International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Phytochemical Analysis of *Alstonia boonei* Stem Bark: Antibacterial and antifungal Efficacy & Characterization via Chromatography and NMR

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Abstract

This study aimed at isolating and identifying the bioactive compounds found in the stem bark of *Alstonia boonei*, The crude extract was subjected to various chromatographic separations, isolation and purification. Structure elucidation of the isolated pure compounds was achieved by through NMR experimentation and the compounds were identified as lupeol **(1)**, and β-amyrin **(2).** The crude extract and the isolated pure compounds were tested for their antibacterial and antifungal properties against *Candida albicans, Aspergillus fumigatus, Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Salmonella enterica*. Compounds **1** and **2** showed moderate mean zones of inhibition of (8.4±1.2) mm**,** (5.0±2.5) mm and (8.3±1.4) mm respectively. The crude extract showed very high zones of inhibition of (9.4±0.7) mm. Compounds **1** and **2** showed high zones of inhibition against *Candida albicans* and *Aspergillus fumigatus* of (12.6±0.2) mm and (12.3±0.7) mm, respectively; This justifies the use of the plant in treatment of various ailments.

Keywords: Phytochemical Analysis; *Alstonia boonei*, Stem Bark; Antibacterial and antifungal Efficacy; Chemical compounds

1. Introduction

In Africa, traditional medicine has been part of the people's culture, and indigenous knowledge of medicinal plants is a source of new ideas for modern pharmaceutical science. Likewise, in Uganda, phytotherapy still remains an important role in meeting the primary health care needs of more than 80% of the population. Research shows that "many people" take a wide range of natural products in addition to the conventional therapeutic products to manage various ailments [1]. The increasing population of Uganda to more than 40 million makes people more vulnerable to diseases and infections due to congestion in many areas. This makes herbal medicine a better and cheaper alternative source of primary health care, especially in rural areas where modern medical services are scarce and expensive for the low income earners [2].

Even with the wide spread of antibiotics and immunization campaigns, infectious illnesses remain the main cause of morbidity and mortality worldwide [3]. The issues are made worse by wide spread of antibiotic resistance, the introduction of brand new diseases such as COVID-19 in addition to the recurrence of old ones, and the death of potent new therapies [4].

Antimicrobial agents may come from plants[5]. The fact that current medications like quinine, vincristine, digoxin, emetine, artemisinin, among others, have been derived from plants shows the enormous potential that still remains for the development of many more new pharmaceuticals [6]. Due to this, ethnopharmacology of medicinal plants has attracted more interest in the field of research and development of new medications [7]. Two thirds of the world's population [8] are thought to rely on traditional herbal treatments because of the scarcity, high cost, and varied adverse effects of pharmaceuticals. This supports the hunt for substitute items made from plants used in folk medicine even more [9].

Uganda is well renowned for its extensive ethno botanical diversity, particularly with regard to medicinal plants, which are historically used to treat illnesses and maybe a valuable source for the development of novel and biodegradable medications [1]. Although infectious diseases are wide spread in Uganda, there are hundreds of different types of medicinal plants that have historically been used to treat them [10]. Many of these herbs have been used for a long time without incident, which may be a subliminal indication of their effectiveness. Unfortunately, only 5-15 percent of higher plants worldwide have been vigorously examined for the presence of bioactive chemicals by the year 2019 [11].

Traditional medicine has employed the stem bark of *Alstonia boonei* plant to treat various ailments like malaria, urinary tract infections, fever sleeplessness, chronic diarrhea and rheumatic aches. However, it is still unknown the substances behind the physiological effects of this old-age treatment. This has made it difficult for this plant to become standardized and developed as well as keeping localized recognition and acceptance some of *Alstonia boonei's* medicinal properties caused by this plant. Thus, much as it is a very important tree the phytochemical compounds behind its medicinal properties were not known and therefore there was a need for research to find out the compounds, hence this study.

This study has produced crucial molecular structures of the contained phytochemicals for the preparation of synthetically enhanced therapeutic agents as well as potential anti-microbial medication development. The isolated and elucidated compounds might also be employed as standards for herbal preparations from *Alstonia boonei*. The crude extract's anti-bacterial activity was also examined against a variety of species. As a first step towards its acceptance and advancement as a therapeutic agent, the bioactivity results of *Alstonia boonei* will offer preliminary scientific justification for the traditional medicinal usage of this ethnomedicine

2. Experimental Section

2.1 Plant collection and identification

After being identified and authenticated by a plant taxonomist, fresh stem bark of *Alstonia boonei* was gathered from Mabira woodland in Uganda. The stem bark sample was carried to the Makerere University Herbarium in plastic bags, where it was assigned the voucher number 51161 for future reference.

2.2 Extraction preparation

The stem bark was finely chopped and allowed to air dry for a month under shade at room temperature at Kyambogo University. An electric grinder was used to convert the dry bark into a fine powder. One kilogram of powdered plant material was extracted three times over a period of 48 hours using five litres of dichloromethane: methanol (1:1). After filtering the extract using cotton wool and Whatman No. 1 filter paper, it was concentrated at about 40°C in a rotary evaporator until dry. To get rid of any possible water residues, the dried extract was transferred to sample glass bottles and placed in a desiccator with anhydrous

sodium sulphate. The dried extracts were then stored in refrigerator sealed in safe glass bottles with tight caps for later analysis.

2.2.1 Extraction and isolation of compounds from the stem bark of *Alstonia boonei*

The airdried stem bark extract (1kg) of *Alstonia boonei* was extracted using dichloromethane: methanol (1:1). The crude extract weighed 41.5 g, representing 4.15 % of the starting materia

2.3. Qualitative Phytochemical analysis

Phytochemical screening was carried out according to the method described by Mehmood et al. [12]. The colour changes of the contents were observed and scored as present (+) or absent
(-). Briefly, Flavonoids were tested as follows: 5.0 mL of the extract was mixed with 2 mL of 2% NaOH solution followed by a few drops of dilute hydrochloric acid. An intense yellow color became colorless upon addition of diluted acid for the methanol extract. Alkaloids were tested as follows: 5.0 mL of the extract was mixed with dilute hydrochloric acid, then 1-2 drops of Wagner's reagent were added along the sides of the test tube. The presence of a reddish precipitate confirmed the presence of alkaloids in the methanol extract. In testing for tannins, 2.0 mL of the plant extract was mixed with 4 mL of 10% NaOH and shaken well. This confirmed the presence of tannins due to the formation of an emulsion. Steroids were assayed as follows: 1 mL of the extract was mixed with an equal volume of chloroform, and a few drops of concentrated H2SO4 were added. The appearance of a brown ring indicated the presence of steroids. Glycosides were tested as follows: 2 mL of the extract was mixed with 3 mL of chloroform and 10% ammonia solution. The formation of a pink color indicated the presence of glycosides. Phenols were tested as follows: 2 mL of distilled water followed by a few drops of 10% ferric chloride were added to 1 mL of the oil extract. The formation of a blue or green color indicated the presence of phenols. The presence of saponins was determined as follows: 1 g of methanol crude extract was weighed using an analytical balance and then mixed with 5 mL of distilled water in a test tube. The mixture was shaken vigorously, and five drops of olive oil were added using a dropper. The formation of stable foam was taken as an indication of the presence of saponins.

2.4 Loading the extract into the packed column and the bioactive fractions used to isolate and purify active substances

The crude extract 31.5 g was adsorbed in 350 g of silica gel and concentrated with methanol.
The sample was allowed to dry, and was then ground into fine powder. The sample was then loaded into the column. The crude extract was eluted with n-hexane with increasing polarity using ethyl acetate. **Lupeol (1)** precipitated as white amorphous solids with 10% EtOAc and 90% n-hexane and was seen as a single spot using 254 nm UV lamp. **β-amyrin (2)** was first seen as green solids and was ran through Sephadex LH-20 and was finally seen as white amorphous solids with 30% EtOAc and 70% n-hexane. It was visualized as a single spot under 254 nm UV lamp.

2.4.1 Column chromatography

This followed the method of Meyers[13]. Briefly, Silica gel (70-230 mesh) column chromatography was used to separate the crude extract. A glass stirring rod was used to quickly combine 350 g of silica gel with n-hexane to create uniform suspension or slurry and the mixture was agitated to remove air bubbles. A glass column was then filled with silica gel slurry. 31.5 g of the crude extract were dissolved in 100 ml n-hexane to create a sample that would be loaded onto the column. This was then concentrated on a rotatory evaporator. It was then poured and spread on a clean aluminium foil using a glass rod and allowed

to dry for some time. On the column layer bed, the dried silica extract mixture was stacked, the mobile phase was n-hexane, and the column was initially eluted with n-hexane. The polarity was then elevated by adding ethyl acetate in 5% increments in n-hexane. 250 ml fractions were collected in 250 ml glass beakers. At 40^0C, a rotary evaporator was used to concentrate the collected fractions until they were dry. To isolate the pure compounds, the fractions were subjected to further fractionation using sephadex LH-20 with dichloromethane and methanol (1:1) as the eluent system

2.4.2 Thin layer chromatography (TLC)

TLC was used to analyze the concentrated fractions that were obtained from column chromatography according to the method of Tchinda et al[14]. In a nutshell, a spot of each fraction of hexane: ethyl acetate (9:1, 8:2,7:3 and 6:4) was carefully put to a thin layer chromatographic plate that had a silica coating before being allowed to dry. A suitable solvent was applied to the plate that is ethyl acetate and n-hexane at varying ratios above five minutes, allowing the compounds in the area to rise via capillarity attraction due to their different retention factors. After that the plate was taken out of the solvent and allowed to dry. Following iodine vapor staining, the locations of various compounds were visualized by fluorescence under UV light of 254nm. Similar TLC fractions were mixed according to their RF values.

2.5 Determining the bioactive chemicals 'structures

The method of Sro [15] was used in this process. Briefly, to ascertain the structures of bioactive compounds, NMR spectroscopy was used. NMR experiments including Homonuclear correlation spectroscopy (COSY), Heteronuclear Multiple Bond Correlation (HMBC) and Heteronuclear Single Quantum Coherence (HSQC) were used. In addition, one dimensional (ID) and two dimensional (2D) proton (^{1}H) and carbon (^{13}C) NMR techniques were applied in structure elucidation. Each sample consisted of 10 mg of the pure compound diluted in 1 ml of a suitable deuterated solvent, filtered through a pasteur pipette with a glass wool stopper, and discharged into an Aldrich Z412845mm NMR tube with a concentric label. Filtration is used to rid of any undissolved sample particles and dust from the solution that could mess with the NMR spectra's resolution line form. Bruker AV-600 instruments were used to record the 1D and 2D spectra. To clarify the structures of the isolated compounds, all the spectra were analyzed and the findings were compared with the information published in the literature.

2.6 Bioassay of pure compounds and the crude extract

The crude and the isolated pure compounds were tested for their anti-microbial activities against six known bacteria, including *Salmonella entercia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes* and fung, namely: *Candida albicans*, *Aspergillus fumigatus.* These were chosen because of their availability and their occurrence. All these bacteria and fungi were tested for the zones of inhibition, minimum inhibitory concentration and the minimum bacterial concentration and minimum fungicidal concentration, and their results are shown in the following sections here below. For MIC, this was determined by adding agar and then inoculating with bacteria or fungi and incubating them at 24^0c. The MBC and MFC were determined as the concentrations that gave clear solutions of both bacterial and fungal cultures.

2.6.1 Bacterial and Fungal Cultures

This was achieved following the methods of Cleven et al.[16] and Zheng et al.,[17]. Briefly, cultures of six pathogenic bacterial strains and two fungi namely *Staphylococcus aureus*, *Escherichia Coli*, *Enterobacter aerogenes, Pseudomonas aeruginosa*, *Salmonella entercia* and *Salmonella typhi* and two

fungi *Candida albicans* and *Arspergillus fumigatus* were maintained in the laboratory in Luria Broth (2% w/v) regularly for four days at 37°C before use in experiments. For the experiments, a portion (100 µl) of the overnight culture was mixed in the tests and control for inoculation. This was done using a sterile pipette tip selecting a single colony from agar plates. The tip of a sterile pipette was dropped into the liquid agar plus antibiotic and swirl. The culture was loosely covered with sterile aluminium foil. For activity testing, bacterial and fungal cultures were stored at 4°C and sub cultured after every 8th day in solid agar plates.

2.6.2 Screening of antibacterial and anti-fungal activity

Antimicrobial activity of *A.boonei* extract and pure compounds was evaluated by agar disc diffusion method[18]. Molten agar was used as media for bacteria. For this purpose six different concentrations of each extract and pure compounds were coated on sterile filter paper discs (Whatman No. 1) of 6 mm in sizes. These extracts and ingredients coated discs dried under laminar flow cabinet. Before experiment inoculum size was determined and adjusted to prepare a final colony number as 10^8 CFU/ml (Colony Forming Unit) in sterile agar plates. Bacterial inoculum was spread evenly on to the surface of agar plate with sterile rubber pad spreader before the coated discs were positioned on the inoculated agar surface. Each extract and pure compound was assayed in triplicate. For comparison, known drugs were used i.e. tetracycline an antibacterial and ketoconazole an antifungal were also used in parallel. These were chosen due to their wide use in treatment of both bacterial and fungal infections with less side effects. All treated and untreated plates were incubated for 24 h at 37°C. The antibacterial and antifungal activities were assessed on the size of the inhibition zone diameter obtained surrounding the filter paper discs. The same procedure was followed for testing antimicrobial activity of pure compounds **1** and **2**. These pure compounds were solubilized in dichloromethane and their increasing concentrations of 5% were used for microbial testing. The anti-microbial activities of these compounds were assessed both in broth dilution and agar disc diffusion assay. Minimum fungicidal concentration (MFC) was determined as the highest dilution (lowest concentration) at which no growth occurred on the plate [19]

3. Results and Discussion

3.1. Qualitative phytochemical composition of methanolic extract of *Alstonia boonei*

Seven compounds were detected qualitatively in *Alstonia boonei.* These included

Alkaloids, Flavonoids, Tannins, Glycosides, Steroids, Phenols, and Saponins as shown in Table 1. Showing phytochemicals in methanolic extract of *Alstonia boonei*

Phytochemicals	**Methanolic extract**
Alkaloids	+
Flavonoids	+
Tannins	+
Glycosides	+
Steroids	+
Phenols	+
Saponins	+

Where; - = absent and + = present.

3.2. Characterization of compounds isolated from the stem bark of *Alstonia boonei.*

The air dried and ground extract of *Alstonia boonei* was extracted using CH_2Cl_2 / MeOH in a ratio of (1:1) by cold percolation at room temperature. The crude extract was subjected to a combination of

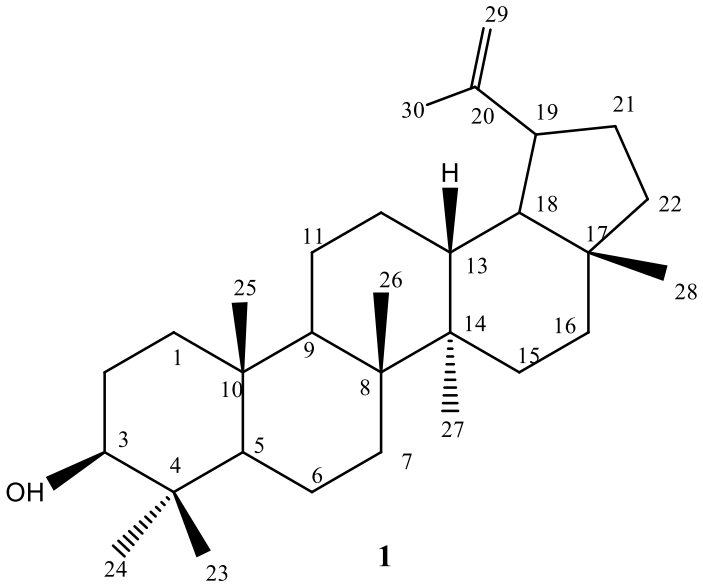
chromatographic techniques which resulted in isolation of two compounds, which are discussed here below.

Compound **(1) (Fig. 1, Table 2)),** *Lupeol* was isolated as white amorphous solid; the ^{1}H NMR spectrum showed the presence of seven methyl singlets at δ_H 0.74, 0.80, 0.83, 0.95, 0.95, 1.04 and 1.68 ppm and an oxymethine proton signal at δ_H 3.17 (1H, m, H-3) ppm. A vinylic proton at δ_H 1.68 (H-30) and the presence of two exo-methylene protons (δ_H 4.69. 4.55, H-29) showed that the compound had the lupine triterpene skeleton. In arrangement with this, the ^{13}C NMR spectrum showed the presence of thirty carbon atoms which confirmed that it was a true triterpene with the olefinic carbons appearing at δc 150.7 (C-20) and δc 109.3 (C-29) consistent with the terminal double bond. The signal at δc 79.0 with characteristic peak confirms a highly de-shielded carbon due to the presence of oxygenation at C-3 in the triterpene. In the HMBC spectrum, the methine proton signal at δH 3.17 (H-3) showed cross peaks with a methyl carbon signal (δc 28.0, C-23) by J2 correlation and a methyl carbon signal (δc 18.46, C-6) by J3 correlation. The sextet methyl signal at δH 2.37 (H-19) showed cross peaks with two methylene carbon signals δc 29.5 (C-21) and δc 109.34 (C-29)], a methine carbon signal [δc 48.25 (C- 18), a methyl carbon signal [δc 19.30 (C-30)] and a quaternary carbon signal [δc 150.7 (C-20)]. The pair of broad singlets of olefinic proton at δH 4.55 and 4.70 showed cross peaks with a methylene carbon signal [δc 48.10 (C-19) and δc 19.30 (C-30)] by J3 correlation The NMR data was in complete agreement with the literature report for lupeol [20].

Table 2: Showing ^{13}C NMR data compound 1

Position	**δc- literature** (Abdullahi *et al.*, 2013)	**δc-experimental**
1	38.7	38.65
2	27.4	27.5
3	79.0	79.00
4	38.9	38.82
5	55.5	55.54
6	18.5	18.46
7	34.2	34.43
8	40.9	40.70
9	50.5	50.52
10	37.2	37.19
11	21.0	21.25
12	25.2	25.12
13	38.1	38.3
14	42.9	42.7
15	27.1	27.1
16	35.5	35.82
17	43.0	42.8
18	48.3	48.25
19	48.0	48.10
20	150.0	150.7
21	29.9	29.5
22	40.0	40.29

23	28.0	28.0
24	15.5	15.56
25	16.1	16.19
26	16.0	16.05
27	14.8	14.80
28	18.0	18.04
29	109.0	109.34
30	19.5	19.30



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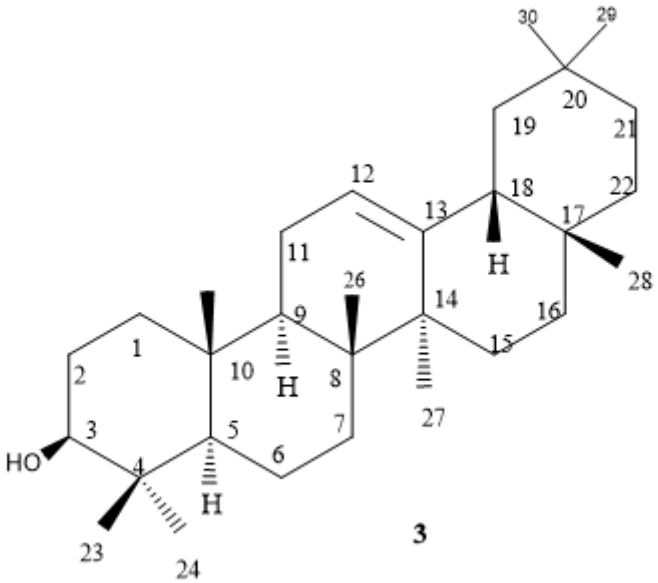
Compound **(2)** was isolated as white amorphous solid. The ^{1}H NMR showed the presence of seven methyl singlets δ_H 0.79, 0.83, 0.87, 0.94, 0.97, 1.00 and1.13ppm, one olefenic proton at δ_H 5.2. 1t and an oxygenated proton at δ_H 3.24dd all suggestive of an olealane type triterpenoid. The ^{13}C NMR spectrum showed the presence of thirty carbon atoms which confirmed that it was a true triterpene. All the proton and carbon signals were assigned based on the ^{1}H NMR, COSY, HSQC, HMBC and ^{13}C NMR. The spectroscopic data was in complete agreement with those previously reported [22](Table 3, Fig. 3)

Table 3: Showing 13C NMR data for compound 2

Position	**δ_H(H,JHz)**	**δc- literature(HSQC)(Viet et al., 2021).**	**δc- experimental (HSQC)**	**HMBC**
1	1.54	38.60	38.90	
2	1.51	27.20	27.30	1, 2, 24,
3	3.26(m,1H, H-3)	79.00	79.20	
4		39.80	39.50	
5		55.20	55.10	
6		18.40	18.60	
7		32.50	32.60	
8		41.70	41.60	
9	1.95d	47.60	47.60	

10		37.00	37.10	
11	1.81	23.70	23.70	
12	5.21 (s, 1H,	121.70	121.80	
13		145.20	145.40	
14		42.80	42.60	
15		26.90	26.70	
16		26.20	26.20	
17		32.70	32.80	
18		47.20	47.20	
19		46.80	47.0	
20		31.10	31.30	
21		34.70	34.80	
22		37.10	37.30	
23	0.78s	28.10	28.30	1, 24
24	1.16s	15.60	15.70	2, 4
25	1.02s	15.50	15.60	1, 5, 3, 24
26	0.89s	16.80	16.80	4, 9, 11, 15
27	0.96s	26.00	26.0	4, 5, 9
28	0.81s	28.40	28.30	1, 3, 5, 27
29	0.86s	33.30	33.10	4, 5, 9
30	086s	23.50	23.70	7, 9, 15



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3.3. Testing the isolated compounds and the crude extract against standards.

All the isolated pure compounds and the crude extract were tested for their antibacterial and antifungal activities and their results were compared with the known drugs for anti-bacterial tetracycline was used while for anti-fungal ketoconazole was used as the positive control. In both cases DMSO was used as the negative control and didn't show any activity. For simplicity compound 1 was given number 1, compound 2 was given number 2, compound 3 was given number 3, the crude extract was given number 4, positive

controls (tetracycline and ketoconazole) were given number 5 and negative control DMSO was given number 6.

They were all tested for their zones of inhibition in (mm) against selected bacteria species and fungi species and their results are shown in Table .5 for bacteria species and in Table 6 for fungi species.

Table 5: Showing the mean zones of inhibition (mm) of isolated compounds 1, 2 and the crude extract 4 and control samples 5 and 6 against selected bacteria species

Sample	**Bacteria**					
	Salmonella entercia	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia Coli***	***Salmonella typhi***	***Enterobacter aerogenes***
1	7.5±0.6	8.5±0.6	9.5±0.6	7±0.4	10±0.4	8±0.4
2	7.5±0.6	7.5±0.6	10.5±0.7	9.5±0.6	7±0.4	7.5±0.6
3	10.5±0.7	9.5±0.6	8.5±0.7	9.5±0.6	9±0.7	9.5±0.6
4	12.5±0.7	12.25±0.9	12±0.9	11.5±0.7	12±0.9	11.5±0.7
5	0	0	0	0	0	0

Lupeol **1**, β-amyrin **2** and crude extract **3,** tetracyline **4,** DMSO **5**

Table 6: Showing the mean zones of inhibition (mm) of isolated compounds 1, 2 and the crude extract 3 and control samples 4 and 5 against selected fungi species

Samples	**Fungi**	
	Candida Albicans	***Aspergillus fumigatus***
1	12.75±0.5	12.5±0.3
2	12.75±0.5	11.75±0.5
3	13.25±0.9	13±0.7
4	13.25±0.9	13.5±0.9
5	0	0

Lupeol **1**, β-amyrin **2** and crude extract **3,** ketoconazole **4,** DMSO **5**

Their MIC, MBC and MFC were recorded in mm and µg/ml (Table 7 and Table 8).

Table 7: Showing MIC and MBC in µg/ml results of isolated compounds and crude extract against selected bacteria

Samples	***Salmonella entercia***		***Staphylococcus aureus***		***Pseudomonas aeruginosa***		***Escherichia Coli***		***Salmonella typhi***		***Enterobacter aerogenes***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	25	50	25	50	25	25	75	-	18.75	50	75	75

2	25	25	50	50	37.25	50	75	75	37.5	50	50	50
3	12.5	25	37.5	37.5	50	75	18.75	37.5	50	50	75	-

Lupeol **1**, β-amyrin **2** and crude extract **3**

Table 8: Showing MIC and MFC in µg/ml results of isolated compounds and crude extract against selected fungi.

Samples	***Candia Albicans***		***Aspergillus fumigatus***	
	MIC	MFC	MIC	MFC
1	25	25	25	50
2	25	25	37.5	50
3	12.5	12.5	25	25

Lupeol **1**, β-amyrin **2** and crude extract **3**

The MICs were considered as the concentrations that inhibited the growth of bacteria and fungi while MBC and MFC were the concentrations that killed the bacteria and fungi respectively and were the concentrations that gave clear suspensions.

Lupeol, and β-amyrin showed moderate zones of inhibition while the crude extract showed relatively higher zones of inhibition. This proves the use of *Alstonia boonei* plant in the treatment of fungal infections however for a better activity it has to be used in conjunction with other medicinal plants or medicine for treatment of bacterial infections.

Lupeol, and β-amyrin showed high zones of inhibition against fungi species while the crude extract showed very high zones of inhibition for fungi species. This is because the crude possesses the synergistic effects than the individual pure compounds. This proves the use of *Alstonia boonei* in the treatment of various diseases due to its antimicrobial properties.

The study reveals the antibacterial and antifungal activities of isolated pure compounds and crude extract from the *Alstonia boonei* plant. The results suggest the potential of these compounds and extracts as antimicrobial agents, potentially beneficial for pharmaceutical and medicinal purposes. The study used tetracycline and ketoconazole as positive controls and DMSO as a negative control. The zones of inhibition, MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), and MFC (Minimum Fungicidal Concentration), were measured to assess the efficacy of the compounds and the crude extract.

The results showed that Lupeol and β-amyrin exhibited moderate zones of inhibition against selected bacterial species. The crude extract displayed higher zones of inhibition compared to individual compounds, suggesting potential synergistic effects of the compounds present in the extract. The study underscores the potential of *Alstonia boonei* as a source of antimicrobial agents, and further investigation into the specific mechanisms of action is warranted to better understand their effectiveness. Synergistic effects observed in the crude extract suggest that exploring combinations of compounds from natural sources could lead to the development of more potent antimicrobial agents. Clinical trials would be necessary to evaluate the safety and efficacy of these compounds and extracts in humans.

4. Summary/ Conclusions

Three compounds were elucidated from the stem bark of *Alstonia boonei* including lupeol **(1)**, and β-amyrin **(2)**. All the pure compounds showed antimicrobial properties.

The crude extract was very active against candida than any other compound. A synergy (crude extract) was found to have more antimicrobial than the isolated compound followed by β-amyrin and lastly Lupeol.

EXPERIMENTAL SECTION

Physical and spectroscopic data of compounds isolated from the stem bark of Alstonia boonei

Lupeol (1)

White amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 4.69 (1H; s, H-30a), 4.56 (1H,s, H-30b), 3.17 (1H,m,H-3); 2.37 (1H,m,H-19), 1.38 (2H,m, H-21), 1.55 (2H,m,H-2) and the methyl protons δ_H 0.74, 0.80, 0.83, 0.95, 0.95, 1.04 and 1.68. ^{13}C NMR ($CDCl_3$, 125 MHz) δc 151.1 (C-20), 109.1 (C-30), 78.8 (C-3), 55.2 (C-5), 50.4(C-9), 48.3 (C-18), 48.0(C-19), 43.0 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.8 (C-4), 38.7 (C-1), 38.1 (C-13), 37.1 (C-10), 35.6 (C-16), 34.3 (C-7), 29.8 (C-21), 27.8 (C-23), 27.5 (C-2), 27.4 (C-15), 25.2 (C-12), 20.9 (C-11), 19.1 (C-29), 18.30 (C-6), 17.8 (C-28), 15.9 (C-25), 15.8 (C-26), 15.2 (C-24), 14.30 (C-27).

β-amyrin (2)

*β-amyrin is a w*hite amorphous solid: ^{1}H NMR ($CDCl_3$, 500MHz): δ_H 5.21 (t, J=3.8Hz H-12), 3.24 (dd, J=10.5 H-3) δ ^{13}C NMR (125MHz, $CDCl_3$): 38.9 (C-1), 27.3 (C-2), 79.2 (C-3), 39.5(C-4), 55.1 (C-5), 18.6(C-6), 32.6 (C-7), 41.6 (C-8), 47.6 (C-9), 37.1 (C-10), 23.7 (C-11), 121.8 (C-12), 145.4 (C-13), 42.4 (C-14), 26.7 (C-15), 26.2 (C-16), 32.8 (C-17), 47.2 (C-18), 47.0 (C-19), 31.3 (C-20), 34.8 (C-21), 37.3 (C-22), 28.3 (C-23), 15.7 (C-24), 15.6 (C-25), 16.8 (C-26), 26.0 (C-27), 28.3 (C-28), 33.1 (C-29), 23.7 (C-30)

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