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Phytochemistry, proximate and elemental compositions of extracts from the leaves of *Rothmannia longiflora* and *Rothmannia hispida*

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ABSTRACT

Medicinal plants have unique therapeutic properties and are therefore used in rural and urban areas for the treatment of various disease conditions and ailments. The phytochemistry, proximate and elemental compositions of methanolic, aqueous and ethanolic extracts from the leaves of Rothmannia longiflora and Rothmannia hispida were carried out using standard procedures. Phytochemical screening revealed the presence of alkaloids, glycosides, saponins, tannins, reducing compounds and polyphenols in both species and in all the extracts. Hydroxymethyl anthraquinones were detected in methanolic and aqueous extracts while flavonoids were detected only in ethanolic extract. Proximate analysis revealed significant ($p<0.05$) differences in the contents of Rothmannia longiflora and Rothmannia hispida. The proximate contents (in %) were moisture: 58.7 ± 0.07 and 84.47 ± 0.02, protein : 9.19 ± 0.01 and 3.20 ± 0.01, fat: 15.43 ± 0.11 and 0.80 ± 0.11, ash: 11.30 ± 0.07 and 2.32 ± 0.02, crude fibe r: 1.73 ± 0.15 and 1.42 ± 0.02, carbohydrate: 3.65 ± 0.29 and 7.79 ± 0.01 for Rothmannia longiflora and Rothmannia hispida respectively. Elemental composition results showed that Rothmannia longiflora contained (in ppm) sodium (Na) 16.37 ± 0.11, calcium (Ca) 10.02 ± 0.02, magnesium (Mg) 68.53 ± 0.11 and manganese (Mn) 0.02 ± 0.01. T he results unraveled the pharmacological basis of the therapeutic applications of Rothmannia longiflora and Rothmannia hispida in traditional medical practice and as potential sources of useful drugs.

Key words: Phytochemistry, phytomedicine, proximate composition, *Rothmannia hispida*, *Rothmannia longiflora*.

INTRODUCTION

There has been a revival of interest in the use of medicinal plants in developing countries recently. One of the reasons for this revival is that herbal medicines are safer and with less side effects when compared with synthetic drugs. In addition, there are many advantages of medicinal herbs especially when on considers the economic status of developing/underdeveloped countries, the proximity of herbs to the rural dwellers and their distance from the metropolis and a health centre [1]. The natural choice therefore is a search for newer drugs with better and cheaper from plant origin, which are abundant in our environment. The governments of some countries have recognized these advantages and are even combining traditional medical practices with orthodox medicine for proper health management [1-3].

WHO [4] has emphasized development of new drugs from plant sources for the management of various ailments and disease conditions. To be of use medicinally, a plant must be able to alter a disease condition. Thus a medicinal plant is defined as any plant which can be used to traet any disease [2] or a plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [5]. In essence, the medicinal potentials of these plants lie in their phytochemical constituents that produce a definite pharmacological action when applied to the living system [6]. It has recently been reported [7] that a herbal plant, *Althementhera repens,* exhibits a comparable anthelminthic activity against microfilaria of *Simulium yahence* as a synthetic drug, ivermectin. Similarly, Ogbonna *et al* [8] reported on the phytochemical and elemental contents of *Tetracarpidium conophorum* and indicates that the phytochemicals of this plant drug confers upon the plant-drug its pharmacological actions. These reports and others indicates that there is an urgent need to intensify phytochemitry and phytotherapy research into any relevant aspects of local plants that are used by traditional herbalists; this may unravel the pharmacological basis of their therapeutic applications in health care services.

Rothmannia (Family: Rubiaceae) are usually small trees or shrubs widely distributed in the forest of tropical Africa. They are found from Gambia east to Sudan, and southwardly to Tanzania and Angola [9]. The family contains several genera and hundreds of species. Rothmannia itself contains more than one hundred species [10]. The leaves of the genus Rothmannia are arranged in threes, the flowers are funnel-shaped and scented while the fruits are either cylindrical or club-like in shape. Some of the species found in tropical Africa include *R. annae, R. capensis, R. globosa, R. hispida, R, longiflora, R. lujae, inter alia.* [10]. Most species yield black dye [2].

Rothmannia hispida and *Rothmannia longiflora* can grow into tall trees of about nine to ten meters [9]. The leaves of these two species are more hairy than the leaves of other species. The distinguishing features between the two are their fruits. The fruit of *R. hispida* is cylindrical while that of *R. longiflora* is club-shaped or spherical. Fruits of both species produce black or indigo dye, which have been used traditionally for years for dyeing mats and for drawing tribal marks on the body of fattened women.

In Nigeria, *R. hispida* and *R. longiflora* are known as ‘‘Okukin’’ by the Ibibios of Akwa Ibom State, ‘‘Asuri’’ by the Binis of Edo State and ‘‘Asogbodu” by the Yoruba. These two species are variously called “Uri” “Uriohia” or Owuruokummo” by the Ibos (Smoke of devil).

Rothmannia longiflora Salisb itself is a small tree or shrub with a height of about 9 meters, most times with climbing stems. The leaves are opposite, and glabrous with triangular stipples and elliptical blades, 6 -18 cm by 2 - 8cm., pinately veined and arranged in 4 - 5 pairs of lateral veins having auxiliary domatia [9]. The flowers are bisexual with five merous, solitary, terminal and resting on short auxiliary branches and sweetly scented. The fruit is globose to ellipsoidal berry with the dimensions of 3.5 - 7cm by 5 - 6cm, green - blackish in colour. The fruit is marked with 10 indistinct ribs, multiple seeded, glabrous with persistent calyx. The seeds are lens - shaped, 6 - 8mm by 1 - 1.5mm long and brown-red in colour [9].

Rothmannia hispida (K. Schum) Fagerl (Syn. *Randia hispida*) is a shrub or small tree of about 10 meters tall. The fruits are 6 - 11cm long. The wood, when cut and exposed to air, takes on a blue colour [9]. The leaves of *R. hispida* are more hairy than the leaves of other species. In tropical Africa and in Nigeria, leave sap and fruit juice of *R. hispida* and *R. longiflora* are commonly used to make black designs on the body and to blacken tattoos. They are also used in colouring fibers of vegetable origin eg. local mats. In Nigeria, a dye and an ink-like extract called “katambiri” is made from finely crushed seeds. The fruits of *R. longiflora* are edible. The stems of these plants are used in making shafts of long-handled chisels in Sierra Leone, used in harvesting bunches of oil palm. The stems are also used in making spear handles and as chewing stick in Ghana [9].

In Africa, *R. hispida* and *R. longiflora* are used traditionally for the treatment of fever and as an analgesic. A decoction of the leaves, twigs, bark and roots is applied internally or externally in lotions, washes and baths. The roots are used in treating bowel complaints in Nigeria; throat abscesses, toothache and leprosy in DR of Congo [9]. The leaves of the *R. longiflora* are *R. hispida* are used as enema against kidney pain and diarrhea and for the treatment of diabetes. Drinking of the leaf juice is used to relieve pain during labour and child birth. It is also used for the treatment of fever, filariasis, dysentery, itching skin diseases, ulcers, and as an emetic [2], [9]. In the southern part of Nigeria, the leaves of *R. hispida* and *R. longiflora* are used traditionally for the treatment of diabetes mellitus, skin infections and for the eradication of intestinal worms.

Recently, the physiological basis for the therapeutic application of *R. hispida* as an antidiabetic agent has been elucidated [11], and the cytoprotective potential, possibly useful for the treatment of gastric ulcer has also been reported [12]. Previously, Asomaning [13] reported antimalarial effect of *R. longiflora* while Bringmann *et al.* [14] isolated a bioactive compound, 4- oxonicotinamide-1-(1-β-D-ribofuranoside). In traditional medicine, the mode of therapeutic application of the leaves is usually by oral administration of cold water or cold alcoholic extract obtained by soaking *R. hispida* or *R. longiflora* leaves in locally brewed gin.

Although the leaves of *Rothmannia longiflora* and *Rothmannia hispida* have been used for the treatment of various ailments in different countries, there has been paucity of information on the scientific basis of their healing powers. Thus the objective of this work is to evaluate phytochemicals, proximate and mineral contents of the leaves of *R. longiflora* and *R. hispida* in order to ascertain the pharmacological basis of their therapeutic application in traditional medical practice.

MATERIALS AND METHODS

Procurement of samples
The leaves of the samples were collected from the Botanical Garden of the University of Calabar. They were identified at the Department of Biological Sciencesof the same University as *Rothmannia longiflora* and *Rothmannia hispida* respectively, of the family Rubiaceae.

Preparation of extracts
In the preparation of methanolic extract, the collected leaves were washed free of dirt and debris and shade-dried for two weeks in the Research laboratory of the Department of pharmacology, University of Calabar, Nigeria. The dried sample was electrically ground to a powdery form and Soxhlet extracted by the methods of Braide [15] and Agil *et al* [16] as modified by Udia *et al* [17]. Aqueous extract was prepared by soaking 400g of fresh leaf sample in 2 liters of distilled water for 18 hours. The mixture was filtered using Whatman's No.1 filter paper. The filtrate was concentrated in an oven (Grieve LR-271C, The Grieve Corporation, Illinois, USA) at a temperature of 45-50^{0}C to a solid paste and used for the experiment.

Analysis
Proximate analysis carried out were adopted from previous work [8] as described by AOAC [18]. Phytochemical screening and elemental analysis were carried out by methods of previously described [5, 18]. The percentage of carbohydrate was calculated using the formula: 100 – (% of ash + % of moisture + % of protein). The caloric values of the samples were obtained by multiplying the values of protein, lipid and carbohydrate by the factors 4, 9 and 4 respectively followed by taking the sum of the products. These methods were adopted with little modifications from Hussain et al [19].

RESULTS

Phytochemical compositions

Table 1: Phytochemical components of methanolic extract of the leaves of *Rothmannia longiflora*

S/No.	Chemical constituents	Score indication
1	Alkaloids	+
2	Glycosides	+
3	Saponins	+
4	Flavonoids	-
5	Reducing compounds	++
6	Tannins	+
7	Polyphenol	+++
8	Phlobatinins	+
9	Hydroxymethyl anthraquinones	+

+ = Present; ++ = Present in excess; +++ = Present in much excess; - = Absent

The qualitative phytochemical screening of the methanolic extract of leaves of *Rothmannia longiflora* indicate the presence of alkaloids, glycosides, saponins, tannins, reducing compounds, polyphenol, phlobatinins and hydroxymethyl anthraquinones (Table 1).

The phytochemical constituents of ethanolic and aqueous extracts from leaves of *R. hispida* are shown in Table 2. The leaf extracts contain pharmacologically active components including alkaloids, glycosides and tannins. High levels of reducing compounds and polyphenol are also present.

There was little difference in the phytochemicals detected in both species and in the methanolic, aqueous and ethanolic leaf extracts. Alkaloids, glycosides, saponins, tannins, reducing compounds and polyphenol were detected in both species and in all the extracts. Hydroxymethyl anthraquinones were detected only in methanolic and aqueous extracts while flavonoids was detected only in the ethanolic extract of *Rothmannia hispida*. A higher level of saponins was detected in aqueous extract of *Rothmannia hispida* compared with other extracts.

Table 2: Phytochemical composition of ethanolic and aqueous extracts from leaves of *Rothmannia hispida*

S/No.	Chemical constituents	Ethanolic extract	Aqueous extract
1	Alkaloids	+	+
2	Glycosides	+	+
3	Saponins	+	++
4	Flavonoids	+	-
5	Reducing compounds	+++	++
6	Tannins	++	+
7	Polyphenol	+++	++
8	Hydroxymethyl anthraquinones	+	-

+ = Present; ++ = Present in excess; +++ = Present in much excess; - = Absent

Comparative proximate compositions

Table 3 shows the results of the comparative proximate compositions of the leaves of *Rothmannia longiflora* and *Rothmannia hispida*. There was significant (p<0.05) difference in all the parameters analyzed except crude fiber which was 1.73 ± 0.15% and 1.42 ± 0.02% for *Rothmannia longiflora* and *Rothmannia hispida* respectively. Moisture contents was significantly (p<0.05) higher in *Rothmannia hispida* than in *Rothmannia longiflora*, i.e. 84.47 ± 0.2% and 58.7 ± 0.07% respectively. There was significantly (p<0.05) higher contents 0f protein, fat and ash in *Rothmannia longiflora* compared to *Rothmannia hispida*. The contents of protein, fat and ash were 9.19 ± 0.01% and 3.20 ± 0.01%, 15.43 ± 0.11% and 0.8 ± 0.01%, 11.30 ± 0.07% and 2.32 ± 0.02% for *Rothmannia longiflora* and *Rothmannia hispida* respectively. The carbohydrate contents of *Rothmannia longiflora* was 3.65 ± 0.29%, this value was significantly (p<0.05) lower than that of *Rothmannia hispida* which was 7.79 ± 0.1%. The caloric value of *Rothmannia longiflora* was significantly (p<0.05) higher than that of *Rothmannia hispida,* i.e. 190.96±2.19kcal and 51.16 ± 0.53kcal respectively.

Table 3: Comparative proximate compositions of *Rothmannia longiflora* and *Rothmannia hispida* (g/100g dry weight)

S/No.	Parameter	*Rothmannia longiflora* (%)	*Rothmannia hispida* (%)
1	Moisture	58.7 ± 0.07	84.47 ± 0.2
2	Protein	9.19 ± 0.01	3.20 ± 0.01
3	Fat	15.43 ±0.11	0.80 ± 0.01
4	Ash	11.30 ± 0.07	2.32 ± 0.02
5	Crude fiber	1.73 ± 0.15	1.42 ± 0.02
6	Carbohydrate	3.65 ± 0.29	7.79 ± 0.1
7	Energy value (kcal)	190.96 ± 2.19kcal	51.16 ± 0.53 kcal

Results are mean ±SEM of three determinants

Elemental composition

The elemental contents of the leaves of *Rothmannia hispida* are shown in Table 4. These include Na, Ca, Mg and Mn with values (in ppm) of 16.37 ± 0.11, 10.02 ± 0.02, 68.53 ± 0.11 and 0.02 ± 0.01 respectively. Mn content was the lowest while Mg content was the highest in the sample studied.

Table 4: Elemental composition of the leaves of *Rothmannia longiflora*

S/No.	Parameter	Value (ppm)
1	Na	16.37 ± 0.11
2	Ca	10.02 ± 0.02
3	Mg	68.53 ± 0.11
4	Mn	0.02 ± 0. 01

Results are mean ±SEM of three determinants

DISCUSSION

The therapeutic effects and economic uses of Rothmannia spp. have mainly been attributed to the leaves, stems and the fruits. The leaves and the fruits of *R. hispida* and *R. longiflora* are used as dye [9]. The leaves Rothmannia spp. are used for the treatment of various ailments including diabetes mellitus [2, 9]. The results of this research work indicate that extracts of *R. hispida* and *R. longiflora* contain potent pharmacologically active components. This is in agreement with the definition of a medicinal plant as one which can be used to treat any disease [2] since one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [5]. Thus it is possible that the use of *R. hispida* and *R. longiflora* in traditional medicine for the treatment of diabetes mellitus and other ailments may be dependent on the presence of pharmacologically active plant components in these leaves.

The proximate data for *R. hispida* leaves revealed the presence of low ash, protein, fat and fibre; but high moisture and carbohydrate contents. The results reveal that *R. hispida* is not a rich source of lipid and can be beneficial to patients in whom a high fat content is a risk factor. Although dietary fats increases the palatability of food by absorbing and retaining flavours [20], excess fat consumption is implicated in certain disorders such as atherosclerosis, cancer and aging [20]. The low crude fibre content in *R. hispida* is consistent with the fact that the plant drug is not used as vegetable food. However, it is known that dietary fibres are employed in the treatment of diseases such as gastrointestinal disorders, cancer, obesity and diabetes mellitus [21].

The phytochemical screening indicated that the leaves of *R. longiflora* and *R. hispida* contain alkaloids, glycosides, saponins, reducing compounds, polyphenol, flavonoids, anthraquinones and hydroxymethyl anthraquinones. These phytochemical components are known to exhibit medicinal and physiological activities [2, 22]. The absence of flavonoids in *R. longiflora* and hydroxymethyl anthraquinones in *R. hispida* is inconsistent with the report of Lewis and Elvin – Lewis [2] who noted the presence of these components in *Rothmannia* species. Flavonoids and flavonoid containing plants exhibit antibacterial, anti-inflammatory, anti- anaphylactic antiallergic, antiviral, anti-estrogenic, antineoplastic, antithrombic, vasodilatory, antispasmodic, hepatoprotective and antidiabetic activity [15, 23 - 28]. The antioxidant activity of flavonoids and their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals is one of the most important functions of flavonoids [24]. Alkaloids exhibit pronounced pharmacological effects on various organs of the body and are useful for the treatment of various ailments and disease conditions. The medicinal use of alkaloids includes treatment of malaria, amoebic dysentery and cancer [2, 29] .

The presence of other phytochemical components was noted. Saponins, although non-toxic, have been noted to exhibit cytotoxic effect and growth inhibition against a wide variety of cells, thus imposing on them anti-inflammatory and anticancer properties. Saponins also exhibit tumour inhibiting activity on primates [30]. The results of this study indicate that the leaves of *R. longiflora* and *R. hispida* contain pharmacologically active plant components. These components contribute to the antidiabetic property and medicinal value and use of these plant drugs and thus they can be potential sources of useful drugs. The effects of the leaves results from the pharmacologically active components present in the herbs.

The results from the present work show that *Rothmannia longiflora* has a higher amount of magnesium compared to other elements in the leaf. In descending order in terms of quantity, other elements were sodium, calcium and manganese. The presence of these elements in *Rothmannia longiflora* indicates that the leaf may be effective in muscular function and synthesis of enzymes, and for body metabolism [31]. Calcium is essential for bone formation and strength, hormone secretion and normal physiological function of the body [31]. In addition to other body functions, magnesium is a co-factor in enzymes activity. It is probable that it could enhance acetyl-coA activity resulting in a decrease or absent of aceto-acetic acid formation which is associated with metabolic complications in diabetes [32]. It is known that in diabetic patients, derangement of metabolism resulting from a critical reduction of insulin is the cause of ketoacidosis and coma, the most important of the acute diabetic complications [33, 34]. The presence of magnesium in *Rothmannia longiflora* suggests that it could be effective in ameliorating diabetic associated metabolic complications. Furthermore, the effectiveness of Rothmannia spp could transcend beyond their therapeutic usage in the treatment of DM and other disease conditions to be effective in other physiological functions including bone development, balance extracellular fluid composition and muscular co-ordination.

CONCLUSION

The present study has shown the phytochemical, proximate and elemental compositions of the leaves of *Rothmannia longiflora* and *Rothmannia hispida*. This in part indicates the pharmacological basis of the therapeutic application of these herbs in traditional medical practice. As a rich source of phytochemicals in addition to the presence of essential elements, *Rothmannia longiflora* and *Rothmannia hispida* are potential sources of useful drugs. Further studies are needed to isolate, characterized and elucidate the pharmacologically active components these plants for industrial usage.

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