Journal of Drug Design and Medicinal Chemistry
2017; 3(5): 71-76
http://www.sciencepublishinggroup.com/j/jddmc
doi: 10.11648/j.jddmc.20170305.12
ISSN: 2472-355X (Print); ISSN: 2472-3576 (Online)

In-vitro Antimicrobial Properties and Phytochemical Constituents of *Anthocleista djalonensis* Leaf Extracts

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To cite this article:

Ijeoma Solomon Okoro, Sampson Dominic Umoh, John Vershima Anyam, Manasseh Msugh-Ter Manyi, Cynthia Adegbe. *In-vitro* Antimicrobial Properties and Phytochemical Constituents of *Anthocleista djalonensis* Leaf Extracts. *Journal of Drug Design and Medicinal Chemistry*. Vol. 3, No. 5, 2017, pp. 71-76. doi: 10.11648/j.jddmc.20170305.12

Received: August 21, 2017; **Accepted**: September 18, 2017; **Published**: November 20, 2017

Abstract: This study investigated the claims of traditional practitioners in the use of *Anthocleista djalonensis* for the treatment of various diseases and infections in Benue State, Nigeria. The leaves of the plant were collected; air dried; pulverized and successively extracted using hexane, ethyl acetate, ethanol and methanol by microwave assisted method. The phytochemical analysis of the leaf extracts of *Anthocleista djalonensis* revealed the presence of glycosides, saponins, terpenes, sterols flavonoids, anthraquinones, resins and balsams in *Anthocleista djalonensis* leaf. The antimicrobial screening of the hexane, ethyl acetate, ethanol and methanol extracts were carried out on Methicillin *Resistant Staphylococcus aureus, Vancomycin Resistant enterococci, Staphylococcus aureus, Helicobacter pylori, Candida albicans, Candida krusei, Candida tropicalis, Escherichia coli, Proteus mirabilis,* and *Pseudomonas aeruginosa* using agar-well diffusion method. The antimicrobial studies showed that all the extracts exhibited activities against *Methicillin Resistant Staphylococcus aureus, Vancomycin Resistant enterococci, S. aureus, H. pylori, C. albicans, and C. krusei* with significant zones of inhibition ranging from 16 - 20 mm for hexane extract, 22 - 28 mm for ethyl acetate extract, 20 - 24 mm for ethanol extract and 20 - 23 mm for methanol extract against test microbes. *E. coli, P. aeruginosa, P. mirabilis and C. tropicalis* showed resistance to the extracts; Minimum Inhibitory Concentration (MIC) of the extracts against the stated microbes were 5 mg/mL, 5 mg/mL, 5 mg/mL, 5 mg/mL, 2.5 mg/mL and 5 mg/mL respectively for hexane extract. 1.25 mg/mL, 1.25 mg/mL, 0.62 mg/mL, 0.62 mg/mL 0.62 mg/mL and 1.25 mg/mL respectively for ethyl acetate extract; Ethanol and methanol extracts recorded 1.25 mg/mL against all the stated test microbes. The minimum bactericidal/fungicidal concentration of the extracts against *Methicillin Resistant Staphylococcus aureus, Vancomycin Resistant enterococci, S. aureus*, *H. pylori, C. albicans* and *C. krusei* ranged from 5 mg/mL to 1.25 mg/mL. The results support the use of *Anthocleista djalonensis* in traditional medicine.

Keywords: Antimicrobial, Phytochemicals, *Anthocleista djalonensis* and Traditional Medicine

1. Introduction

Increase in hazards posed by drug-resistant strains of bacteria has attracted attentions recently in the scientific community; thus demanding continuous effort to solve this. The efforts made in the isolation, concentration, purification and mass production of penicillin was followed by the development of Streptomycin, tetracycline, chloramphenicol and other agents, and their synthetic modification has been prominent in the development of new antimicrobial agents [1]. The mechanism of action of antimicrobial agents can either be by selection of toxicity, inhibition of cell membrane synthesis and function, inhibition of protein synthesis or by inhibition of nucleic acid synthesis [1]. Microorganism resistance to a

certain drug may also imply resistance to other drugs that share same mechanism of action. Such a relationship exists mainly between agents that are chemically closely related or that have a similar mode of binding or action [1]. Thus a thorough understanding of the target site (biology) and the chemistry of the agent are necessary during the development or investigation of a new agent [2]. For *in-vitro* antibacterial investigation, pH, moisture content, length of inoculums and stability of antimicrobial agents are important parameters [1, 3] to consider. In this study, the traditional use of *Anthocleista djalonensis* in treatment of microbial infections in Benue State, Nigeria was investigated.

2. Materials and Methods

Methods adopted in this work have been described in Aliyu *et al*. [4]; Desta [5]; Lalitha [3]; Tor-Anyiin *et al.* [6] and Akinyemi and Ogundare [7].

2.1. Sample Collection

Fresh leaves of *Anthocleista djalonensis* were collected in September, 2015 from Tse-gwaza village in Konshisha local government Area of Benue State and identified by Mr. Ikoyobo John Technical Officer, Department of Wild life and Range Management, College of Forestry and Fisheries, Federal University of Agriculture, Makurdi, Nigeria.

2.2. Extraction

The pulverized plant material (400 g) was successively extracted with 2000 mL of n-hexane, ethyl acetate, ethanol and methanol using micro-wave assisted extraction method. The mixture was irradiated for 30 minutes at defrost of 70-80 watts and a controlled pressure at 3 minutes intervals after which it was filtered. The filtrate was collected and concentrated using a rotary evaporator at 40°C. Concentrates were placed in a fumehood to drive off traces of solvent.

2.3. Antimicrobial Activities

Antimicrobial activities of the leaf extracts of *Anthocleista djalonensis* were determined using some pathogenic microbes in accordance with established standard procedures and conditions. The microbes were obtained from the Department of Medical Microbiology, Ahmaedu Bello University Teaching Hospital, Zaria, Nigeria.

2.3.1. Preparation of Culture Media

The extracts were screened for antimicrobial activity using the diffusion method. The Muller-Hinton Agar was used as the growth medium for the test microbes. The medium was prepared according to the manufacturer's instruction, sterilized at 121°C for 15 minutes, poured into sterile petri dishes and allowed to cool and solidify.

2.3.2. Preparation of Culture and Inoculation

A measure 0.5 mg of the extract was weighed and dissolved in 10 mL of dimethyl sulphoxide (DMSO) and a concentration of 5 mg/mL was obtained. The sterilized medium was seeded with 0.1 mL of test microbe, the inoculums of the microbe was spread evenly over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6 mm in diameter, a well was cut at the centre of each inoculated medium. 0.1 mL of the solution of the extract of the concentration of 5 mg/mL was then introduced into each well on the inoculated medium. The inoculated medium was incubated at 37°C for 24 hours, after which the plate of the medium was observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimetres.

2.3.3. Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the extracts was carried out using the broth dilution method. Muller Hinton broth was prepared, 10 mL was dispensed into test tubes and was sterile at 121°C for 15 minutes and the broth was allowed to cool. Normal saline was prepared; 10 mL was dispensed into sterile test tubes and was incubated at 37°C for 6 hours. McFarland's turbidity standard scale number 0.5 was prepared to give turbid solutions. Dilution of the test microbe was done in the normal saline until the turbidity matched that of McFarland's scale by visual comparison. At this point, the test microbe had a concentration of about $1.5x10^8$ cfu/mL. Two fold serial dilution of the extract in the sterile broth was made to obtain concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.62 mg/mL and 0.313 mg/mL.

Having obtained different concentrations of extract in the sterile broth, 0.1 mL of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37°C for 24 hours, after which the test tubes of the broth was observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration.

2.3.4. Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Muller-Hinton agar was prepared, sterilized at 121°C for 15 minutes, poured into sterile petri dishes and was allowed to cool and solidify. The contents of the Minimum inhibitory concentration (MIC) in the serial dilution were then sub-cultured into the prepared medium. Incubation was made at 37°C for 24 hours after which the plates of the medium were observed for colony growth. The MBC/MFCs were the plates with the lowest concentration of the extract without colony growth.

3. Results and Discussion

The phytochemical screening result revealed that terpenes, sterols and resins are present in the hexane extract. Resin was present in the ethyl acetate extract, saponins, flavonoids, resins and anthraquinones are present in the ethanol extract and glycosides, saponins, terpenes, sterols, flavonoids, resins and balsams were present in the methanol extract. However, tannins, phlobatannins and phenols were absent. A number of interesting outcomes have been found with the use of a mixture of natural product extracts to treat diseases [8]. The

presence of terpenes, sterols, flavonoids, anthraquinones, glycosides, resins and balsams in the leaf extract of *Anthocleista djalonensis* is in agreement with previous reports of Ojialkor and Okoye, [9] and Akinyemi and Ogundare, [7]. These constituents have been known to exhibit medicinal properties as well as physiological activities [10]. Saponins have a tendency to eradicate microbes and this makes them good agents for treating yeast and fungal infections. They are important for their cardio-tonic activities and antimicrobial properties. They can also be used in nutrition, herbal medicines and cosmetics [11]. Flavonoids are associated with a broad spectrum of health promoting agents; they are indispensable components in nutraceuticals, pharmaceuticals, medical and cosmetic applications. This is attributed to the antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic properties coupled with their capacity to moderate key cellular enzyme functions. The methanol extract contains glycoside content. Cardiac glycosides have been used as stimulant in case of cardiac failure [12]. The presence of terpene in the methanol extract indicates that it could be effective against bacterial infections. To a very great extent, the soil texture, geographical location, the level of humidity in a place, the climate condition, the maturity of the plant, time of collection of the plant and the method of extraction adopted are possible reasons for variation in chemical compositions.

Table 1. *Phytochemical Screening Test Result of the Leaf Extracts of Anthocleista djalonensis.*

S/N	Secondary metabolites	Hexane	Ethyl acetate	Ethanol	Methanol
1	Tannins	–	–	–	–
2	Phlobatannin	–	–	–	–
3	Glycosides	–	–	–	+
4	Saponins	–	–	+	++
5	Terpenes	+	–	–	+
6	Sterols	++	–	–	+
7	Flavonoids	–	–	++	++
8	Phenols	–	–	–	–
9	Resins	+	+	+	+
10	Balsams	–	–	–	+++
11	Anthraquinones	–	–	++	–

Key; - = Absent + = Present ++ = Moderately Present +++ = Highly Present

Table 2. *Antimicrobial Activity Test Result for the Leaf Extract of Anthocleista djalonensis.*

Test organism	Hexane	Ethyl acetate	Ethanol	Methanol
Methicillin Rest Staph aureus	S	S	S	S
Vancomycin Rest Enterococci	S	S	S	S
Staphylococcus aureus	S	S	S	S
Escherichia coli	R	R	R	R
Helicobacter pylori	S	S	S	S
Proteus mirabilis	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R
Candida albicans	S	S	S	S
Candida krusei	S	S	S	S
Candida tropicalis	R	R	R	R

Key; S= Sensitivity R= Resistance Rest= Resistant Staph= Staphylococcus

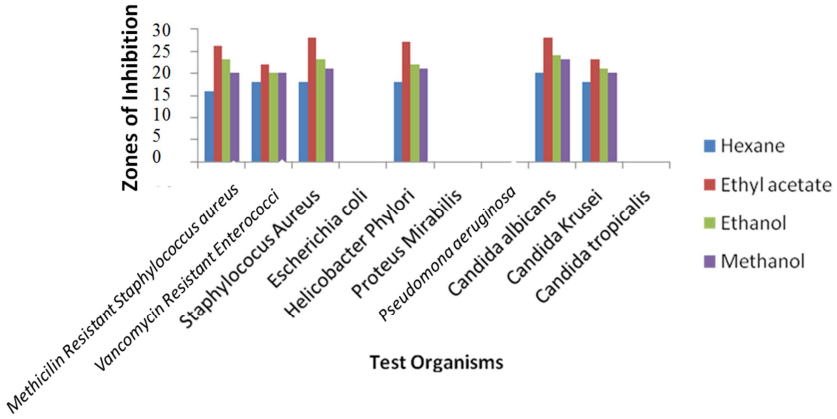

Figure 1. *A Chart Showing the Zones of Inhibition of the Leaf Extracts of Anthocleista djalonensis Against Test Microorganisms (mm).*

The extracts inhibited microbial organisms by producing zones of inhibition ranging from 16-28 mm for *Methicillin Rest Staph aureus, Vancomycin Rest enterococci, staphylococcus aureus, Helicbacter pylori, Candida albicans*

and Candida krusei. The activity of the extract was more potent against *C. albicans* (20 mm, 28 mm, 24 mm, 23 mm) and less potent against Methicillin Rest Staph aureus (16 mm, 26 mm, 23 mm, 20 mm) of the hexane, ethyl acetate, ethanol and methanol extracts respectively. The ethyl acetate extract was more potent against all test microbes with *Methicillin Rest Staph aureus (26 mm), Vancomycin Rest enterococci*, (22 mm), *S.aureus* (28 mm), H. *phylori* (27 mm) *C. albican*s (28 mm) and *C. krusei* (23 mm); *E.coli*, P.mirabilis, P. aeruginosa and C. tropicalishowever showed zero activity. The hexane extract demonstrated the weakest activity against the test microbes.

Table 3. *Minimum Inhibition Concentrations of Hexane Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	_	0*	+	++	+++
Vancomycin Rest Enterococci	_	0*	+	++	+++
Staphylococcus aureus	_	0*	+	++	+++
Escherichia coli					
Helicobacter pylori	_	0*	+	++	+++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	_	0*	+	++
Candida krusei	_	0*	+	++	+++
Candida tropicalis					

Key; - = No turbidity (no growth)0*= MIC+ = Turbid (light growth)++ = Moderate Growth+++ = High Turbidity (high growth)

Table 4. *Minimum Inhibition Concentration of Ethyl acetate Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	_	_	0*	+	++
Vancomycin Rest Enterococci	_	_	0*	+	++
Staphylococcus aureus	_	_	_	0*	+
Escherichia coli					
Helicobacter pylori	_	_	_	0*	+
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	_	_	0*	+
Candida krusei	_	_	0*	+	++
Candida tropicalis					

Key; - = No turbidity (no growth)0*= MIC+ = Turbid (light growth)++ Moderate Growth +++ = High Turbidity (high growth)

Table 5. *Minimum Inhibition Concentration of Ethanol Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	_	_	0*	+	++
Vancomycin Rest Enterococci	_	_	0*	+	++
Staphylococcus aureus	_	_	0*	+	++
Escherichia coli					
Helicobacter pylori	_	_	0*	+	++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	_	0*	+	++
Candida Krusei	_	_	0*	+	++
Candida tropicalis					

Key; - = No turbidity (no growth)0*= MIC+ = Turbid (light growth) ++ = Moderate Growth +++= High Turbidity (high growth)

Table 6. *Minimum Inhibition Concentration of Methanol Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph aureus	_	_	0*	+	++
Vancomycin Rest Enterococci	_	_	0*	+	++
Staphylococcus aureus	_	_	0*	+	++
Escherichia coli					
Helicobacter pylori	_	_	0*	+	++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	_	0*	+	++
Candida Krusei	_	_	0*	+	++
Candida tropicalis					

Key; - = No turbidity (no growth)0*= MIC+= Turbid (light growth)++= Moderate Growth+++= High Turbidity (high growth)

Table 7. *Minimum Bactericidal/Fungicidal Concentration of Hexane Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	0*	+	++	+++	++++
Vancomycin Rest Enterococci	0*	+	++	+++	++++
Staphylococcus aureus	0*	+	++	+++	++++
Escherichia coli					
Helicobacter pylori	0*	+	++	+++	++++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	0*	+	++	+++	++++
Candida Krusei	0*	+	++	+++	++++
Candida tropicalis					

Key; - = No colony growth0*= MBC/MFC,+= Scanty colony growth++= Moderate colony growth+++= heavy colony growth++++= Extremely heavy colony growth

Table 8. *Minimum Inhibition Concentration of Ethyl acetate Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	_	0*	+	++	+++
Vancomycin Rest Enterococci	0*	+	++	+++	++++
Staphylococcus aureus	_	_	0*	+	++
Escherichia coli					
Helicobacter pylori	_	_	0*	+	++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	_	0*	+	++
Candida Krusei	_	0*	+	++	+++
Candida tropicalis					

Key; - = No colony growth0*= MBC/MFC,+= Scanty colony growth++= Moderate colony growth+++= heavy colony growth++++= Extremely heavy colony growth

Table 9. *Minimum Bacterial/Fungicidal Concentration of Ethanol Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph Aureus	_	0*	+	++	+++
Vancomycin Rest Enterococci	0*	+	++	+++	++++
Staphylococcus aureus	0*	+	++	+++	++++
Escherichia coli					
Helicobacter pylori	0*	+	++	+++	++++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	0*	+	++	+++
Candida Krusei	0*	+	++	+++	++++
Candida tropicalis					

Key; - = No colony growth0*= MBC/MFC,+= Scanty colony growth++= Moderate colony growth+++= heavy colony growth++++= Extremely heavy colony growth

Table 10. *Minimum Bacterial/Fungicidal Concentration of the Methanol Leaf Extract of Anthocleista djalonensis Against Test Microbes.*

Test microbes	5 mg/mL	2.5 mg/Ml	1.25 mg/mL	0.62 mg/mL	0.313 mg/mL
Methicillin Rest Staph aureus	0*	+	++	+++	++++
Vancomycin Rest enterococci	0*	+	++	+++	++++
Staphylococcus aureus	0*	+	++	+++	++++
Escherichia coli					
Helicobacter pylori	0*	+	++	+++	++++
Proteus mirabilis					
Pseudomonas aeruginosa					
Candida albicans	_	0*	+	++	+++
Candida krusei	0*	+	++	+++	++++
Candida tropicalis					

Key; - = No colony growth0*= MBC/MFC,+= Scanty colony growth++= Moderate colony growth+++= heavy colony growth++++= Extremely heavy colony growth

The MIC (Minimum Inhibition Concentration) of the hexane extract was found to be 2.5 mg/mL for *Methicillin Rest Staph aureus, Vancomycin Rest enterococci, S. aureus, H. pylori, C. krusei* and 1.25 mg/mL for *C. albicans*. The

MIC of the ethyl acetate was found to be 1.25 mg/mL, 1.25 mg/mL, 0.62 mg/mL, 0.62 mg/mL, 0.62 mg/mL and 1.25 mg/mL for *Methicillin Rest Staphaureus, Vancomycin Rest enterococci, S. aureus, H. pylori, C. albicans and C. krusei* respectively. The ethanol and methanol extracts were found to have MICs of 1.25 mg/mL for all test microbes as mentioned above. The minimum bactericidal/fungicidal concentrations of the hexane extract showed that the extract completely killed the test microbes at a concentration of 5 mg/ml for *Methicilin Rest Staph aureus, Vancomycin rest enterococci, S. aureus, H. pylori, C. albicans* and *C. krusei.* The MBC/MFC of the ethyl acetate extract against test microbes was 2.5 mg/mL, 5 mg/mL, 1.25 mg/mL, 1.25 mg/mL, 1.25 mg/mL and 2.5 mg/mL for *Methicillin Rest Staph aureus, Vancomycin Restenterococci, S. aureus, H. pylori, C. albicans* and *C. krusei* respectively. The ethanol extract had an MBC/MFC against test microbes as 2.5 mg/mL, 5 mg/mL, 5 mg/mL, 5 mg/mL, 2.5 mg/mL and 5 mg/mL for *Methicillin Rest Staph aureus, Vancomycin Rest enterococci, S. aureus, H. pylori, C. albicans* and *C. krusei* respectively. While the MBC/MFC of the methanol extract against test microbes was found to be 5 mg/mL for *Methicillin Rest Staph aureus, Vancomycin Rest enterococci, S. aureus, H. pylori, C. krusei* and 2.5 mg/mL for *C. albicans*.

4. Conclusion

The activity exhibited by the leaf extracts of *Anthocleista djalonensis* against gram positive and gram negative pathogenic microorganisms corroborates with the observed phytochemical constituents, hence provides scientific justification for its traditional use.

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