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THE ANTIBACTERIAL AND PHYTOCHEMICAL ASSESSMENT OF THE ETHANOLIC EXTRACTS OF PARTS OF *CLEOME RUTIDOSPERMA* PLANT

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ABSTRACT
This work examines the antibacterial and the phytochemical properties of the ethanolic extracts of the various plant parts of *Cleome rutidosperma* (Capparaceae). A weighed amount of the air-dried roots, seeds, stems and leaves of *Cleome rutidosperma* was macerated and extracted completely with 96% ethanol. A phytochemical analysis of the extracts was carried adopting standard methods. Their antibacterial actions against *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*) *and Pseudomonas aeruginosa* (*P. aeruginosa*) and their minimum inhibitory concentration (MIC) at different concentrations were determined using the agar well diffusion methods. From the results, the percentage yield of extracts was as follows: seed > leaves > root > stem. Each plant component studied contained steroids, saponins and triterpenoids, though the stem and leave contained a higher amount of saponins. Tannins and reducing sugars were mainly found in the seed and leave. Antibacterial activities against *S. pneumonia* were noted as follows: root (10.50 mm) = leaves (10.50mm) > seed (9.50mm) > stem (8.50mm). There was no significant susceptibility against *S. aureus* and *P. aeruginosa*. The MIC (at 12.5mg/ml) were: root (7.00mm) = leaves (7.00mm) > seed (6.75 mm) > stem (6.25mm). Since *S. pneumoniae* is a causative organism of otitis media as well as conjunctivitis, the folkloric use of *Cleome rutidosperma* in the treatment of these ailments is justifiable. However, a higher antibacterial activity of the plant will be achieved with the whole plant.

KEYWORDS: *Cleome rutidosperma*, antibacterial, phytochemical, ethanolic extract.

INTRODUCTION
The United Nations acknowledged 2010 as the International Year of Biodiversity in an effort to affirm the relevance of nature to health and biomedical science. It is noteworthy that more than half of all drugs put in place since the previous quarter century resulted from nature.[1] A good number of our best and significant medications were found in plants with an extensive antiquity of use by mankind. Universally, the World Health Organization[2] approximates that in various emerging nations as much as 80 % of the people depend on traditional medical practice from nature as a principal basis for health maintenance. Plants naturally contain numerous phytochemical fragments such as vitamins, terpenoids, phenolic, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids and other metabolites which are a rich source of free radical scavengers[3] which act as antioxidant composites having anti-inflammatory, antibacterial, antiviral activities, etc.[4] Phytochemicals secluded from plant bases have been used for the hindrance and dealing with several diseases.[5] By traditional stories, Cleome *rutidosperma* is claimed to be used to treat conjunctivitis, otitis media, convulsions, spasm, pain, various skin diseases, etc. Ethanolic extract of its whole plant has been reported to exert a broad-spectrum antibacterial action against *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, these organisms, being the causative agents for such diseases as otitis media, conjunctivitis, etc.[6-10] In Ghana, Gabon and Democratic Republic of Congo, leaf sap from Cleome *rutidosperma* is applied to cure an earache and deafness. It is used as antimalarial by traditional healers in Cameroon and is also used in the treatment of paralysis, epilepsy, convulsion and spasm.[11,12] Extracts from *Cleome rutidosperma* have been studied for its central nervous system (CNS) depressant effect after it was found to have been used by the local people in the Philippine for that purpose.[13] Okoro *et al* examined the effects of oral administration of aqueous extracts of *Cleome rutidosperma* leaves at different doses (125, 250 and 500 mg/kg body weight) orally for 28 days to consider its

blood glucose, biochemical parameters, lipid profile, antioxidant enzymes actions and hepatic glucose regulating enzyme activities in streptozotocin (STZ)-induced diabetic rats in comparison with glibenclamide (600 µg /kg body weight as a standard. Their studies suggested that *Cleome rutidosperma* leaves aqueous extracts exhibited antihyperglycaemic, antihyperlipidaemic and antioxidant effects and consequently could prevent various complications of diabetes. The study also provided evidence for the traditional usage of the plants in the control of diabetes.[14] Khuntia and Mohanty explored the antifungal potential of different solvent extracts of aerial parts of *Cleome rutidosperma* on *Aspergillus niger, Penicillium notatum, Candida albicans, Helminthosporium solani* in comparison with clotrimazole. They found that its ethanolic extract exhibited prominent antifungal activity against all selected strains and indicated a potential usefulness of *Cleome rutidosperma* aerial parts as an antifungal agent.[12] The diuretic action of crude aqueous extract and broad-spectrum antibacterial activities of whole plant ethanolic extract has also being been reported.[15] Mondal and Suresh studied the wound healing outcome of the petroleum ether, chloroform, methanol and aqueous extracts of *Cleome rutidosperma* root in rats using excision and incision models correspondingly. They measured the wound curing actions by the rate of wound closure, time of epithelialisation and wound breaking strength using nitrofurazone (0.2% w/w) in simple ointment I.P. as a reference drug. The results of their study showed that the rats applied with methanol and aqueous extracts of *C. rutidosperma* exhibited a quicker rate of wound healing when judged with the other extracts under evaluation. The chloroform extract of the selected plant also showed encouraging results but the effects were seen to be of lesser extent than the corresponding methanol and aqueous extracts. In their investigation, the petroleum ether extract did not produce significant results. However, their studies justified the use of *C. rutidosperma* roots for wound healing action according to the legend.[16]

The purpose of the present study is to compare the antibacterial and the phytochemical properties of the ethanolic extracts of diverse plant parts: seeds, roots, leaves and stems of *Cleome rutidosperma* to investigate the folkloric use of a whole crude extract of the plant in the treatment of some bacterial infections.

MATERIALS AND METHODS

Materials

The following materials were used as procured and include: NUTRIENT AGAR, MULLER-HINTON AGAR (Titan Biotech, India), ETHANOL, 96% (JHD, China), DIMETHYLSULPHOXIDE (DMSO), CIPROFLOXACIN (Sigma, USA).

Methods

Plant Sample Collection and Identification

The plant materials were collected from the premises of the University of Port Harcourt, Port Harcourt. It was identified and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt. A voucher specimen no. UPH/3/107 was assigned to it as it was deposited in the herbarium.

Processing of sample

The plant sample was washed to remove sand. The respective parts (roots, leaves, seeds and stem) of the sample was separated and air-dried for two weeks. Each component was milled into a coarse powder (Binatone, China).

Collection of test microorganisms

Clinical isolates of *S. aureus, P. aeruginosa* and *S. pneumoniae* were obtained from microbiology laboratory of University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. They were isolated and properly identified in the Pharmaceutical Microbiology laboratory, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The test isolates were maintained on nutrient agar slant, refrigerated at 4^{o}C and were subcultured onto nutrient broth. A standard inoculum was prepared in 0.1% peptone water and incubated for 24 h at 37^{o}C to 0.5 McFarland standards of 1 x 10^{6} CFU/ml.

Preparation of plant extracts

The method of Bose *et al*.[15] was adopted with slight modification. The pulverized plant parts were exhaustively extracted with 96% ethanol using maceration method for 3 days. The solvent was allowed to evaporate using the rotatory-evaporator and was further dried in porcelain dishes at 60^{o}C on a water bath (Model TT-6, Techmel & Techmel, USA). The dried residue was stored in air-tight universal containers, labelled appropriately until ready to use.

Phytochemical screening of *Cleome rutidosperma* plant parts.

The qualitative determination of some chemical constituents (phytochemical screening) of the ethanolic extracts of the various plant parts (leaf, root, stem and seed) of *Cleome rutidosperma* was undertaken by the standard methods of Sofowora.[17]

Preparation of stock solution and dilutions of crude leaf, root, stem and seed extracts

One gram quantity each of the extract concentrates was dissolved in 10 ml of Dimethyl sulfoxide (DMSO) to obtain 100mg/ml. From the stock solution, four 2-fold serial dilutions were made to obtain 50, 25, 12.5, 6.25 mg/ml used for the minimum inhibitory concentration determination. Ciprofloxacin solution (10mg/ml) and DMSO were used as positive and negative controls respectively.

Antimicrobial Testing

To assay for antimicrobial activities of ethanolic extracts of *Cleome rutidosperma* plant parts, agar-well diffusion method was deployed. 1ml of the standardized culture of the selected strains of bacteria was seeded into molten Mueller Hinton agar using pour plate technique. For each diverse part, 5 wells were made on the agar plate (3 for the extract and 2 for controls) with a 6 mm sterilized cork borer at equal distance apart. A 0.1ml volume of the stock solution of the different plant part extract was introduced into the corresponding labeled well.[18] A diffusion period of 1 h was allowed on the bench and after which the plates were incubated at 37^{o}C for 20 h. The plates were observed and the average inhibition zone diameter (IZD) in nearest millimetres excluding the size of cork borer was measured around the wells.

Determination of minimum inhibitory concentration (MIC) of plant extracts

An agar well diffusion assay method was used to determine the MIC. A four 2 fold serial dilution of the extracts (2:2) was performed. A previously prepared molten Mueller Hinton agar was seeded with the test microorganism and poured into a petri dish to solidify. A 6mm sterile cork borer was used to aseptically bore wells on the agar and 0.1 mL of different concentrations of *Cleome rutidosperma* leaf extract was introduced into the well.[19] The antibacterial agent was allowed to diffuse in the agar for 1 h before incubating for 24 h at 37^{o}C. This same procedure was repeated for seed, root and stem extract at same concentrations.

RESULTS AND DISCUSSION

After exhaustive extraction of the diverse plant parts (root, seed, stem and leaves) of *Cleome rutidosperma*, the yield of the extract was in the order: seeds > leaves > roots > stems indicating that the highest yield was obtained from the seeds while the stems gave the least yield. The results of the phytochemical screenings are presented in Table 1, showing the presence of saponins, steroids and triterpenoids in all plant parts assessed as reported by previous investigators[20], however, the stems and leaves contain more saponins. The seeds and leaves contain all the phytochemical constituents screened while the stems and roots were devoid of tannins and reducing sugars.

Table 1: Phytochemical screening of the extract of the different parts of *Cleome rutidoserpma*

Phytochemical test	Root	Seed	Stem	Leaves
Saponins	+	+	++	++
Steroids	+	+	+	+
Tannins	_	+	-	+
Triterpenoids	+	+	+	+
Reducing sugars	-	+	_	+

Key: (+) = Present; (++) = More abundant; (-) = Absent.

The antibiotic susceptibility testing (AST) using the extracts from different parts of *Cleome rutidosperma* against *S. pneumoniae*, *S. aureus* and *P. aeruginosa* showed varying results in terms of the ability of the plant extract to effectively inhibit the growth of these organisms. There was evidence of antibacterial activity of the respective extracts from the various parts of the plant against *S. pneumonia* with the highest inhibitory zone diameter (IZD) of 10.5 mm exhibited by the extract from the roots and leaves. It has been reported that secondary metabolites like tannins, saponins, flavonoids probably are accountable for the observed antibacterial activity of plants.[15] The antimicrobial activity of the leave extract is an evidence of the report that it has a high concentration of phytochemical constituents attributed to its larger surface area as well as the metabolic function.[21]

Table 2: Antibiotic susceptibility testing and Minimum Inhibitory Concentrations (MICs) of ethanolic extracts of stems, leaves, seeds and roots of *C. rutidosperma* against *Streptococcus pneumonia.*

Plant extract	Concentrations of extract (mg/ml) / MIC* (mm)				
	100	50	25	12.5	6.25
Roots	10.5	9.5	8	7	-
Seeds	9.5	8.5	8	6.75	-
Stems	8.5	7.5	7	6.25	-
Leaves	10.5	9.5	8	7	-
	*MIC values are the mean of triplicate determination.				

The result of antibiotic susceptibility testing and Minimum Inhibitory Concentrations (MICs) of ethanolic extracts of *Cleome rutidosperma* plant parts against *Streptococcus pneumonia* are shown in Table 2. The antibiotic susceptibility testing at 100mg/ml and MIC obtained with a successive dilution of plant parts extracts revealed the highest activity from roots and leaves extracts. The MIC gave varied activity with concentration up to 12.5mg/ml from the ethanolic leaves and roots extracts (7mm) was in line with the result of

antimicrobial activity / antibiotic susceptibility testing using these plant parts while least activity (6.25mm) was exhibited by the stems extract.

CONCLUSIONS

The ethanolic extract of the different parts of *Cleome rutidosperma* having revealed its activity against *Streptococcus pneumonia*, justified its folkloric use in the treatment of otitis media and conjunctivitis. However, a higher antibacterial activity of the plant will be achieved with the whole plant.

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