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Original Research Article

Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants

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A B S T R A C T

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Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug discovery. In the present study, principal phytoconstituents of 20 Cameroonian medicinal plants were identified in order to relate their presence with bioactivities of the plants. Screening of the plants was performed using standard methods and resulted in the detection of the presence of tannins, flavonoids, phenolics, saponins, steroids, cardiac glycosides and alkaloids. Flavonoids were present in 18 of 20 plants while alkaloids were present in only four of the selected plants. It is evident from the study that *Bridelia ferruginea*, *Justicia obliquifolia*, and *Morinda lucida* registered the highest therapeutic efficacy, possessing majority of phytochemical classes of compounds while *Spilanthes filicaulis* recorded the lowest therapeutic potential due to absence of majority of phytoconstituents. These results validate the exploitation of the studied medicinal plants, according to high amounts of active principles and their potential in medications, allelopathy, agroecosystems, dietary supplements or cosmetics industries. Further studies are needed with these plants to evaluate their allelopathic potentials, isolate, characterize and elucidate the structures of the bioactive compounds responsible for their antimicrobial activity, allelopathic activity, and other medicinal values.

Introduction

The tropical rainforest is home to a significant array of rich bio-resources and accounts for 25 % of plant-extractable allopathic medicines used by humans (Yorek *et al.*2008; Ndah *et al.*, 2013). Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. The long term use of herbs in medicine is a sure indication of their value and usefulness in the future. In modern medicine, the importance of medicinal plants is increasing (Iordăchescu and Dumitriu, 1988) with pharmaceutical and cosmetic industries increasingly using plant resources from rural or unpolluted areas.

Nature has been a source of medicinal agents for thousands of years and generally produces many secondary metabolites which constitute important leads for the development of new environmentally friendly microbicides, pesticides, herbicides and many pharmaceutical drugs (Bobbarala *et al*., 2009). Traditional societies in Africa and elsewhere have always used plants to promote healing (Idu *et al*., 2005; Bussmann, 2006; Teferi *et al*., 2009) and about 80 % of the world's population depends on the use of traditional medicine for health care (WHO, 1993). Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Doughari, 2008).

As science advanced, however, it became possible to determine rigorously the active components of these extracts through painstaking and laborious chemical methods. This rational approach to the discovery of drugs inaugurated an era of bio-prospecting that is, raiding nature's storehouses of plant and microbiological life. Bio-Prospecting literally involves exploring the forests, diving in the oceans and digging in the dirt to obtain environmental samples. The study of the compounds discovered by these methods has become a major area of research in organic chemistry, biological science and has led to the isolation and identification of thousands of different structures, mostly extracted from plants and more recently from micro-organisms, with the animal kingdom contributing rather sparsely to the total (Fujii *et al*., 1991; Duke *et.al*., 2000; Nazir *et al*., 2007). Over the last few decades, the biological and pharmacological potentials of organic substances from many indigenous plants have been well understood. For instance, phenolic compounds have been associated with antimicrobial (Narayana *et al*., 1999), anti-inflammatory, antiviral, and cytotoxic activities (Chhabra *et al*., 1984). However, the bioactive constituents conferring these properties on many plant species have also been implicated in allelopathy (Nazir *et al*., 2007).Allelopathy has been defined as the effect(s) of one plant on other plants through the release of chemical compounds in the environment (Rice, 1984). Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates and their derived compounds, can have allelopathic activity that varies over growing seasons (Rice, 1984; Rizvi *et al*.,1999).

It has been indicated that phenolic acids are the most commonly occurring natural products noted for allelopathic activities (Singh *et al*., 2003). Mungole *et al*. (2010) have also included alkaloids, coumarin, flavonoids, saponnins and volatile constituents of the essential oils as being allelopathic agents. Generally, the presence of different phytochemicals in crude plant extracts has been linked to the detrimental effects of leachates, root exudates or decomposing residues of such plants on the other vegetation or succeeding crops (Chung *et al*., 2005; Mubashir and Wajaht, 2011). Phytochemical analyses of several species of medicinal plants and allelopathic activities of the crude chemical compounds on crops and plants have yielded positive results (Fujii *et al*., 2004). Of the different plant families studied, Viles and Reese (1996) indicated that members of the Asteraceae family have great potential for inhibitory activities. Thus, the increased interest in the isolation and identification of the chemical compositions of organic products associated with biological activities with particular emphasis on germination, growth and yield of crops has stimulated research on plants having both medicinal and allelopathic properties (Hegazy and Farrag, 2007).

Cameroon is one of the richest floristic regions of the world, well known for the different understory plant biomass and medicinal species (Sunderland *et al*., 2003; Focho *et al*., 2009). It has diverse habitats with humid tropical forest covering 54 % of the southern part of the country, mountain forest and savannah in the highlands and sub-Sahelian savannah and near desert in the far North (Sunderland *et al*., 2003). These diverse habitats harbour more than 9,000 species of plants, 160 species of which are endemic. The majority of the endemic taxa are concentrated around Mount Cameroon and other highland areas such as the Mount Bamboutos Caldera in south western Cameroon.

The caldera is endowed with different plant species valuable for health care needs of the larger populations and fodder for the livestock (Ayonghe and Ntasin, 2008; Harvey *et al*., 2010; Fonge *et al*., 2013). Like many other developing nations, where several classes of plant secondary metabolites have been implicated in allelopathy and biological control, similar observations have also been documented in Cameroon (Cho-Ngwa, 2010; Fongod, 2004). Worldwide, utilization of the allelopathic characteristics of both plants and crop cultivars for the control or management of other vegetation in crop based agroecosystems is common. However, very little is known on the allelopathic interactions of medicinal species growing in association with traditional crops in the Wabane municipality of the Mount Bamboutos Caldera of south western Cameroon. Most of these known medicinal plants have not been studied empirically in detailed for the active chemical compounds. It is now necessary to selectively screen some of the medicinal plants for their allelopathic or bioactive principles that could be exploited for novel candidate molecules for drug discovery and bioherbicides for weed control. The aim of this work is to present for the first time, the phytochemical screening of bioactive compounds found in 20 of the most wide spread and used medicinal plants in the western flank (Wabane Municipality) of the Mount Bamboutos Caldera of South Western Cameroon. The obtained result is a key aspect in making recommendations concerning the cultivation of certain species, whose active principles can be valued as phytoterapeutical products, food supplements, cosmetics and agroecosystem chemical products.

Materials and Methods

Plant material

An inventory comprising about 200 species of medicinal plants identified in the Mount Bamboutos Caldera was made by a research team lead by the first author. From these, a total of 20 most commonly used species in primary health care were selected and analyzed in terms of their active principles. The plants were harvested in different localities of the western flank of the Mount Bamboutos Caldera of Cameroon and identified and authenticated at the Limbe Botanic Garden (LBG) by the botanist, Mr. Litonga Ndive Elias with voucher specimens deposited. Detailed information on each medicinal plant is given in Table 1.

Preparation of extracts

Fresh plant materials were collected for each plant and dried at room temperature in an aerated laboratory for three weeks. The dried materials were ground using a mill with 2 mm sieve attached to it to yield a fine powder. One hundred grams of each powder was weighed and macerated three times in 1000 mL of acetone for 48 hours. The mixture was filtered using Whatman filter paper No. 1 and the filtrate concentrated under reduced pressure by rotary evaporation (BUCHI Rotavapor R-200, Switzerland) at appropriate temperature. Residual solvent was removed by drying in air at room temperature (23 – 25 °C) and the extract weighed and stored at -20 °C until used. An aliquot of each crude extract obtained was used for phytochemical tests while the remaining fraction was kept for further studies.

Qualitative tests: phytochemical screening

The concentrated residues from the acetone extracts were used to detect the secondary plant metabolites including alkaloids, flavanoids, steroids, saponins, glycosides, phenolics and tannins using standard methods with some modifications (Trease, 1989; Christen, 2000; Young and Woodside, 2001; MacNee, 2005)

Test for saponins (Frothing test)

Saponins were tested by dissolving one half gram (0.5 g) of the crude extract in a test tube containing 3 mL of hot distilled water and then the mixture was shaken vigorously for one minute and persistent foaming observed indicated the presence of saponins.

Test for flavonoids (Cyanidine test)

One half gram (0.5 g) of the crude extract was dissolved in methanol and 2 mL of concentrated hydrochloric acid added. A spatula full of magnesium turnings was added and the mixture observed for effervescence. A brick red colouration observed indicated the presence flavonoids.

Test for steroids (Lieberman-Burchard test)

About one half gram (0.5 g) of the crude extract was dissolved in 0.5mL dichloromethane to give a dilute solution and then 0.5 mL of acetic anhydride added, followed by three drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

Test for tannins (Ferric chloride test)

One half gram (0.5 g) of the crude extract was dissolved and added to a tube containing 20 mL of boiling distilled water and then boiled for an hour. A few drops of ferric chloride was added and allowed to stand for proper colour development. A blue-black colouration indicated the presence of tannins.

Test for Alkaloids (Dragendorf's test)

The sample was dissolved in dichloromethane and then spotted on a thin layer chromatographic plate which was developed in 20 % hexane in ethylacetate. The presence of alkaloids in the developed chromatogram was detected by spraying with freshly prepared Dragendorf's reagent in a fume chamber. A positive reaction on the chromatogram indicated by an orange or darker coloured spot against a yellow background is confirmatory evidence that the plant extract contained alkaloids.

Test for cardiac glycosides

An extract of the plant was added to 2 mL of glacial acetic acid plus one drop of ferric chloride. The set up was underplayed with 1 ml of concentrated sulphuric acid. There was the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer which indicated the presence of cardiac glycosides.

Test for phenolics

To 1 mL of the plant extract, one drop of 5 % $FeCl_3$ (w/v) was added. Formation of greenish precipitate indicated the presence of phenolics.

Table.1 List of 20 Cameroonian medicinal plants used in phytochemical tests

S.No	Plant Name	Familly	Life form	Part used
1	*Ageratum sp*.	Asteraceae	Herb	Whole
2	*Eremomastax speciosa* Benth	Acanthaceae	Herb	Leaf
3	*Justicia obliquifolia* Hochst	Acanthaceae	Herb	Leaf
4	*Ageratum conyzoides* L.	Asteraceae	Herb	Leaf
5	*Emelia coccinea* (Sims) G. Don	Asteraceae	Herb	Whole
6	*Spilanthes filicaulis* (Schum. And Thonn) C.D. Ada	Asteraceae	Herb	Whole
7	*Aframomum melegueta* K. Schum	Zingiberaceae	herb	Seed
8	*Piper umbellatum* L.	Piperaceae	Herb	Leaf
9	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae	Herb	Leaf
10	*Laportea ovalifolia* (Schum & Thonn.) Chev.	Urticaceae	Herb	Whole
11	*Harungana madagascariensis* Lam. & Poir.	Hypericaceae	Tree	Bark
12	*Impatiens macroptera* Hook F. Rank	Balsaminaceae	Herb	Leaf
13	*Melanthera scandens* (schumach and Thonn.)Robety	Asteraceae	herb	Leaf
14	*Echinops gingateus* Var. lelyi C.D. Adams	Asteraceae	Shrub	Root
15	*Vernonia hymenolepis* A.Rich	Asteraceae	Shrub	Leave
16	*Vernonia comferta* Benth	Asteraceae	Shrub	Leaf
17	*Momordica foetida* Schum	Cucurbitaceae	Climber	Leaf
18	*Bridelia ferruginea* Benth.	Euphorbiaceae	Tree	Bark
19	*Morinda lucida* Benth	Rubiaceae	Tree	Bark
20	*Commelina benghalensis* Linn.	Commelinaceae	Herb	Whole

Results and Discussions

After performing the analysis of bioactive compounds of the studied medicinal plants extracts, results obtained are as shown in Table 2. The phytochemicals, steroids, alkaloids, phenolics, Cardiac glycosides, tannins, saponins, flavonoids, were detected as present in the medicinal plants in different proportions and classes. The results from the phytochemical screening of the studied medicinal plants extracts have shown that flavonoids are found in eighteen of the twenty plants, with *Eremomastax speciosa, Justicia obliquifolia, Momordica foetida* and *Bridelia ferruginea* extracts being very rich in these compounds.

Steroids are found in most of the plant extracts, except for those obtained from *Aframomum melegueta, Harungana madagascariensis* and *Echinops giganteus*. In contrast, the extract from *Justicia obliquifolia and Emelia coccinea* were very rich in steroids. The highest contents of alkaloids were found in *Bridelia ferruginea* followed *Piper umbellatum* while seventeen plant extracts did not contain this type of compounds. Saponins were present in six studied plants, with *Bridelia ferruginea* habouring the highest content. *Piper umbellatum* and *Morinda lucida* are moderately rich in tannins, while sixteen of the plant extracts did not contain these compounds.

Cardiac glycosides were found highly expressed in the analyzed extracts of *Justicia obliquifolia, Morinda lucida* and *Piper umbellatum*. Phenolics are present in great quantities in *Aframomum melegueta, Harugana madagascariensis* and *Bridelia ferruginea*. Analyzing the results further, it can be observed that the studied medicinal plants containing the largest number of bioactive compounds were *Bridelia ferruginea, Justicia obliquifolia,* and *Morinda lucida* while *Spilanthes filicaulis* registered the lowest presence of phytochemicals.

The studied bioactive compounds have a broad range of biological activities. For example, phytochemicals such as saponins have anti-inflammatory effects (Vinha and Soares, 2012), hemolytic activity, and cholesterol binding properties (Nyarko and Addy, 1990). Glycosides are known to lower blood pressure (Marinkovic and Vitale, 2008.) and tannins exhibit antioxidant, antimicrobial and antiviral effects (Sayyah and Hadidi, 2004).

The plant extracts were also revealed to contain steroids, which are known to produce an inhibitory effect on inflammation (Savithramma and Linga, 2011) and alkaloids that have been reported to exert analgesic, antispasmodic and antibacterial activities (Nyarko and Addy, 1990). The phytochemical screening results of the extracts are consistent with the results reported by Alghazeer and El-Saltani (2012), where authors mentioned the presence of tannins, alkaloids, saponin and terpenoids in screened medicinal plants. Phenolic acids are the most commonly occurring natural products noted for allelopathic activities (Singh *et al*., 2003).

Mungole *et al*. (2010) have also included alkaloids, coumarin, flavonoids, saponnins and volatile constituents of the essential oils as being allelopathic agents. Generally, the presence of different phytochemicals in crude plant extracts has been linked to the detrimental effects of leachates, root exudates or decomposing residues of such plants on the other vegetation or succeeding crops (Chung et al., 2005; Mubashir and Wajaht, 2011). It is difficult to compare the data with the literature because several variables influence the results.

According to some authors, the quantity and the composition of bioactive compounds present in plants are influenced by the genotype, extraction procedure, geographic and climatic conditions, and the growth phase of the plants (Ciulei and Istodor, 1995; Trease and Evans, 2002). Plant cells produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism (carbohydrates, lipids and proteins).

Table.2 Phytochemical constituents of twenty (20) Cameroonian medicinal plants

Plants	**Phytochemical constituents**						
	Steroids	Alkaloids	Cardiac glycosides	Phenolics	Tannins	Flavonoids	Saponins
Ageratum sp.	+	-	-	+	-	+	-
Eremomastax speciosa (Benth)	+	-	+	-	-	+++	+
Justicia obliquifolia (Hochst)	+++	-	+++	-	-	+++	+
Ageratum conyzoides(Linn.) (Cufod.) Dandy	+	-	-		-	++	
Emelia coccinea (Sims) G. Don	+++	-	-	+	-	++	
Spilanthes filicaulis (Schum. And Thonn) (C.D. Ada)	+	-	-	-	-	+	-
Aframomum melegueta (k. Schum)	-	+	-	+++	+	+	+
Laportea ovalifolia (Schum & Thonn.) (Chev.)	++		+	-	-	++	-
Harungana madagascariensis (Lam. & Poir.)	-	+	+	+++	-	+	++
Impatiens macroptera Hook F. Rank	+	-	+	-	-	++	-
Melanthera scandens (schumach and Thonn.)Robety	+	-	+	-	-	-	-
Echinops gingateus Var.lely D.C	-	-	+	+	-	+	-
Vernonia hymenolepis A.Rich	++	-	++	++	-	++	++
Vernonia comferta Benth	+	-	-	-	-	+	-
Momordica foetida Schum	++	-	++	+	-	+++	+
Bridelia ferruginea Benth.	+	+++	++	+++	+	+++	+++
Morinda lucida Benth	++	-	+++	+	++	++	-
Commelina bengalensis Linn.	+	-	-	-	-	++	-
Piper umbellatum L.	+	++	+++	+	++	-	-
Bryophyllum pinnatum (Lam.) Oken	++	-	-	+	-	+	-

Legend: +=Low concentration, ++ = Moderate concentration, +++ = High concentration, - = Absent

Most natural products are compounds derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity (alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, etc.) (Pal, 2007). These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides, and herbicides (Okwu, 2005; Ramawat and Dass, 2009; Ramu and Mohan, 2012). Similar analyses have been conducted in areas that have a long tradition in the cultivation and utilization of medicinal plants, such as Pakistan (Dai and Mumper, 2010) and India (Ravishankar and Bhagyalakshmi, 2007).

A phytochemical screening, including qualitative analyses of bioactive compounds, was performed for a total of 20 medicinal plant species. All the plants investigated have bioactive compounds namely saponins, tannins, steroids, alkaloids, cardiac glycosides, phenolics and flavonoids. Among the medicinal plants that were studied in this paper, *Bridelia ferruginea*, *Justicia obliquifolia*, and *Morinda lucida* have the highest contents of the analyzed active principles.

This result validates the exploitation of the studied medicinal plant species, according to their high amount of active principles and their potential in medications, allelopathic studies, agroecosystems, dietary supplements or cosmetics industries. Further studies are needed with these plants to evaluate their allelopathic potentials, isolate, characterize and elucidate the structures of the bioactive compounds responsible for their antimicrobial activity, allelopathic activity, and other medicinal values.

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