Journal of Clinical Investigation and Studies

Research Article ISSN: 2631-4002

Chemical characterization and investigation of the bio-effects of the leaves of Al-stonia boonei (Apocynaceae) on some selected microorganisms

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Abstract

The bark of *Alstonia Boonei* is used in traditional herbal practices majorly in the western part of Nigeria and widely in West Africa; *A. boonei* is known for its significant therapeutic properties such as antirheumatic, anti-malarial, antipyretic, antidiabetic, anthelminthic, antimicrobial and antibiotic properties; it is also applied in the treatment of cardiac dysfunctions, hepatitis, and heart diseases. The chemical constituents of the ethanolic extract of the bark of *A. boonei* was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) technique and varying compounds were identified which include Methoxyacetic acid (13.68%), Eugenol (3.86%), Caryophyllene (5.67%), Acetic acid (8.41%), Cyclodecane (16.12%), Benzene dicarboxylic acid (19.03%), Phenol-d6- (5.85%), n-Hexandecanoic acid (18.62%) and other constituents (8.76%). The extract displayed strong antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Escherichia coli, Salmonella typhi,* and *Proteus mirabilis.* The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. The presence of these bioactive compounds in the bark of *A. boonei* could be the reason behind its bacterial extermination effects as well as its concomitant use in the treatment of diseases and infections in herbal medicine in Nigeria.

Introduction

The plant tree *Alstonia Boonei* also known as tree of God, is an herbal medicinal tree plant of West African origin. This tree plant is of the family *Apocynaceae*; there are about twelve species of genus *Alstonia*, two of these species are indigenous to Africa and other species are scattered all over the world [1]. The plant tree *Alstonia boonei* is also called many names according to locality; it is called Onyame dua (in akan, Ghana), Ahun, Egbu-ora, Ukhu, Ukpukulu (in Yoruba, Igbo, Edo, and Urhobo respectively in Nigeria) [2]. Moreso, it is also called quite few names in English, like cheese wood, blackboard tree, Dita bark, Australian quinine, scholar tree, pattern wood, stool wood and few more others [2]. *Alstonia boonei* is a deciduous plant, traditional healers practicing along the west coast of Africa are well acquainted with this tree; it grows into giant tree of about 35 meters high and about 1.2 meter in diameter thriving very well in damp river banks [3]. It has bole which is often deeply fluted to 7 m, small buttresses present; by color, it has grey or greyish-green bark, rough, slash rough-granular, ochre-yellow, exuding a copious milky latex; its branches and leaves are in whorls. The leaves are dark shiny-green and its fruits are paired with slender follicle up to 16 cm with brown floss at each end [4].

Generally, all the parts of *Alstonia Boonei* are useful; the back of *Alstonia* is one of the effective analgesic herbs available in nature [5]. Therapeutically, the bark of *Alstonia boonei* has been found to be very useful because it possesses antirheumatic, anti-malarial, antipyretic, antidiabetic, anthelminthic, antimicrobial and antibiotic properties [2]. Though the freshly cut part of the bark of the tree is more effective during decoction, however the dried part is also effective [6]. It is remarkable to highlight some health benefits of *Alstonia boonei*, according to Adotey, et al. (2012), some of the health benefits of *Alstonia boonei* are listed below:

i. A sweetened decoction taken at least 4 times daily can serve as painkillers for painful menstruation, which may be associated with ovarian cyst or uterine fibroid in women.

ii. The painful urethritis common with gonococcus or other microbial infections in men can also be relieved.

iii. Gynecological problems such as pelvic inflammatory diseases ranging from lower abdominal and pelvic congestions can also be relieved.

iv. Pains associated with malaria fever can be relieved by the exertion of a mild antibacterial effect from Alstonia boonei.

v. Taken to combat rheumatic and arthritic pains by exhibiting anti-pyrexia and anti-malaria effects.

vi. More so, a mild hypoglycemic effect can be exerted on diabetic patients when a cold decoction made from Alstonia is taken orally two to three times daily.

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Key words: *alstonia boonei, GC-MS analysis, antibacterial activity, chemical characterization*

Received: March 22, 2025; **Accepted:** April 09, 2025; **Published:** April 14, 2025

It is noteworthy that *Alstonia Boonei* has many uses, ranging from health to culinary. For example, a cold infusion (made from either fresh or dried bark of *Alstonia*) can be administered orally to expel roundworms, threadworms, and other intestinal parasites in children [7]. The fresh bark of *Alstonia* could be used in preparing herbal tinctures, it is mainly useful as an effective antidote against Snake bite, Rat or Scorpion poison [8]. The administrating of this to women after birth helps in expelling any retained products of conception [9]. Tinctures prepared from other plants mixed with the tinctures of *Alstonia* can be used in the treatment of Asthma [10]. The bark decoction of *Alstonia Boonei* mixed with the tinctures of other plants can be used in the treatment of dislocation or fractures [11].

Material and methods

2.1 Collection and identification of plant materials

The bark of *Alstonia boonei* was collected from an open field at the Staff quarters of the University of Cross River State Calabar, Nigeria, and authenticated in the Department of Plant and Biotechnology.

2.2 Extraction of plant materials

The fresh bark of *Alstonia boonei* was rinsed in clean water and then left to air dry at room temperature. The sample was dried for 14 days at room temperature to remove moisture and preserved for analysis. This prolonged drying period ensures thorough evaporation of water content, preventing microbial growth and maintaining sample integrity. Controlled drying at ambient temperature mitigates the risk of thermal degradation or denaturing of sample composition. Thereafter, the sample was pounded to resize to smaller particles, and then an electric blender was used to blend it into very fine particle sizes, and a Soxhlet extractor was used to extract the powder formed using ethanol. The extract was then inserted into a water bath where it was left for a few hours until all of the ethanol had evaporated, leaving only the extract in the beaker.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The method Raju and Kumar (2020) used was followed for conducting GC-MS analysis of the *Alstonia boonei* bark [12]. The analysis was performed using BUCK M910 Gas chromatography equipped with HP-5MS section (30 m long × 250 µm in width × 0.25 µm in thickness of film). Spectroscopic identification by GC–MS used an electron ionization framework that employed high-energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas at a flow rate of 1 mL/min. The initial temperature was set at 50 – 150 °C with an increasing rate of 3 °C/min and a holding time of around 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the pre-prepared 1% solution of the extract diluted with specific solvents was injected in a splitless mode. The relative quantity of the compounds present in each of the extracts was expressed as a percentage based on the peak area generated in the chromatogram.

2.4 Identification of components

The components of the extracts were identified and validated by comparing the mass spectra of the peaks with those from kinds of literature after matching the peaks with computer Wiley MS libraries.

2.5 Bioassay

Six chosen bacteria (three gram-positive and three gram-negative) were cultured for 24 hours to test the bark extract of *A. boonei's in vitro* antibacterial properties. *Salmonella typhi, Bacillus cereus, Escherichia coli, Proteus mirabilis, Staphylococcus aureus*, and *Enterococcus faecalis* were the six bacterial organisms utilized. All test organisms were clinical isolates of human pathogens from stock cultures at the University Teaching College Hospital in Calabar, Nigeria. The Whatman No. 1 filter paper was cut into circular discs with a diameter of 5 mm using a single-hole punch office paper perforator. To remove any remaining preservatives, the paper discs were cooked in distilled water for an hour. The cooked paper discs were then let to drain dry before being wrapped in aluminum foil and autoclaved for 15 minutes at 121°C to sterilize them. But within 48 hours of manufacture, they were put to use. The Disc Diffusion Technique was used to ascertain each test microorganism's susceptibility to the extract [13]. The surface of a sterile solid medium suitable for the test organism was aseptically coated with a loopful of each test sample organism. The extract-bearing paper discs were carefully positioned on the surface of the inoculation medium at a certain distance from one another after the inoculums had been equally distributed over the medium's surface using a flamed glass puck and a flamed pair of forceps. In an incubator set to 37 °C, the infected plates were incubated for 24 hours. Every day, their growth and the existence of inhibitory zones surrounding the paper discs were assessed. A transparent millimeter rule was used to measure the inhibition zone's diameter, which indicated the degree of sensitivity. By comparing the various extract concentrations with distinct zones, the Minimum Inhibitory Concentration (MIC) was ascertained by choosing the lowest concentration.

Results and discussion

As shown in Figure 1, the chromatogram of the ethanolic extract of the *A. boonei* bark depicts very closely related peaks; however, the more distinctive peaks are presented below.

Values are in mm and include the diameter of the paper disc (5 mm).

Data are means of triplicate determinations.

Table 2 lists the antimicrobial activities of the ethanolic extract of *Alstonia boonei* bark. The extract demonstrated strong inhibition of six microorganisms, including three gram-negative bacteria (*E. coli, S. typhi, and P. mirabilis*) and three gram-positive bacteria (*S. aureus, E. faecalis, and B. cereus*). The extract effectively inhibited these organisms at varying concentrations, with the highest antimicrobial activity against *S. typhi* and *P. mirabilis*. The overall order of the extract's biological activity against the test organisms was:

S. typhi > *P. mirabilis* > *E. coli* > *E. faecalis* > *S. aureus* > *B. cereus*

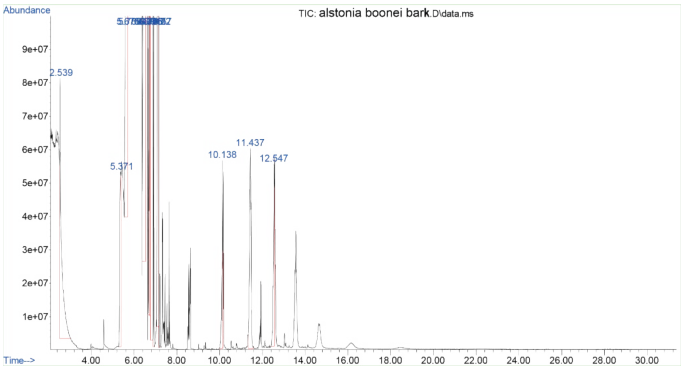

Figure 1: GC-MS Chromatogram of ethanolic extract of *A. boonei*

Table 1: Phytochemicals identified from the GC-MS analysis of the bark extract of *A. boonei*

Chromatogram peak	Compound name	Molecular formula	Molecular weight g/mol	Nature of compound
1	Methoxyacetic acid	$C_7H_{14}O_3$	146	Carboxylic
2	Eugenol	$C_{10}H_{12}O_2$	164	Phenol
3	Caryophyllene	$C_{15}H_{24}$	204	sesquiterpene
4	Acetic acid	$C_2H_2Cl_2O_2$	128	Carboxylic
5	Cyclodecane	$C_{10}H_{20}$	140	Alkane
6	Phenol-d6-	C_6D_6O	310	Phenol
7	n-Hexandecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic
8	1,2-Benzenedicarboxylic acid	$C_8H_60_4$	166	Phthalic
9	Eicosane	$C_{20}H_{42}$	282	Alkane
10	Nonadecanoic acid	$C_{19}H_{38}O_2$	296	Fatty acid

Table 2: Antimicrobial activities of the bark of *A. boonei*

Test microorganism	Concentration (%)				
	25	50	75	100	MIC (%)
Staphylococcus aureus	7.33	10.62	12.88	14.33	25
Enterococcus faecalis	7.67	11.67	13.33	14.67	25
Bacillus cereus		8.68	11.33	13.5	50
Escherichia coli	7.78	11.66	12.92	14.67	25
Salmonella typhi	8.13	12.38	13.33	15.67	25
Proteus mirabilis	8.12	11.46	13.21	15.33	25

The extract's Minimum Inhibitory Concentration (MIC) ranged from 50% to 100%. The pathogens' sensitivity to the extract may have been caused by the high levels of phenols (13.68%) and alkaloids (18.62%), as indicated by the GC-MS phytochemical screening in Table 1. Since the compounds found in the extract are primarily bioactive, they may have worked in concert to play an antimicrobial role (Figures 2-11). Alkaloids are plant bases that have specific physiological properties when used in herbal medicine [14]. Many of them possess antibacterial and antimalarial qualities [15]. Additionally, it has been observed that phenolic compounds exhibit strong antifungal and antibacterial properties in humans [16]. The examined bacteria are commensals of humans and have been implicated in wound infection [17]. These results support the use of *A. boonei* in traditional herbal treatments in South Eastern Nigeria to treat a variety of ailments, including boils, deep and deadly wounds, syphilis, and gonorrhea. The extracts' ability to inhibit *S. typhi* and *S. aureus* implies that they could be used to treat typhoid fever and Sexually Transmitted Infections (STDs).

Additionally, the mechanism of inhibitory action of these phytochemicals on the microorganisms may be due to the integrity of the cell membrane and structural component synthesis. The use of plants or plant extracts in controlling diseases has several advantages, including their being pathogen-specific, biodegradable, inexpensive, readily available, and more environmentally friendly than synthetic chemicals [18].

MIC = Minimum Inhibitory Concentration = Zone of no inhibition

Figure 2: Methoxyacetic acid

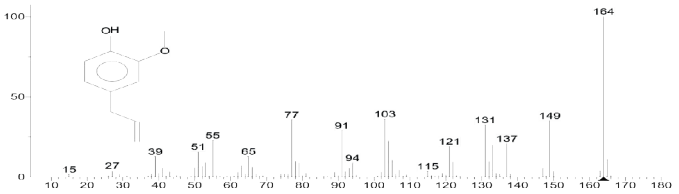

Figure 3: Eugenol

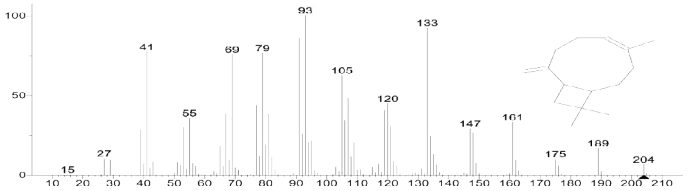

Figure 4: Caryophyllene

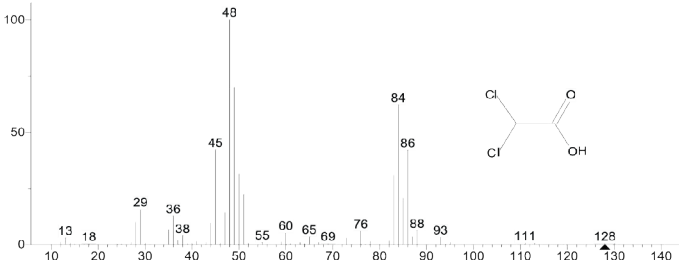

Figure 5: Acetic acid

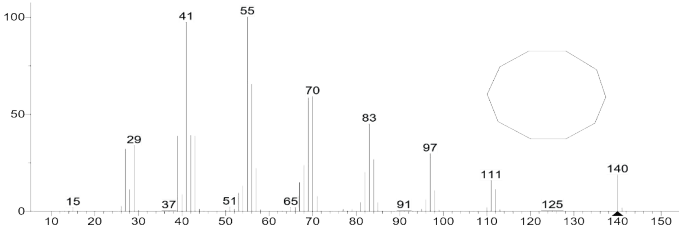

Figure 6: Cyclodecane

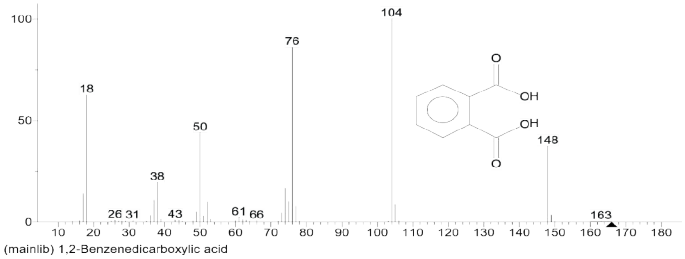

Figure 7: 1,2-Benzenedicarboxylic acid

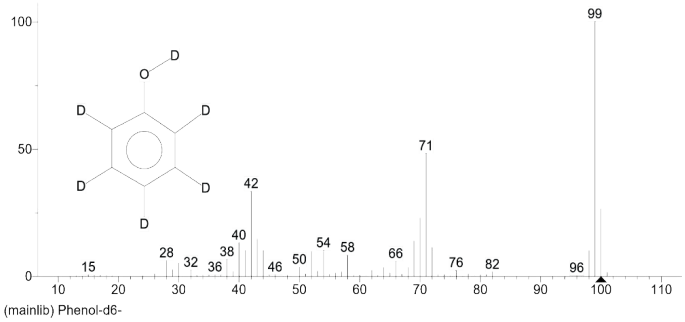

Figure 8: Phenol-d6-

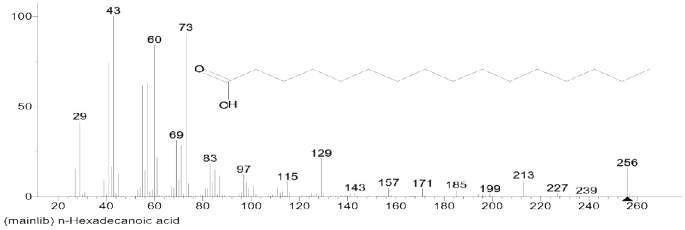

Figure 9: n-Hexandecanoic acid

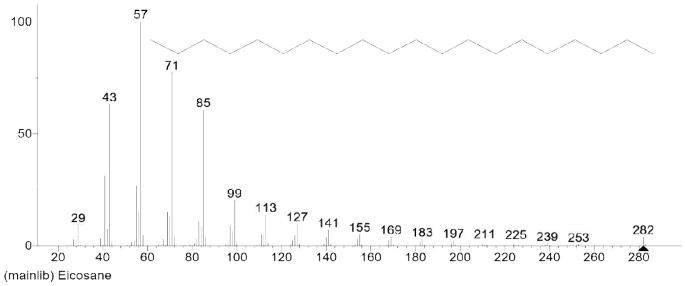

Figure 10: Eicosane

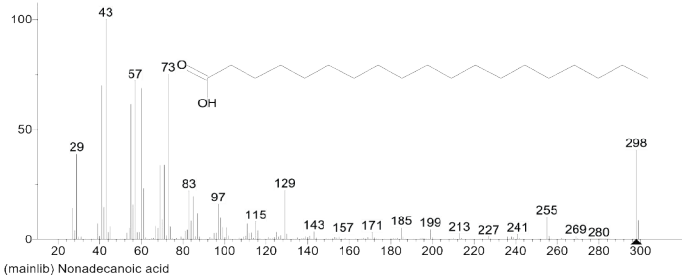

Figure 11: Nonadecanoic acid

Conclusion

The GC-MS results of the bark of *A. boonei* have given insight on the chemical constituents of the plant in herbal medicine for the treatment of diseases and infections especially in South Eastern Nigeria.

Acknowledgments

The author is grateful to the Department of Plant science and Biotechnology, University of Cross River State, Calabar, Nigeria for their assistance in identifying and authenticating the plant sample.

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