



## **Antibacterial Properties of the Leaf Extracts of *Anthocleista djalonensis* A. Chev on Some Pathogenic Organisms**

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### **Authors' contributions:**

*All the authors have cordially supported to the work and preparation of manuscript. Author AIA have designed the entire study and protocols with interpretations of the results and prepared the first draft of the manuscript. Authors AIA and AOO managed the analyses of the study and computational work respectively. Author AIA guided in the entire research and documented the final draft of the manuscript. All the authors have read and approved the final manuscript.*

**Original Research Article**

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### **ABSTRACT**

**Aims:** To determine the antibacterial effect of methanol, petroleum ether and hot water extracts of *Anthocleista djalonensis* A. Chev on some pathogenic bacteria and to establish the use of the leaf extract in herbal medicine.

**Study Design:** *In vitro* assay of antibacterial properties.

**Place and Duration of Study:** Department of Microbiology, Federal University of Technology, Akure, Nigeria, between November 2011 and January 2012.

**Methodology:** Collection of bacterial isolates; preparation of plant extracts; phytochemical screening; *in vitro* susceptibility test (agar well diffusion assay); minimum inhibitory concentration; antibiotics sensitivity test (disc diffusion assay); rate of killing of plant extracts; sodium and potassium ion leakage.

**Results:** The results of the phytochemical screening showed the presence of tannins, saponins, flavonoids, steroid, terpenoid and cardiac glycosides. All the leaf extracts inhibited all the test organisms except *Escherichia coli* which was not inhibited by petroleum ether extract. The methanol extract had the highest effect on the test organisms. The minimum inhibitory concentrations (MICs) of the extracts ranged between

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10 mg/mL and 40 mg/mL. The result of the antibacterial activity of the leaf extracts compared favourably with the activity of standard antibiotics. It was observed that the number of bacterial cells was decreasing with increase in time of interaction between the extracts and the bacterial cells at a concentration 50 mg/mL of the extracts. There was also increase in the number of sodium and potassium ion leaked from the bacterial cells by the leaf extracts.

**Conclusion:** The results of the study indicate the antibacterial potential of *Anthocleista djalensis* A. Chev which may be a source of new bioactive compounds for drug development. The results obtained also establish the use of the plant in traditional phytomedicine for the diseases caused by the microorganisms.

**Keywords:** Antibacterial; phytochemical screening; rate of killing; ion leakage.

## 1. INTRODUCTION

The increasing rate of infection by antibiotics-resistant microorganisms had caused worldwide concern which has led to a rising interest in the research for natural products from plants for the discovery of new antimicrobials. Many of these plants have been known to synthesize active secondary metabolites [1]. Santo et al. remarked that the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs [2]. Therefore, plant materials remain an important resource to combat serious diseases in the world to cover the basic health needs in the developing countries.

Infections have increased, within the recent years to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem [3]. Antimicrobial resistance on enteric pathogen is of great public health concern in the developing world. Researchers have also reported an increasingly wide spread use of antibiotics in food and animals contributing to high dissemination of resistant enteric infections to humans [4].

*Anthocleista djalensis* A. Chev belongs to the family Gentianaceae and the Yoruba people of South west Nigeria refers to it as “Ewe Shapo” [5]. The tree grows up to 15 m tall; bole up to 40 cm in diameter; twigs sometimes with 2 erect spines or small cushions above the leaf axils [6]. The Genus *Anthocleista* comprises 14 species [7]; the West Africa species have the same vernacular names (Cabbage tree) and are used by local practitioners for the same medicinal purpose. Several members of the genus are used for similar medicinal purposes. It is very difficult to differentiate between the dried bark of the different species [8]. Some of the ethno-medical uses of the extract of *Anthocleista djalensis* leaves, roots and stem bark include treatment of wound, constipation, diarrhoea, dysentery, abdominal pain [9], hepatitis, jaundice, cirrhosis, fungal skin infection, filarial worm infections, acute inflammation and boils on skin [10].

In recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem [3]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [11,12]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds [13].

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Bacterial Isolates

The bacterial isolates used were collected from the Microbiology Laboratory, Obafemi Awolowo University Teaching Hospital complex, Ile-Ife, Osun State and they include: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*.

### 2.2 Standardization of the Test Organisms

A loop full of test organism was inoculated on nutrient broth and incubated for 24 h. Exactly 0.2 ml from the 24 h culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3- 5h to standardize the culture to 0.5 McFarland standards ( $10^6$ cfu/ml) before use according to the method of Oyeleke et al. [14].

### 2.3 Collection and Extraction of Plant Material

The plant leaf was collected from the forest and wild life reserve of the Federal University of Technology, Akure, Nigeria and was authenticated by Prof. Akinyele of the Department of Crop Science and Pest Management, where a voucher specimen was submitted. The extraction of the plant material was done according to the method of Harbone, (1998) with slight modification. The leaves were air dried for three weeks and pulverized. Exactly 400 g of the powdered leaf were macerated separately for 72 h using methanol, petroleum ether and hot water followed by sieving with a muslin cloth and filtered with No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated *en vacuo* using rotary evaporator (Resona, Germany).

### 2.4 Phytochemical Screening

The extracts were screened for anthraquinone, alkaloid, tannin, saponin, phlobatannin, steroid, flavonoid, terpenoid and cardiac glycoside as described by Trease and Evans [16].

### 2.5 Experimental Procedure

#### 2.5.1 Phytochemical properties of the leaf extracts of *anthocleista djalensis*

The result of the phytochemical screening of the methanol, petroleum ether and hot water leaf extracts of *Anthocleista djalensis* shows the presence of tannins, saponins, flavonoids, steroid, terpenoid and cardiac glycosides

#### 2.5.2 Antibacterial activity

##### *2.5.2.1 Determination of antibacterial activities by leaf extract*

The antibacterial activity of the plant extracts was determined using agar dilution method described by Madigan et al. [17]. Exactly one milliliter of each of the standardized test organism was transferred into different sterile petri dishes. Mueller Hinton agar was then poured on these inocula and the plates swirled for even dispersion of the organism in the agar. After solidification of the agar, a 6 mm diameter cork borer was used to make wells in

to each plate and the prepared extract was introduced. The plates were incubated at 37°C for 24 h. Clear zones around the bored holes are indicative of the inhibition of the organisms by the extract. The activity indices (AI) were calculated as the division of zone of inhibition of the extract by that of the standard drug- Gentamycin [18].

#### 2.5.2.2 Determination of the minimum inhibitory concentration

This was carried out using the agar dilution method described by Doughari et al. [19]. Different concentrations of the extracts (30 mg/mL, 25 mg/mL, 12.5 mg/mL, 10 mg/mL, 6.25 mg/mL, and 5mg/mL) were used. Plates were incubated at 37°C for 24 h, after which they were observed for clear zones around the wells, indicating inhibition. The concentration below in which there was no zone was noted as minimum inhibitory concentration (MIC).

#### 2.5.2.3 Antibiotics sensitivity test

The Kirby - Bauer test also known as disc diffusion method was used to determine the effect of standard antibiotics on the bacterial isolates as described by Willey *et al.*, [20]. Standard antibiotics disc were placed aseptically on agar plates already seeded with the test organisms using sterile forceps. The plates were then incubated at 37°C for 24 hours. Zones of inhibition around the antibiotics disc were measured in millimeters.

#### 2.5.2.4 Determination of the rate of killing of leaf extract

This was done according to the method of Ogundare and Akinyemi [21] to determine the rate of killing of the test organisms by the extracts. Exactly 5 ml of the standardized broth culture was introduced into 5mls of 50mg/mL concentration of each of the extracts. The suspension was mixed and then plated using pour plate method at 0, 1, 2, 4, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24h. The plates were incubated at 37°C for 24 h after which observation was made for microbial growth. The numbers of colonies were counted using the digital colony counter.

#### 2.5.2.5 Determination of sodium and potassium ion leakage

Exactly 0.5 mL each of the standardized organism was added to 4.5 mL of the prepared concentration of the leaf extracts and then incubated for 18 hours. The solution was centrifuged at 7 000 revolution per minute (r. p. m.) and the supernatant analyzed using a flame photometer at 589 nm and 766 nm for sodium and potassium ion leakage respectively. The sodium and potassium ion leakage was determined using the method of Hugo and Russel [22].

### 3. RESULT AND DISCUSSION

*Anthocleista djalensis* leaves are extensively used to treat diarrhoea, wound, abdominal pain, boils on skin and fungal skin infection. The result of the phytochemical screening of the methanol, petroleum ether and hot water leaf extracts of *Anthocleista djalensis* shows the presence of tannins, saponins, flavonoids, steroid, terpenoid and cardiac glycosides which may be attributed to different solvents used in the extraction as reported by Srinivasan et al. [23,24] and probably account for the antibacterial activity of the extracts may be due to the presence of metabolites revealed in the phytochemical screening, which possess pharmacological activities responsible for the use of plants in traditional phytomedicine to treat diseases caused by pathogenic microorganisms [9].

The result of the antibacterial activities of the leaf extracts as shown in Table 1 reveals that the highest inhibition by the methanol extract was recorded with *Pseudomonas aeruginosa* with a zone of inhibition value of 9.67 mm while the least zone of inhibition value of 2.00 mm was recorded for the hot water extract on *Klebsiella pneumoniae* and *Escherichia coli*. Petroleum ether did not inhibit the growth of *Escherichia coli* out of all the test organisms while the hot water inhibited the test organisms at an inhibition zone ranging from 2.00 to 4.00 mm. The result shows that methanol extract possessed more potent antibacterial activity than the petroleum ether extract and the hot water extract. Previous studies showed that the cold water and ethanol extract of the roots have remarkable activities against some bacterial isolates [9] while the methanol extract of the leaf and root had high antimycobacterial activity [25]. The commercial antibiotics were observed to be more effective in inhibiting the test organisms as shown on Table 3. Doughari et al. [19] reported that the state of administration of an antimicrobial agent affects the effectiveness of such agent, and that antibiotics being in a refined state and plant extracts in crude state, may record higher antimicrobial activity. Also, the small molecular size possessed by antibiotics as reported by Mailard [26] aids their solubility in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism. However, the antibacterial activities of the crude leaf extracts well with standard antibiotics, which if purified may exhibit higher zones of inhibition on the test organisms and may serve as a substitute to the commercially available antibiotics to which bacteria are developing resistant.

**Table 1. Antibacterial activity of the leaf extracts of *Anthocleista djalensis***

Organisms	Zone of inhibition (mm) at the concentration of 50.0 mg/ml		
	MET	PET	HWT
<i>Staphylococcus aureus</i>	6.00 ± 0.00	7.67 ± 0.58	2.67 ± 0.58
<i>Klebsiella pneumoniae</i>	6.67 <sup>a</sup> ± 0.58	6.33 <sup>d</sup> ± 0.58	2.00 <sup>c</sup> ± 0.00b
<i>Proteus mirabilis</i>	8.67 <sup>b</sup> ± 0.58	7.67 <sup>c</sup> ± 0.58	3.33 <sup>c</sup> ± 0.58
<i>Salmonella typhi</i>	7.67 <sup>a</sup> ± 0.58	6.33 <sup>b</sup> ± 0.58	2.33 <sup>b</sup> ± 0.58
<i>Pseudomonas aeruginosa</i>	9.67 <sup>c</sup> ± 0.58	8.00 <sup>c</sup> ± 0.00	4.00 <sup>c</sup> ± 0.00
<i>Escherichia coli</i>	8.00 <sup>ab</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00	2.00 <sup>b</sup> ± 0.00

**Key:** MET- Methanol Extract, PET- Petroleum Ether Extract, HWT- Hot Water Extract

Values are mean inhibition zone (mm) ± Standard deviation of three replicate

<sup>a-d</sup> Means in the same column not sharing a common letter are significantly different ( $P \leq 0.05$ ) by Duncan's multiple range test

The minimum inhibitory concentration (MIC) tests of the leaf extracts on the test organisms as shown on Table 2 indicated that methanol and petroleum ether extracts were more active (MIC, 10-25 mg/mL) than the hot water extract (MIC, 30 mg/mL). The inhibitory effect of the leaf extracts on the test organisms is also seen from the result of the rate of killing of the organisms by the extracts (Figs. 1a, 1b). The number of organisms present at each hour declined at each hour with increased time of exposure of the test organisms to the extracts. However, a tidal effect was not exhibited by the extracts at a concentration of 50 mg/ml which may be as a result of the concentration or the crude nature of the extracts, but there was a decrease in the number of colonies over a period of 24 hours. Sodium and potassium ion were leaked by the leaf extracts from the cell of the organisms as represented in Fig. 2. Sodium ion from all isolates was leaked to a high value of 1215 ppm while potassium ion leaked to a value of 242 ppm. This implies that the extract probably induced antibacterial

effects through the leakage of intracellular materials which is evidenced in its membrane damaging action. The potassium ion did not leak as much as the sodium ion from the cells of the organisms. This may be attributed to the molecular mass of the ions, which might have aided the high number of sodium to escape the cells than the potassium ions [27]. Sodium and potassium ions have been known to affect osmotic balances in the cell and their leakages might cause cell lyses and eventual death.

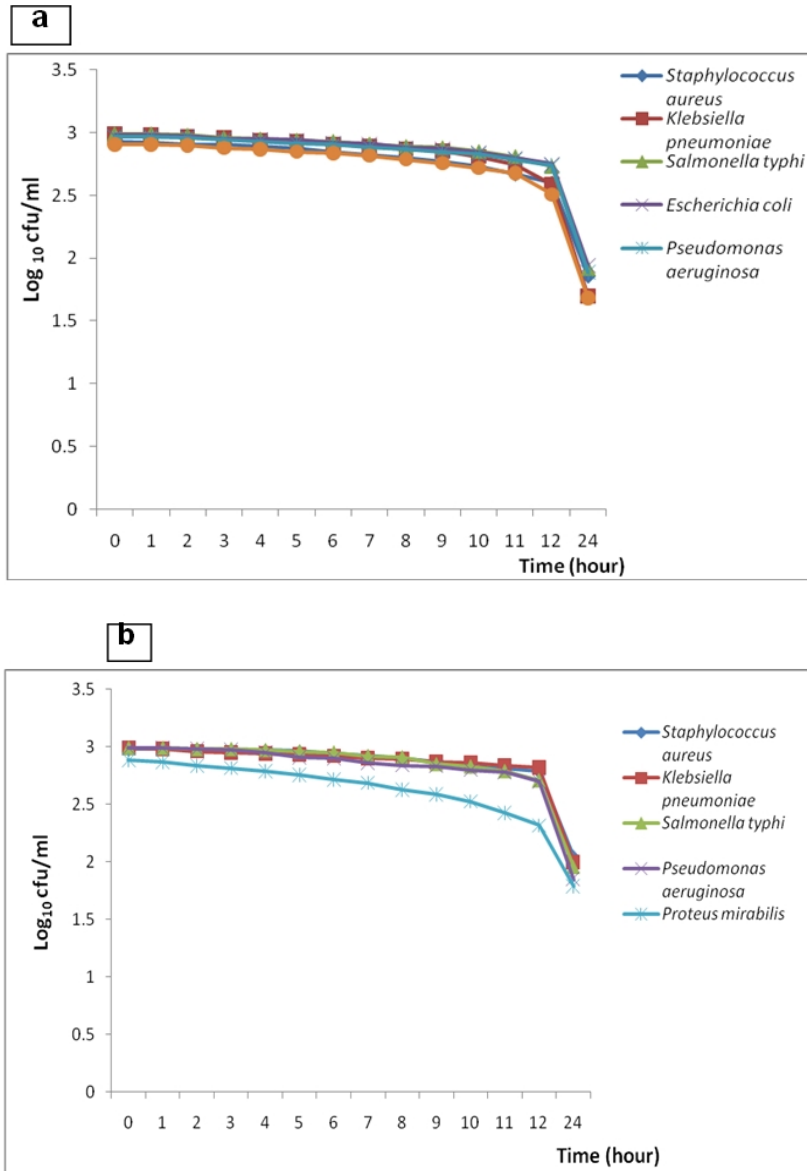


Fig. 1. Rate of killing of bacterial isolates by (a) methanol extract and (b) petroleum ether extract of extract of *Anthocleista djalensis*

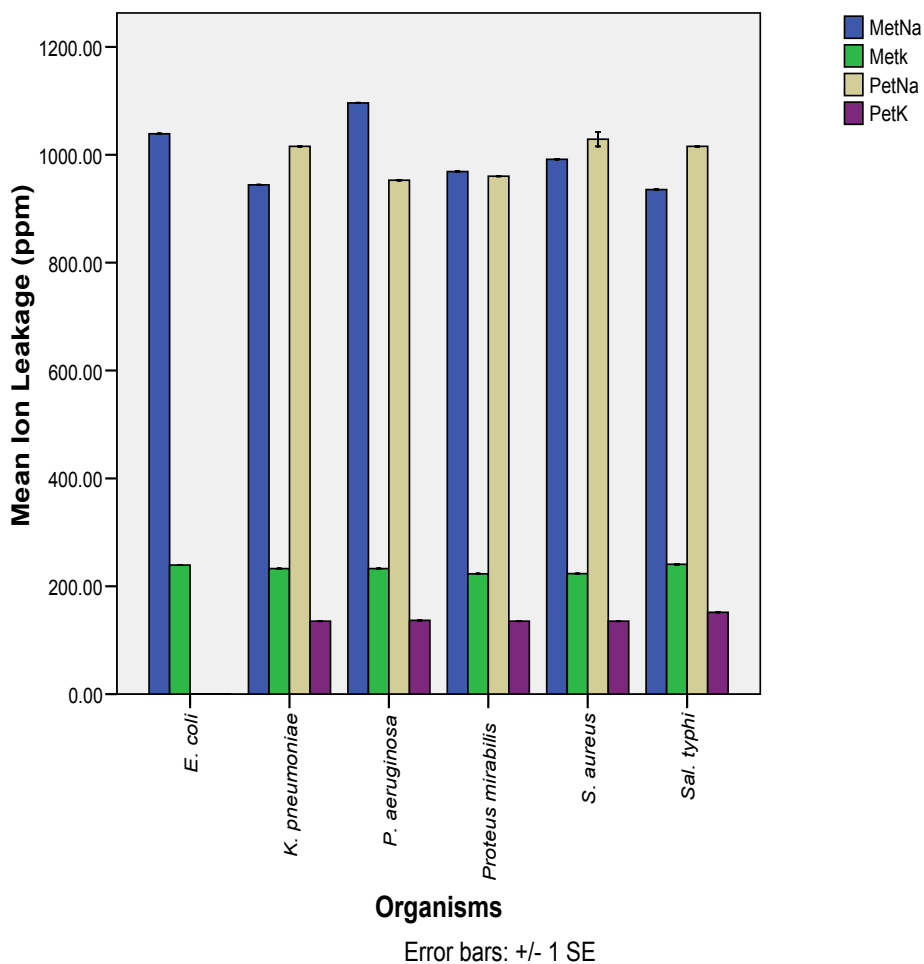


Fig. 2. Sodium and Potassium ion leakage from bacterial cells by leaf extracts

Table 2. Minimum inhibitory concentration (mg/mL) of the leaf extracts

Organisms	MET	PET	HWT
<i>Staphylococcus aureus</i>	20	10	30
<i>Klebsiella pneumoniae</i>	10	25	40
<i>Proteus mirabilis</i>	12.5	12.5	30
<i>Salmonella typhi</i>	10	12.5	30
<i>Pseudomonas aeruginosa</i>	20	12.5	30
<i>Escherichia coli</i>	12.5	20	30

Table 3. Antibiotics sensitivity test on bacterial isolates

Gram Positive Organisms	Zones of inhibition (mm)							
	COT	CXC	ERY	GEN	AUG	STR	TET	CHL
<i>Staphylococcus aureus</i>	12.67 <sup>c</sup> ±1.15	–	11.33 <sup>b</sup> ±1.15	3.67 <sup>a</sup> ±0.58	12.67 <sup>d</sup> ±1.15	10.33 <sup>d</sup> ±0.58	–	9.33 <sup>b</sup> ±1.15
Gram Negative Organisms	AUG	OFL	GEN	NAL	NIT	COT	AMX	TET
<i>Klebsiella pneumoniae</i>	–	–	16.0 <sup>d</sup> ±0.00	10.33 <sup>b</sup> ±0.58	–	6.33 <sup>b</sup> ±0.58	6.33 <sup>c</sup> ±0.58	5.00 <sup>a</sup> ±1.00
<i>Proteus mirabilis</i>	–	5.67 <sup>b</sup> ±0.58	4.33 <sup>b</sup> ±0.58	–	14.33 <sup>d</sup> ±0.58	12.00 <sup>d</sup> ±0.00	4.33 <sup>c</sup> ±0.58	6.33 <sup>c</sup> ±0.58
<i>Salmonella typhi</i>	6.33 <sup>c</sup> ±0.58	–	–	–	9.33 <sup>c</sup> ±1.15	–	–	–
<i>Pseudomonas aeruginosa</i>	–	10.67 <sup>f</sup> ±1.15	9.33 <sup>d</sup> ±1.15	–	–	–	–	–
<i>Escherichia coli</i>	–	10.33 <sup>de</sup> ±0.58	–	9.33 <sup>c</sup> ±1.15	9.33 <sup>c</sup> ±1.15	–	2.33 <sup>b</sup> ±0.58	7.33 <sup>d</sup> ±1.15

Values are mean inhibition zone (mm) ± Standard deviation of three replicate

<sup>a-d</sup> Means in the same column not sharing a common letter are significantly different ( $P \leq 0.05$ ) by Duncan's multiple range test

**Key:** COT- Cotrimazole (25µg); CXC- Cloxacillin (5µg); ERY- Erythromycin (5µg); GEN – Gentamycin (10µg); AUG- Augmentin (30µg); STR- Streptomycin (10µg); TET- Tetracycline (10µg); CHL- Chloramphenicol (10µg); OFL- Ofloxacin (5µg); NAL- Nalidixic Acid (30µg); NIT- Nitrofurantoin (200µg); AMX- Amoxicillin (30µg).



#### 4. CONCLUSION

The result showed that methanol and petroleum ether extracts exhibited considerable inhibitory activity against the test organisms as demonstrated by the diameters of the zones of inhibition. The result from this work established its use traditionally as an antimicrobial. The methanol and petroleum ether extracts of the leaf are potential sources of new antibacterial agents for the treatment of diseases caused by the pathogenic organisms. The result of this study suggest that the extracts be purified further and tested for their antibacterial potential as against the use of crude extracts. Also, further isolation and characterization of the active components contained in the leaf extracts be carried out.

The biodiversity of tropical forest plant species, coupled with the chemical diversity found within each plant leads one to the conclusion that tropical plants are perhaps the most valuable source of new bioactive chemical entities.

#### CONSENT

Not applicable.

#### ETHICAL APPROVAL

Not applicable.

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#### COMPETING INTEREST

Authors have declared that no competing interests exist.

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