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Comparative Evaluation of Phytochemicals and Antibacterial Activity of the Roots and Leaves of *Anthocleista vogelii* on Some Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, OEE and UOE designed the study. Author UOE performed the statistical analysis. Authors RUBE, UOE, RSJ and AOO wrote the protocol and wrote the first draft of the manuscript. Authors RUBE and OEE managed the analyses of the study. Authors RUBE, UOE, RSJ and AOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Anthocleista vogelii have been shown to possess a number of medicinal properties. The primary aim of this study was to evaluate the roots and leaves of *A. vogelii* for phytochemicals using crude qualitative analysis and gas chromatography-mass spectrophotometer (GC-MS) techniques, in addition to the antibacterial activity of its aqueous and ethanolic extracts against four clinical isolates namely *Staphylococcus aureus*, *Esherichia coli, Proteus species* and *Pseudomonas aeruginosa*. Isolation of clinical isolates, biochemical tests, antimicrobial sensitivity and screening for phytochemicals were all carried out using standard methodologies. Resulting replicate mean readings were analysed using analysis of variance and student t-test. The results of the crude screening showed that the leaves and roots contained alkaloids, glycosides, saponins, flavonoids,

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reducing compounds and polyphenols. However, GC-MS analysis revealed the presence of 13 and 14 phytochemicals in the leaves and root, respectively. In addition to terpenens, phytosterol, oxalate, steroid, tannin, phenols, saponins, alkaloids, anthocyanides, flavonoids, phytate, cardiac glycoside and cyanogenic glycoside found in the roots, the leaves also had coumarin. Alkaloid (22.6 conc. units) was the most abundant phytochemical in root but third most abundant in the leaves (15.6 conc. units). In the leaves, the most abundant phytochemical was phytate (20.4 conc. units) while the second was terpenes (20.7 conc. units). The results of the antibacterial sensitivity revealed varying activities against the test clinical isolates. For the leaves, the highest zone of inhibition of 18.00±0.00 mm was obtained with 100 mg/ml ethanolic extract against *Proteus species* while the least zone of 11.51±0.02 mm was recorded against *E. coli*. However, the highest zone of inhibition was 32.67±0.67 mm with 200 mg/ml of the aqueous extract against *Proteus* species for the root. Based on the findings of this study, the leaves and root of *A. vogelii* are rich in phytochemicals with promising antibacterial potentials that is worth exploiting further.

Keywords: Anthocleista vogelii; phytochemicals; clinical isolates; antibacterial; GC-MS.

1. INTRODUCTION

The discovery of penicillin from the fungus *Penicillium notatum* in the early 1940's by Alexander Flemings brought about hope and relief for the morbidity and mortality of infectious diseases. In addition to saving lives, they have also played pivotal roles in achieving major advances in medicines and surgery. The gains of this novel discovery were almost immediately threatened by the emergence of antibiotics resistance by microorganisms. Sadly, this was rightly warned by Alexander Flemings in 1945 in this letter about future abuse of the antibiotic [1]. As rightly pointed out earlier [1], microorganisms have successfully developed resistance to all classes of antibiotics. Even the most recently introduced ceftaroline is no longer effective against *Staphylococcus* as they have already developed resistance. The challenge of antibiotic resistance is now a major global public health challenge. Despite this challenge, antibiotics production is still largely dominated by microorganism especially the genus *Streptomyces*. Studies have shown that resistance still remain an evolutionary property of target microbes and this has lead researcher to seek alternatives that are cheaper and more efficient sources of antimicrobials agents [2-3]. One of such promising alternatives is the use of medicinal plants bioactive compounds in the management of infectious diseases that cause about 17 million annual deaths [2,4].

Over the past few decades, there has been a renaissance of interest in medicinal plants in the treatment and management of infectious diseases for which existing drugs are facing resistance [3-4]. This interest is anchored on a number of reasons such as being safe, less expensive, readily available and effective [5]. Medicinal plants have a promising future because there are about half a million plant species around the world of most of which are yet to be unexploited [6-7]. Their potential is based on the phytochemicals present in their leaves, barks, roots and fruits that are capable of been exploited as lead compounds in the development of new drugs [8]. These bioactive compounds belong to one of the many phytochemical groups such as alkaloids, saponins, terpenoids, tannins, flavonoids, steroids, essential oils, quninones and have been found to be present in a number of medicinal plants [9-11]. *Anthocleista vogelii* have been shown by studies to possess a number of interesting properties including antibacterial and antidiabetic properties [5].

Locally called Benin rope, *A. vogelii* and other *Anthocleista* species are trees and shrub-like plants that belong to the Gentianaceae family and is well distributed throughout the tropical regions of Africa [11]. The traditional medicinal use of *Anthocleista* species is in the treatment of stomach ache, fever, constipation, inflammatory diseases, diabetes and wounds [5]. The genus *Anthocleista* has other uses apart from medicinal, and these include making dyes, stains, inks, tattoos and mordants [5]. Although a number of studies have shown that the genus possesses a number of properties such as antidiabetic and antimicrobial activity against causative agents of typhoid, candidiasis and other mycosis, few studies exist that have examined the antimicrobial activity of *A. vogelii* against clinical isolates [5]. The aim of this study was therefore to comparatively evaluate the antimicrobial activities of the leaves and roots of

A. vogelii against clinical isolates in addition to phytochemical screening.

2. MATERIALS AND METHODS

2.1 Collection of Study Plant

The leaves and roots of *A. vogelii* used in this study were collected in July, 2017 with the help of villagers from the Obong University community, Obong Ntak, Etim Ekpo LGA, Akwa Ibom State, Nigeria. They were identified by Mr. Frank Okpopoye of the University of Calabar Botanical Garden, Calabar, Cross River State. The plants were then transported immediately to the laboratory for further analysis [9-10]. Voucher numbers of the collected plant samples were OU004 and OU005 for the leaves and roots, respectively.

2.2 Collection and Identification of Clinical Isolates

The clinical isolates used in this study were obtained from the Microbiology laboratory of the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State and were characterised using morphological and biochemical tests as previously described [12-13]. These included Gram staining, motility test, and biochemical tests that included catalase, citrate, coagulase, indole, oxidase and sugar fermentation, urease, methyl red, and Voges proskauer.

2.3 Preparation of Leaves Extracts

The freshly harvested leaves and roots were allowed to air dry and chopped into smaller pieces and then oven dried for 2 hours at 60^{o}C. After drying, the leaves were then grounded into a powder using a mortar and pestle, and stored in universal bottles at room temperature away from moisture. The aqueous and ethanolic extracts were then prepared as previously described [9-10]. Briefly, 10g of the leaves powder were extracted in 100 ml of distilled water, and100 ml of 90% ethanol, respectively. These were then allowed to stand for 72 hours and then heated in a water bath at 70^{o}C to allow the solvents to evaporate resulting in the crude extracts that were slurry in viscosity. These were then stored at 4^{o}C in a refrigerator with aluminium foil wrapped around the sample bottles containing it until required for use.

2.4 Phytochemical Screening

The various extracts of the leaves and roots were screened for the presence of phytochemicals as previously described by [14] but with some little modifications [15-17].

2.5 Quantification of Phytochemicals

2.5.1 Tannin, saponin, alkaloid and flavonoid

Tannin content of the samples was quantified using the Folin Denis Colorimetric method (Kirk and Sawyer, 1989). Saponin was quantified using the double solvent extraction gravimetric method while the alkaline precipitation gravimetric method was used for alkaloid. Flavonoid was quantified using the acid reflux and ethyl acetate method [18].

2.5.2 Reducing sugar, glycosides and polyphenol

Reducing sugar was estimated using Benedict's quantitative test and the formular of AOAC (2002). Glycoside was estimated using the method described previously [19]. Polyphenol content of the sample was determined using the spectrophotometric method described [20].

2.6 Antibacterial Sensitivity Test

The antibacterial sensitivity test was carried out using agar disk diffusion methods previously described [21]. Briefly, a manual borer was used to obtain disks of about 5mm of sensitivity disk from Whatman filter paper 1. The discs were heated in a hot air oven for 30minutes at 60^{o}C to sterilise them. After sterilisation, these discs were soaked in each of the extracts (aqueous and ethanolic) concentrations (50, 100, 200 mg/ml) for 15 minutes and were gently placed on Nutrient Agar plates inoculated with the test organisms. The plates were then incubated at 37^{o}C overnight. Following incubation, the diameters of the zones of inhibitions were measured in millimetre.

2.7 GC-MS Analysis

This was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold - Perkin Elmer Turbomass 5.1 spectrometer. All other operating conditions were set according to manufacturer's instructions.

2.8 Statistical Analysis

Replicate readings obtained were analyzed for significance using Analysis of Variance (ANOVA)

at 95% confidence level. All the analyses were done using Microsoft Excel 2007 Version.

3. RESULTS

The results of the study are presented in Tables 1 to 5. Table 1 shows the results of the crude phytochemical screening of the roots and leaves. Tannin was the only phytochemical present in leaves of the plant but not in the roots. Glycosides, saponins, flavonoids, reducing compounds and polyphenol were present in both leaves and roots of *A. vogelii*. Ethanolic extract showed more abundance of the phytochemicals than the aqueous extract. Reducing compounds and polyphenol were the most abundant phytochemicals in both extracts. Phlobatannins, anthraquinones and hydroxymethyl anthraquinones were all not detected in the leaves and roots of our study plant.

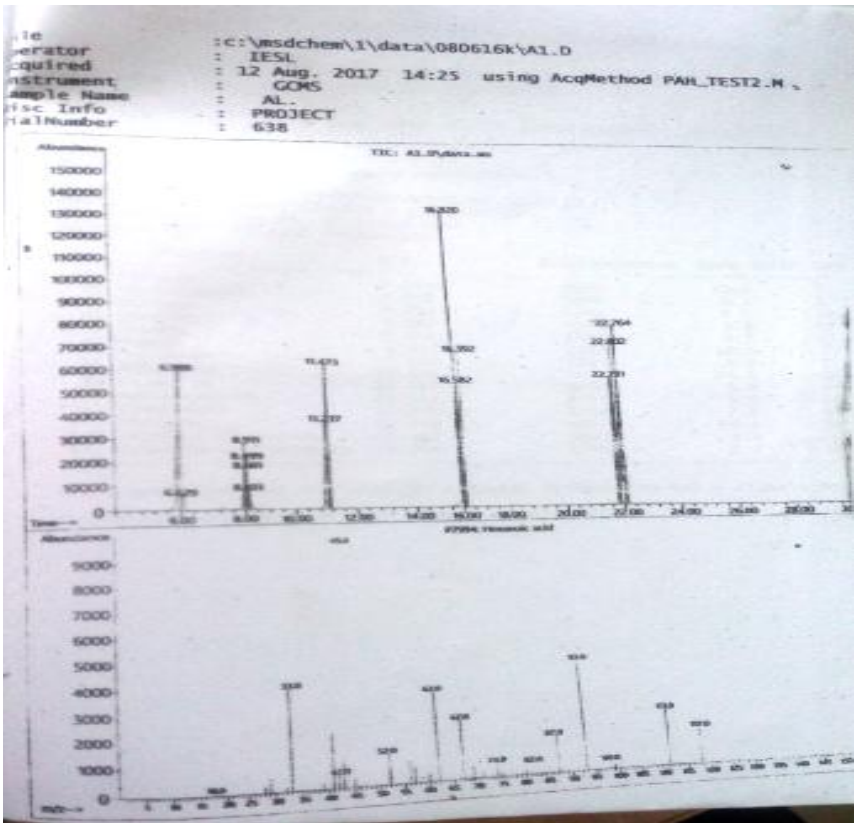

Fig. 1. GC-MS chromatogram of the roots and leave extracts. That of the leaves is presented on top and that of the leaves below

Table 1. Preliminary phytochemical screening of the leaves and roots of *A. vogelii*

Parameters	Leaves		Roots	
	Ethanolic	Aqueous	Ethanolic	Aqueous
Alkaloids	++	+	+	+
Glycosides	+	+	++	+
Saponins	++	+	++	+
Tannins	+	+	-	-
Flavonoids	++	+	+	++
Reducing compounds	++	+	+++	+
Polyphenol	+++	+	+++	++
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-
Hydroxymethyl anthraquinones	-	-	-	-

Keys: + = present, ++ = present in excess, +++ = present in much excess and - = absent

The result of the quantification of the phytochemicals is presented in Table 2. Analysis of variance showed significance ($p < 0.05$). The phytochemicals estimated were those that gave positive results from the crude preliminary screening. From the results, the most abundant phytochemical in the leaves was reducing compounds while it was polyphenol in the roots. The order of abundance was reducing compounds > flavonoids > polyphenol > tannins > glycosides > alkaloids > saponins in the leaves while it was polyphenol > flavonoids > reducing compounds > saponins > glycosides > alkaloids in the roots.

The result of the quantification of the phytochemicals is presented in Table 2. Analysis of variance showed significance ($p < 0.05$). The phytochemicals estimated were those that gave positive results from the preliminary screening. From the results, the abundant phytochemical in the leaves was reducing compounds while it was polyphenol in the roots. The order of abundance was reducing compounds > flavonoids > polyphenol > tannins > glycosides > alkaloids > saponins in the leaves while it was polyphenol > flavonoids > reducing compounds > saponins > glycosides > alkaloids in the roots.

The results of the antimicrobial of the leaves and roots aqueous and ethanolic extracts of our study plant are presented in Tables 3 and 4. The results show various degrees of sensitivity amongst the test clinical isolates. Table 3 shows the antimicrobial activity of the leaves of the study plant. The ethanolic extracts of the leaves showed more antimicrobial activity than the aqueous extracts. The highest zone of inhibition of 18.00±0.00 was obtained with 200 mg/l ethanolic extract against *Proteus species* while the least zone of 11.51±0.02 mm against *E. coli*.

Table 4 shows the antimicrobial activity of the root of the study plant. The least concentration of 50mg/ml was showed the least of antimicrobial activity. Consistently, the aqueous extracts showed much better and consistent activity with increasing concentration of test extracts. The highest zone of inhibition was 32.67±0.67 with 200mg/l against *Proteus* species. The second most sensitive isolates with the aqueous isolate was *S. aureus* which gave an inhibition of 20.00±0.01 with 200 mg/ ml. The least zone was obtained with 100 mg/ml against *E. coli*.

Table 2. Crude quantitative estimation of the phytochemical in leaves and roots of *A. vogelii* (Mean±SD)

Phytochemicals	Leaves (%)	Roots (%)
Alkaloids	1.35±0.01[a]	1.14±0.02[a]
Glycosides	1.80±0.10	1.47±0.01
Saponins	0.21±0.10	1.40±0.10
Tannins	4.48±0.02	ND
Flavonoids	9.28±0.02	9.34±0.01
Reducing compounds	9.80±0.10	4.57±0.01
Polyphenol	1.08±0.02	10.48±0.02

Analysis of variance showed significance (Mean±SD) at $p < 0.05$ as represented by the superscript ([a]) across all the phytochemicals. ND = Not detected. Student t test did not show any significant difference between the leaves and the roots ($p > 0.05$)

Fig. 1 shows the results of the chromatogram of the GC-MS. Table 5 shows the results of the interpreted GC- MS analysis carried out on the roots and leaves of *A. vogelii*. The results revealed a total of 13 and 14 phytochemical components in the leaves and roots, respectively alongside their concentrations. These include terpenens, phytosterol, oxalate, steroid, tannin, phenols, saponins, alkaloids, anthocyanides, flavonoids, phytate, cardiac glycoside and cyanogenic glycoside for the roots. The phytochemicals for the leaves were same except that in addition to those found in the roots, coumarin was also detected. Alkaloid was the most abundant phytochemical in root but third most abundant in the roots. In the leaves, the most abundant phytochemical was phytate while the second was terpenes.

Table 3. Antimicrobial activity of leaves extract of *A. vogelii*

Isolates	Ethanol (mm)			Aqueous (mm)		
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	-	11.51±0.02	13.33±1.52	-	-	14.33±2.3
S. aureus	13.67±2.52	10.00±0.00	13.00±0.01	-	-	11.33±0.57
Proteus sp.	17.50±2.50	17.00±0.00	18.00±0.00	16.67±2.89	14.33±0.57	14.67±2.08
Pseudomonas sp.	11.67±0.57	15.33±1.52	16.00±0.00	-	-	15.20±0.21

Key: - = no antimicrobial activity

Table 4. Antimicrobial activity of roots extract of *A. vogelii*

Isolates	Ethanol (mm)			Aqueous (mm)		
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	11.67±1.57	13.67±1.53	11.67±1.57	12.33±0.55	12.00±0.00	16.00±0.00
S. aureus	-	15.33±2.08	13.00±2.00	14.00±1.73	18.00±1.00	20.00±0.01
Proteus sp.	-	12.00±0.00	13.33±0.01	14.00±1.73	9.67±2.50	32.67±0.67
Pseudomonas sp.	-	13.33±2.08	15.00±1.00	-	15.64±0.02	17.67±2.08

Key: - = no antimicrobial activity

Table 5. GC-MS analysis of the phytochemical components of the ethanolic leaves and roots of *A. vogelii*

Compounds	Leaves (conc. units)	Roots (conc. units)
Terpenes	18.6	20.7
Phytosterol	8.2	8.2
Oxalate	1.5	3.1
Steroid	5.9	5.9
Tannin	8.3	15.3
Phenol	18.5	10.5
Saponin	3.7	1.5
Alkaloid	15.6	22.6
Coumarin	3.2	-
Anthocyanins	5.8	8.3
Flavonoid	2.5	0.8
Phytate	20.4	8.5
Cardiac glycoside	5.3	7.4
Cyanogenic glycosides	2.7	5.3

4. DISCUSSION

The results of the study show that the ethanolic and aqueous extract of the leaves is rich in phytochemicals such as alkaloids, glycosides saponins, tannins, flavonoids, reducing compounds, and polyphenols. Our findings agree with those of a previous study [22] that showed the presence of total polyphenol, alkaloids, terpenes and steroids in the stem and leaves of *Anthocleista schweinfurthii* but anthocyanins, leucoanthocyanins, flavonoids, garlic and catechic tannins, coumarins, quinones and saponins were absent. However, in our study, phlobatannins, anthraquinones and hydroxymethyl anthraquinones were absent. Ojiake and Okoye [23] showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, saponins and reducing compounds and these components are also present in leaves of our study plant.

On quantitative analysis, the most abundant was reducing compounds followed by flavonoids while polyphenol was the least abundant. Ojiake and Okoye [23] showed that on quantification, the most abundant phytochemical in their study was saponins with 4% which was higher than our saponin concentration of 0.2%. Akinyemi and Ogundare [24] in their study showed the presence of tannins, saponins, flavonoids, steroid, terpenoids and cardiac glycosides. Although the present study did not evaluate the leave extract for steroids and terpenoids, the rest were also detected in our study. Jegede [25] evaluated the leaves and stem bark of *A. vogelii*, and found five phytochemicals which were phenols, sterols, terpenes, alkaloids, flavonoids and saponins and also found in our study. According to Ngbolua et al. [22] in their study qualitative screening test for the phytochemical showed the presence of total polypenols, alkaloids, terpenes and steroids in the stem and leaves of *A. schweinfurthii.* However, they did not detect the presence of anthraquinones, saponins, and tannins. Eze and Omeh et al. [26] in their qualitative and quantitative phytochemical screening of the stem and leaf extracts of *Anthocleista vogelii* found the presence of saponins, flavonoids, tannins, alkaloids, flavonoids, sterols and hydrogen cyanide (HCN) and these are active components present in the plant that makes it medicinal.

When compared with the crude extraction method, GC-MS was far better and more sensitive than crude screening and estimation in quantitative and quantification screening of phytochemicals.

The results of the antibacterial activity showed that the extracts showed varying degrees of activity against the test isolates used in this study. The aqueous extracts of the root showed much higher activity with increasing concentration of test extracts. The highest zone of inhibition was 32.67±0.67 mm with 200 mg/ml against *Proteus* species. Musa et al. [27] showed that the ethanolic, aqueous and chloroform extracts of the leaves of *A. vogelii* showed inhibition against *E. coli, S. typhi* and *S. aureus*. The recorded an inhibition ranges of ≤10 mm which was less than our findings. The antibacterial activity shown in our study by the extracts was further confirmed by Eze et al. [26] who reported promising antibacterial effect on the test organisms (*Salmonella typhi, Escherichia coli* and *Staphylococcus aureus*) when compared to control antibiotics (Chloramphenicol) [24]. Furthermore, the minimum inhibitory concentrations (MICs) of the extracts ranged between 10 mg/ml and 40 mg/ml with *Anthocleista djalonens*, a close and related species to our study plant [24]. Ojiako and Okoye [23] reported inhibitions for *Staphylococcus aureus* of 10.17 mm, *Escherichia coli* 13.30 mm and *Candida albicans* 14.97 mm for ethanolic extract and zones of 7.07 mm for *Staphylococcus aureus*, 9.23 mm for *Escherichia coli* and 12.63 mm for *Candida albicans* with aqueous extracts. These readings were all within the lower limits of obtained results for both leaves and roots extracts.

5. CONCLUSION

The findings in this study confirmed that the fact that *A. vogelii* root and leaves have an abundance of phytochemicals. GC-MS was better in capturing the phytochemicals than crude extraction techniques. Furthermore, the antibacterial activity recorded in our study against the clinical isolates by the roots and leaves further buttress the fact that the plant holds a lot of potential in the search for new and safer antimicrobials. Thus further studies are strongly advocated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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