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Determination of Mineral Composition, Anti-microbial Activity and Anti-nutrient Screening of *Cleome gynandra (*Cat's Whiskers)

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Authors' contributions

Authors MEK and UDY designed the study, wrote the protocol, determined the mineral composition, anti-microbial activity and wrote the first draft of the manuscript. Author DK performed the anti-nutrient screening and managed the literature searches. All authors read and approved the final manuscript, thus the work was carried out in collaboration between all authors.

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ABSTRACT

The aim of this research is to investigate the mineral, anti-nutrients composition and the anti-microbial activity of the ethanol fraction of the leaf, stem and roots of *Cleome gynandra*. (Cat's whiskers).
Matured whole plant of *C. gynandra*, collected from Mubi North Local Government Area, Adamawa State Nigeria, extracted with 95% ethanol, showed presence of Ca, Na, Mg and Fe. Samples contained high Na and low Ca, Mg, and Fe concentrations. Heavy metals of Zn, Mn, Cr, Cu, and Pb, were not detected. The anti-microbial activity of the extract using agar diffusion method showed zones of inhibition (mm) against *Salmonella typhi* (11.00±0.1,8.00±0.1,9.00±0.1*), Pneumonia spp* (11.00±0.2,12.00±0.1, 8.00±0.2*), Pseudomonas aeruginosa,* (11.00±0.3,8.00±0.1,ND) and *Staphylococcus aureus* (8.00±0.1,11.00±0.2, & ND), for the leaf, stem and root respectively. The anti-nutrient component showed phytate and oxalate contents were less than is nutritionally

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significant, while tannins were in traces. Toxicological analysis could be done on the plant, for it has good medicinal potentials.

Keywords: Cleome gynandra; Spectrophotometrically analysed; Salmonella typhi; Pneumonia spp; Pseudomonas areuginosa; Staphylococcus aureus.

1. INTRODUCTION

In Nigeria, as in other tropical African countries where the daily diet is dominated by the same staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids [1]. Vegetables are important foods both from an economic and nutritional point of view. Vegetables are living entities and respiring when they are freshly harvested, have high water contents and abundant cellulose [2]. Most of these vegetables have both nutritional and medicinal values and have been used for centuries as remedies for human diseases because they contain components of therapeutic value [3,4].

Medicinal and aromatic plants have played a vital role in alleviating human sufferings [5]. A medicinal plant is any plant in which one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Plants have always been of important therapeutic uses and have been used to cure human and other animal diseases [6]. Traditional medicine is an integral part of the African culture and traditions, and varies between different cultural groups and regions [7]. The awareness of the importance of medicinal plants in human health care in developing countries has resulted in research into traditional medicine with a view to integrating it with modern Orthodox medicine and they have been of great importance to the health of individuals and communities [8,9].

Many of these indigenous plants are used as spices and foods. They are sometimes added to foods for pregnant and nursing mothers for medicinal purposes [10,11,12]. Medicinal plants are generally used in traditional medicine for the treatment of many ailments [13,14]. The different parts used include leaves, roots, stem, flowers, fruits, seeds, and bark. It is to this effect that this work is carried out on the leaves, stems, and roots of *Cleome gynandra,* in order to evaluate the nutritional and medicinal values of the plant [15].

Cleome gynandra (Cat`s whiskers), (Kinaski, in Hausa) of *capparaceae* family is found throughout the tropics and subtropics. It occurs probably in all countries of tropical Africa, where it is mainly found near human settlements. The plant is an erect, annual herb that grows up to 150 cm tall, it is strongly branched with tap root and few secondary roots, the stem is densely glandular, leaves alternate, palmetly compound with (3, 5, 7) leaflets almost sessile. Flowers are bisexual white or tinged with purple; pedicel? 1-5-2-5 cm long with 4 sepals, 4 petals, 6 stamens, and a purple ovary. Fruits are long narrow, cylindrical capsules up to 12 cm x 1 cm, seeds are subglobose, 1-1.5mm in diameter, grey to black, irregularly ribbed. *Cleome* comprises 150-200 species, about 50 of them occurring in Africa [16].

The Plant is highly reputed for its numerous nutritional and medicinal uses [17,18]. The tender leaves, young shoots and occasionally flowers are boiled or cooked as stew. The leaves care also boiled and water discarded while the paste is made into balls with groundnut cake in Kenya and Zimbabwe [19]. Sometimes the bitter leaves are cooked with milk or other leafy vegetables such as cowpea leave for food. Extracts of the leaves are used in Mubi North L.G.A. of Adamawa State, North East Nigeria in form of concoction to treat typhoid and anaemia. Liquid extract of roots is used as medicine for chest pain and leaves extract to treat diarrhoea [16]. The leaves when rubbed into the skin relieve pneumonia [2]; and the leaves extract is also used as eyewash [2].

In the present study, extract of the leaf, stem and root of *Cleome gynandra* were analyzed for their mineral constituents and were also screened for anti-nutrients and antimicrobial activity against gram-positive and gram-negative bacteria (*Salmonella typhi, Pneunonia spp., Pseudomonas aeruginosa,* and *Staphylococcus aureus,)* with the view of confirming or disproving the acclaimed curative properties of the plant.

2. MATERIALS AND METHODS

Fresh *cleome gynandra* leaf, stem and root were collected in Mubi North Local Government Area of Adamawa State. The plant was identified and authenticated by prof. Saquip of the Adamawa State University, Mubi according to [20]. The

Forestry Herbarium Index (FHI) number 074 was assigned and a specimen of the plant was deposited in the herbarium. Samples were air dried in the laboratory. The plant was separated into the various parts, leaves, stems and roots and each part was separately pounded to obtain fine powdered sample and sieved with 2 mm mesh size sieve. The fine powdered samples were stored separately in clean and dry sample containers for extraction.

2.1 Extraction

30 g of each of the fine powdered sample was accurately weighed and dissolved in 200 ml of 95% ethanol for 72 hours before filtration. The concentrated extracts were obtained by rotary evaporation and kept for analysis as described by [21].

2.2 Determination of Mineral Constituents

5.0 g of each sample was weighed and placed in crucibles and transferred to a muffle furnace to incinerate for four (4) hrs at 500℃ until the ash was carbon free (white ash). The samples were then removed, cooled in desiccators and then reweighed to obtain the percentage ash content. The ash content was used for the elemental analysis. 10 ml of 0.1 M HCl was added to each of the ashed samples. The mixture was filtered into a 100 ml volumetric flask. The crucible and the filter paper (whatman number one) were washed three times with 10 ml of 0.1 M HCl solution. The absorbance of each element was determined using Atomic Absorption Spectrometer (AAS) Bulk 210 model. Standard solutions of each element were prepared and their absorbances were determined before the sample analysis. The respective concentrations of the elements in the samples were obtained from the standard calibration curves by extrapolation.

2.3 Anti-microbial Analysis

The bioactive analysis of the extracts was carried out against *Salmonella typhi, Pneumonia spp., Pseudomonas aeruginosa,* and *Staphylococcus aureus* obtained from the General Hospital and New Life Hospital Mubi. The anti-bacterial activities were tested by agar diffusion method.

The ethanolic extracts of the sample obtained after extraction were reconstituted to get the final concentration of 100mg/ml for the test.

2.4 Sensitivity Test

Wells of 6 mm diameter were made on the nutrient agar using a standard gauge (sterile cork borer). The test organisms were inoculated into the nutrient agar after thorough mixing with Peptone water. 0.3 ml of the reconstituted extracts was carefully introduced into the wells. Antibiotic discs for both gram positive (Ampiclox) and gram negative (Gentamycin) organisms were also used as standard controls. The plates were all allowed to stand for 30 minutes at room temperature for diffusion of the substance to proceed before the growth of organisms commenced. The plates were incubated at 37^0C for 24 hours. The zone of inhibition which indicates anti-microbial activity was measured and recorded [3].

2.5 Anti-nutrient Components Evaluation

2.5.1 Phytate analysis

4.0 g of each sample were soaked in 100 ml of 2% HCl for 5 hours and filtered. 25 ml of the filtrate were placed in a 250 ml conical flask and 5ml of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with standard ferric chloride solution until a brownish-yellow colour persisted for 5 minutes [22].

2.5.2 Oxalate analysis

2.0 g of each sample each was dissolved in 100 ml of 1.5 NH_2SO_4. The solutions were then carefully stirred with magnetic stirrer for 1hour and then filtered. 25 ml of each filtrate was measured and titrated hot (80-90℃) against 0.1 N $KMnO_4$ solutions until a faint pink colour which persisted for at least 30 seconds [23].

2.5.3 Tannin analysis

1.5 g of each powdered sample was boiled in 5 ml of distilled water in a test tube. The solutions were filtered. 3 drops of 0.1% ferric chloride was added to each of the filtrate and observed for brownish green or blue-black colouration [24,25,8].

3. RESULTS

The result of the mineral composition (Fig. 1), the antimicrobial activity and the anti-nutrient tests (Tables 1 and 2) are presented below:

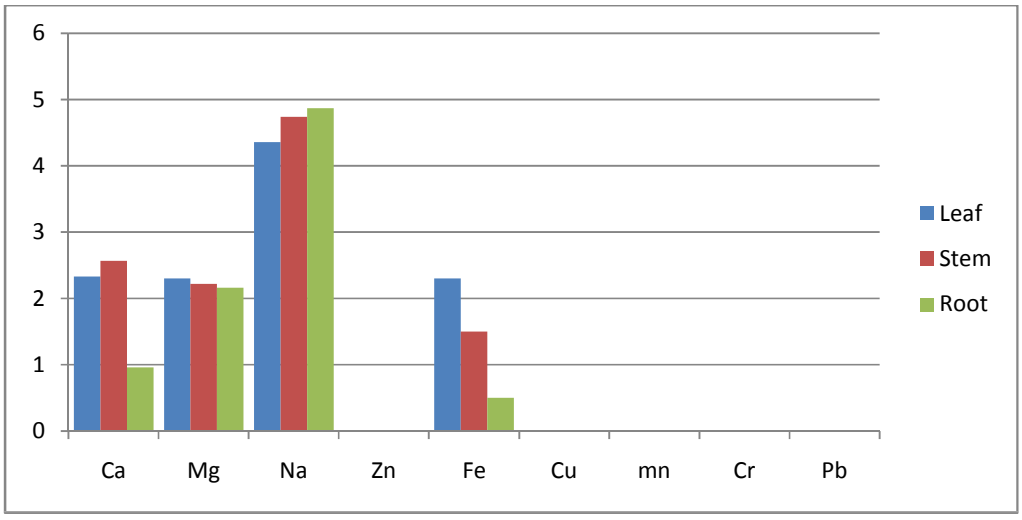

Fig. 1. Results of Mineral constituents; concentration in (ppm), all concentrations are results of triplicates with 0±0.5 Standard error

Table 1. Result of antibacterial tests

	Zone of inhibition in (mm)			
Samples	***S. typhi***	***P. spp***	***P. aerugenosa***	***S. aureus***
Leaf	11.00±0.1	11.00±0.2	11.00±0.3	08.00±0.1
Stem	08.00±0.1	12.00±0.1	08.00±0.1	11.00±0.2
Root	09.00±0.1	08.00±0.2	ND	ND
Standard control	Ampiclox x 50 (14.00)	Ampiclox x 50 (14.00)	Gentamycin x50 (30.00)	Gentamycin x 50 (30.00)

ND = Not detected, Mean of Three Trials ± Standard Error

Table 2. Result of anti-nutrients tests

Samples	**Anti-nutrients (mg/100g)**		
	Phytate	**Oxalate**	**Tannin**
Leaf	0.85 ±0.01	1.58±.002	Traces
Stem	0.69±0.01	0.50±0.01	Traces
Root	0.65±0.02	0.38±0.01	Traces

Mean of Three Trials ± Standard Error

4. DISCUSSION

The results obtained from the mineral content evaluation in Fig. 1 showed that *Cleome gynandra* is a good source of minerals such as Ca, Mg, Na and Fe, while Zn, Cu, Mn, Cr and Pb were not detected.

The structural and functional roles of mineral composition of some medicinal plants to biosystems have been well established [26,27]. For instance the presence of phosphorus has been exploited for synthesizing numerous phytochemical compounds in plants [28]. However the results of this work shows good correspondence with results presented earlier for some medicinal plants of northern Nigerian origin in regards to the accumulation of certain minerals (Fe, K and Mn) in fruity parts of plants than in the stems and leaves [27].

Ca plays an important role in building and maintaining strong bones and teeth, large parts of human blood and cellular fluids. Also it is necessary for normal functioning of cardiac muscles, blood coagulation, clotting and regulation of cell permeability. Ca deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, pre-menstrual tension, and cramping of the uterus [29].

Mg was also present, which plays a vital role in the formation and function of bones, muscles and prevents high electrolyte disorders, high blood pressure and depression in enzyme activities. Its deficiencies interfere with transmission of nerve and muscle impulses, causing irritability and nervousness, thus leading to heart diseases [29]. Na strictly helps in ionic balance of water and acid (osmotic regulation) in the body, and maintain tissue excitability, helps normal muscle

contraction, and formation of gastric juice in the stomach. Deficiency causes nervousness, mental disorientation, low blood sugar, insomnia and coma [29]. The presence of Fe (iron) plays an important role in the formation of blood haemoglobin and cytochromes. Deficiency leads to anaemia, and hyperthyroid [29].

Cr, Cu, Pb, Mn and Zn are heavy elements or "heavy metals." These elements were not detected in *Cleome gynandra* or their levels were so small that they could not be detected by the equipment used. Small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amount of up to 0.5mg/l of any of them may cause acute or chronic toxicity (poisoning). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lung, kidney, liver and other vital organs [13].

Pb, if present leads to toxicity in the body; causing respiratory problems and restricts the flow of blood into the body system. The Environmental Protection Agency (EPA) has detected and declared that there is no safe level of lead intake. It can cause high blood pressure and reduce haemoglobin production, necessary for O_2 transportation, thus interfering and can interfere with normal cellular Ca metabolism [30].

Cu was not detected but it is an important compound of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin; an iron oxidizing enzyme in blood. Deficiency has been associated with cardiac abnormalities in human and animals such as anaemia and Neutrogena [29]. Cr is of benefit only to those people working for performance enhancement in physical activities. Also in the core factors that dictate our overall health and well being. Excess of Cr leads to malignant growth in the respiratory tract. Cr is one of the most toxic elements from vegetables [13].

The result obtained for antimicrobial activity Table 1, showed moderate zone of inhibition for the tested organisms. The root of C*leome gynandra* was not active against *P. aerugenosa and S. aureus* but the leaf and stem were active against all the tested organisms.

The plant is active against S. typhi that causes typhoid, paratyphoid, non-typhoid fever and enteric fever such as gastrointestinal illness, followed by bacteraemia and diarrhoea [31]. This explains their usage traditionally to cure numerous diseases in Mubi North L.G.A.

The plant is also active against *Pneumonia spp* that causes infection of wound in burned patients and hospitalised individuals.

Leaf and stem are active against *S. aureus* that causes minor skin infection such as impedigo boils, scalded skin syndrome and abscesses, to diseases such as pneumonia and meningitis. The leaf and stem are also active against *P. aerugenosa* which cause diseases such as sour throat, scarlet fever and kidney disease [32].

Results of the anti-nutrient analysis presented in Table 2 showed that phytate and oxalate were present in small amounts while tannins were not detected or in traces. The values obtained in this study for phytate and oxalate is far below the range of 4-9 mg/L thus is insignificant [33]. Above this range, phytate could be of nutritional danger resulting in the reduction in the availability of many other essential dietary minerals [33].

Oxalate at the above mentioned range could have serious effects on human nutrition and health particularly by decreasing Ca absorption and adding to the formation of kidney stone [33]. Therefore, the reduced oxalate and phytate content in the vegetable makes it good for consumption, without any observable health hazards. This could add to the reasons why most families in Northern Nigeria use *C. gynandra* as a constant source of vegetable for soup preparation.

5. CONCLUSION

C. gynandra contains chemical constituents responsible for antibacterial activities. This therefore justifies the use of the plant in folklore medicine for treatment of such disease, as ear infection, blood replenishing (in case of anaemia), rheumatism and diarrhoea / dysentery / typhoid. Mineral content evaluation showed presence of Ca, Na, Mg, Fe, while Zn, Pb, Cr, Cu, and Mn were absent. The plant could therefore be used as a medicinal herb and nutritional vegetable.

6. RECOMMENDATION

It is advised that toxicological analysis be done on this plant and if found safe, then its inclusion

in the list of excellent medicinal and vegetable sources in the community could be contemplated. The activity of the extracts could further be tested on other microorganisms of importance

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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