Research Article

In Vitro Qualitative and Quantitative Phytochemical Analysis and Antibacterial Activities of Ethanolic Extracts of *Anthocleista vogelii* on Some Bacteria Responsible for Wound and Enteric Infection

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ABSTRACT

The qualitative and quantitative phytochemical screening of the stem and leaf extracts of *Anthocleista vogelii* were carried out and indicated the presence of saponins, flavonoids, tannins, alkaloids, flavonoids, sterols and hydrogen cyanide (HCN), which are active components present in the plant that makes it medicinal. The quantitative phytochemical analysis showed high concentrations of hydrogen cyanide, HCN (14.95% and 5.46%), alkaloids (2.78% and 2.20%) and Tannins (2.50% and 1.28%) for leaf and stem extracts respectively. A total of 100g of the powdered leaf yielded 0.533g (5.33%) and 0.643g (6.43%) for ethanolic and aqueous extracts respectively while, the stem yielded 0.822g (8.22%) and 0.620g (6.20%) for ethanolic and aqueous extracts respectively. The antibacterial activities of plant extracts judged by their various zones of inhibition showed that both ethanolic and aqueous extracts of the leaves have good antibacterial effect on the test organisms (*Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*) when compared to the control antibiotics (Chloramphenicol). The antibacterial activity studies carried out showed that the plant extract has antibacterial properties. It showed sensitivity towards disease causing organisms like *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. This result shows that the plant *Anthocleista vogelii* could be used for herbal medicine.

Keywords: *Anthocleista vogelii,* Antibacterial, Enteric, Infection, Phytochemical, Wound.

INTRODUCTION

Plants have formed the basis of traditional systems of medicine that have been in existence for thousands of years and continue to provide mankind with new remedies. Medicinal plants have been used for centuries for human diseases because they contain compounds of therapeutic value. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind, even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.[1-3]

The acceptance of traditional medicine as an alternative form of healthcare and the development of resistance to the available antibiotics has led to widespread investigation of the antimicrobial activities of medicinal plants.[4]

Herbal medicine is readily available in our diverse vegetation, cheap and has the potential for introducing new templates into modern medicine.[5] According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs, about 80% of individuals from developing countries have used traditional medicine, which has compounds derived from medicinal plants; therefore such plants should be investigated to better understand their properties, safety and efficiency. The increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggest that in order to find active compounds, a systematic study of the medicinal plants is important.[5]

Phytochemicals are grouped into two main categories[6] namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds. [6, 7] Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidals[8], antibacterial, antifungal[9], anticonstipative[10], spasmolytic[11], antiplasmodial[12] and antioxidant[13] activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contains. Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. It is calculated that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States.[14] In addition, antibiotics are sometime associated with adverse effects including hypersensitivity, immune-suppressant and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents.

Anthocleista vogelii belongs to the family Loganiaceae. It is widely spread in tropical Africa, Cameroon, Sudan, and Sierra Leone. It is also found in Northern, Western and Eastern Nigeria particularly in swampy areas near streams and closed forest. Locally the plant is called "Kwari" in Hausa, "Apaoro" in Yoruba, "Oriweni" in Bini, "Orimi" in Benin, "Impoto idele" in Enugu dialect of Ibo language in

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Nigeria.[15] The, the shrub is believed to be endowed with antibacterial properties in therapeutic treatment of enteric ailments and wound infections. Thus, it is used as medicine for the treatment of diarrhea, dysentery and other veneral diseases.[15]

Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[16] Contrary to the synthetic drugs, antimicrobials of plant origin which is not associated with many side effects have enormous therapeutic potentials in handling many infectious diseases. The survey of medicinal plants with folkloric antibacterial properties used in Nigeria indicates that *Anthocleista vogelii* among a few others has shown very high promise.

Hence the aim of this study was to determine the phytochemical constituents and to investigate the antimicrobial properties so as to ascertain their uses in traditional medicines.

MATERIALS AND METHODS

Preparation of Crude Extracts

Aqueous Extract

One hundred grams of air-dried powder each of the leaf and stem was weighed and soaked separately in 750ml of aliquots of distilled water in a conical flask. The mixture was rocked gently to form a homogenous suspension and allowed to stand for 24hours at room temperature undisturbed. Each portion was filtered through a clean cheese cloth and later using a sterile filter paper (Whatman filter paper No. 1). The filtrates were collected in different beakers and weighed. The filtrates were evaporated to dryness using water bath at controlled temperatures. After evaporation, the beakers were reweighed and the differences in their weights before and after evaporation were calculated and record.

Ethanolic extracts

One hundred grams of leaf sample powder were soaked in 750ml aliquots of ethanol in a conical flask. The mixture was rocked gently to form a homogenous suspension and allowed to stand for 24 hours. This was then filtered through a clean cheese cloth and later with No 1 Whatman filter paper. The filtrate was then evaporated to dryness in a water both to obtain ethanolic extract.

Phytochemical Screening

Qualitative analysis of phytochemicals

Phytochemical tests were carried out first to establish the presence or otherwise of some specific phytochemicals. The chemical tests were carried out with the standard specimens using standard procedures to identify the constituents as described by.[17,18] The seeds were screened for alkaloids, saponins, tannins, flavonoids, phenols, sterols and hydrogen cyanide (HCN).

Quantitative analysis

Alkaloids

5 g of the plant sample was prepared in a beaker and 200 ml of 10% CH_3CO_2H in C_2H_5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture was then filtered and the extract is allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which is then dried and weighed.

Flavonoids

Extracted 10 g of the plant sample with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight.[19, 20]

Saponins

20 g of each ground plant samples were put into a conical flask and 100 ml of 20% ethanol was added to the plant sample. The said sample is heated over a water bath for 4 h at about 55°C with continuous stirring. The extracted mixture is then filtered and the residue is then re-extracted again with 200 ml of 20% ethanol. The collective residues are reduced to 40 ml over a hot water bath. The concentrated is then transferred to a separating funnel and 20 ml of diethyl ether is added to the plant extract and the shaken vigorously. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. Sixty milliliter of n-Butanol was added and combined n- Butanol extract were washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.[19, 20]

Tannins

500 mg of plant sample was weighed and transferred to 50 ml flask. Then added 50 ml of distilled water and stirred for 1 h. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm wavelength within 10 min. [17, 18]

Hydrogen cyanide (HCN)

Dried ground (1 g) of *A. vogelii* leaf was weighed out separately and macerated with 50 ml of distilled water and left to stand for 24 hours. The mixture was filtered and 1ml of the filtrate was transferred out. Alkaline picrate solution (4 ml) was added on each sample, boiled for 5 minutes and allowed to cool. Absorbance was measured at 490 nm.

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Phenols

Plants sample was boiled for 15 min with 50 ml of $(CH_3CH_2)_2O$. 5 ml of the sample was pipette into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. The sample was made up to the mark and left to react for 30 min for color development and measured for 505 nm wave length using a spectrophotometer.[17, 18]

Selected Test Microorganisms

The *in vitro* screening for antibacterial properties of *Anthoclesita vogelii* was carried out using selected pathogenic microorganisms of the wound and enteric infection origin, which were pure cultures of *Escherichia coli* and *Salmonella typhi and Staphylococcus aureus* obtained from the Microbiology Laboratory of Federal Medical Center Umuahia Abia State.

Antibacterial Assay

A loopful of bacteria was collected from a 18-24 hours culture plate. It was serially diluted and dilution 10^{-2} was used for inoculation. Freshly prepared nutrient agar were poured into 9cm sterile Petri dishes, and allowed to solidify at room temperature. The solidified agar plate was bored with sterile 5mm cork borer to create 5 wells on the agar plate about 10mm deep. A loopful of bacteria suspension from the dilution 10^{-2} was streaked onto the plate. 0.1ml of 12.5mg/ml, 25mg/ml, 50mg/ml, and 100mg/ml of crude extracts was added to each of the well on the agar plate, and the antibiotic (control) was added to fifth well. The concentrations were achieved by reconstituting the crude extracts in their solvents of extraction. Chloramphenicol was used as the control. The plates were incubated for 24hours at $37^{\circ}C$. The resulting zones of inhibition in (mm) were then measured using a transparent ruler and the result was recorded as the mean value.

Statistical analysis

The experimental results were expressed as mean of two replicates.

RESULTS

Phytochemicals are plant-derived chemical compounds which are non-essential nutrients, some of which show potential health-promoting properties. The results of the phytochemical properties are shown in tables 1 and 2.

Qualitative and Quantitative analysis

Table 1 show that alkaloids, flavonoids, saponins, HCN, sterols, tannins and phenols were all present in both leaves and stem samples.

The quantitative phytochemical analysis of *Anthoclesita vogelii* of both leaf and stem extracts (Table 2), shows the various percentage composition of phytochemicals observed in the preliminary test. HCN was seen to have the highest concentration (14.95 and 5.46%) in the leaf and stem extracts respectively while sterols were seen to have the least concentration of 0.42 and 0.29% for leaf and stem extracts respectively.

Table 1: Qualitative phytochemical analysis of the plant *Anthoclesita vogelii*

Phytochemical	Leaves	Stem
Saponin	++	++
Alkaloid	++	+
Tannins	+	+
Flavonoid	+	+
Phenol	++	+
HCN	+++	++
Sterols	+	+

Table 2: Quantitative phytochemical analysis of plant *Anthoclesita vogelii*

Phytochemical	Leaves (%)	Stem (%)
Sterols	0.42	0.29
HCN	14.95	5.46
Phenol	0.19	0.12
Saponin	0.86	0.75
Tannins	2.50	1.28
Alkaloid	2.78	2.20
Flavonoid	1.48	1.80

Table 3 shows the total yields of extracts of the leaf and stem of *Anthoclesita vogelii.* The ethanolic extract of the leaf had a higher value of 0.643g (6.43%) compared to the aqueous extract 0.533g (5.33%). The aqueous extract of the stem showed a higher yield of 0.822g (8.22%) whereas the ethanolic extract had a lower yield of 0.620g (6.2%).

Table 3: Mean yield of extracts obtained from leaves and stem of *Anthocleista vogelii*

	Leaves	Stem
Aqueous extract	0.533g (5.33%)	0.822(8.22%)
Ethanol extract	0.643g (6.43%)	0.620 (6.2%)

Antibacterial Assay

Table 4 shows the inhibitory effect of ethanolic and aqueous extracts of *A. vogelii* against *Salmonella typhi.* Ethanolic and aqueous extracts at 100mg/ml had the same mean zone of inhibition (14.0mm), while at 25mg/ml; ethanolic extract had a slightly higher zone of inhibition (9.0mm) compared to aqueous extract (8.0mm). At a concentration of 12.5mg/ml; both ethanolic and aqueous extracts did not inhibit the growth of *S. typhi.*

Table 5 shows the inhibitory effect of ethanolic and aqueous extracts of *A. vogelii* against *Escherichia coli.* Ethanolic and aqueous extracts at 100mg/ml had

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different mean zones of inhibition (12.0 and 14.0mm respectively), while at 25mg/ml; ethanolic extract did not have any effect on the indicator organism, whereas, aqueous extract at the same concentration had 8.0mm zone of inhibition. At a concentration of 12.5mg/ml; both ethanolic and aqueous extracts did not inhibit the growth of *E. coli*.

Table 4: Inhibitory Effect of Ethanolic and Aqueous Extract concentration of *Anthocleista vogelii* on growth of *Salmonella typhi*

	Concentration	R_1	R_2	Mean
Ethanol Extract	12.5mg/ml	-	-	-
	25mg/ml	10.0	8.0	9.0
	50mg/ml	11.0	13.0	12.0
	100mg/ml	14.0	14.0	14.0
	Control	16.0	14.0	15.0
Aqueous Extract	12.5mg/ml	-	-	-
	25mg/ml	9.0	7.0	8.0
	50mg/ml	12.0	12.0	12.0
	100mg/ml	14.0	14.0	14.0
	Control	16.0	15.0	15.5

Table 5: Inhibitory Effect of Ethanolic and Aqueous Extract concentration of *Anthocleista vogelii* on growth of *Escherichia coli*

	Concentration	R_1	R_2	Mean
Ethanolic Extract	12.5mg/ml	-	-	-
	25mg/ml	-	-	-
	50mg/ml	10.0	8.0	9.0
	100mg/ml	10.0	11.0	10.5
	Control	12.0	12.0	12.0
Aqueous Extract	12.5mg/ml	-	-	-
	25mg/ml	8.0	8.0	8.0
	50mg/ml	12.0	13.0	12.5
	100mg/ml	14.0	14.0	14.0
	Control	15.0	15.0	15.0

Table 6: Inhibitory Effect of Ethanolic and Aqueous Extract concentration of *Anthocleista vogelii* on growth of *Staphylococcus aureus*

	Concentration	R_1	R_2	Mean
Aqueous Extract	12.5mg/ml	-	-	-
	25mg/ml	7.0	5.0	6.0
	50mg/ml	7.0	9.0	8.0
	100mg/ml	11.0	13.0	12.0
	Control	14.0	16.0	15.0
Ethanol Extract	12.5mg/ml	-	-	-
	25mg/ml	10.0	8.0	9.0
	50mg/ml	12.0	12.0	12.0
	100mg/ml	13.0	13.0	13.0
	Control	15.0	15.0	15.0

Table 6 shows the inhibitory effect of ethanolic and aqueous extracts of *A. vogelii* against *Staphylococcus aureus*. At 12.5mg/ml; both ethanolic and aqueous extracts did not inhibit the growth of *S. aureus*. Ethanolic and aqueous extracts at 100mg/ml had different mean zones of inhibition (12.0 and 13.0mm respectively), while at 25mg/ml; ethanolic extract and aqueous extract showed clear zones of inhibition (6.0 and 9.0mm respectively).

DISCUSSION

In this study, the phytochemical constituents and antibacterial activities of leaf and stem plant parts of *Anthocleista vogelii* was evaluated. The qualitative and quantitative phytochemical screening of the leaf and stem of *A. vogelii* revealed that it contains saponins, flavonoids, tannins, steroids, Alkaloids, HCN as shown in Table 1. Similar results were shown by. [21] Saponins, alkaloids, steroid and flavonoids have been discovered to be starting materials for most synthetic drugs. The Alkaloids are specifically used as analgesics, stimulants, anesthetic and antibacterial. [22]

Meanwhile, quantitatively, the phytochemical screening test (Table 2) showed that tannin (stem: 2.50%, leaf: 1.28%), HCN (leaf: 14.95%, stem: 5.46%) and alkaloid (leaf: 2.78%, stem: 2.20%) had high concentrations compared to the other phytochemicals, which is in agreement with[23] that showed the presence and in high amount alkaloids and tannins in leaves of *A. vogelii*.

The solvent extraction of leaves of *A. vogelii* using ethanol and water as solvents yielded extracts which weighed 0.533g (5.33%) and 0.643g (6.43%) respectively, whereas, the stem had 0.822g (8.22%) and 0.620g (6.20%) yield for ethanolic and aqueous extracts respectively as shown in Table 3. The percentage yield showed that ethanolic extraction of the leaves yielded more than aqueous extraction. While the reverse was the case in stem extracts as aqueous extracts showed more yield compared to ethanolic extracts.

Antibacterial properties of extracts of *Anthoclesita vogelii* using different extraction solvents against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* were investigated (Tables 4-6). Ethanolic extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 9.0, 12.0 and 14.0 mm respectively); while aqueous extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 8.0, 12.0 and 14.0 mm respectively) against *S. typhi*. This result showed similarity in the antibacterial activity of both extracts. More so, Ethanolic extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 0.0, 9.0 and 10.5 mm respectively); while aqueous extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 8.0, 12.5 and 14.0 mm respectively) against *E. coli*. Furthermore, Ethanolic extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 6.0, 8.0 and 12.0 mm respectively); while

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aqueous extracts of *A. vogelii* at 12.5, 25.0, 50.0 and 100mg/ml showed zones of inhibition (0.0, 9.0, 12.0 and 13.0 mm respectively) against *S. aureus.* These antibacterial effects of the plant extracts were in complete agreement with[24], which showed antibacterial activities of *A. vogelii* petroleum ether extracts.

These results show that aqueous and ethanolic extracts of leaves and stem of *A. vogelii* could be used in checkmating infections caused by some pathogenic bacteria and thus could be used in the treatment of wound and enteric infections.

CONCLUSION

The qualitative and quantitative phytochemical screening of the leaf and stem ethanolic and aqueous extracts of *Anthocleista vogelii*, indicated the presence of active ingredients; Saponins, Flavonoids, Tannins, HCN, Steroids, Alkaloids and Phenols. These extracts were tested with cultured disease causing organisms and their various sensitivities observed at different concentrations. Extracts showed a good sensitivity towards these organisms. Although full characterization and identification of these present active ingredients by modern and sophisticated techniques like spectroscopic methods were not delved into, results from this work give a good clue to the use of the plant for herbal purposes. This piece of work has also puts this plant forward to be harnessed as a product for developing modern drugs for the cure of some diseases, as it is currently used locally by traditional healers/herbalists to treat stomach upset, dysentery and diarrhea due to food poisoning and in treating wound infections.

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