Phytochemical Screening, Proximate analysis and Antimicrobial Activity of aqueous extract of Megaphrynium macrostachyum seeds

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ABSTRACT: The phytochemical screening, proximate analysis, minerals composition and antimicrobial activity of the seeds of *Megaphrynium macrostachyum* belonging to the family of *Marantaceae* was investigated. The phytochemical screening of the seeds of *Megaphrynium macrostachyum* showed that the extract contained alkaloids, tannins, carbohydrate, and terpenes that could be responsible for the observed antimicrobial activities. The bioactives compounds of seed was extracted, using aqueous (water) and was investigated for antimicrobial activity on some microorganisms using agar well diffusion method. The aqueous extract showed little significant effect against both the gram positive and gram negative organisms tested. The mineral element analysis of the plant showed that it contains Ca (37.95±1.62 mg/l), Mg (1.34±0.02 mg/l), Zn (2.67±0.01 mg/l), K (6.44±0.28 mg/l), Na (5.85±0.21 mg/l), Mn (6.86±0.04 mg/l), and Fe (0.40±0.08 mg/l). Proximate analysis revealed that *Megaphrynium macrostachyum* contains moisture (16.60%), protein (10.78 %), fat (6.30%), ash (10.38%), crude fiber (8.26%) and carbohydrate (47.68%).From our findings, the proximate, minerals element composition and antimicrobial property shown by this plant extract(*Megaphrynium macrostachyum)* is a good evidence of its nutritional and medicinal importance.

Keywords: Antimicrobial, *M. macrostachyum,* Minerals composition, Phytochemical, Proximate analysis.

Introduction

Natural products have traditionally played a pivotal role in drug discovery as medicinal plants are the most exclusive sources of life saving drugs for the majority of the world's population Sagwan *et al*; 2010. Medicinal plants as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health. However there is need to know which constituents in the medicinal herb are responsible for therapeutic uses. Therefore the need arises to extract, isolate and identified the phytoconstituent (bioactive components) responsible for its therapeutic use. These bioactive components are often referred to as phytochemicals ('phyto-' from Greek - *phyto* meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests (Liu, 2003). Phytochemicals like flavonoids and phenols are strong antioxidants and have an important role in the health care system (Dhan Prakash et al., 2007). Screening of active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and alzheimer's disease (Soma *et al*., 2010). Screening of various natural organic

compounds and identifying active agents is the need of the hour, because successful prediction of drug like properties at the onset of drug discovery will pay off later in drug development.

Megaphrynium macrostachyum (K.Schum.) (Family: *Marantaceae*) is found in the rainforest of West and Central Africa (Jennings et al., 2001). A perennial semi-woody herb, rhizomatous, forming extensive clumps, with stems to 2½ m high bearing a single large leaf 30–60(-90) cm long by 12–30 cm wide; flowers, borne on the petiole below the leaf, whitish with red or purple calyx. The leaves are harvested from the forest and used fresh in wrapping food in order to preserve the food. It is distributed from Guinea and Sierra Leone to DR Congo, Cabinda (Angola), Sudan and Uganda. The leaves are much used for wrapping food, packing and thatching. In Central Africa, for instance, they are often used for wrapping cassava sticks before cooking. The leaves are made into a range of other articles, such as disposable baskets, plates, cups, pots, containers, funnels, fans and parasols, and they are used as cushion under sleeping mats. In Gabon the leaves are used for wrapping clothes to keep them dry in the rain. In DR Congo they are used for covering clay walls. The entire or split petiole and strips from its bark are used for tying and for making mats, baskets, brooms and other utensils. It is also made into bracelets and other ornaments. Young leaves are cooked with oil and water and eaten as a vegetable. The fruit pulp is eaten and used as a sweetener in food preparation. The fruits are also used to wean babies. The roasted seeds are eaten in DR Congo; they are said to taste like maize. In traditional medicine in Cote d'Ivoire, the leaf sap is drunk for the treatment of epilepsy and madness, and the leaves and fruits are sometimes prescribed as an antidote for poisonings. In Cameroon a leaf decoction is taken against jaundice. The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases, the result of different studies provide evidence that some medicinal plants might indeed be potential source of new antibacterial agents. NCCLS (2000). Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, the first step towards this goal is in vitro antibacterial activity. Tona *et al* (1998). The extracts of higher plant can be very good source of antibiotics against various bacterial pathogen. Kone *et al* (2004). Plant based antimicrobial compounds have enormous therapeutics potential as they can serve the purpose without any side effects that are often associated with synthetic antibacterial compounds.

This research work is therefore focused on the determination of mineral element composition, proximate, phytochemical contents and antimicrobial activity of *Megaphrynium macrostachyum* in order to evaluate its pharmacological and nutritional values.

Materials and Methods

The seeds of *Megaphrynium macrostachyum* were harvested from Oniwafin farm land, Tapa in Ibarapa North local Government in Oyo state, Nigeria during the month of April, 2013. And was identified and authenticated at Herbarium Unit of Botany Department, University of Ibadan, Oyo state, Nigeria. The seeds were sun-dried and screened to remove undesirable materials such as stones and other impurities, after which they were dehulled, milled into powder and the powder kept in an air-tight polythene bags until needed for analysis.

Figure 1: *Megaphrynium macrostachyum seeds*

Preparation of aqueous plant extracts

The solvents extraction was done by cold extraction; 50 g of dried plant was soaked in 200ml of water. The mixture was kept undisturbed at room temperature for 72 hrs in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. The extracts was concentrated to dryness in rotary pressure evaporator at 40^{o}c and stored for further antimicrobial study

Test organisms used

Two gram-positive organisms *Staphylococcus aureus*, *Bacillus subtilis and* two gram-negative organisms *Pseudomonas aeruginosa, Escherichia coli* were obtained and confirmed at the research laboratory of the Department of Microbiology, Faculty of science, University of Ibadan.

Phytochemical Screening

The aqueous extract of the plant was tested for the presence of phytochemicals qualitatively. Secondary metabolites such as phytosterols, polyphenols (tannins, flavonoids), saponins, alkaloids, saponin glycosides, steroids and triterpenoids, glycosides, hydrolysable tannins, phenols and volatile oils were screened for using the procedures described by Sofowora (1993) and Harbone (1998).

Proximate analysis

The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of the sampleswas determined using methods of the Association of Official Analytical Chemists (AOAC, 1984). All determinations were done in triplicates. The proximate values were reported in percentage. Determination of moisture content was done by weighing the sample in crucible and drying in oven at 105 0C, until a constant weight was obtained, determination of ash content was done by ashing at 550 0C for about 3 hours. The kjeldah method was used to determine the protein content by multiplication of the nitrogen value with a conversion factor of 6.25. The crude fibre content of the samples was determined by digestion method and the crude fat was done by Soxhlet extraction method. Total soluble carbohydrate was determined by the difference of the sum of all the proximate composition from 100 %.

Mineral element analysis

The mineral contents of the plant seeds: potassium and sodium were determined using flame photometer, while calcium, magnesium, iron, zinc and manganese were determined using atomic absorption spectrophotometer as described the methods of the Association of Official Analytical Chemists (AOAC, 1990) after appropriate digestion by acids. All the determinations were done in triplicates.

Antibacterial activity assay

Agar well diffusion method

The antibacterial activity of *Megaphrynium macrostachyum* was evaluated by the aqueous extracts through agar well diffusion method. Shyamala and Vasantha (2010). 24 hours broth cultures of test organisms were used for assay. Cultures were spreaded in the Meuller-Hinton agar surfae. After drying, wells were made on the seeded plates using sterile metal borer (8mm). The plates were allowed to dry for 5 min. Sterile water solubilised extracts (100, 200 and 300 µl) were dispensed into each well. Sterile water and streptomycin (10 µl) was used as negative and positive controls. The plates were incubated overnight at 37˚C.

Statistical analysis

All data generated were analyzed using descriptive statistic (Olawuyi, 1996). Statistical values that were calculated include mean and standard deviation.

Results and Discussion

Phytochemical screening

The phytochemical screening of the seeds of *Megaphrynium macrostachyum* as shown in table 1 revealed that the extract contained alkaloids, tannins, carbohydrate, and terpenes that could be responsible for the observed antimicrobial activities.

Table 1: Phytochemical screening of *M. macrostachyum* seeds

Phytochemicals	***M. macrostachyum***
Alkaloids	+
Saponin	+
Terpenoids	+
Flavonoids	-
Steroids	-
Cardiac glycoside	-
Reducing sugar	+

Proximate composition

Proximate analysis revealed that *Megaphrynium macrostachyum* contains moisture (16.60%), protein (10.78 %), fat (6.30%), ash (10.38%), crude fiber (8.26%) and carbohydrate (47.68%). The crude protein content of the seeds (10.78±0.010) is within the range as reported by other scientist. *Hexalobus crispiflorus* (7.11 ± 0.02%), *Clitandra togolana (*10.81 ± 0.25%) by Akoja and Amoo (2011) and Guinea peanut 10.38 ± 0.08% by Ogunlade *et al*; (2011). However, the concentration is relatively lower compared to 43.1% for luffa cylindrical kernel by Olaofe *et al;* (2008). Availability of such moderate contents of protein are helpful in maintaining proper growth and development

in adults, children, and pregnant which require good quantity of protein daily. (Aletor and Adeogun, 1995). The values obtained for the moisture content of the seeds (16.60±0.000) varied significantly from those obtained for castor, jack bean, pigeon pea and cowpea, African oak and Guinea peanut, *Pterygota macrocarpa* and *Hexalobus crispiflorus* seeds as reported by Akoja and Amoo (2011), Ogunlade *et al*; (2011), Amoo and Agunbiade (2009), This suggested a strong correlation between moisture contents and fibre, which could be of interest to human health as the fibrous are easily digested and disintegrated. The fat content of the seeds is lower when compared to 74.40 ± 0.57% of *Pterygota macrocarpa*; Amoo and Agunbiade (2009). 35.86±0.41% of *Hexalobus crispiflorus*; Ogunlade *et al*; (2011). 43.2% of calabash kernel; Olaofe *et al;* (2008). However, these values are higher than 1.95 ± 0.04% for jack bean Arawande and Borokini (2010). The seeds contain a considerable amount of fibers which is necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al*., 2000).

Ash content determined is significantly high which suggest that the selected plant seeds could be good sources of mineral elements. The carbohydrate contents is higher than that of groundnut flour; 31.5%; Oyenuga, V.A (1968). African oak (*A.africana*; 45.92 ± 0.72%) and African locust bean; 41.10%; Elemo *et al* (2011), Carbohydrates are known to be important components in many foods, and the digestible carbohydrates are considered as an important source of energy. Our findings revealed that the two seeds are very good sources of carbohydrate with high energy values which gives the needed energy for good living of human and livestock

Table 2: Proximate (%) results of the *M. macrostachyum* seeds

Proximate composition %	***M. macrostachyum***
Moisture content	16.60±0.000
Crude protein	10.78±0.010
Crude fat	6.30±0.000
Crude fibre	8.26±0.002
Ash	10.38±0.010
Carbohydrate	47.68±0.010
Available Energy (KJ/100g)	1226.92

Mineral element composition

The mineral element analysis of the plant showed that it contains Ca (37.95±1.62 mg/l), Mg (1.34±0.02 mg/l), Zn (2.67±0.01 mg/l), K (6.44±0.28 mg/l), Na (5.85±0.21 mg/l), Mn (6.86±0.04 mg/l), and Fe (0.40±0.08 mg/l). (Table 3). Minerals element composition of the plant revealed that it contains minerals that are required for normal growth and muscles and skeletal development. Deficiency of these nutrients and minerals are known to affect the performance and health in both humans and livestocks Merck. (2005).

Table 3: Mineral element composition of *M. macrostachyum* seeds (mg/l)

Mineral elements	***M. macrostachyum***
Zn	2.67±0.01
Ca	37.95±1.62
Na	5.85±0.21
Fe	0.40±0.08
K	6.44±0.28
Mg	1.34±0.02
Mn	6.86±0.04

Antimicrobial activity

The antimicrobial activity of *M. macrostachyum* using agar well diffusion method revealed that aqueous extract of the plant seeds showed little significant effect against both the gram positive and gram negative organisms tested. Table 4. Generally, antimicrobials provide the main basis for the therapy of microbial infections, and their effectiveness depends largely on the ability of such antimicrobial compound to stop or inhibit the growth of any microorganism in the body system they infect. However the high genetic variability of microorganisms enables them to rapidly evade the action of antimicrobials by developing resistance. Stephen and Ehiagbonare (2011). In the present study, the antimicrobial activity of aqueous extracts of the seeds of *M. macrostachyum* was tested against four pathogenic bacteria. Antimicrobial activity of the extracts of the seeds *M. macrostachyum* was first time investigated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphyllococcus aureus*, For all the tested microorganisms aqueous extracts of *M.macroctachyum* was not active against P.aeruginosa and maximum inhibition was observed in *B. subtilis* followed by *S. aureus*, this implies that the higher concentrations of the extracts were observed to be more active against the tested bacterials as shown in table 4.The low effect of this aqueous extracts agrees with the assumption that bioactive substances that are less soluble in water could dissolve in organic solvent (Oyagade *et al*., 1999).

Table 4: Antimicrobial activity (zone of inhibition, mm) of Aqueous extracts *M. macrostachyum* seeds against clinical pathogens.

Plants extracts(µl)	*S. aureus*	*B. subtilis*	*P.aeruginosa*	*E. coli*
100	R	R	R	R
200	05	8	R	R
300	10	11	R	4
streptomycin (10µg/disc) *	16	18	R	16

*R – Resistant., * Standard*

Conclusion

The results obtained from the present study support the use of this plant parts in the traditional treatment of diseases in Nigeria. The proximate and antimicrobial results justified that the extracts of M. *macrostachyum* could be used in providing precursors for the synthesis of useful antiseptics drugs to maintain a healthy life.

Recommendations

Several plants are currently being investigated to know their antimicrobial and medicinal properties. The present study reveals that seed of M. *macrostachyum* have great potentials as an antimicrobial agent due to the presence important phytochemicals and their ability to inhibit the growth of some of the selected organisms. I, therefore, recommend that:

1. Other solvents should be used for the extraction of the bioactive compound that present in this seed as it will help to extract the organic component of the phytochemical which is more potent against microbes.

2. Further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s) and toxicological studies of the plant extract should be carried out.

3. The potentially useful phytochemical structures present in these plants be synthesized chemically and used as antibiotics/chemical preservatives.

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