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Table of Contents: Volume 12 Number 20 25 July, 2018

ARTICLES

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Journal of Medicinal Plants Research

Full Length Research Paper

Medicinal plants used in the treatment of livestock diseases in Berbere district of Bale zone, Oromia region, Ethiopia

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An ethnoveterinary study of medicinal plants used by local people of Berbere district was carried out from June 25 to September 5, 2015. The study was focused on utilization of medicinal plants to treat various livestock health problems by people of the study area. The data were gathered using semi-structured interview, participant observation and personal interviews. A total of 69 informants (55 male and 14 female) in the age between 30 and 89 years were purposively selected from eight kebeles. Twenty four medicinal plants, which are distributed in 18 genera and 17 families have been collected and identified. These medicinal plants were collected from natural habitat [23 (94.6%)], whereas 1 (5.6%) from home gardens. The most frequently harvested medicinal plants parts was shrub accounting for 11 species (45.8%) for livestock. Leaves [13 (52%)] were frequently used plant parts for preparation of livestock remedies. Agricultural expansion was the major threat to medicinal plants. Creating awareness of young generation on the usage of traditional medicine and conservation is recommended.

Key words: Medicinal plants, traditional utilization, Berbere district, South East Ethiopia.

INTRODUCTION

The importance of traditional medicine (TM) and its contribution to health care among humans worldwide cannot be under estimated. These medical systems are mainly dependent on various plant species and plant based products. Before the introduction of modern veterinary practice, traditional healers were usually the only people approached to attend to these livestock diseases. The various traditional practices included prevention of diseases, recognition of toxic plants, surgical intervention and crude vaccination methods. The current account of medicinal plants of Ethiopia, as documented for National Biodiversity Strategy and Action Plan by Tanto et al. (2002), shows that about 900 plant species were reported to be used in the traditional medicine.

Many researchers in Ethiopia have revealed the loss of valuable medical plants due to population pressure, agricultural expansion and deforestation (Abebe, 2001; Getachew and Shiferaw, 2002; Yirga, 2010). Moreover, documenting traditional medical knowledge is important to assist discovery of new sources of drugs (Hassan et al., 2014). This study, therefore, attempts to identify

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ethnoveterinary medicinal plants used for the treatment of livestock ailments in Berbere district, Bale zone, South Eastern Ethiopia.

MATERIALS AND METHODS

Description of study area

Geographical location

Berbere district is situated between 06°33' N and 06°75'N and 039°95' E and 040°29'E. It is located at about 526 km southeast of Addis Ababa, in Bale zone of Oromia Regional State. This district has 17 kebeles which are characterized by undulating highlands in the north and lowlands in the south (Figure 1).

Topography and climate

According to OIDA (2003), the area is characterized by flat lands and moderately steep rolling hills with valley bottoms. The altitude of the district ranges between 900 and 2100 m a.s.l. There are three agro-ecological zones represented in this district.

The majority, 70% of the district is classified as lowland (Gammoojjii), with 25% midland (Badda- Daree) and only 5% is considered as highland (Baddaa) (BDARO, 2010).

Livestock

Livestock population in Berbere district is high because of large coverage size of pasture land of the district. According to the Berbere District Livestock Development Agency, the numbers of livestock in the district include: 1,320,500 cattle, 18,940 sheep, 5,645 camels, 131,000 goats, 13,654 poultry, 5,480 mules, 895 horses, and 11,850 donkeys. In the district, livestock make a substantial contribution to the rural economy. Most rural farming, transport and source of income are directly or indirectly linked with them. The contribution of their products like milk, meat and egg to the regional and national economy is very low mainly due to poor management, inadequate and low quality feed supply and the prevalence of various animals' diseases.

There are three veterinary clinics in the district, located in Harawa, Chekata and Darasa kebeles. The number of cattle and clinics are not balanced. Therefore, there is no doubt that people of the district use ethnoveterinary medicine to treat various livestock ailments. The most important animal diseases in Berbere district include: trypanomiasis, lamp skin disease, bovine pasturalosis, gastro intestinal parasite, external parasite, black leg, blotting, phasolosis and African horse sickness (BDLDA, 2010).

Land use, soil and agriculture

A survey of the land in this district shows that 20.5% is arable (4.3% is under cultivation), 36.4% is pasture, 41.7% is forest or heavy vegetation cover, and the remaining 1.4% is considered swampy, degraded or otherwise unusable (BDARD, 2010a). The major crops include maize and teff, while sorghum and wheat are minor crops. The area has a high potential to produce cash crop like coffee and khat (BDARD, 2010b).

Vegetation

There are two types of vegetation in the area. One is deciduous bush land and thicket that forms ecotone between montane forest and deciduous forest. The second one is *Acacia-Commiphora* bush land. This type of vegetation is mainly characterized by *Acacia* species like *Acacia mellifera* and *Acacia senegal.* The ecotone taxa include *Euphorbia* species and *Carissa spinarum* (OFEA, 2010).

Reconnaissance survey and selection of study sites

A reconnaissance survey of the study area was conducted from June 25 to July 5, 2015. The study sites were selected depending on recommendation from elder, local authorities and altitudinal range. Thus, the study was carried out in eight kebeles from two agro ecological zones. They are Burkitu, Chekata, Darasa, Gabe Keku, Gora Heddo, Haro Nannoo, Harawa and Sirima.

Sampling of informants

A total of 69 individuals (55 males and 14 females) in the ages of 30 and above were selected from eight kebeles purposively following Tongco (2007). Of the total informants, 30 key informants (25 males and 5 females) were systematically selected based on recommendation from elders and local authorities (Development Agents and Kebele Administration Leaders). The choice of informants was following the suggestion made by Martin (1995). Local healers were also considered as key informants since they are expected to have intensive knowledge of medicinal plants. The informants were selected from the local people of the study area to see the general knowledge of medicinal plants of the people depending on their willingness to participate.

Ethnobotanical data collection

Ethnobotanical data were collected from July 6, 2015 to September 4, 2015. The standard data collection methods like that of Martin (1995), Alexiades (1996), and Cotton (1996) have been followed to obtain indigenous knowledge of the local community on health, local classification of soil and plants, use, conservation and threats of medicinal plants. The techniques employed for data collection were group discussion, semi-structured interviews, guided field walks and observations with informants.

Market survey

Market survey was done on three markets in order to see medicinal plants in trade following Martin (1995). Three markets were encountered in the study area. One is found in Darasa in which the market day was held on Monday; Haro Dumal, in which the market day was on Thursday and Chekata in which the market day was on Wednesday.

Specimen collection and identification

At the end of each interview, sample specimens of the plants cited for their medicinal use were collected, numbered, pressed and dried for identification. The local names and growth habits of the medicinal plants were recorded for each plant species.

Preliminary identification was done in the field. The voucher specimens which could not be identified in the field were taken to Jimma University herbarium and identified by using taxonomic keys, Flora of Ethiopia and Eritrea and the specimens that could not be identified using the taxonomic keys, Flora of Ethiopia and Eritrea were taken to the National Herbarium (ETH), Addis Ababa University and compared with already identified specimens. The specimens were deposited at Jimma University herbarium and the ETH.

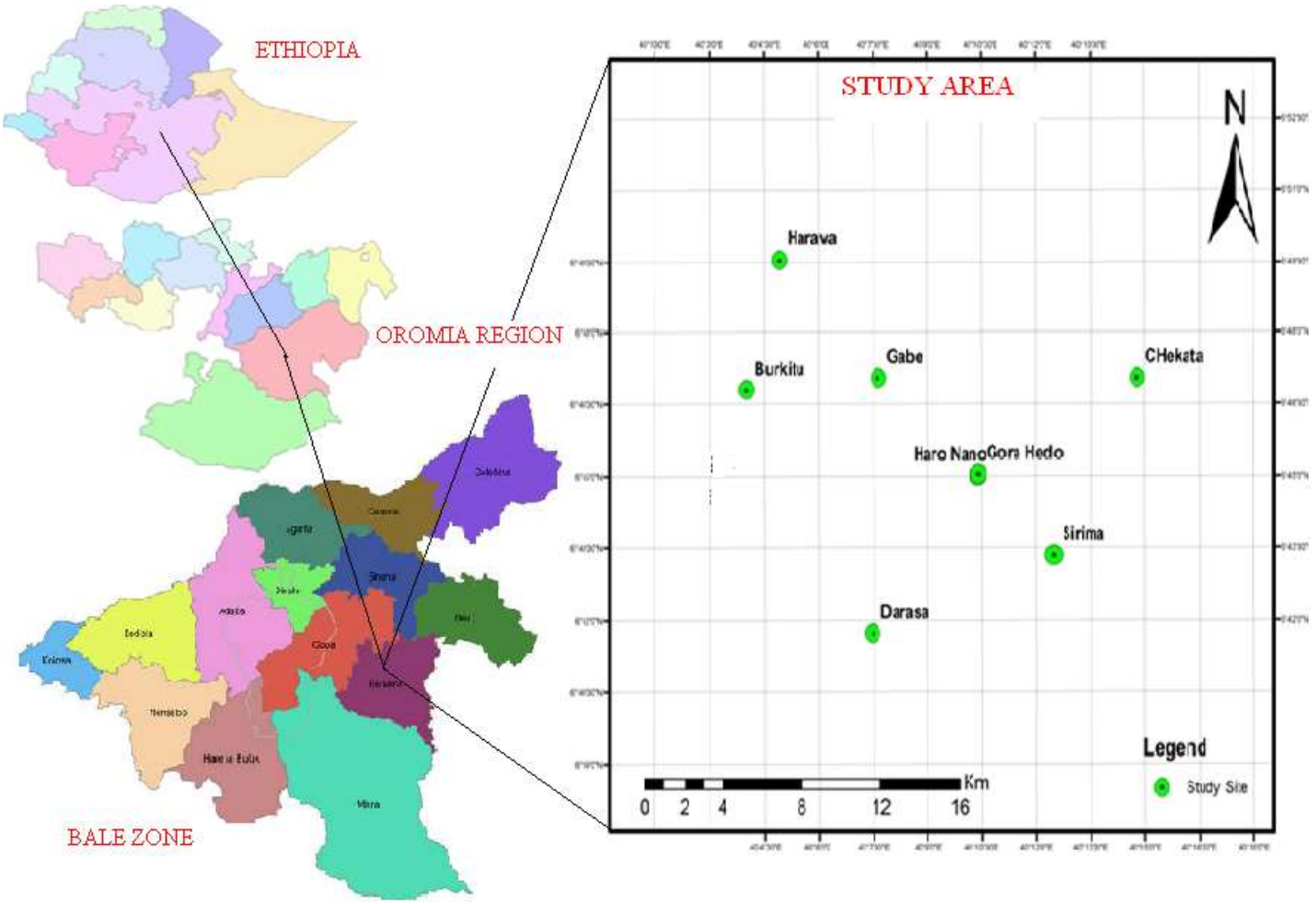

Figure 1. Map showing study area.

RESULTS AND DISCUSSION

Medicinal plants used to treat livestock health problems

In comparison to human diseases, livestock diseases are treated with less number of plant species in the study area. A total of 14 livestock ailments were identified that are treated by traditional medicinal plants in the area. Medicinal plants collected and identified in the study area which are used for livestock health problems were 24 species. They were grouped into 18 genera and 17 families. Regarding the families of medicinal plants used in livestock health problem, family Fabaceae represented by three species (16.66%) and it was the leading family. Regarding the occurrence of the medicinal plants documented for treating livestock ailments, all were obtained from the wild and this shows that the attempt of local community is not promising for cultivating livestock remedies in home gardens. Various studies conducted in Ethiopia reported that the majority of medicinal plants were harvested from the wild, for instance the studies of Yineger et al. (2008).

Growth form and part used for livestock medicinal plants in the study area

Out of the 24 plant species used for treating livestock ailments in the area, 11 (45.8%) species were represented by shrubs. The growth forms indicate that shrubs constitute most of the medicinal plants used for treating livestock ailments in the study area. This result shows that people rely more on shrubs and herbs because they are relatively common in the area when compared to tree species. A study conducted by Teklehaymanot and Giday (2007) and Adefa and Abraha (2011) indicated that the shrubs are the major plants parts harvested followed by herbs (Tables 1 and 2)

Regarding the plant parts documented for preparing various medicines to livestock ailments, leaves accounted for the highest represented by 13 species

Table 1. List of livestock disease in the study area.

S/N	Local name	Medical name
1	Nyaaqarsa	****
2	Jogii	Trypanomiasis
3	Darabaa	Froze Blotting
4	Furtu	Black leg
5	Jigoo	Mastitis
6	Goflaa	Oedema
7	Kilisi	African horse sickness
8	Burka	***
9	Silmi	Ticks
10	Raammoo gara	Intestinal parasite
11	Madaa afaani	Mouth sore
12	Budaa	Evil eye
13	Madaa	Wound
14	Nyaakarsa lafee	Bone TB

Table 2. Habit of medicinal plants used to treat livestock disease.

Habit of the species	No. of the species	Percentage
Climber	5	20.8
Epiphyte	3	12.5
Herb	3	12. 5
Shrub	11	45.8
Tree	2	8.4

(52%) followed by roots, 10 (40%) species. It has been noted that collection of leaves alone could not pose a lasting danger to the continuity of an individual plant when compared with the collection of roots, bark, stem or whole plant. The finding of Yirga (2010) revealed that leaves are the most (34.2%) used parts of medicinal plants followed by roots (30.9%) and barks (8.2%) and (10) indicated that 48% of livestock remedies were prepared from leaves in Tehuledere district, South Wollo, Ethiopia. However, Balemie et al. (2004) obtained a result that the Kereyu Oromo people mostly prepared livestock remedy from roots. Also, the findings of Yineger et al. (2007) showed that roots were the major plants part used for veterinary medicine (41.54%) when compared with leaves (36.15%) in Bale National park. There are instances where different parts of the same plant were being used for different purposes. There are also cases where more than one plant is used to treat a particular ailment (Figure 2).

Method of preparation and mode of application of livestock medicine

Preparation method of livestock medicine includes various techniques such as crushing, pounding, powdering, boiling and crushing and pounding. Majority of the method contributed by crushing which accounted for 15 medicines (60%) followed by pounding which accounted for 5medicines (20%). The findings of Hunde et al. (2006) revealed that crushing (17%) is the leading remedy preparation in Boosat sub-district, Central Eastern Ethiopia (Figure 3).

The routes of application like oral, ocular, anal, nasal and dermal have been documented in associatin with various livestock ailments. The common adopted route of application was oral which accounted for a total of 13 (52%) preparations followed by 11 (44%) preparation through dermal routs and similar studies revealed that oral routes of administration was more prominent (Yineger et al., 2008).

Knowledge on medicinal plants

Ethnomedicinal knowledge is concentrated in the elderly and relative members of the community and is difficult to transfer from the elders to the young generation. The majority of the respondents (79%) preferred to transfer their indigenous knowledge to their family verbally and

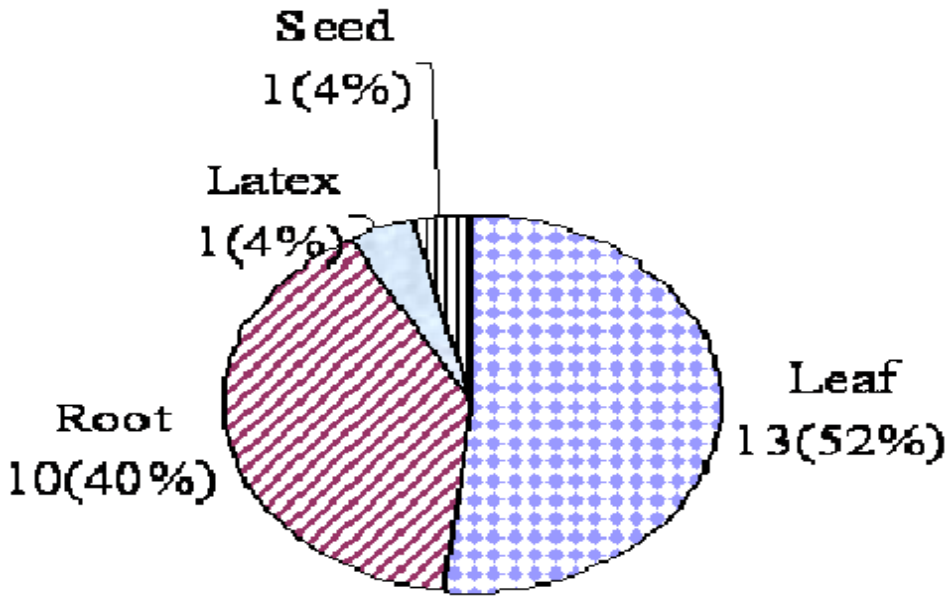

Figure 2. Plant parts used for the treatment of livestock ailments.

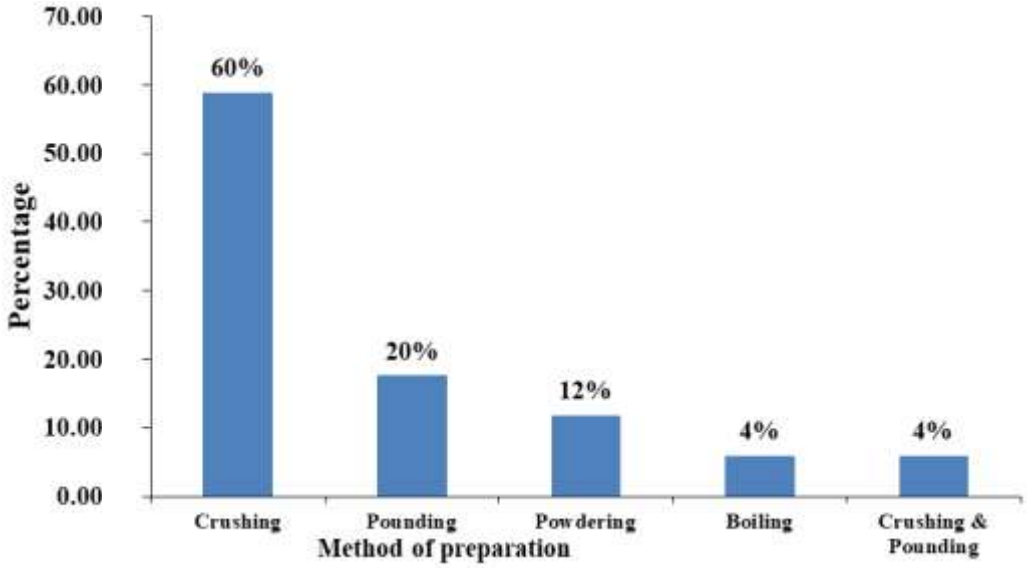

Figure 3. Method of remedy preparation of livestock traditional medicine.

he remaining informants (21%) by showing the medicinal plant in the field and through demonstration including remedy preparation methods. The indigenous knowledge transfer is poor which may cause erosion of the practice and knowledge. The study revealed that, medicinal plant knowledge and transfer of knowledge to the young generation is affected by modernization due to having access to modern education and health services. This might be related to the diminishing of interest of the young generation on indigenous knowledge. The same idea was generated by Yineger and Yewhalaw (2007) based on a study from Sekoru district, Jimma zone, Southwestern Ethiopia.

Ranking of medicinal plants

In the study area, a number of medicinal plants were found to be multipurpose species being utilized for a variety of uses. Direct matrix ranking showed that, of the

total medicinal plants, *Premna schimperi* Engl is the most multipurpose use medicinal plant followed by *A. mellifera* (Vahl) Benth.

Threat and conservation of medicinal plants of the study area

Threats

Medicinal plants are at increasing risk from destruction of their habitats (agricultural expansion, fire, construction, overgrazing, and urbanization) and over harvesting of known medicinal species (Cunningham, 1992). According to Roberson (2008), about 15,000 medicinal plant species may be threatened with extinction world widely due to habitat loss and over harvesting and it is estimated that the earth is losing one potential major drug every two-year. In the study area, the people also rely on medicinal plants for various purposes such as charcoal, medicine, firewood, construction and food. The major threat to medicinal plants in the study area was agricultural expansion which accounted for 55%, charcoal production (15%) and fire wood accounted for 18%. The result of Giday et al. (2003) indicated that intense deforestation became the major threat to medicinal plants in Zay people. Also Yineger and Yewhalaw (2007) reported that deforestation (40%) and agricultural expansion (12.5%) were the most threat to medicinal plants of Sekoru district of Jimma zone. Findings of Lulekal et al. (2008) reported deforestation (90%) as the principal threat to medicinal plants in Mana Angetu sistrict, Eoutheastern Ethiopia.

Similar study by Yineger et al. (2008) from Southwestern Ethiopia and Yirga (2010) from Alamata, Southern Tigray showed that deforestation is the major threat to medicinal plants. This indicates that special focus should be given for conservation of these plants since they are being widely exploited for purposes other than their medicinal value. Availability of medicinal plants has been affected by a dramatic decrease in the area of native vegetation due to agricultural expansion, deforestation, fire, overgrazing and charcoal and firewood (Cunningham, 1996; Giday et al., 2003).

Conservation of medicinal plants in the study area

The influences of human on the natural habitat of medicinal plants are the problems for the conservation of medicinal plants and associated knowledge. Even though there are many problems plus high population growth and thus there are over exploitation of medicinal plants for different purposes and for daily activities, the significant numbers of the local people of the area knew the importance of conserving the plants in both *ex-situ* and *in-situ* conservation methods.

The effort to conserve medicinal plants in the district was observed to be very poor. Some informants have started to conserve medicinal plants by cultivating at home gardens, though the effort was minimal. About 5.5%, of the medicinal plants collected were reported as found cultivated at home gardens. The result of Lulekal et al. (2008) indicated that only 5.7% of medicinal plants were cultivated in home garden showing minimal effort of medicinal plant conservation in Mena Angetu district.

Yinegeret et al. (2008) suggested that natural resources could be utilized best in sustainable way if management practices are complete. In fact, such valuable activities require appropriate action and changes by the full range of societies and stakeholders involved in the conservation, production and management as well as use of medicinal plants. Since an action on conservation and sustainable use of medicinal plants need involvement of various sectors and greater public support, it needs a continuous task of creating public awareness (Schippmann et al., 2002).

Conclusions

A study on medicinal plant utilization in area revealed that the communities commonly use medicinal plants for maintaining their livestock healthcare and they have rich traditional knowledge on use, preparation and application of local plants to cure various ailments animals. The study has resulted in 24 medicinal plants species which include 23 angiosperms and one gymnosperm spread in 18 genera and 17 families. The families of medicinal plants Asteraceae represented the largest number of medicinal plants. Out of the 24 medicinal plants, 23 species occur in wild and one is found in home gardens and cultivated lands.

Shrubs found the dominant growth form of medicinal plants used for preparation of livestock traditional remedies followed by herbs and trees. Leaves were the most used plant parts for the preparation of livestock remedies. Traditional medicine preparation mostly involves a single plant and the method of preparation was mainly crushing followed by pounding; the mode of administration was mainly internal in which oral administration is the common route.

Depletion of indigenous knowledge among the people of the district was serious because of disinterest of young generation to gain the knowledge, oral based knowledge transfer, unavailability of the species and influence of modern education. The main threat for medicinal plants in the area arises from agricultural expansion, firewood, charcoal production and construction. In addition, the multipurpose use of some of the medicinal plants led to overexploitation followed by the depletion of the natural population became a highly threatening factor for the medicinal plants in the study area. Threat exerted on medicinal plants was high due to the utilization of root.

Even though there was no conservation measure taken in the area, the people of the district had started conservation either in their original place or in home gardens.

RECOMMENDATIONS

Based on the results of the study, the following recommendations are forwarded:

(1) Special consideration and all possible endeavors must be made to use the traditional medicinal plants in the study area.
(2) The indigenous knowledge and skill of preparing traditional medicine of indigenous people must be encouraged and protected. This could be the way through which such people could exercise their knowledge boldly.
(3) Identifying effective medicinal plants and promoting their production and cultivation. This is a task to be accomplished through genuine collaboration between local administrators and local people.
(4) Encouraging people to grow medicinal plants in the home gardens, mixing with crops in farmlands, as live fences and on degraded land.
(5) The uses of medicinal plants for the treatment of different ailments indeed need to be confirmed through scientific investigations to identify those that may provide alternatives for modern drugs.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Plants and their metabolites against *Streptococcus mutans*

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Oral diseases represent a major public health problem, especially for economically marginalized communities with limited access to health services. In addition, the constant increase in bacterial resistance to many of the antibiotics contributes to worsen the problem. In this context, great importance had been given to natural compounds for the discovery of new drugs that contribute to the prevention and control of oral affection. The present study proposed a systematic review of articles that used the techniques of agar diffusion and broth dilution to measure the efficiency of plant samples against *Streptococcus mutans,* one of the main agents involved in the development of dental cavities. Families and plant species most used in the study, the concentration and polarity measurements of the samples used in the tests, the disk and well variants in the agar diffusion technique, as well as the most outstanding results presented by the articles are reported. The review highlights the bacteriostatic effect of natural products against *S. mutans* and strengthened parameters that could validate the best strategy for the identification of natural products with antimicrobial action, having as object the *S. mutans*. The agar diffusion test should not be neglected as screening test but scientific measurements should be taken into consideration to obtain plant extracts which are likely to undergo clinical usage against *S. mutans*.

Key words: Medicinal plant, plant extract, antimicrobial, qualitative technique, quantitative technique.

INTRODUCTION

It has been a while since the study of natural products against microorganisms has gained interest by researchers and pharmaceutical industry. The bacterial resistance and side effects of the antimicrobial drugs available in the market have contributed to this fact (Ramakrishna et al., 2011). Moreover, the ecological awareness, traditional knowledge appreciation, and lack of access of marginalized communities to pharmaceutical medicines make the rational search for new compounds still important (Rates, 2001; Halberstein, 2005; Pelkonen et al., 2014).

Tooth decay has been considered one of the most

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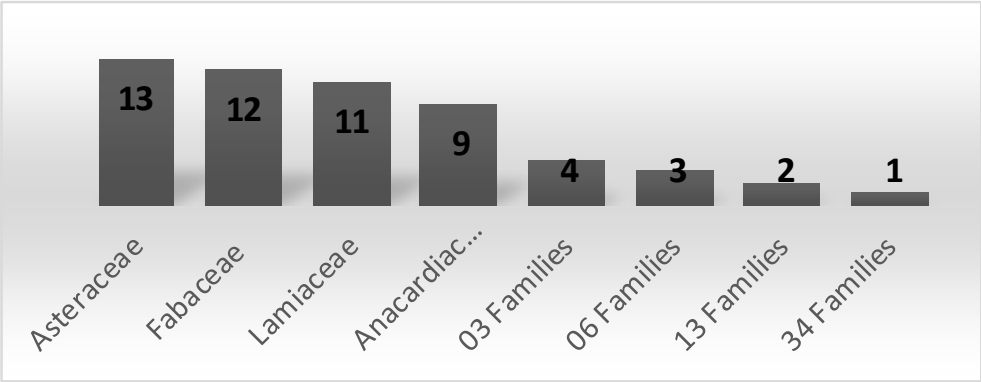

Figure 1. Number of species by family reported in peer reviewed articles found in the literature between January, 1st, 2006 to December 31th, 2016.

prevalent disease in the world (Petersen, 2003) being *S. mutans* one of the greatest risk factor for its development (Nishikawara, 2006).Trying to solve the oral diseases problem, antibacterial effect study of natural products or their derivatives has been conducted (Kouidhi et al., 2015).

Two main techniques have been used in natural products against bacteria research: The agar diffusion (Kirby Bauer) technique, considered a qualitative method, and the broth dilution technique which is defined as quantitative or semi-quantitative method depending on the objective of the studies (Valgas et al., 2007). Both techniques have been used to pre-select plant species, initial concentration to be used, extraction solvents, or isolated molecules giving support to more specific methods related to mode of action of antimicrobial substances (Balouiri et al., 2016). Although the broth dilution is considered the *gold* test for antimicrobial resistance profile (Campana et al., 2011), agar diffusion has an important role in detection of subpopulation of bacteria with resistance to antimicrobials (Matthew, 2015) and it is considered an adequate and simple tool to evaluate bacterial resistance in clinical diagnosis (Schumacher et al., 2018). Therefore, the purpose of this paper is to review and compare different methodologies used to verify the antimicrobial effect of medicinal plants and their derivatives on *S. mutans*.

MATHERIALS AND METHODS

The study is characterized by a systematic review including peer reviewed articles published in international journals using as index tool the "Periodicos CAPES" (http://www.periodicos.capes.gov.br/index.php) which gives integrated access to international indexers such as PubMed, Web of Science, LILACS, SciELO, and SCOPUS. The criteria used to select the articles included: (1) "Plant extract," "*Streptococcus mutans,*" and "medicinal plants" as key words; (2) English, Spanish, and Portuguese languages; (3) published between January, 1st 2006 and December, 31th 2016. Following selection, articles were classified by plant taxonomical classification (family and species), methodology approach (agar diffusion or broth dilution techniques), extract or substance unit measure (mg, %, etc.) used to standardize samples, solvents used for plant substances extraction such as apolar (hexane, benzene, chlorophorm, butane, and petroleum ether), aprotic polar (dichloromethane, ethyl acetate, and acetone/propane) and protic polar (ethanol, butane, methanol, and water), and isolated substances with anti-*S. mutans* activity. The criterion used to consider a "good result" for agar diffusion technique was an inhibition halo of ≥ 18 mm since from the articles selected this diametre seemed to reflect sweetable concentrations in broth dilution. In the case of broth dilution, the minimal inhibitory concentration (MIC) values ≤100 µg/mL (Cos et al., 2006) and the ratio between minimal bacterial concentrations (MBC) and MIC (MBC/MIC) ≥16 were considered "good results." MBC is ≥16 times the MIC value means that the microorganism is tolerant following criteria analyzed by Sherris (1986). Articles with description of antibacterial techniques were described but no results were excluded from the analysis. Data systemizing and graph building were done using Microsoft Excel 2010 software and results were expressed in absolute values and frequencies.

RESULTS AND DISCUSSION

Through refined search using the keywords "plant extract," "*Streptococcus mutans,*" and "medicinal plants," a total of 129 available articles were found. According to selection criteria, 28 articles were selected to this review giving 135 plant species classified in 60 botanical families tested against *S. mutans*. Asteraceae, Fabaceae, Lamiaceae, and Anacardiaceae families were the most frequent (Figure 1 and Table 1) and 30 plant families had relevant results by agar diffusion, MBC/MIC, or both (Table 2). Among them, *Rhus standley* (Anacardiaceae), *Amphipterygium adstringens* (Anacardiaceae), *Aloe vera* (Asphodelaceae), *Mikamia glomerate* (Asteraceae), *Tagetes lucida* (Asteraceae), *Bixa orellana* (Bixaceae), *Bursera simaruba* (Burseraceae), *Drymariagracilis* (Carophyllaceae), *Cnidoscolus multilobus* (Euphorbiaceae), *Glyycyrrhiza uralensis* (Fabaceae), *Liquidambar macrophylla* (Hamamelidaceae), *Cinamomum vera* (Lauraceae), *C. zeylanicum* (Lauraceae), *Persea americana* (Lauraceae), *Eysenhardti apolystachya*

Table 1. Plant species used for anti-*Streptococcus mutans* tests reported in peer reviewed articles published from January 1st, 2006 to December 31th, 2016.

S/N	Family	n	Species
1	Acanthaceae	1	*Justicia spicigera* Schlechtendal
2	Alliaceae	2	*Allium cepa; Allium sativum*
3	Amaranthaceae	2	*Achyranthes aspera; Beta vulgaris* L.
4	Anacardiaceae*	9	*Amphipterygium adstringens; Anacardium humile; Cotinus coggygria; Pistacia atlantica;Rhus coriaria; Rhus standleyi* Barkley; *Schinus terebinthifolius; Semecarpus anacardium; Spondias purpurea* L.
5	Annonaceae	2	*Annona hypoglauca; Annona senegalensis*
6	Apiaceae	1	*Trachyspermum ammi*
7	Apocynaceae	1	*Calotropis gigentica*
8	Asphodelaceae (Liliaceae)	1	*Aloe barbadensis* miller (*Aloe vera*)
9	Aspleniaceae	1	*Phyllitis scolopendrium*
10	Asteraceae (Compositae)*	13	*Cichorium intybus; Parthenium hysterophorus; Tagetes lucida; Wedelia chinensis; Calendula oficinalis L.; Helichrysum litoreum; Heterotheca inuloides* Cass; *Lostephane heterophylla* (Cav.) Benth.; *Mikania glomerata; Milleria quinqueflora L.; Senecio sessilifolius (*H. et A.) Hemsley; *Coreopsis mutica* DC.; *Cirsum mexicanum* DC.
11	Betulaceae	1	*Alnus acuminata* Kunth
12	Bixaceae	1	*Bixa Orellana* L.
13	Boraginaceae	4	*Cordia* cf. Exaltata; *Cordia nodosa; Cordia sp.; Tournefortia hartwegiana* Standley
14	Brassicaceae	1	*Eutrema japonicum*
15	Bromeliaceae	1	*Ananas comosus*
16	Burseraceae	1	*Bursera simaruba* (L.)
17	Caprifoliaceae	1	*Sambucus mexicana* Presl
18	Caryophyllaceae	1	*Drymaria gracilis* Cham. & Schehlechtendal
19	Celastraceae	1	*Celastrus paniculatus*
20	Chrysobalanaceae	1	*Parinari curatellifolia*
21	Clusiaceae (Calophyllaceae)	4	*Garcinia lancifolia; Garcinia kola; Moronobea coccinea;Mammea americana*
22	Combretaceae	1	*Terminalia chebula*
23	Convolvulaceae	1	*Ipomoea alba*
24	Crassulaceae	1	*Sedum dendroideum* Moc & Sessé
25	Cucurbitaceae	1	*Momordica charantia* L
26	Cupressaceae	1	*Juniperus communis*
27	Ebenaceae	3	*Diospyros guianensis; Euclea divinorum; Euclea natalensis*
28	Equisetaceae	2	*Equisetum arvense; Equisetum hyemale*
29	Euphorbiaceae	3	*Cnidoscolus multilobus* (Pax.) I.M. Johnston; *Croton campestris; Croton draco Schlechtendal*
30	Fabaceae (Leguminosae)*	12	*Bauhinia purpurea; Clitoria ternatea; Copaifera langsdorffii; Erythrina lysistemon; Glycyrrhiza glabra* L; *Glycyrrhiza uralensis; Libidibia ferrea* L.; *Haematoxylon brasiletto; Lysiloma candidum Brandegee; Olneya tesota; Eysenhardtia polystachya* (Ort.) Sarg.; *Prosopsis juliflora* (Swartz) DC.
31	Fagaceae	2	*Quercus elliptica; Quercus infectoria*
32	Gentianaceae	2	*Centaurium erythraea; Centaurium erythraea*
33	Geraniaceae	1	*Pelargonium peltatum*
34	Hamamelidaceae	1	*Liquidambar macrophylla*
35	Lamiaceae (Labiateae)*	11	*Hoslundia opposita; Mentha arvensis L.; Ocimum basilicum; Ocimum sanctum (tenuiflorum);* *Ossimum gratissimum; Perilla frutescens; Rosmarinus officianalis; Salvia officianalis; Thymus vulgaris L.; Mentha viridis L.; Mentha x piperita L.*
36	Lauraceae	4	*Cinnamomum cassia; Cinnamomun zeylanicum* Ness.; *Cinamonum verum; Persea americana* Mill.

Table 1. Cont'd.

37	Lythraceae (Punicaceae)	2	*Lafoensia pacari; Punica granatum*
38	Malpighiaceae	1	*Byrsonima crassifolia* (L.)
39	Meliaceae	2	*Azadirachta indica; Cedrela odorata* L.
40	Mimosaceae	1	*Stryphnodendron adstringens*
41	Mirtaceae	1	*Syzygium aromaticum*
42	Moraceae	1	*Morus alba*
43	Moringaceae	1	*Moringa oleifera*
44	Myristicaceae	1	*Myritica fragrans*
45	Myrtaceae	3	*Myrciaria dubia; Psidium guajava; Rhodomyrtus tomentosa*
46	Papaveraceae	1	*Argemone mexicana* L.
47	Pedaliaceae	1	*Dicerocaryum senecioides*
48	Phyllanthaceae	1	*Emblica offcinalis*
49	Piperaceae	2	*Piper nigrum* L.; *Piper sanctum (Miq.)*
50	Poaceae	1	*Cymbopogon citratus*
51	Rosaceae	3	*Eriobotrya japonica (Thunb.) Lindl; Prunus serotina* var. capuli Karst; *Rosa canina*
52	Rubiaceae	3	*Psychotria sp.; Zanthoxylum compactum; Zanthoxylum piperitum*
53	Salicaceae	2	*Casearia javitensis; Caseria spruceana*
54	Sapotaceae	3	*Englerophytum magalismontanum; Madhuca longifolia; Mimusops elengi*
55	Selaginellaceae	1	*Selaginella lepidophyla* (Hook. &Grev.) Spring
56	Smilacaceae	1	*Smilax sp.*
57	Solanaceae	2	*Datura stramonium* L.; *Solanum cf. lanceifolium*
58	Sterculiaceae	1	*Chiranthodendron pentadactylon* Lam.
59	Verbenaceae	2	*Lantana camara; Verbena carolina* L.
60	Zingiberaceae	2	*Zingiber mioga; Zingiber Officinale*
		135	

*Families most reported; n, number of species reported for each family.

(Leguminosae), *Haematoxylon brasiletto* (Leguminosae), *Cedrela odorata* (Meliceae), *Myrcia riadubia* (Myrtaceae), *Syzygium aromaticum* (Myrtaceae), *Argemone mexicana* (Papaveraceae), *Piper sanctum* (Piperaceae), *Punica granatum* (Punicaceae), and *Datura stramonium* (Solanaceae) results have met international journals standard requirements. It is worth noting that some "good results" reported for agar diffusion was not correlated to a "good result" in MBC/MIC.

Natural products scientific experts have been standardizing parametres to stablish sweatable antimicrobial techniques for plants extracts or substances investigation. In this case, MIC and MBC have been the most recommended strategies (Ríos and Recio, 2005; Cos et al., 2006). From the literature review, only 19 articles met the established criteria here. Results reported involved 135 plants using qualitative techniques such as agar diffusion (20%), quantitative techniques (MBC and/or MIC) (43.69%), and a combination of both (36.28%) (Figure 2). The fact that almost 80% of the results were from quantitative techniques indicates the tendency to improve the quality of results following high scientific impact journals requirements. Regarding the results reported by several of studies analyzed, a combination of both seemed to be the best choice. In this case, in the researchers' opinion, researchers can associate a technique still used in clinical diagnosis (Kirby-Bauer) to a more accurate quantitative procedure: The qualitative method to screen plant extract and the quantitative method to establish extract concentrations.

Analyzing the agar diffusion technique, different variants were reported: Disc-variant, well-variant, and cilinders-variant. They were used in 52.9, 45.43 and 1.62% of the articles reporting agar diffusion as the method of choice, respectively. Comparing the two more-frequent reported, disc-variant and well-variant, and considering the best results (inhibition zone from ≥16), no significant statistical difference ($p=0.35$) was seen between them in relation to halo size results. However, according to Valgas et al. (2007) and Silveira et al. (2009), the sensibility of diffusion method well-variant is superior to disc-variant for two reasons: (a) The presence of suspended particulate matter seems to interfere less with the diffusion of the antimicrobial substance into the agar; and (2) the precipitation of substances (that is, cationic) in the disc may prevent diffusion of antimicrobial substances into the agar. Thus, despite the restriction usage of agar diffusion by natural products researchers, based on easy execution and low coast (Silveira et al., 2009), it is believe that this approach should still be

Table 2. Plant species with relevant anti-*S.mutans* activity by agar diffusion and/or microdilution technique.

Family	Species	Part of the plant	Extract/solvent used	Technique used (unit of measurement)	Concentration (halo ≥18 mm)	MIC	MBC	Reference
Alliaceae	*Allium sativum*	Bulb	Aqueous crude extract	AD-DV (mg/g)	NI	ND	ND	OHara et al. (2008)
				AD-WV (%); MIC/MBC (mg/mL)	0.2	6.25	12.5	Jain et al. (2015)
Amaranthaceae	*Achyranthes aspera*	Root	Ethyl Acetate	AD-DV (mg/disco)	5	ND	ND	Jebashree et al. (2011)
	Pistacia atlantica	Leaf	Aqueous	AD-DV/WV - (mg/ml) MIC/MBC - (µg/mL)	40, 80, 100	60	90	Roozegar et al. (2016)
	Rhus coriaria	Peel fruit	Aqueous	AD-WV; MIC/MBC (mg/ml)	100	1.56	6.25	Vahid-Dastjerdi et al. (2014)
	Rhus standleyi Barkley	Aerial parts	Aqueous	MIC/MBC (µg/mL)	ND	32.5	125	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	65	250	
	Schinus terebinthifolius	Leaf, stem bark	n-hexane	AD-DV; MIC (mg/mL)	20 mg/Ml (initial concentration)	3.25	ND	Pereira et al. (2011)
	Amphipterygiumadstringens Schiede ex Schlechter	Stem bark	Aqueous	MIC/MBC (µg/mL)	ND	67.5	>1000	Rosas-Piñón et al. (2012)
Apocynaceae	*Calotropis gigentica*	Leaf	Ethanol	AD-WV (%)	1.25 and 20	ND	ND	Sharma et al.(2015)
Asphodelaceae (Liliaceae)	*Aloe Vera*	Leaf	Crude (gel)	AD-WV (%) MIC (µg/mL)	50 and 100	12.5	ND	Fani and Kohanteb. (2012)
Asteraceae	*Mikania glomerata*	Certified dried aerial parts	Sequence of dichloromethane, methanol/H_2O (9:1), and n-hexane	MIC/MBC (µg/mL)	ND	6.25	12.5	Andrade et al. (2011)
	Tagetes lucida	Aerial parts	Ethanolic	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
Bixaceae	*Bixa Orellana L.*	Leaf	Methanol	AD-C-PV	NC	62.50		Medina-Flores et al. (2016)
		Seeds	Methanol	MIC (µg/mL)		31.25		
Burseraceae	*Bursera simaruba* (L.) Sarg.	Stem bark	Aqueous	MIC/MBC (µg/mL)	ND	100	62.5	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	750	
Caryophyllaceae	*Drymaria gracilis* Cham. & Schehlechtendal	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	67.5	500	Rosas-Piñón et al. (2012)
Chrysobalanaceae	*Parinari curatellifolia*	Stem	Ethanol	AD-WV; MIC/MBC (mg/ml)	100	6.5	25	Oshomoh and Idu (2012)
Clusiaceae	*Garcinia lancifolia*	Fruit	Crude (juice)	AD-WV (mg/ml)	5	ND	ND	Policegoudra et al. (2012)
		Branch	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Leaf	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Fruit	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Leaf	Dichlorometane	AD-WV (mg/ml)	5	ND	ND	

Table 2. Contd.

Combretaceae	*Terminalia chebula*	Fruit	Hexane Ethyl acetate Ethanol Methanol	MIC (mg/mL)	ND	0.76	ND	Jebashree et al. (2011)
Compositae	*Iostephane heterophylla*(Cav.) Benth.	Roots	Aqueous	MIC/MBC (µg/mL)	ND	67.5	125	Rosas-Piñón et al. (2012)
Convolvulaceae	*Ipomoea alba* L.	Aerial parts	Organic	AD-WV/MIC/MBC (mg/ml)	8.34	<0.04	≤0.04	Silva et al. (2014)
			Chloroform fraction	AD-WV/MIC/MBC (mg/ml)	10.78	ND	ND	
Euphorbiaceae	*Cnidoscolus multilobus* (Pax.) I.M. Johnston	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	15.6	<250	
Fabaceae	*Glycyrrhiza uralensis*	Root	Deglycyrrhizinated licorice root extract	MIC/MBC (µg/mL)	ND	8	8	Ahn et al. (2012)
Geraniaceae	*Pelargonium peltatum*	Leaf	Aqueous	AD-DV (mg/ml)	≥200	ND	ND	Hurtado et al. (2013)
Hamamelidaceae	*Liquidambar macrophylla*	Leaf	Ethanolic	MIC/MBC (µg/mL)	ND	67.5	500	Rosas-Piñón et al. (2012)
Lamiaceae	*Ocimum sanctum (*tenuiflorum*)*	Leaf	Ethanol	AD-WV (%)	5 and 10	ND	ND	Pai et al. (2015)
Lauraceae	*Cinamonum verum* J. Presl	NI	Hexane extraction	MIC/MBC (µg/mL)	200	ND	ND	OHara et al. (2008)
	Cinnamomun zeylanicum Ness.	Bark	Aqueous	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
	Persea americana Mill.	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	32.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	65	500	
Leguminosae	*Eysenhardtia polystachya* (Ort.) Sarg.	Wood	Aqueous	MIC/MBC (µg/mL)	ND	78	500	Rosas-Piñón et al. (2012)
	Haematoxylon brasiletto Karst	Stem barck	Aqueous	MIC/MBC (µg/mL)	ND	10.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	12.5	125	
Lythraceae	*Lafoensia pacari*	Leaf, roots, stem	Ethanol	AD-DV; MIC (mg/ml)	20 mg/mL initial concentration	1.0	ND	Pereira et al. (2011)
Meliaceae	*Cedrela odorata* L.	Seed	Aqueous	MIC/MBC (µg/mL)	ND	60	500	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	32.5	250	
Myrtaceae	*Myrciaria dubia*	Seeds	Methanol	AD-WV (w/v)	1:1			Camere-Colarossi et al. (2016)
		Pulp	Methanol	MIC (µg/mL)	ND	62.5	ND	
	Psidium guajava	Leaf	Ethyl Acetate	AD-DV (mg/disco)	2.5	ND	ND	Jebashree et al.(2011)
			Ethyl acetate, hexane, etanol, and methanol	MIC (mg/mL)	ND	<0.076	ND	
	Syzygium aromaticum (L.) Merr. & Perry	Fruit	Aqueous	MIC/MBC (µg/mL)	ND	25	250	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	125	

Table 2. Contd.

Papaveraceae	*Argemone mexicana* L.	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	78	500	Rosas-Piñón et al. (2012)
Phyllanthaceae	*Emblica offcinalis*	Fruits	Crude extract	AD-WV (%)	0.2	25	50	Jain et al. (2015)
			Organic extract	MIC/MBC (mg/mL)	0.2	50	100	
			Aqueous extract		0.2	12.50	50.00	
Piperaceae	*Piper sanctum* (Miq.)	Leaf	Ethanolic	MIC/MBC (µg/mL)	ND	62.5	<500	Rosas-Piñón et al. (2012)
Punicaceae	*Punica granatum* L.	Pericarp	Aqueous	MIC/MBC (µg/mL)	ND	12.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	250	
			Ethanol 70% Glucolic etxract	AD-CV (%)	100≥3	ND	ND	Argenta et al. (2012)
Salicaceae	*Caseria spruceana*	Leaf	Organic	AD-WV/MIC/MBC (mg/ml)	200	≥12.5	≥12.5	Silva et al. (2014)
Solanaceae	*Datura stramonium* L.	Aerial parts	Aqueous	MIC/MBC (µg/mL)	ND	100	>1000	Rosas-Piñón et al. (2012)
Zingiberaceae	*Zingiber officinale*	Rhizomes	Organic solvent	AD-WV (%)	0.2	12.50	25.00	Jain et al. (2015)
				MIC/MBC (mg/mL)				

AD-WV, Agar diffusion-well variant; AD-DV, agar diffusion-disc variant; AD-CP, agar diffusion - cup plate variant; ND, not done; NI, not informed; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; NC, information is not clear

accepted for screening of natural products taking into consideration the fact that this technique is also considered a “gold test” for clinical diagnosis (Schumacher et al., 2018).

A point of concern on medicinal plant folk usage validation is the preparation and solubilization and/or dilution of plant material. The expression of units in the analyzed results in the articles was quite varied: mg/mL (54.52%) followed by percentage (23.67%). It is important to note that 15.95% used mg/disco, 4.78% of articles did not mention any amount of measurement, and 1.06% used proportion criterion (volume/volume or weight/volume). When the best results (≥18.5 mm inhibition zone) was considered, the ones expressed in mg/mL (63.33%) were the most promising when compared to results expressed in percentage (13.33%). Furthermore, analyzing the diferences in agar diffusion technique, the more frequent was mg/mL usage on DV (62.43%) and percentage unit on WV (45.08%) (Table 3). Hence, taking into consideration these aspects, two points which deserve attention were considered: (1) the standardization of international units requested by journal editors (Cos et al., 2006) recognizing that mg/mL is still the most accurate measurement for *in vitro* tests instead of percentage, and (2) percentage seems to better represent the folk method or it is the best choice for some plant material processing (that is, resin, pasty, or gelatinous substances) and it should not be neglected.

Taking into consideration the best plants results against *S. mutans* by agar diffusion technique, three vegetal products were distinguishable in their results (≥ 30 mm inhibition zone): *Aloe vera* gel, *Garcinialancifolia* fruit juice, and *Allium sativum* bulbo juice. The *Aloe vera* gel at 50 and 100% showed inhibition zone of 30 and 54 mm, respectively, and antibacterial action was confirmed by promising results showed in MIC technique (12.5 µg/mL) (Fani and Kohanteb, 2012).The *G. lancifolia* fruit juice at 5 mg/mL gave 47 mm inhibition zone (Policegoudra et al., 2012) and the bulbo juice from *A. sativum* at 100 mg/mL showed 30 mm of inhibition zone (OHara et al., 2008). It is important to note that in the case of *A. sativum* (Jain et al., 2015), although the anti-*S. mutans* potential has been also confirmed by MIC (6,250 µg/mL) and MBC (12,500 µg/mL), the concentration showed in these two quantitative techniques are not in the parameters established by high impact publications since they use (Cos et al., 2006) reference which, unfortunately, makes uncertain the validation of these species.

From the articles selected (n=43) using broth dilution as technique for searching natural products

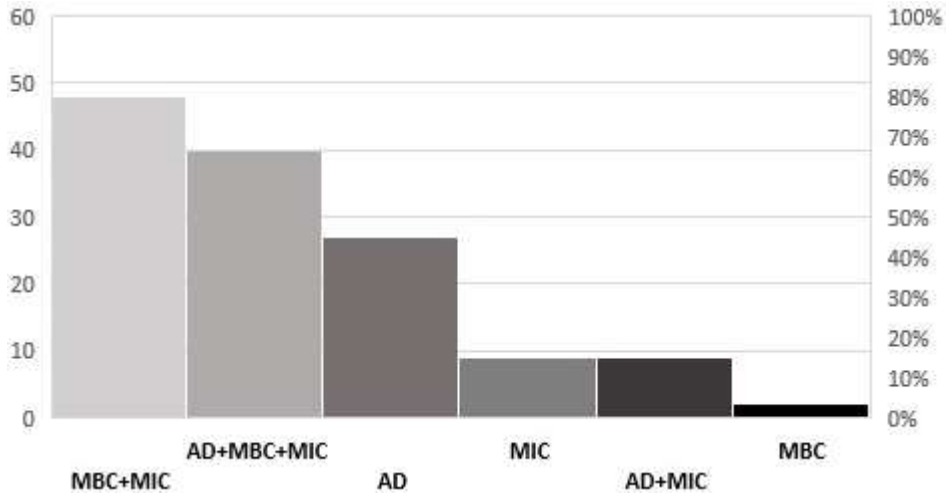

Figure 2. Qualitative and quantitative techniques reported in peer reviewed articles found in databases from January 2006 to December, 2016. AD, Agar diffusion technique; MIC, Minimal inhibitory concentration technique; MBC, Minimal bacterial concentrations technique.

Table 3. Frequency of results reported in articles using agar diffusion technique indexed in database in the period of January, 2006 to December, 2016.

AD variant	mg/mL	%	mg/disco	Proportion (v/v or w/v)	Not reported	Total
DV						
Total	123(62.43%)	14 (7.10%)	60(30.45%)	0	0	**197**
18,5-54 mm	9(75%)	2(16.66%)	1(8.33%)	0	0	**12**
WV						
Total	74(43.78%)	73(43.19%)	--	4(2.36%)	18(10.65%)	**169**
18,76-47 mm	10(55.55%)	2(11.11%)	--	2(11.11%)	4(22.22%)	**18**
Total	**216**	**91**	**61**	**6**	**22**	**396**

DV, Disc variant; WV, well variant; v/v, volume/volume; w/v, weight/volume

with anti- *S. mutans* activity, few (n=17) showed MIC and MBC in association. A ratio between MBC/MIC (r) was obtained in order to evaluate the quality of antibacterial results (Table 4). A ratio ≥16, indicating bactericidal tolerance, was found only for three species ethanolic extracts: *Cnidoscolus multilobus*, *Tournefortia hartwegiana*, and *Coreopsis mutica*. However, since MIC was considered "good results," in the researchers' opinion, it would be interesting to isolate molecules with antimicrobial action from these species. Microbial tolerance may be influenced by bactericidal activity such as antagonism of molecules, technical factors, or microorganism characteristics (Sherris, 1986; Traczewski et al., 2009).

Also, considering ideal MIC values, it was observed that for MIC ≥ 100 µg/mL, MBC values were 3 times higher; while for MIC ≤ 100 µg/mL, MBC values were next to 6 times higher then MIC. From the study point of view, the MIC values were higher than clinical valuable concentrations suggested in the literature (Cos et al., 2006) to reach a bactericidal effect and it means that the natural products tested had a tendency to be bacteriostatic against *S. mutans*.

Following the same criteria cited earlier (MIC ≤ 100 µg/mL and r=1) and establishing the best products reported in literature, *Glycyrrhiza uralensis* deglycyrrhizinated licorice root extract (Ahn et al., 2012), *Mikania glomerate* ent-Kaurenoic acid-rich extract (Moreira et al., 2016b), *Ipomoea alba* chloroform fraction (Silva et al., 2014), and *Pistacia atlantica* aqueous extract (Roozegar et al., 2016) showed the best results. Considering isolated substances and criteria established here, only saponin class showed important results. Saponins isolated from the seeds of *Madhuca longifolia* and *Bauhinia purpurea* were tested against two *S. mutans* strains and promising results were found for both (Jyothi and Seshagiri, 2012) (Table 5).

The polarity of plant metabolites is also a point of concern since it can interfere on substances diffusion and/or solubilization. Non-polar or other samples difficult

Table 4. MBC and MIC ratio of plant extracts with MIC ≤ 100 µg/mL tested against *S. mutans* reported in peer reviewed articles (January, 2006 to December 2016).

S/N	Species	Plant family	Part of the plant	Type of extract/solvente used	MIC µg/mL	MBC µg/mL	r	References
1	*Mikania glomerata*	Asteraceae (Compositae)	Aerial organs	ent-Kaurenoic acid-rich extract ♣	6.50	12.50	1.92	Moreira et al., 2016
2	***Glycyrrhiza uralensis***	Fabaceae	Root	deglycyrrhizinated licorice root extract♥	8.00	8.00	1.00	Ahn et al., 2012
3	*Haematoxylon brasiletto*	Fabaceae (Leguminosae)	Branch, barck	Áqueous	10.50	125.00	11.90	Rosas-Piñón et al., 2012
4	*Haematoxylon brasiletto*	Fabaceae (Leguminosae)	Branch, barck	Ethanol	12.50	125.00	10.00	
5	*Punica granatum L.*	Punicaceae	Pericarp	Áqueous	12.50	125.00	10.00	
6	*Cnidoscolus multilobus (Pax.) I.M. Johnston*	Euphorbiaceae	Leaves	Ethanol	15.60	< 250.00	16.03	
7	*Syzygium aromaticum (L.) Merr. & Perry*	Myrtaceae	Fruit	Áqueous	25.00	250.00	10.00	
8	*Rhus standleyi Barkley.*	Anacardiaceae	Aerial parts	Áqueous	32.50	125.00	3.85	
9	*Persea americana Mill.*	Lauraceae	Leaves	Áqueous	32.50	125.00	3.85	
10	*Heterotheca inuloides Cass.*	Compositae	Aerial parts	Ethanol	32.50	125.00	3.85	
11	*Cedrela odorata L.*	Meliaceae	Seed	Ethanol	32.50	250.00	7.69	
12	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	< 40.00*i	≤40.00*i	1.00	Silva et al., 2014
13	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	60.00*ii	80.00*ii	1.33	
15	*Pistacia atlantica*	Anacardiaceae	Leaves	Áqueous	60.00	90.00	1.50	Roozegar et al., 2016
16	*Cedrela odorata L.*	Meliaceae	Seed	Áqueous	60.00	500.00	8.33	Rosas-Piñón et al., 2012
17	*Syzygium aromaticum (L.) Merr. & Perry*	Myrtaceae	Fruits	Ethanol	62.50	125.00	2.00	
18	*Punica granatum L.*	Punicaceae	Pericarp	Ethanol	62.50	250.00	4.00	
19	*Cinnamomun zeylanicum Ness.*	Lauraceae	Barck	Áqueous	62.50	250.00	4.00	
20	*Cnidoscolus multilobus (Pax.) I.M. Johnston*	Euphorbiaceae	Leaves	Áqueous	62.50	250.00	4.00	
21	*Tagetes lucida*	Asteraceae	Aerial parts	Ethanol	62.50	250.00	4.00	
22	*Piper sanctum (Miq.)*	Piperaceae	Leaves	Ethanol	62.50	< 500.00	8.00	Rosas-Piñon et al., 2012
23	*Bursera simaruba (L.)*	Burseraceae	Branch, barck	Ethanol	62.50	750.00	12.00	
24	*Tournefortia hartwegiana Standley.*	Boraginaceae	Aerial organs	Ethanol	62.50	> 1000.00	16.00	
25	*Coreopsis mutica DC.*	Compositae	Aerial organs	Ethanol	62.50	> 1000.00	16.00	
26	*Rhus standleyi Barkley.*	Anacardiaceae	Aerial organs	Ethanol	65.00	250.00	3.85	
27	*Persea americana Mill.*	Lauraceae	Leaves	Ethanol	65.00	500.00	7.69	
28	*Iostephane heterophylla (Cav.) Benth.*	Compositae	Root	Áqueous	67.50	125.00	1.85	
29	*Drymaria gracilis Cham. & Schehlechtendal*	Caryophyllaceae	Leaves	Áqueous	67.50	500.00	7.41	
30	*Liquidambar macrophylla*	Hamamelidaceae	Leaves	Ethanol	67.50	500.00	7.41	
31	*Amphipterygium adstringens*	Anacardiaceae	Branch, barck	Áqueous	67.50	> 1000.00	14.81	
32	*Argemone mexicana L.*	Papaveraceae	Leaves	Áqueous	78.00	500.00	6.41	
33	*Eysenhardtia polystachya (Ort.) Sarg.*	Fabaceae (Leguminosae)	Wood	Áqueous	78.00	500.00	6.41	
35	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	m100.00	160.00	1.60	Silva et al., 2014
36	*Bursera simaruba (L.)*	Burseraceae	Branch, barck	Áqueous	100.00	500.00	5.00	Rosas-Piñon et al., 2012
37	*Datura stramonium L.*	Solanaceae	Aerial organs	Áqueous	100.00	1000.00	10.00	

r, Ratio between MBC and MIC; *m, Mean of reported values;♣ The final product was originated from soluble fraction of dichloromethane extraction, partitioned with n-hexane; ♥Dried roots were passed thrhough heating in distilled water (20:1 [v/w]) for 2 h, heating (78°C) in 95% ethanol (95% ethanol:residue ratio of 15:1 [v/w]) for 2 h, column (6.5 cm - 60 cm) filled with Diaion HP-20 adsorbent equilibrated with distilled water, 50% ethanol and 99% etanol. *The same extract was tested for two different bacterial concentration: i – 1×10^2; ii – 1×10^3

Table 5. Minimal inhibitory concentration and minimal bactericidal concentration of plant compounds with MIC ≤ 30 µg.mL^{-1} tested against *S. mutans*.

Species	Family	Part of the plant	Compound	*S. mutans* strain	MIC (µg/ml)	MBC (µg/ml)	MBC / MIC
Madhuca longifolia	Sapotaceae	Seed	Saponin	MTCC 890	18.30	34.40	1.88
				MTCC 497	23.60	39.60	1.68
Bauhinia purpurea	Fabaceae	Seed	Saponin	MTCC 890	26.40	43.00	1.63
				MTCC 497	26.30	38.00	1.44
			Ent-kaurenoic acid	ATCC25175	10	20	2

MIC, Minimal inhibitory concentration; MBC, Minimal bactericidal concentration.

to diffuse in the medium, should be avoided in diffusion methods (Valgas et al., 2007). In this review the most frequent category used was polar solvent but it is worth noting that a combination of solvent or crude extracts obtained directly from the plant without solvent addition was also described. By agar diffusion technique, from non-polar solvent extraction, only two showed ≥ 18 mm inhibition zone: The hexanic extract from bark of *Cinamonum verum* (OHara et al., 2008) and the hexanic extract from bark/leaves of *Schinuster ebinthifolius* (Pereira et al., 2011).

Both articles reported the use of dimetylsulfoxide as solvent to solubilize the powdered extracts. Thus, although both hexanic extracts were active at higher than clinically acceptable concentrations, the results published by Ohara et al. (2008) and Pereira et al. (2011) suggested that solvent such as dimetylsulfoxide can help some molecules to diffuse into agar medium and make possible the use of agar diffusion technique for apolar substances as suggested by Valgas et al. (2007).

The antibacterial property of a variety of natural products is documented; nonetheless, the great variability of secondary metabolites composition makes the studies in this area always laborious.For anti-*S. mutans*, it seems there is a restriction in this variety of substances. From the peer reviewed sources used in this work, the only pure substance report was saponins extracted from four species: *Madhuca longifolia, Bauhinia purpurea, Celastrus paniculatus,* and *Semecarpus anacardium* by Jyothi and Seshagiri (2012) with promising results only for saponins isolated from *M. longifolia.* The antimicrobial broad spectrum of saponins has been reported (Avato et al., 2006; Qin et al., 2016), however the amphiphilic characteristics of this class, with the diversity which it carries, plays an important role in the antimicrobial property itself or in its solubilization in aqueous based medium used in the techniques employed were considered.

Conclusion

The present systematic review showed a comprehensive examination of medicinal plants under the perspective of anti-*S. mutans* activity. Results showed that agar diffusion technique is still widely used for medicinal plants antimicrobial activity screening being either well-variant or disc-variant worthwhile as screening tests. The remarkable results from *A. vera*, *G. lancifolia* and *A. sativum* by agar diffusion or from *G. uralensis*, *M. glomerata*, *I. alba*, and *P. atlantica* by MIC/MBC show that although taxonomic criterion may be considered a leader for antimicrobial activity, ethnobotanical criterion should also be considered an excellent guide for in vitro studies. The unit of measurement used (µg/mL) is scientifically considered more accurate; however, it is worth noting that percentage as unit of measurement warrants validation since it mimics the popular usage of plants. Finally, the standardization of antimicrobial protocols for medicinal plants antimicrobial tests is needed in order to obtain more accurate results and make the comparision between natural products and controls easier.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Quantitative analyses of phytochemical and trace elements contents of daily detox, herbal tea consumed in Nigeria

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Tea is one of the commonest drinks in most homes. Many people consume tea due to its unique taste and associated health benefits. Several medical disorders such as cancer, cardiovascular diseases and diabetes mellitus have been linked to the excessive generation of free radicals and oxidative stress. Studies conducted on Daily Detox, a tea consumed by many Nigerians have been limited to qualitative assessment of phytochemicals, but quantitative measurement of phytochemical, microelement, macro elements and heavy metal contents of the tea have not been explored. This study was designed to bridge this gap. Two packs of the daily detox made from *Agerantus conyzoides* (common name, Goat weed) and *Loranthus bengwensis* (common name, African Mistletoe) each containing 21 tea bags in dust form, supplied by the manufacturer were used for analyses. Quantitative measurements of terpenoids, trypsin inhibitors, tannin, phenol, alkaloids and carotenoids were performed using standard methods. Copper, zinc, iron, sodium, potassium, cadmium, nickel, chromium and manganese were estimated using standard methods. Quantitative values of phytochemicals obtained from the herbal tea were: Terpenoids (325.2 µg/g), trypsin inhibitors (16115.5 µg/g), tannin (39.4 µg/g), phenol (55.0 µg/g), alkaloids (1.9 µg/g), flavonoids (3.0 µg/g) and carotenoids (205.5 µg/g). Macro and micro elements measured from the herbal tea were: Copper (16.9 µg/g), zinc (82.9 µg/g), iron (2742.7 µg/g); sodium (2442.9 µg/g); potassium (22132.8 µg/g); chromium (18.9 µg/g) and manganese (340.4 µg/g). Lead was (9.9 µg/g) while nickel and cadmium levels were undetected. The metabolic roles of these chemicals are discussed in relation to their health benefits.

Key words: Herbal tea, phytochemicals, herbal medicine, traces elements.

INTRODUCTION

Tea is one of the commonest drinks in most homes. Many people consume tea due to its unique taste and associated health benefits. Several medical disorders such as cancer, cardiovascular diseases, diabetes

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mellitus as well as other chronic and non-chronic diseases have been linked to excessive generation of free radicals thereby causing oxidative stress. Consumption of tea, especially green tea (GT) has been associated with reduced occurrence of oxidative process with all the associated medical consequences (Butt and Sultan, 2009). The health benefits derivable from tea have been attributed to its high content of polyphenols; and these have been reported to possess antioxidant, anti-inflammatory and antiviral properties (Butt and Sultan, 2009). Polyphenol present in tea has also been implicated in increasing the activities of detoxifying enzymes, stimulating immune function and decreasing platelet aggregation (Butt and Sultan, 2009; Frankel, 2008). Many plants with medicinal benefits have been identified in Nigeria and some can easily be extracted by boiling and drank as tea with a view to treating various local medical conditions.

Tea because of its desirable aroma, taste and smell is one of the commonest consumed beverages globally (Zhu, 2002). Tea leaves are good sources of minerals and trace elements such as zinc, manganese, iron, copper, magnesium, titanium, aluminium, strontium, bromine, sodium, potassium, phosphorous, iodine and fluorine (Jha and Mann, 1996; Srividhya and Raj, 2011). In addition, tea leaves or tea infusion contains tanning substances, alkaloids, carbohydrates, amino-acids, enzymes, vitamins, very little protein and aroma-forming substances as well as polyphenols (Jha and Mann, 1996; Friedman, 2007). Some of the important tea polyphenols are flavanols, predominantly catechins which effectively kill bacteria, reduce the growth of cancer suppress plaque and cavity formation and prevent excessive build-up of blood cholesterol due to their strong antioxidant activity (Oguni, 2002). It is believed that tea catechins can react with reactive oxygen species which may play central roles in carcinogenesis by terminating chain oxidative reactions (Srividhya and Raj, 2011). Other polyphenols of importance present in tea is epigallocatechin-3-gallate (EGCG), which also has significant antioxidant properties (Chandra and Mejia, 2004); cholesterol lowering effect (Maron et al., 2003), hepatoprotective effects (Hasegawa et al., 1995) and anticancer activities (Fujiki, 2005; Bettuzzi et al., 2006). The polyphenols present in tea possess antioxidative activities, useful for fighting the deleterious effect of environmental and endogenous free radicals (Ostrowska and Skrzydlewska, 2001; Srividhya and Raj, 2011).

The medicinal value of herbal tea depends on their phytochemical constituents. Phytochemical analysis of herbal tea showed that anti diarrhoea herbal tea contained tannin, saponin, flavonoid and phenols; anti-diabetic herbal tea contained alkaloids, volatile oils, flavonoids and phenols while slimming tea contained alkaloids, saponis, flavonoids and phenols, but devoid of volatile oils and tannins (Omogbai and Ikenebomeh, 2013).

Description of medicinal plants contained in Daily Detox

Daily Detox is made basically from two popular medicinal plants. These are: *Agerantus conyzoides* (Common name: Goat weed,) and *Loranthus bengwensis* (Common name: African Mistletoe).

Agerantus conyzoides

This plant is commonly called Goat weed. It belongs to the family, Asteraceae. The genus 'Ageratum' is derived from the Greek words 'ageras' which signifies the non-aging characteristics of the plant. It is an annual herb which has been widely used in herbal medicine in both tropical and sub-tropical regions of the world (Brojendro et al*.,* 2013). Phytochemical analysis of the medicinal plant showed that it contained monoterpenes, sesquiterpenes, benzofuran, chromene, chromone, coumarin, flavonoids, alkaloids, tri-terpenes and steroids (Ekundayo et al., 1988; Adebayo et al., 2013; Singh et al*.,* 2013). As a widely grown medicinal plant, it has been used locally for the treatment of various ailments and medical conditions which include wound dressing, skin diseases, diarrhoea, dysentery, rheumatic fever, sleeping sickness, toothache, gynaecological disorders amongst others (Brojendro et al*.,* 2013). Furthermore, the plant has also been shown to possess pharmacological properties which include analgesic, antimicrobial, anti-inflammatory, anti malaria and anti-cancer properties (Brojendro et al*.,* 2013).

Loranthus bengwensis

This is commonly called African Mistletoe. It belongs to the Loranthesceae family. Report has shown that the plant is specific to Africa and is often grown within the tropical regions (Ahmed and Mohammad, 2014). The plant is usually a host to other trees (Becker, 1998) and its chemical composition varies depending on the tree that hosts its growth (Osadolor , et al, 2014). Phytochemicals which have been identified in the plant include flavonoids, tannins, saponis, lectins (carbohydrate binding protein), polypeptides, polysaccharides and tri-terpines . Pharmacological properties have been reported to include anti- cancer, anti-inflammatory and anti-diabetic (Becker, 1998; Ahmed and Mohammad, 2014).

While daily detox is currently consumed by many Nigerians, none of its acclaimed benefits have been evaluated using randomized clinical trial. Studies evaluating the quantitative and qualitative contents of phytochemicals, macronutrients, trace elements and heavy metal of this tea are scarce. Few published studies evaluating its medicinal constituents have been limited to

qualitative analysis, but limited information on quantitative measurement of its phytochemicals, heavy metals, macro- and micro-elements. This study was designed to bridge this gap. The information generated from this study could be helpful in developing protocol for clinical studies

MATERIALS AND METHODS

Selection of product

Herbal tea (Daily Detox) was supplied by the manufacturer of the product, Heritage Herbal Medicine Ventures Limited, Lagos, Nigeria. The product had neither been listed nor registered by the National Agency for Food Drug Administration and Control (NAFDAC) and none of its medical claims have been evaluated using randomized clinical trial. Two packs of the product, each containing 21 tea bags in dust form were supplied and used for the analysis.

Laboratory analysis

Laboratory analysis was performed at Jagee Laboratory, a laboratory accredited by the Nigerian Institute of Science Laboratory Technology, Ibadan, Nigeria. All reagents used for the analysis were of the analytical grade. The following parameters were quantitatively measured.

Terpenoids

Terpenoids was measured by the method described by Ferguson (1956). One gram (1 g) of tea dust was weighed and put into a conical flask containing 10 ml of petroleum ether. The mixture was left for 15 min followed by intermittent shaking. At the end of 15 min, it was filtered and the resulting absorbance was measured at 420 nm using UV-visible spectrophotometer. The intensity of colour developed is directly proportional to the concentration of terpenoids present in the herbal tea.

Flavonoids

Flavonoid was measured by the method described by Boham and Kocipai (1974). Ten gram (10 g) of sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The flavonoid was calculated as follows:

Mg/100 g flavonoids = Weight of crucible + filtrate after drying - weight of empty crucible

Trypsin inhibitor

Trypsin inhibitor was measured using the procedure described by Kakade et al. (1974). One gram of sample was mixed with 100 mL of 0.009 M HCl. The mixture was shaken at ambient temperature for 2 h and centrifuged (10000 × g, 20 min). The resulting supernatant was used for the estimation of trypsin inhibitor. The extract from each sample was diluted with distilled water to obtain a dilution whereby 1 ml extract produced trypsin inhibition activity of between 40 and 60%. One millilitre (1 mL) of the extract was incubated with 1 mL trypsin solution at 37°C for 10 min. A 2.5 mL of pre-warmed substrate (BAPNA) was added and after exactly 10 min at 37°C the reaction was stopped with 0.5 mL of acetic acid (30%, v/v). The absorbance was measured at 410 nm against a blank using the spectrophotometer.

Tannin

This was measured using the method described by the Association Official Analytical Chemists (1990). Two hundred milligrams (200 mg) of the sample was extracted with 10 mL of 70% aqueous acetone (v/v) for 24 h at room temperature. The extracts were centrifuged at 3000 rpm for 20 min and the supernatant was analyzed for tannins. In a 10 mL test tube containing 0.5 mL Folin-Denis reagent, was added 0.5 mL of the tannins extract and 1 mL of saturated sodium carbonate solution. The volume was made up to 10 mL with distilled water. After 30 min, tannin content was measured at 760 nm with the spectrophotometer against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound.

Phenol

Phenol was analyzed using Folin-Denis and Folin-Ciocalteu method as described by Box (1990). The method was based on the reduction of molybdic acid in the presence of phenols to a blue colour which is measured spectrophotometrically. In a test tube containing the mixture of 1 mL of organic extract, 10 mL of water (deionized) and 2 mL of Folin Denis reagent was added 2 mL of saturated sodium carbonate solution and incubated in the dark at room temperature for 1 h. The resulting colour was measured spectrophotometrically at 640 nm. The total phenolic concentration was calculated using a pre- prepared calibrated curve prepared using standard phenolic compound.

Carotenoids

Total carotenoids were estimated using the method described by Sass-Kiss et al. *(2005).*10 g of sample was extracted using 20 ml of mixed extraction solvent *(hexane/acetone/ethanol) in the ratio* 2:1:1 respectively. After stirring for 30 min, the supernatant was recovered. Re-extraction was repeated for the second time using 10 ml of the extraction solvents. The mixture of the two hexane phases was used for the determination of total carotenoids by spectrophotometry at 420 nm. Concentrations of carotenoids were measured in reference to the calibration curve using β-carotene as standard and the results are expressed in µg/g.

Alkaloids

This was determined using the method described by Harborne (1973). Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered and allowed to stand for 4 h. It was then filtered and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2 M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.

Table 1. Quantitative values of phytochemicals obtained from Daily Detox.

Parameter	Value (µg/g)
Terpenoids	325
Trypsin Inhibitor	16115
Tannin	39
Phenol	55
Carotenoids	205
Flavonoids	2.96
Alkaloids	1.92

Table 2. Quantitative values of micro, macro elements and heavy metals measured from Daily Detox.

Element	Value (µg/g)	WHO limits (µg/g)
Copper	16.9	-
Zinc	82.87	25
Iron	2741.7	100
Sodium	2442.9	-
Potassium	22132.8	-
Lead	9.92	10
Chromium	18.9	50
Manganese	340	-
Nickel	**ND	70
Cadmium	**ND	3

** Not detectable.

Measurement of micronutrients

The mineral contents were measured using Atomic Absorption Spectrophotometry (AAS) after appropriate sample digestion had been made.

RESULTS AND DISCUSSION

The data obtained from this study are shown in Tables 1 and 2. Quantitative concentration of various phytochemicals obtained from the herbal tea were: Terpenoids, 325.2 µg/g; trypsin inhibitors, 16115.5 µg/g; tannin, 39.4 µg/g; phenol, 55.0 µg/g; alkaloids,1.9 µg/g; flavonoids, 3.0 µg/g and carotenoids, 205.5 µg/g. The concentration of macro and micro elements measured from the herbal tea were: Copper, 16.9 µg/g; zinc, 82.9 µg/g; iron, 2742.7 µg/g; sodium, 2442.9 µg/g; potassium, 22132.8 µg/g and manganese 340.4 µg/g. The concentrations of lead and chromium were 9.9 and 18.9 µg/g, respectively. Nickel and cadmium were undetected.

Generally, herbal tea had been reported to contain different phytochemicals including flavonoids, catechins, alkaloids, saponins, terpenoids, carotenoids, phenols and tannins (Sharma et al., 2011; Ikenebomeh, 2013). From this study, it was evident that Daily Detox contained different concentrations of flavonoids, terpenoids, carotenoids, alkaloids, phenols and tannins. Phytochemicals identified from the studied herbal tea was consistent with those previously reported in herbal tea (Sharma et al., 2011; Omogbai and Ikenebomeh, 2013). This implies that the investigated herbal tea could be a very good source for these phytochemicals.

The level of flavonoids detected in the herbal tea was 3.0 µg/g. Flavonoids have been reported to be one of the major constituents of herbal tea (Ikenebomeh, 2013). Flavonoids are vital in the scavenging of oxygen-derived free radicals. *In vitro* studies have shown that flavonoids possess anti-inflammatory, anti-allergic, anti-viral, and anti-carcinogenic properties (Middleton, 1998). Flavonoids prevent injury from free radicals by reacting with the reactive oxygen species thus stabilizing the reactive oxygen species, scavenging superoxides and peroxynitrites, inhibiting oxidation of low density lipoprotein (LDL) thereby protecting against atherosclerosis (Ikenebomeh, 2013).

The level of carotenoids in the herbal tea was 205.5 µg/g. Carotenoids are important constituents of most herbal tea. They are precursors of vitamin A. Consuming

diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Faulks and Southon, 2005). Increased carotenoid intakes have been linked with reduced risk to cancer and cardiovascular disease. This justifies the health benefits derived from consumption of tea.

One the major phytochemicals which is rarely quantitated in herbal tea is trypsin inhibitor (TI). From this study, 16115.5 µg/g of TI was quantitated in the herbal tea. The concentration of TI contained in the herbal tea was higher than all the other phytochemicals combined. There could be possible antagonistic effects of TI on other phytochemicals when this tea is consumed especially on a regular basis. This could limit the beneficial effects of the phytochemicals due to antagonistic effects of TI on metabolic process. This hypothesis could be elucidated using further research. Furthermore, although, published studies reporting the acceptable level of TI are scanty, yet in the researchers' opinion, this value was considered to be excessively high. Trypsin inhibitor has been reported to inhibit the physiological activities of trypsin, a proteolytic enzyme, which is physiologically essential in the digestion of proteins (Hama and Khalid, 2007; Gu et al., 2014). Study conducted on animal models have shown that TI induced oxidative stress, limited the activities of both enzymatic and non-enzymatic antioxidants, increased the formation of lipid peroxidation and generation of free radicals (Gu et al., 2014).

The herbal tea, Daily Detox, contained various levels of essential micronutrients. The human body requires both metallic and non-metallic mineral elements within certain permissible limits for growth and good health. Many of these elements play a very important function in the metabolic processes and in the general wellbeing of humans.

The observed zinc content of this herbal tea was 82.9 µg/g. This is higher than WHO recommended limit of 25 µg/g for herbal tea. From this study, it implies that the herbal tea could be a good source of zinc, but excessive consumption could be damaging to the body. Zinc is an indispensable trace element in the body (Dosa et al., 2014). It is required for numerous enzymatic and cellular processes including protein synthesis, intracellular signalling, in addition to functioning as antioxidant and anti-inflammatory agent (Shannon et al., 2011; Maret, 2013; Cruz et al., 2015; Bonaventura et al., 2015). It plays significant role in ageing (Stefanidou et al., 2006), normal growth and development, testicular maturation, neurological function, wound healing and immuno-competence (European Food Safety Authority (EFSA),;European Food Safety Authority, 2006), thyroid functions and glucose metabolism (Zargar, 1998) as well as endocrine system (Mahdizadeh et al., 2014).

The level of manganese quantitated in the herbal tea was (340.4 µg/g). This implies that Daily Detox could serve as a good source of this micronutrient. There is no formal Recommended Dietary Allowance (RDA) for manganese. However, an estimated safe and adequate dietary intake (ESADDI) of 2 to 5 mg/day for adults was established by the US National Research Council (Freeland-Graves et al., 1982), and the Scientific Committee for Food (SCF) of the European Union estimated 1 to 10 mg/day as an acceptable range of intake (Scientific Committee on Food, 1993). Manganese has been reported to play essential role in various enzymatic activities in numerous species. Insufficient intake of manganese can result in adverse effects such as impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn, and defects in lipid and carbohydrate metabolisms (European Food Safety Authority, 2006). On the contrary, evidence of manganese deficiencies in man is poor. A specific deficiency syndrome has not been described in humans (Scientific Committee on Food, 1993; World Health Organisation, 1996; Deepak, 2011).

The level of iron quantitated in the herbal tea was 2742.7 µg/g. This suggests that the herbal tea could be a very good source of iron. For adequate metabolic process, there should be a balance between intake of iron and those lost through normal body physiological processes.

Iron is an essential trace element that has important metabolic functions - such as oxygen transport and storage as well as many redox reactions. Insufficient intake results in deficiency leading to anaemia, adverse outcomes of pregnancy, impaired psychomotor development and cognitive performance and reduced immune function (European Food Safety Authority, 2006).

The level of sodium and potassium quantitated from the herbal tea was 2442.9 and 22132.8 µg/g, respectively. This implies that the tea could be good source of these essential elements. Sodium and potassium do play essential roles in cell metabolism and enzymatic function. However, the observed higher content of potassium over sodium in this herbal tea suggests that it could be helpful in controlling hypertension since consumption of potassium - rich food and drinks has been linked to reduction of blood pressure.

The level of copper quantitated in Daily Detox was 16.9 µg/g. Copper is an essential micronutrient which is useful for several biological functions in human (Deepak, 2011). It is a constituent of many enzymes such as tyrosinase, cytochrome oxidase, peptidyl and glycyl-amidatingmonooxidase, caeruloplasmin and other ferroxidases (Uauy et al., 1998) as well as Cu/Zn superoxide dismutase (Uauy et al., 1998).

The level of lead (9.9 µg/g) quantitated in the herbal tea was within the WHO recommended level. This implies that lead toxicity might not be a possible consequence from consuming this herbal tea. Furthermore, the undetected levels of Nickel and Cadmium are suggestive that these might not be possible causes of toxicity when

the herbal tea is consumed

Conclusion

Consumption of food and food products rich in phytochemicals have been associated with reduction in oxidative stress and its associated health disorders. Information obtained from this study showed that the herbal tea (Daily Detox) contained various arrays of phytochemicals, macro and micro elements. Premised on this, it could be a very good source for these chemicals; and could be helpful in improving general health status of consumers. The high level of trypsin inhibitor obtained in this tea calls for caution among consumers. The value of lead quantitated from the tea was within the acceptable limit recommended by WHO. The quantitated levels of cadmium and nickel suggest that these metals might not be possible causes of toxicity when the herbal tea is consumed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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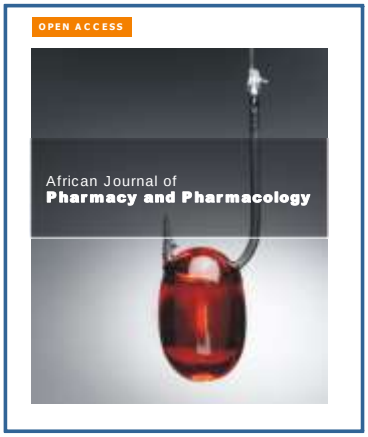

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