of *A. comphylacantha* showed activity against *S. pneumonia*. The highest activity was observed in *V. amygdalina* and *M. feotida* at 24 and 21 mm inhibition diameter respectively against S*. pneumonia.* Therefore, all methanol plant extracts (100%) were active on *K. pneumoniae* (a gram negative bacterium) and 75% of the methanol extracts were active on *S. pneumonia* (a gram positive bacterium) (Table 3).

Almost all the plant extracts showed activity against the test pathogens. Of the four diethly ether plant extracts, 2 (*V. amygdalina* and *M. foetida*) had activity on both bacteria (Table 4). *Solanum macrocaporn* was only active *against K. pneumoniae,* while *A. comphylacantha* was only active against *S. pneumoinae* and 2 were active only on one bacterium. The biggest zone of inhibition was observed in both *S. macrocarpon* and *V. amygdalina* at 20 mm diameter against *K. pneumoniae* and *S. pneumonia*, respectively. Both methanol and diethyl ether extracts of *M. foetida* and *V. amygdalina* were active on both bacteria species. The most active, however, was *V. amygdalina.* Since plant medicines have many compounds in them, this makes parasites less prone to developing resistance to them. Hence an effective broad spectrum antibiotic could be formulated and developed from a combination of these plants.

Minimum inhibitory concentration (MIC)

The plants which were considered for minimum inhibitory concentration were those whose methanol or ether extracts showed activity against at least one bacterial species (Table 5).

DISCUSSION

Majority of the respondents were below 55 year of ages, this was an indication that the older people are the ones who have knowledge on medicinal plant use and the knowledge of preparations may disappear with time, hence necessitating documentation of medicinal plants so that the knowledge is not lost with death of the old people. Most of the respondents were peasant farmers; therefore, there income is low and hence they cannot afford the cost of allopathic medicine. So, they resort to the use of herbal medicine which is cheaper and readily available to them. This, therefore, calls for testing of the medicinal plants to determine their efficacy and safety to save the local people from taking 'non authenticated' and unsafe medications.

Majority of the respondents were married. This could be because the married people are the ones with children below 5 years and hence they use more herbs since URTIs mainly affect children below 5 years. Kibuule and Kagoya (2015) also reported that most of the affected are the children below 5 years. Very few of the people interviewed had attained tertiary education. This could be because most people who have reached that level do not use herbal medicine possibly since they can afford allopathic medicine possibly due to having better jobs.

Herbal remedies are prepared in several rather standardized ways which usually vary based upon the utilized plant, and sometimes, what condition is being treated. Many studies have reported use of leaves, oral administration and decoctions being highest in use compared to other plant parts (Tabuti et al., 2012). In this study, most commonly used plant parts were also leaves, while decoction was the most common form of preparation. All preparations were administered orally. The use of leaves encourages sustainable utilization and conservation of plants hence increased availability of the plants to the local people. The local communities can be encouraged to use the medicinal plants as alternatives, particularly those that have been proven efficacious and safe.

Table 3. Antibacterial screening on methanol plant extract against *K. pneumonia* and *S. pneumonia.*

Plant extracts	Inhibition diameter (to the nearest mm)	
	K. pneumoniae	***S. pneumoniae***
S. marcrocarpon	12	13
V. amygdalina	13	24
A. comphylacantha	15	Not active
M. foetida	12	21

Table 4. Antibacterial screening on diethyl ether extract against *K. pneumoniae* and *S. pneumonia.*

Plant extracts	Inhibition diameter (to the nearest mm)	
	K. pneumoniae	***S. pneumoniae***
S. macrocarpon	20	Not active
V. amygdalina	15	20
A. comphylacantha	Not active	14
M. foetida	13	12

Table 5. Minimum inhibitory concentration (MIC) of plant extracts against *K. pneumoniae.*

Methanol extracts	Minimum Inhibitory Concentration (MIC) ***K. pneumoniae***				
	1 g/ml	**0.5 g/ml**	**0.25 g/ml**	**0.125 g/ml**	**0.0625 g/ml**
S. marcrocarpon	12	0.0	0.0	0.0	0.0
M. foetida	11	0.0	0.0	0.0	0.0
A. comphylacantha	14	10	0.0	0.0	0.0
V. amygdalina	13	12	10	0.0	0.0
	Diethyl ether extracts				
S. marcrocarpon	18	15	10	10	0.0
M. foetida	12	0.0	0.0	0.0	0.0
V. amygdalina	14	11	10	0.0	0.0
	Minimum Inhibitory Concentration (MIC) *S. pneumoniae*				
	1 g/ml	0.5 g/ml	0.25 g/ml	0.125 g/ml	0.625 g/ml
S. marcrocarpon	11	10	10	0.0	0.0
M. foetida	20	17	15	13	10
V. amygdalina	23	20	18	15	10
	Diethyl ether extracts				
M. foetida	12	10	0.0	0.0	0.0
A. comphylacantha	13	11	10	10	0.0
V. amygdalina	19	17	14	11	10

It was found that the selected plants had antibacterial activity against at least one of the tested bacteria. In previous studies, both Vernonia amygdalina and M. feotida had showed antibacterial activities against E. coli and S. aureas (Lovet et al., 2015; Olukayode and Adebola, 2008). In this study, the plants showed activity on more test organisms including *K. pneumoniae.* The results of (MIC) also showed that the extracts were more active on gram positive bacterium. Generally, methanol extracts were more active than ether extracts. Therefore, probably the active ingredients in the plants were mostly the polar components since methanol extracts a larger proportion of polar compounds (Narhstedt, 2014). *V. amygdalina* and *M. foetida* had a broad spectrum activity for both di ethyl ether and methanol extracts. Amongst the 4 plants, *V. amygdalina* had the greatest activity and

hence has a great potential to be developed into a drug for URTIs. *S. marcrocarpon* had the greatest activity against *K. pneumoniae* and hence a standardized herbal medicine including a mixture of *S. marcrocarpon, V. amygdalina* and *M. foetida* could be further scientifically evaluated to standardize a medicine that would be used against URTIs at household level in the local communities.

A standard drug (antibiotic), ceftriaxone was also used to act as a positive control and sterile distilled water was used as a negative control. This was done to test the viability of the experiment. In the determination of MIC, the agar well dilution method was used because of its simplicity as compared to the broth dilution method. The concentrations of the different extracts were diluted by serial dilution method to the fifth dilution (0.0625 g/ml). It was observed that different concentrations of the extracts were the MIC for the different bacteria. The ether extract of *S. marcrocarpon* had the highest MIC (0.125 g/ml) against *K. pneumoniae*. *M. foetida* had the lowest MIC against *K. pneumonia* at 0.5 g/ml both in the diethyl ether and methanol extracts. This implied that *M. foetida* has good activity however it needs to be used in high concentrations. Hence it is essential to determine the safety profile of this plant. *M. foetida* and *V. amygdalina* had showed a low MICs for both extracts against *S. pneumoniae.* This is an indication that a formula consisting these two plants could lead to a more effective remedy against respiratory tract infections. We are making efforts to have a study on the combined or synergistic effects of these plants against the test pathogens in the near future. This could lead to an affordable alternative in management of upper respiratory infections. Generally, the 4 extracts had lower MIC for *S. pneumoniae,* a gram positive bacterium.

Conclusion

Plants extracts tested have antimicrobial activity against gram positive and gram negative bacteria and therefore can be of great medicinal function. Developing countries, like Uganda should therefore take advantage of such medicinal plants to formulate medications to treat upper respiratory infections especially in the rural areas. This further verifies that indigenous knowledge is important both in Primary Health Care and in Drug Development. There is need to intensify research in plant medicines/formulae that can be incorporated in the national health system.

Conflict of interests

The authors have not declared any conflict of interests.

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