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Research Article

In Vitro Antibacterial Activity of *Gynandropsis gynandra* on some Pathogenic Enteric Bacterial Isolates

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Abstract

Plants contain many biologically active compounds that have potential for development as medicinal agents. The study was conducted to investigate the antimicrobial activities of *Gynandropsis gynandra* (leaves and stem) and its major bioactive constituents (phytochemicals). The aqueous and ethanol extracts from the leaves and stem of the plant was tested using well Diffusion method for their antimicrobial activity against some members Enterobacteriales family (*Escherichia coli, Shigella* sp*, Klebsiella* sp and *Salmonella typhi*) isolated from diarrheic stool sample from Murtala Muhammad specialist Hospital, Kano. Preliminary phytochemical analyses showed that both stem and leaf extracts contain alkaloids, tannins, terpenoid, Anthraquinones, reducing sugar, amino acid, flavonoids, saponins, cardiac glycosides, resin and phenols. The result shows that the extracts demonstrated higher antibacterial activity against the isolates tested with the average zone of inhibition of 16.60 mm, 16.54 mm, 14.73 mm and 13.95 mm for *Shigella* sp*, E. coli*, *Klebsiella* sp and *S. typhi* respectively. The result also demonstrated that leaf extract is more effective than the stem extract. The ethanolic extracts of the plant showed higher zones of inhibition against test organisms (17.74 mm) compared to aqueous extracts (13.15 mm). There is no significant different on the susceptibility of the organisms tested against the extracts at $p<0.05$. The results of the present study have provided the justification for therapeutic potential of *Gynandropsis gynandra* leaves and stem extracts.

Keywords: Bacteria, *Gynandropsis gynandra*, Kano, Phytochemicals, Antibacterial activity

Introduction

Medicinal plants have been sources of a number of important compounds which have been discovered during last century. In the light of their established therapeutic efficacy, the pharmaceutical industries are using crude extracts of medicinal plants for manufacturing drugs [1]. Research conducted on medicinal plants have served the dual purposes of bringing up new therapeutic agents and providing useful leads for studies directed towards the synthesis of drugs on the basis of the chemical structures of the natural products. Modern pharmaceutical industries still rely to some extent on the bioactive principle, obtained from plants [2]. Eighty percent of the world population still depends on herbal medicine as their main source of medicinal therapy [3]. Today many scientists and medical experts around the world are emphasizing the value of herbal remedies for health. Only a small fraction of earth's plants have been investigated scientifically leaving an enormous unexplored potential. From the foregoing, it is apparent that more organized efforts are required for bioassay directed isolation studies of natural products from medicinal plants [4].

The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [5]. Phytochemicals are naturally occurring compounds in the medicinal plants [6]. Large populations of the world, especially in developing countries depend on the traditional system of medicine to treat variety of diseases [7]. Several hundred genera of plants were utilized traditionally for medicinal purposes. The world health organization [8] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents [9].

The plant *Gynandropsis gynandra* L. (Family; *Cleomaceae*) (Syn *Gynandropsis pentaphylla* L., *Cleome gynandra* L.) is commonly known as "*Gasaya"* in Hausa and 'Cat's whiskers' or Spider flower in English [10]. It is herb indigenous to the tropical and subtropical regions. The leaves and seeds of the plant are used in indigenous medicine in many countries. It has been used for several years in Indian traditional medicine as an anthelmentic and antimicrobial agent [11]. Leaves are applied externally over the wounds to prevent the sepsis. The decoction of the root is used to treat fevers. Leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried. The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for ear ache and eye wash. Stems are used as analgesic and anti-inflammatory agent [10]. A decoction or infusion of boiled leaves and/or roots is administered to facilitate childbirth, treat stomach-ache, constipation, and conjunctivitis or thread-worm infection. The seeds and roots also have antihelmintic properties [12]. The whole plant is also used in the treatment of malaria, piles, rheumatism and in tumor [13]. Considering the therapeutic value of the plant, the research was aimed at assessing the extracts (aqueous and ethanolic) from leaves and stem of *Gynandropsis gynandra* for antibacterial activity and phytochemical screening against some enteric bacteria isolated from diarrhea patients in Kano, Nigeria.

Materials and Methods

Plant Materials

The plant materials used in the present research are leaves and stem of *Gynandropsis gynandra* which were collected from Biological garden of Ahmad Bello University, School of Basic and Remedial Studies Funtua, Katsina state at about 07:30 a.m. Identification and authentication of the plant materials has been done there and voucher specimen has been deposited there for future reference. The samples were washed with water to remove dust and rinsed with distilled water and air-dried for 14 days to constant weight. The dried materials were cut into pieces and grinded into powder using a sterile electric blender under laboratory condition. The powder was then kept for future use.

Figure 1: *G. gynandra* plant

Test Organisms

Clinical isolates of *Escherichia coli, Shigella, Klebsiella and Salmonella typhi* were obtained from Department of Microbiology of Murtala Muhammad Specialist hospital for further experiment. Characterization of the isolates was conducted by using three procedures namely Gram staining, cultural characterization using selective/indicative media and biochemical characterization as described by Cheesbrough [14]. The pure isolates of each of the test organism were inoculated in sterile agar slants containing Nutrient agar and transported to the laboratory of Microbiology department of Kano University of Science and Technology, Wudil and refrigerated at 4℃ before use.

Extraction of Plant Material

Aqueous (water) and organic (ethanol) solvents were used for extraction of the active components of the plant part. For aqueous extraction, twenty five gram of each of the grounded leaf and stem were extracted by successive soaking for 5 days using 50 ml of distilled water in a 250 ml sterile conical flask. The extracts were filtered using Whatman filter paper and the filtrates concentrated in water bath at 60°C. The concentrated filtrate, now the extracts were then stored in universal bottles in the refrigerator at 4°C before use. For organic extraction, 25 g of the powdered plant part was extracted in 250 ml of 80% ethanol for 3 days mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator. The solid residues obtained were reconstituted in DMSO at stock concentration, stored in the refrigerator at 4 ^{0}C until used.

Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem of *G. gynandra.* Presence of Alkaloid, saponin, Glycoside, Tannin, flavonoids, resin, steroid, terpenoid, Anthraquinones, terpenoids were determined using procedure described by Sofowora [15].

Antimicrobial Assay

The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1ml of the different standardized organisms were introduced separately and thoroughly mixed with Mueller Hilton Agar in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200mg/ml, 150 mg/ml, 100mg/ml and 50 mg/ml while the 5th well contained the solution used for the research to serve as control, Ciprofloxacin (Chi pharmaceutical limited, Nigeria) 125mg/ml, was used as control in this research. These were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°c for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters [16]. The experiment was conducted in triplicate and average values were calculated.

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used were analyzed using One-Way ANOVAs from statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Ethical Approval

Ethical approval for the research (issue number HMB/GEN/488/VOL. 1) was obtained from Health Service Management Board Kano and Murtala Mohammed Specialists Hospital (MMSH), Kano based on the consent of the Hospitals Ethical Committees.

Results

Phytochemical Screening

Results of preliminary phytochemical screening of the leaf and stem of *Gynandropsis gynandra* are shown in Table 1. Results showed the presence of alkaloids, Anthraquinones, cardiac glycosides, flavonoids, resins, saponin, steroid, and tannin.

Table 1: Phytochemical constituents of leaf and stem extract of *Gynandropsis gynandra*

Phytochemical	Leaf extracts	Stem extracts
Alkaloids	+	+
Anthraquinones	+	+
Cardiac glycoside	+	+
Flavonoids	+	+
Resin	+	+
Terpenoid	-	-
Saponin	+	+
Steroid	+	+
Tannin	+	+

***Key**: + = Positive, - = Negative*

Antibacterial Activity of the Extracts

Leaf Extracts

The antibacterial activity of *G. gynandra* leaves extracts against the tested isolates is presented in Table 2. The result showed that ethanol extract is more effective with average zone of inhibition of 19.06 mm than aqueous extract which has an average zone of inhibition of 13.63 mm. Based on the result, *Shigella* sp is more sensitive to the extract (17.36 mm), followed by *E. coli* (17.04 mm), *Klebsiella* sp (15.80 mm) and least activity was shown against *S. typhi* (15.21 mm). The zone of inhibition shown by Ciprofloxacin (25 mg/ml) ranged between 22 and 24 mm for the tested isolates.

Table 2: Antibacterial Activity of Leaf Extracts against some Pathogenic Enteric Bacteria

		Organisms/Zones of inhibition			
Extracts	**Conc. (mg/ml)**	***E. coli***	***Shigella***	***Klebsiella***	***S. typhi***
	50	12.34±0.70^{b}	12.67±1.20^{b}	10. 34±1.50^{a}	09. 34±0.90^{a}
ALE	100	12.00±1.30^{a}	14.00±1.70^{b}	12. 34±0.50^{a}	12.34±1.40^{a}
	150	13.34±1.00^{a}	14.67±0.80^{b}	12.67±1.90^{a}	12. 67±0.90^{a}
	200	16.34±0.00^{a}	19. 34±1.00^{b}	17. 34±1.80^{a}	16. 34±1.30^{a}
	50	17.67±0.70^{b}	14. 34±1.40^{a}	15.00±1.30^{a}	15. 34±1.30^{a}
ELE	100	18.67±0.90^{b}	17. 34±2.20^{b}	16.00±0.80^{a}	15.67±2.30^{a}
	150	22.34±2.10^{b}	21.67±0.80^{b}	20. 34±1.00^{b}	18.67±1.40^{a}
	200	23.67±1.00^{b}	24.67±0.90^{b}	22. 34±1.20^{a}	21.34±1.50^{a}
Control	125	23	24	23	22

***Key:** ALE = Aqueous Leaf Extract, ELE = Ethanol Leaf Extract. Values having different superscript in the same row are considered significantly different at probability level of p<0.05.*

Stem Extracts

The antibacterial activity of *G. gynandra* stem extracts against the tested isolates is presented in Table 3. The result showed that ethanol stem extract is more effective with average zone of inhibition of 16.43 mm than aqueous stem extract which has an average zone of inhibition of 12.68mm. Based on the result, *E. coli* is more sensitive to the extract (16.04 mm), followed by *Shigella* sp (15.83 mm), *Klebsiella* sp (13.66 mm) and least activity was shown against *S. typhi* (12.70 mm). The zone of inhibition shown by Ciprofloxacin (25 mg/ml) ranged between 22 and 24 mm for the tested isolates.

Table 3: Antibacterial Activity of Stem Extracts against some Pathogenic Enteric Bacteria

		Organisms/Zones of inhibition			
Extracts	**Conc. (mg/ml)**	***E. coli***	***Shigella***	***Klebsiella***	***S. typhi***
	50	10.34±0.50^{b}	11.00±1.30^{b}	08.67±1.00^{a}	08.34±1.20^{a}
ASE	100	11.00±1.70^{a}	11.67±1.30^{a}	13.00±0.80^{b}	10.67±0.70^{a}
	150	14.67±1.20^{a}	15.34±0.80^{a}	13.67±0.90^{b}	12.67±0.50^{b}
	200	16.34±1.70^{b}	17.34±2.10^{b}	14.67±1.30^{a}	13.67±0.80^{a}
	50	17.00±0.00^{c}	16.00±1.40^{c}	10.34±0.00^{a}	12.00±1.00^{a}
ESE	100	19..00±1.00^{c}	16.34±0.90^{b}	12.34±0.80^{a}	12,67±1.30^{a}
	150	19.67±0.50^{c}	17.67±0.70^{b}	18.00±2.20^{b}	14.34±0.60^{a}
	200	20.34±0.70^{b}	21.34±1.00^{b}	18.67±1.30^{a}	17.34±0.80^{a}
Control	125	23	24	23	22

***Key:** ASE = Aqueous Stem Extract, ESE = Ethanol Stem Extract. Values having different superscript in the same row are considered significantly different at probability level of p<0.05.*

Discussion

The presence of phytochemicals in the leaf and stem extracts (Table 1) showed that the extracts possess antibacterial properties. These results are in agreement with similar study by Thenmozhi *et al*. [17] on morpho-anatomical and preliminary phytochemical studies of leaves of *Gynandropsis pentaphylla* L. in which their result revealed the presence of phenolic compounds, flavonoids alkaloid, tannin, glycoside, carbohydrate, saponins, protein and amino acids, and steroids. The result of this study is also inconformity with that of Ajaiyeoba [10] who investigated the phytochemical and antimicrobial studies of *Gynandropsis gynandra* extracts. His results showed that *Gynandropsis* gynandra extracts possessed alkaloids, glycosides, steroidal nucleus and reducing sugar etc. Phytochemical screening by Osama and Awdelkarim [18] revealed that Phenols, Tannins, Flavonoids, Coumarins, Anthraquinones, Alkaloids, Steroids, Saponins are present in all fractions tested for *Gynandropsis gynandra* extract, this result support to the present research. The alkaloid is known to contain antimicrobial agents which accounted for its antimicrobial activity [19]. Flavonoid is believed to contain antioxidant agents and it is reported that it reduce the oxidation of low-density lipoprotein, lower cholesterol level and triglyceride [20]. It is also expressed in plant in respond to microbial attack suggesting their antimicrobial property [21]. Saponins limit the growth and viability of cancer cell by reacting with cholesterol rich membrane of cancer cell [22]. Steroid is important pharmaceutically for production of drugs due to possession of compound showing similarities to sex hormones [23]. Phenolics are reported to possessed antioxidant property which prevents oxidative damage of cell due to present of free radical scavengers [24]. Tannins are known to have potential antiviral activity as well as anticancer agent [25].

Results showed that the activity of the extracts against the test bacteria increased with increase in the concentration with the ethanol extracts demonstrating higher activity than the aqueous extracts. This could be because the active component must be a highly polar compound. It has been observed that the more polar the solvent the higher the yield of extraction [26]. Statistical analysis of the results shows that *Shigella* sp is the most susceptible isolate with average zone of inhibition of 16.60 mm while *S. typhi* is the least susceptible with average zone of inhibition of 13.95 mm. The result also demonstrated that leaf extract is more effective than the stem extract. The result of antibacterial activity of this study is supported by the work of Ajaiyeoba, [10] who found that *Gynandropsis gynandra* extract was active against *Klebsiella* (with average zone of inhibition of 12.5 mm) and *Escherichia coli* (with average zone of inhibition of 16.6 mm). The antibacterial activity of the extracts against the isolates is due to the presence of phytochemical constituents such as alkaloid, flavonoid, tannin and saponin which found to have antibacterial agents. The extracts showed antibacterial activity against the study organisms compared with the positive control (125 mg/ml of Ciprofloxacin)

Conclusion

This study showed that leaf and stem extracts of *Gynandropsis gynandra* possessed phytochemical substances that can be used as components of new antimicrobial agents. Therefore there is need for further investigations in terms of toxicological studies and purification of active components with the view to using the plant in novel drug development. The study has also justified the traditional usage of this plant as health remedy

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