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Proximate Composition, Phytochemical and Antimicrobial Activity of Aqueous and Ethanolic Extracts of *Hunteria umbellata* on some Clinical Isolates

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ABSTRACT: A medicinal plant is any plant which has therapeutic used and are used for drug production. The use of medicinal plants for traditional uses is well known in rural areas and many developing countries. This study was undertaken to evaluate the antibiotic and therapeutic importance of the extracts of *Hunteria umbellata* against selected clinical isolates known to cause diseases to man. Agar well diffusion method was used to determine the antibacterial activity of *Hunteria umbellata.* The proximate composition showed that carbohydrate with 71.55±0.10 % had the highest value. The elemental composition analyzed showed that Fe had the highest amount. The phytochemical screening of both aqueous and ethanolic extracts showed the presence of oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols, cyanogenic glycoside and anthraquinones. The clinical isolates *E. coli, S. pneumonia, S. pyogenes, S. aureus, Staphylococcus* sp., *P. aeruginosa, Micrococcus* sp., *Klebsiella* sp., *Proteus* sp., *Bacillus* sp., *S. epidermidis, Candida albicans, S. cerevisiae.* The zone of inhibition of aqueous and ethanolic extracts in comparison with the conventional antibiotics showed that the extracts had better antibacterial properties. The results obtained showed the important use of *H. umbellata* in ethnomedicine.

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A medicinal plant is any plant which has therapeutic uses and are used for the production of antimicrobial drugs (Bouayed *et al.*, 2008), antioxidant, anti-infections and anti-tumor activities (Akroum *et al.*, 2009). Plants are the source of medicine for many people in different countries, which make diseases to be treated with herbal medicines gotten from various plants. This present day pharmaceutical companies depend on the diversity of secondary metabolites in plants of which at least 12,000 have been isolated and this can be estimated to be up to 10 % of the total of the secondary metabolites (Mallikharjuna *et al.*, 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.*, 2005). *Hunteria umbellate* K. Schum (Apocynaceae) is a tree with evergreen crown of about 15 m – 22 m in height (Oliver, 1986). It is located in the tropical zone of the southern part of Nigeria where it is called local names such as osu (Edo), abeere (Yoruba) and nkpokiri (Igbe *et al.*, 2009). The leaves are broad, abruptly acuminate and broadly lineate The fruit is about 5 cm – 25 cm and consist of globose mericaps of 3 cm – 6 cm long (Keay *et al.*, 1964). The genus *Hunteria* has been used severally for different herbal medicine.

Different kinds of medicine gotten from the extracts of different parts of the plant are presently being used in the treatment of various clinical diseases, although there is little knowledge on their mechanisms of action (Igbe *et al.*, 2010).

Therefore, the objective of this paper is to evaluate the proximate composition, phytochemical and antimicrobial activity of aqueous and ethanolic extracts of *Hunteria umbellata* on some clinical isolates.

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MATERIALS AND METHODS

Sample collection and identification: The plant seed of *H. umbellata* were purchased from a market in Oshodin Local Government Area, Lagos State. The plant seeds were dried for three weeks, thereafter the epicarps were removed and further dried for another three (3) weeks. The air drying was done to protect the bioactive components of the plant seeds. The preliminary identification of the plant was carried out by Dr. E. I. Aigbokhan, Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Preparation of sample: The dried seeds of *H. umbellata* were pulverized using electronic milling machine grinder, Lab. Mill, Serial No. 4745, Christy and Norris Ltd, England. The pulverized seeds were stored in air tight plastic container for further experimentation.

Extraction of plant materials: The extraction was carried out according to the methods of Igbe *et al.* (2009). Four hundred grammes (400 g) of the powdered form were macerated in a sterile grinder and transferred into Pyrex flasks containing 1.5 litre of aqueous (sterile distilled water) and non-aqueous solvent (ethanol) respectively, the suspension was allowed to stay for 24 hrs for the aqueous and 72 hrs for the non-aqueous mixture. At the end of extraction, the homogenate was filtered with a Whatman Filter Paper No. 1 using a glass funnel. The filtrates were labelled accordingly for subsequent use and concentrated using water-bath at 80 ^{0}C to dryness. The dried extract of both solvents were preserved in clean glass containers at 4 ^{0}C for further experimentation.

Proximate and Mineral elemental composition: The proximate and mineral elemental analyses were carried out according to the methods of AOAC (1999).

Determination of the quantitative phytochemical components of the pulverised seeds of H. umbellate: The quantitative phytochemical analyses of the powdered seeds of *H. umbellata* were determined according to the methods of Harborne (1973). Microbiological analyses and source of microbial isolates: Eleven bacterial isolates which include seven Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus* sp., *Staphylococcus epidermidis, Streptococcus pneumoniae, Micrococcus* sp. and *Streptococcus pyogenes*), four Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*., *Klebsiella* sp., *Proteus* sp,) and two yeast which include *Candida albicans* and *Saccharomyces cerevisiae* were collected from Microbiology Laboratory, University of Benin Teaching Hospital (UBTH), Benin City for the bacteriological analyses. The test isolates were transported to the laboratory in an ice pack box for experimentation.

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of *H. umbellata*, where carbohydrate had the highest percentage of 71.55±0.10. Table 2 showed the mineral elemental composition of the seeds of *H. umbellata* which showed the presence of these elements K, Na, P, Mg, Ca, Fe, Mn, Zn and Cu. Table 3 revealed the presence of phytochemicals such as oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols, cyanogenic glycoside and anthraquinones. Alkaloids had the highest percentage for both aqueous and ethanolic extracts. The results of the diameter of the zones of inhibition in Table 4 and 5 of the aqueous and ethanolic extracts of the several extract concentrations of the seeds of *H. umbellata* against the clinical isolates compared with the conventional antibiotics showed that the aqueous and ethanolic extracts of the seeds of *H. umbellata* performed better in the course of the experiment. Medicinal plants are of great importance to the health of individuals and communities. Medicinal plants have been used as healing agents in many parts of the world especially Africa, where access to formal health care is limited. Medicinal plants have played significant roles in maintaining health and sustaining the quality of life. This study was therefore undertaken to evaluate the proximate composition, mineral elemental and the antimicrobial activities of the aqueous and ethanolic extracts of *H. umbellata* on some clinical isolates. The result in Table 1 showed that carbohydrate was the highest among the other proximate parameters which is in agreement with the research carried out by Morakinyo *et al*. (2020). Nutritional and proximate analysis of plants are used to evaluate their nutritional importance as they are being employed by humans for medicinal purposes (Hussain *et al.*, 2009). Carbohydrate content of 72.11±0.80 *Hunteria umbellata* was reported by Morakinyo *et al*. (2020) which supports the result of 71.55±0.10 of carbohydrate content of *Hunteria umbellata* in this study.

Table 1: Proximate composition of the seed of *H. umbellata*

Proximate values	Results in %
Crude protein	12.25±0.70
Residual moisture content	10.20±0.01
Ether extract (lipid)	2.00±0.02
Crude fibre	0.80±0.03
Ash content	3.20±0.01
Carbohydrate	71.55±0.10
Gross Energy (Kcal/g)	495.35

Values in Mean ± SEM

The result of elemental analyses shows the presence of K, Na, P, Mg, Ca, Fe, Mn, Zn, Cu (Table 2). The

presence of these elements in the seeds of *H. umbellata* is an indicator of its nutritional quality.

Table 2: Mineral elemental composition of the seeds of *H. umbellata*

Elements	Concentration (mg/100 g)
K	7.7±0.10
Na	6.5±0.03
P	52.4±0.05
Mg	31.1±0.20
Ca	20.5±0.01
Fe	245.6±0.11
Mn	3.4±0.08
Zn	4.4±0.06
Cu	6.0±0.01

Values in Mean ± SEM

The result of the nutritional composition of *Hunteria umbellata* in this research is in agreement with the result of Morakinyo *et al*. (2020) which showed the presence of minerals such as calcium, phosphorus, zinc and sodium. The presence of these elements in the seeds of *H. umbellata* makes it useful in the prevention and control of diseases, acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction (particularly the cardiac muscle), bones and teeth regulation, cell membrane function (Murray *et al*., 2000). The results in Table 4 revealed the presence of the phytochemical components such as oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols cyanogenic glycoside and anthraquinones. Saponins, alkaloids and phenols were found to be present in high concentrations, which supports the findings of Adeneye and Adeyemi (2009); Morakinyo *et al*. (2020) on the research done on the seeds of *H. umbellata*, and they reported the presence of tannis, alkaloids, cardiac glycosides, flavonoids, saponins, anthraquinone and reducing sugar with alkaloids present in high concentration. The extraction of bioactive components from *H. umbellata* had led to the discovery of potent compounds with low toxicity. The phytochemicals contained in *H. umbellata* in this study has been shown severally to be active against several infections (Owolabi *et al*., 2007). A compound such as alkaloids present in Table 4 is known to be an antimalaria agent, analgesics and can act as stimulants. Glycoside moieties such cyanogenic glycosides, saponins and anthraquinones have been reported to be able to inhibit tumor growth, antiparasitic agents, antidepressant (Ajayi and Ojelere, 2013). Phytochemicals are known to accumulate in different parts of the plant such as in the leaves, flowers, stems, roots, fruits or seeds (Costa *et al*., 1999). Phenol also reported in the present study also plays significant role in the managements of antiaging, antiapoptosis, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function (Han *et al*., 2007). Glycosides which are present in Table 4 have been reported to lower blood pressure (Nyarko and Addy, 1990). The results of phytochemicals therefore report the increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. The results in Tables 4, 5 and 7 showed the antibacterial activity of the aqueous and ethanolic extracts of *H. umbellata* seeds on some clinical isolates such as *E. coli, S. aureus, S. pneumoniae, Staphylococcus* sp., *P. aeruginosa*, *Micrococcus* sp., *Klebsiella* sp., *Proteus sp., Bacillus* sp., *S. cerevisiae, C. albican , S. epidermidis.* The plant extracts showed varying degrees of antimicrobial activities against the test isolates. The antimicrobial activities of aqueous and ethanolic extracts of *H. umbellata* seeds were observed to be dosage-dependent and their activity varied with concentrations against the test isolates as shown in Tables 4, 5 and 7.

The concentrations of the minimum inhibitory concentration and minimum bacteriocidal concentration as shown in Tables 4, 5 and 7 were observed to be very effective against the clinical isolates even at low concentrations. This further proves the antibacterial efficacy of the aqueous and ethanolic extracts of *H. umbellata.*

Table 3: Quantitative phytochemical constituents of *Hunteria umbellata* seeds

Test Organisms	Zone of inhibition (mm)						
	150 mg/ml	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml	MIC mg/ml
E. coli	21.50±0.50	20.00±0.20	16.50±1.50	10.50±0.50	10.00±0.40	9.50±0.50	75
S. pneumonia	24.00±1.00	15.00±1.00	13.00±0.50	11.50±1.50	10.00±1.00	9.50±0.50	75
S. pyogenes	16.50±1.50	19.50±0.50	15.50±0.50	10.50±0.5	0.00	0.00	75
S. aureus	25.00±1.00	23.00±2.00	20.50±5.50	18.50±1.5	17.00±1.00	11.50±1.50	25
Staphylococcus sp.	29.50±0.50	26.50±1.50	21.50±6.5	18.50±0.50	14.00±1.00	13.00±1.00	15
P. aeruginosa	15.50±1.50	13.00±2.00	12.50±0.50	11.00±1.00	0.00	0.00	75
Micrococcus sp.	25.50±0.50	23.50±3.50	22.50±2.50	19.00±2.00	15.50±0.50	11.50±1.50	25
Klebsiella sp.	18.00±2.00	15.50±3.50	13.00±6.00	9.50±0.05	0.00	0.00	75
Proteus sp.	17.00±2.00	15.50±1.50	13.00±1.00	8.50±3.50	0.00	0.00	75
Bacillus sp.	21.50±0.50	19.00±1.00	17.50±0.50	15.50±1.50	13.00±1.00	11.00±1.00	25
S. epidermidis	22.00±2.00	19.50±2.50	19.00±2.00	17.00±1.50	15.50±2.00	10.50±0.50	25
C. albicans	40.00±2.00	35.00±1.00	25.50±0.50	24.5±0.50	22.00±1.00	15.00±2.00	15
S. cerevisias	17.00±2.00	13.50±1.00	13.00±1.00	12.00±0.50	0.00	0.00	50

Table 4: Zones of inhibition of aqueous seeds extract of *H. umbellata* on clinical isolate

Test Organisms	Zone of inhibition (mm)						
	150 mg/ml	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml	MIC mg/ml
E. coli	19.50±0.50	18.00±3.00	15.00±4.00	14.00±4.00	12.00±0.05	9.00±0.00	25
S. pneumoniae	32.00±1.00	28.00±0.04	25.50±0.50	19.00±2.00	13.50±0.04	12.00±0.03	25
S. pyogenes	29.00±0.06	21.50±0.50	18.50±0.50	15.00±1.00	13.50±0.04	0.00	25
S. aureus	26.00±0.10	22.00±0.02	17.00±0.50	15.00±1.50	13.00±0.50	8.50±1.50	25
Staphylococcus sp.	23.50±1.50	19.00±0.50	14.00±1.00	12.50±1.50	10.00±1.00	8.50±0.50	50
P. aeruginosa	24.00±1.00	20.50±1.50	18.00±0.50	16.00±1.00	14.00±0.02	0.00	25
Micrococcus sp.	30.00±2.00	28.00±2.00	25.50±1.50	15.00±1.00	10.50±0.50	8.50±0.50	50
Klebsiella sp.	25.50±1.50	20.00±2.00	15.50±1.50	10.50±1.50	0.00	0.00	75
Proteus sp.	27.00±1.00	25.50±1.50	20.00±2.00	16.00±1.00	14.50±1.50	12.00±0.50	15
Bacillus sp.	24.50±1.50	20.00±1.00	16.50±1.50	14.00±1.00	13.00±1.00	10.00±1.50	25
S. epidermidis	21.50±0.50	19.50±0.50	16.50±1.50	14.00±1.00	12.50±0.50	11.50±1.50	25
C. albians	42.00±1.00	39.50±0.50	28.00±0.00	20.50±2.50	15.50±0.50	13.00±1.00	15
S. cerevisiae	19.00±1.00	17.00±1.00	15.50±0.50	13.00±1.00	9.00±1.50	0.00	50

Despite what many researchers have reported that *C. albicans* are very resistant fungi, this work demonstrated that, aqueous and ethanolic extracts of the seeds of *H. umbellata* were effective against *C. albicans* at concentration of 75 mg/ml. This result is in agreement with the research carried out by Pavendan and Sebastine (2012) where it was reported that the leaf extract of a medicinal plant *E. singampattiana* was effective against *C. albicans.*

The result of this study is in agreement with the research carried out by Selvamohan *et al.* (2012) where the aqueous extracts of three medicinal plants *P. niruri, P. emblica and P. vera* showed inhibitory activity against selected human pathogenic microbes such as *Klebsiella* sp., *P. aeruginosa*, *E. coli* and *S. aureus* which were also used in this study. This research is also in agreement with the research carried out by Adegoke *et al*. (2010) where the ethanolic extract of *Phyllanthus amarus* showed inhibitory effect on *S. aureus*, *P. aeruginosa*, *Klebsiella* sp., and *E. coli.* It was observed in this research work that increase in the concentration of the ethanolic extract increased susceptibility of the test isolates.

Table 5: Zones of inhibition of ethanol seeds extract of *H. umbellata* on clinical isolates

Parameters	Aqueous, (mg/100 g)	Ethanol (mg/100 g)
Oxalate	99.0	53.0
Phytate	66.0	33.0
Tannins	2.0	4.0
Flavonoids	0.00	5.00
Saponins	128.0	91.0
Alkaloids	208.0	270.0
Phenols	81.0	379.0
Cyanogenic glycoside	36.0	55.0
Anthraquinones	84.0	110.0

Table 6: Antibacterial activity of standard antibiotics against test clinical isolates

Antibiotics	*Organisms*											
Inhibition zone diameter (IZD) mm	*B. subtilis*	*Micrococcus* sp.	*S. pneumoniae*	*S. epidermidis*	*S. pyogenes*	*S. aureus*	*Klebsiella sp.*	*E. coli*	*Proteus sp.*	*P. aeruginosa*	*Candida albicam*	*S. cerevisiae*
S	13	11	0	12	9	12	0	11	12	12	10	13
SXT	12	0	10	11	8	13	0	0	0	11	9	0
CH	14	0	10	10	0	14	0	0	0	10	10	0
SP	13	11	12	11	7	15	0	0	0	10	14	11
CPX	11	14	13	10	10	14	14	0	4	9	11	13
AM	13	0	13	13	11	12	0	0	0	0	10	10
AUG	12	0	12	10	0	11	0	0	0	9	0	10
CN	14	0	11	12	0	12	0	0	0	10	0	9
PEF	15	0	12	13	0	13	0	0	0	10	9	11
OFX	15	0	10	10	0	14	10	0	0	12	0	12
MZN	0	0	0	0	0	0	0	0	0	0	18	19
Control	0	0	0	0	0	0	0	0	0	0	0	0

IZD ≥20 mm: Susceptible, IZD 15-19 mm: intermediate, IZD ≤14: resistant; S: streptomycin, SXT: septrin, CH: chloramphenicol, SP: sparfloxacin, CPX: ciprofloxacin, AM: amoxicillin, AU: augmentin, CN: clindamycin, PEF: pefloxacin, OFX: Ofloxacin, MNZ: Metronidazole

Table 7: Minimum bacteriocidal concentration of the extracts of *H. umbellata* seeds on clinical isolates

Test organisms	Aqueous (mg/ml)	Ethanol (mg/ml)
E. coli	150	75
S. pneumoniae	150	50
S. pyogenes	150	75
S. aureus	50	50
Staphylococcus sp.	75	150
P. aeruginosa	150	75
Micrococcus sp.	50	150
Klebsiella sp.	75	75
Proteus sp.	75	50
Bacillus sp.	50	75
S. epidermidis	75	75
C. albicans	75	75
S. cerevisiae	75	75

This confirms the research of Obi and Onuoha (2000) where it was reported that the increase in inhibitory effects as the concentrations of the extract increased may be as a result of the ethanol ability in extracting most of the active ingredients of the plants. However, the ability of the extracts to inhibit the growth of *Staphylococcus aureus, Pseudomonas* sp., *Klebsiella* sp. and *Escherichia coli* indicated that these organisms do not possess the ability of inactivating the active ingredients in the extracts or other mechanisms which include exclusion of the substance from the cell and modification of the target site of the substance.

The antimicrobial activities of the extracts possess antibacterial activity against all the test isolates. In this research ethanol extract showed more activity against the bacterial isolates than the aqueous extract. This may be due to the higher volatility of the ethanol which tends to extract more active compounds from the seeds of *H. umbellata* than aqueous (Ibekwe *et al*., 2001). The results in Table 4, 5 and 7 reveals that the antimicrobial activity of aqueous and ethanolic extract of *H. umbellata* can be compared and said to be better than the activity of the conventional drugs on the test isolates. The result in Table 6 showed the poor performance of the conventional antibiotics on the test isolates. This result showed that the aqueous and ethanolic extracts of *H. umbellata* can be used in the formulation of antibiotic drugs which will have strong inhibitory effect on clinical isolates that have been known to cause diseases. The presence of the phytochemicals in the seeds of *H. umbellata* have been known to cause curative activity against several bacteria and it is not surprising that this plant extracts are used traditionally by herbalist to cure bacteria related ill-health (Dahiru *et al.*, 2006). The findings of Muanya (2008) which is in agreement with the result of this study reported that *Garcinia kola* a medicinal plant has strong antibiotic activities and are very effective against disease-causing microorganisms such as *E. coli, S. aureus, P. aeruginosa, Salmonella* sp., *Streptococcus* sp., *Candida albicans, Vibrio cholera* and *Neisseria gonorrhoea*. This study has further authenticated the antimicrobial potential of *H. umbellata* and justifies its use in the daily diet to treat mankind from certain ailments.

Conclusion: The results of this study showed that *H. umbellata* seeds has medicinal properties which helps in the sustenance of health of individuals in communities. The presence of phytochemicals in this plant seeds plays a lot of roles in its therapeutic effects. Medicinal plants have been used as healing agents in many parts of the world especially Africa where access to formal health care is limited.

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