

1 DETERMINATION OF MINERAL COMPOSITION, ANTI-MICROBIAL ACTIVITY AND
2 ANTI-NUTRIENT SCREENING OF *Cleome gynandra* (CAT'S WHISKERS)

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29 .ABSTRACT

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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* ($11.00 \pm 0.1, 8.00 \pm 0.1, 9.00 \pm 0.1$), *Pneumonia spp* ($11.00 \pm 0.2, 12.00 \pm 0.1, 8.00 \pm 0.2$), *Pseudomonas aeruginosa*, ($11.00 \pm 0.3, 8.00 \pm 0.1, ND$) and *Staphylococcus aureus* ($8.00 \pm 0.1, 11.00 \pm 0.2, \& ND$), for the leaf, stem and root respectively. The anti-nutrient component showed phytate and oxalate contents were less than is nutritionally significant, while tannins were in traces. Toxicological analysis could be done on the plant, for it has good medicinal potentials.

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32 Key words: *Cleome gynandra*, Spectrophotometrically analysed, *Salmonella typhi*,

33 *Pneumonia spp*, *Pseudomonas areuginosa*, and *Staphylococcus aureus*.

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43 **INTRODUCTION**

44 In Nigeria, as in other tropical African countries where the daily diet is dominated by the
45 same staple foods, vegetables are the cheapest and most readily available sources of
46 important proteins, vitamins, minerals and essential amino acids, Okafor; (1983).
47 Vegetables are important foods both from an economic and nutritional point of view.
48 Vegetables are living entities and respiring when they are freshly harvested, have high water
49 contents and abundant cellulose, Okafor; (1979). Most of these vegetables have both
50 nutritional and medicinal values and have been used for centuries as remedies for human
51 diseases because they contain components of therapeutic value, Nastro; (2001) and
52 Narendhirakannan, et al; (2005).

53 Medicinal and aromatic plants have played a vital role in alleviating human sufferings,
54 Baguar; (2001). A medicinal plant is any plant in which one or more of its parts contains
55 substances that can be used for therapeutic purposes or which are precursors for the
56 synthesis of useful drugs. Plants have always been of important therapeutic uses and have
57 been used to cure human and other animal diseases, Oyetayo; (2008). Traditional medicine
58 is an integral part of the African culture and traditions, and varies between different cultural
59 groups and regions, Makhubu; (2006). The awareness of the importance of medicinal plants
60 in human health care in developing countries has resulted in research into traditional
61 medicine with a view to integrating it with modern Orthodox medicine and they have been of
62 great importance to the health of individuals and communities, Sofowora; (1993) and
63 Edeoga, et al.; (2005)

64 Many of these indigenous plants are used as spices and foods. They are sometimes added
65 to foods for pregnant and nursing mothers for medicinal purposes, Okwu; (1999), Okwu;
66 (2001) and Schonfeldt & Pretorius (2011). Medicinal plants are generally used in traditional
67 medicine for the treatment of many ailments, Njoku & Ezeibe: (2007) and Ogukwe et al.;
68 (2004). The different parts used include leaves, roots, stem, flowers, fruits, seeds, and bark.
69 It is to this effect that this work is carried out on the leaves, stems, and roots of *Cleome*

70 *gynandra*, in order to evaluate the nutritional and medicinal values of the plant Gupta and
71 Rao;(2012).

72 *Cleome gynandra* (Cat`s whiskers), (Kinaski, in Hausa) of *capparaceae* family is found
73 throughout the tropics and subtropics. It occurs probably in all countries of tropical Africa,
74 where it is mainly found near human settlements. The plant is an erect, annual herb that
75 grows up to 150 cm tall, it is strongly branched with tap root and few secondary roots, the
76 stem is densely glandular, leaves alternate, palmately compound with (3, 5, 7) leaflets almost
77 sessile. Flowers are bisexual white or tinged with purple; pedicel? 1-5-2-5 cm long with 4
78 sepals, 4 petals, 6 stamens, and a purple ovary. Fruits are long narrow, cylindrical capsules
79 up to 12 cm x 1 cm, seeds are subglobose, 1-1.5mm in diameter, grey to black, irregularly
80 ribbed. *Cleome* comprises 150-200 species, about 50 of them occurring in Africa, Grubben,
81 & Denton; (2004).

82 The Plant is highly reputed for its numerous nutritional and medicinal uses, Anbazhagi;
83 (2009) and Van der Walt; (2009). The tender leaves, young shoots and occasionally flowers
84 are boiled or cooked as stew. The leaves are also boiled and water discarded while the
85 paste is made into balls with groundnut cake in Kenya and Zimbabwe, Samuel, & Brian;
86 (2007). Sometimes the bitter leaves are cooked with milk or other leafy vegetables such as
87 cowpea leave for food. Extracts of the leaves are used in Mubi North L.G.A. of Adamawa
88 State, North East Nigeria in form of concoction to treat typhoid and anaemia. Liquid extract
89 of roots is used as medicine for chest pain and leaves extract to treat diarrhoea, Grubben, &
90 Denton; (2004). The leaves when rubbed into the skin relieve pneumonia, Okafor; (1979);
91 and the leaves extract is also used as eyewash, Okafor; (1979).

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

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98 **MATERIAL AND METHODS**

99 Fresh *cleome gynandra* leaf, stem and root were collected in Mubi North Local Government

100 Area of Adamawa State. The plant was identified and authenticated by Dr. Dogmo of the
101 Forestry Unit Mubi according to Hutchinson and Daziel; (1956). The Forestry Herbarium
102 Index (FHI) number 074 was assigned and a specimen of the plant was deposited in the
103 herbarium. Samples were air dried in the laboratory. The plant was separated into the
104 various parts, leaves, stems and roots and each part was separately pounded to obtain fine
105 powdered sample and sieved with 2 mm mesh size sieve. The fine powdered samples were
106 stored separately in clean and dry sample containers for extraction.

107 Extraction

108 30 g of each of the fine powdered sample was accurately weighed and dissolved in 200 ml
109 of 95% ethanol for 72 hours before filtration. The concentrated extracts were obtained by
110 rotary evaporation and kept for analysis as described by Okogun; (2000).

111 Determination of Mineral constituents

112 5.0 g of each sample was weighed and placed in crucibles and transferred to a muffle
113 furnace to incinerate for four (4) hrs at 500⁰C until the ash was carbon free (white ash). The
114 samples were then removed, cooled in desiccators and then reweighed to obtain the
115 percentage ash content. The ash content was used for the elemental analysis. 10 ml of 0.1
116 M HCl was added to each of the ashed samples. The mixture was filtered into a 100 ml
117 volumetric flask. The crucible and the filter paper (whatman number one) were washed three
118 times with 10 ml of 0.1 M HCl solution. The absorbance of each element was determined
119 using Atomic Absorption Spectrometer (AAS) Bulk 210 model. Standard solutions of each
120 element were prepared and their absorbances were determined before the sample analysis.
121 The respective concentrations of the elements in the samples were obtained from the
122 standard calibration curves by extrapolation.

123 Anti-microbial analysis

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

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

130 Sensitivity test

131 Wells of 6 mm diameter were made on the nutrient agar using a standard gauge (sterile cork
132 borer). The test organisms were inoculated into the nutrient agar after thorough mixing with
133 Peptone water. 0.3ml of the reconstituted extracts was carefully introduced into the wells.
134 Antibiotic discs for both gram positive (Ampiclox) and gram negative (Gentamycin)
135 organisms were also used as standard controls. The plates were all allowed to stand for 30
136 minutes at room temperature for diffusion of the substance to proceed before the growth of
137 organisms commenced. The plates were incubated at 37⁰C for 24 hours. The zone of
138 inhibition which indicates anti-microbial activity was measured and recorded, Nastro; (2001).

139 Anti-nutrient components evaluation

140 Phytate analysis

141 4.0 g of each sample were soaked in 100 ml of 2% HCl for 5 hours and filtered. 25 ml of the
142 filtrate were placed in a 250 ml conical flask and 5ml of 0.3% ammonium thiocyanate
143 solution was added. The mixture was then titrated with standard ferric chloride solution until
144 a brownish-yellow colour persisted for 5 minutes, Reddy & Kove; (1999).

145 Oxalate analysis

146 2.0 g of each sample each was dissolved in 100 ml of 1.5 NH₂SO₄. The solutions were then
147 carefully stirred with magnetic stirrer for 1hour and then filtered. 25 ml of each filtrate was
148 measured and titrated hot (80-90⁰C) against 0.1 N KMnO₄ solutions until a faint pink colour
149 which persisted for at least 30 seconds, Day & Underwood; (1986).

150 Tannin analysis

151 1.5 g of each powdered sample was boiled in 5 ml of distilled water in a test tube. The
152 solutions were filtered. 3 drops of 0.1% ferric chloride was added to each of the filtrate and
153 observed for brownish green or blue-black colouration, Harbone (1973), Evans & Trease
154 (1989), and Sofowora, (1993)

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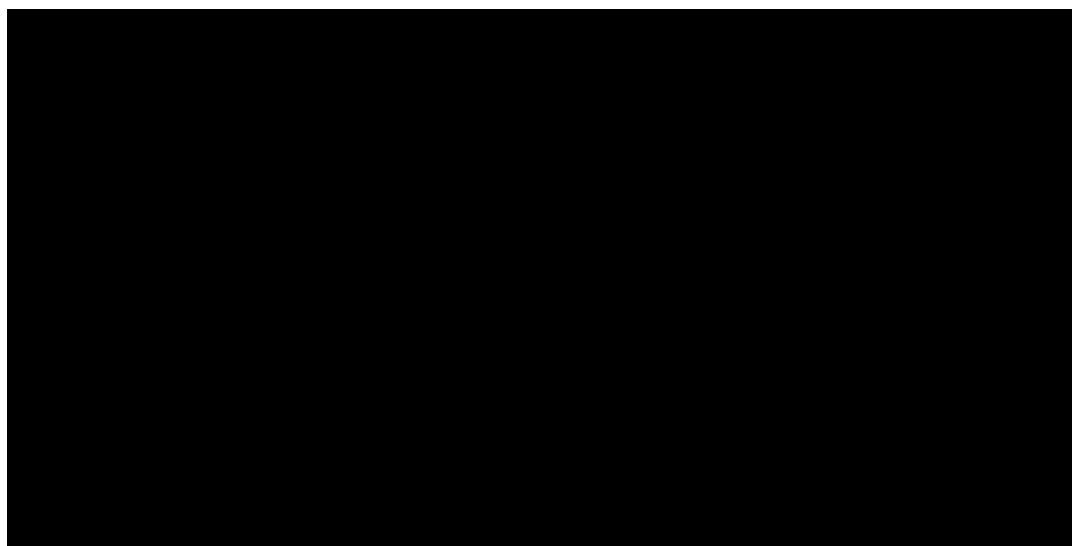
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157 **RESULTS**

158 The result of the mineral composition (figure 1), the antimicrobial activity and the anti-nutrient
159 tests Tables 1 & 2) are presented below:-

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163 Figure: 1 Results of Mineral constituents; concentration in (ppm), all

164 concentrations are results of triplicates with 0 ± 0.5 Standard error.

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Table: 1 Result of antibacterial tests

	ZONE OF INHIBITION IN (mm)			
Samples	<i>S.typhi</i>	<i>P. spp</i>	<i>P. aerugenosa</i>	<i>S. aureus</i>
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)

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

Table: 2 Result of anti-nutrients tests

	Anti-nutrients (mg/100g)		
Samples	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

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Mean of Three Trials ± Standard Error

DISCUSSION

The results obtained from the mineral content evaluation in Figure: 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, O'Dell and Campbell; (1971), Ogugbuaja *et al*; (1997). For instance the presence of phosphorus has been exploited for synthesizing numerous phytochemical compounds in plants, Habibovic *et al*; (2002). However the results of this work shows good correspondence with results presented earlier for some medicinal

197 plants of northern Nigerian origin in regards to the accumulation of certain minerals (Fe, K
198 and Mn) in fruity parts of plants than in the stems and leaves, Ogugbuaja; *et al.* (1997).
199 Ca plays an important role in building and maintaining strong bones and teeth, large parts of
200 human blood and cellular fluids. Also it is necessary for normal functioning of cardiac
201 muscles, blood coagulation, clotting and regulation of cell permeability. Ca deficiency causes
202 rickets, back pain, osteoporosis, indigestion, irritability, pre-menstrual tension, and cramping
203 of the uterus, Siddhuraju & Becker; (2001).

204 Mg was also present, which plays a vital role in the formation and function of bones, muscles
205 and prevents high electrolyte disorders, high blood pressure and depression in enzyme
206 activities. Its deficiencies interfere with transmission of nerve and muscle impulses, causing
207 irritability and nervousness, thus leading to heart diseases, Siddhuraju & Becker; (2001). Na
208 strictly helps in ionic balance of water and acid (osmotic regulation) in the body, and
209 maintain tissue excitability, helps normal muscle contraction, and formation of gastric juice in
210 the stomach. Deficiency causes nervousness, mental disorientation, low blood sugar,
211 insomnia and coma, Siddhuraju & Becker; (2001). The presence of Fe (iron) plays an
212 important role in the formation of blood haemoglobin and cytochromes. Deficiency leads to
213 anaemia, and hyperthyroid, Siddhuraju & Becker; (2001).

214 Cr, Cu, Pb, Mn and Zn are heavy elements or “heavy metals.” These elements were not
215 detected in *Cleome gynandra* or their levels were so small that they could not be detected
216 by the equipment used. Small amounts of these elements are common in our environment
217 and diet and are actually necessary for good health, but large amount of up to 0.5mg/l of any
218 of them may cause acute or chronic toxicity (poisoning). Heavy metals toxicity can result in
219 damaged or reduced mental and central nervous function, lower energy levels, and damage
220 to blood composition, lung, kidney, liver and other vital organs, Njoku & Ezeibe; (2007).

221 Pb, if present leads to toxicity in the body; causing respiratory problems and restricts the flow
222 of blood into the body system. The Environmental Protection Agency (EPA) has detected
223 and declared that there is no safe level of lead intake. It can cause high blood pressure and

224 reduce haemoglobin production, necessary for O₂ transportation, thus interfering and can
225 interfere with normal cellular Ca metabolism, Montague & Peter; (2010).

226 Cu was not detected but it is an important compound of many enzyme systems such as
227 cytochrome oxidase, lysyl oxidase and ceruloplasmin; an iron oxidizing enzyme in blood.
228 Deficiency has been associated with cardiac abnormalities in human and animals such as
229 anaemia and Neutrogena, Siddhuraju & Becker; (2001). Cr is of benefit only to those people
230 working for performance enhancement in physical activities. Also in the core factors that
231 dictate our overall health and well being. Excess of Cr leads to malignant growth in the
232 respiratory tract. Cr is one of the most toxic elements from vegetables, Njoku & Ezeibe;
233 (2007).

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

237 The plant is active against *S. typhi* that causes typhoid, paratyphoid, non-typhoid fever and
238 enteric fever such as gastrointestinal illness, followed by bacteraemia and diarrhoea,
239 Machkaire, *et al*; (2011). This explains their usage traditionally to cure numerous diseases
240 in Mubi North L.G.A.

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

243 Leaf and stem are active against *S. aureus* that causes minor skin infection such as
244 impetigo boils, scalded skin syndrome and abscesses, to diseases such as pneumonia and
245 meningitis. The leaf and stem are also active against *P. aeruginosa* which cause diseases
246 such as sour throat, scarlet fever and kidney disease, Robert, F. Boyd (1995).

247 Results of the anti-nutrient analysis presented in Table 2 showed that phytate and oxalate
248 were present in small amounts while tannins were not detected or in traces. The values
249 obtained in this study for phytate and oxalate is far below the range of 4-9 mg/L thus is
250 insignificant, Andrew Weil; (2011). Above this range, phytate could be of nutritional danger

251 resulting in the reduction in the availability of many other essential dietary minerals, Andrew
252 Weil; (2011).

253 Oxalate at the above mentioned range could have serious effects on human nutrition and
254 health particularly by decreasing Ca absorption and adding to the formation of kidney stone,
255 Andrew Weil; (2011).

256 Therefore, the reduced oxalate and phytate content in the vegetable makes it good for
257 consumption, without any observable health hazards. This could add to the reasons why
258 most families in Northern Nigeria use *C. gynandra* as a constant source of vegetable for
259 soup preparation.

260 **CONCLUSION**

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

268 **RECOMMENDATION**

269 It is advised that toxicological analysis be done on this plant and if found safe, then its
270 inclusion in the list of excellent medicinal and vegetable sources in the community could be
271 contemplated. The activity of the extracts could further be tested on other microorganisms of
272 importance

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