



Partial Purification and Characterization of Albain 1, a Triterpene with Antimicrobial Activity, from the Wood Extract of *Avicennia alba* Blume

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Authors' contributions

This work was carried out in collaboration among all authors. Author MC designed the study, wrote the protocol, carried out all main experiments and wrote the first draft of the manuscript. Author KM determined the probable structure of Albain 1. Authors AR and AS managed the analyses of the study. Authors MC, AD and AS were involved in studies involving pathogenic strains. All authors read and approved the final manuscript.

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ABSTRACT

Secondary metabolites of plants are important resources for development of new drugs. Mangrove plants are very well known sources of wide variety of secondary metabolites. Many of these secondary metabolites from mangroves have been found to possess significant biological activities where human health is concerned. *Avicennia alba* Blume is one such mangrove plant with reports of having many such secondary metabolites of clinical and commercial interests.

Aim: To evaluate antimicrobial activity potential of *A. alba* wood extract and to isolate new bioactive constituent(s) responsible for such biological activity.

Methodology: Preliminary screenings of antimicrobial activities in different organic solvent extracts of *A. alba* wood tissue were done by TLC-bioautography method and phytochemical nature of the

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antimicrobial constituent(s) in the extracts have been studied. One compound exhibiting significant antimicrobial activity, named as Albain 1, has been isolated. MIC value has been determined for Albain 1. The purity and structure of Albain 1 have been determined by HPLC, ¹H NMR, FTIR and HRMS etc. analysis.

Results: ¹H NMR, FTIR and HRMS analysis have found out that the isolated compound Albain 1 is a triterpene and the molecular formula is C₃₀H₄₈O₄. It has exhibited remarkable antimicrobial activity against *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus pumilas* (MIC 125 µg / ml).

Conclusion: The observed antimicrobial activity of the isolated fraction of *A. alba* offer great potentials in pharmaceutical industries.

Keywords: Antimicrobial activity; *Avicennia alba* Blume; secondary metabolites; MIC.

1. INTRODUCTION

Natural plant products have been used for centuries as remedies for human ailments and diseases. In recent years, microorganisms have developed resistance to various antibiotics due to extensive and unwise uses of easily available commercial antibiotics [1]. Drug resistant microorganisms have increasingly been reported all over the world [2]. As a result several types of dreaded diseases have become severe problems in healthcare system. To combat such situation it is necessary to discover new antimicrobial compounds. In this respect the merits of mangrove plants should be considered seriously for the discovery of novel drugs. Mangrove plants live in a very stressful environment. This happens because they live in estuarine/coastal regions serving as a connection between freshwater and highly salty marine ecosystems. As a result of living in these situations, they have undergone many adaptive modifications. They possess physiological modifications to establish water and salt balance which is very essential for their survival. There are modifications or alterations in their metabolic processes, particularly in the polyphenol synthesis pathways, which lead to the synthesis of unique secondary metabolites. It is thought that these unique metabolites protect them from the destructive forces of nature [3,4]. The importance of the mangroves largely stems from these phytochemicals which are known to possess significant biological activities of many different types such as, antitumor, antiulcer, antimicrobial etc. [5,6,7]. Many examples exist where the biological activities of these phytochemical have been exploited successfully in the human healthcare areas and other commercial purposes. *Avicennia alba* Blume, a tropical mangrove under the family Avicenniaceae, has been reported to exhibit several medicinal properties such as antimicrobial, antioxidant, anticancer, anti-inflammatory activities etc. [8,9,10,11]. Earlier

surveys pointed out that the plant extract of *Avicennia alba* is a rich source of secondary metabolites such as tannins, alkaloids, glycosides, steroids, polyphenols, naphthoquinones, triterpenes etc. [12,13]. The resin of this plant is used in birth control, ulcer treatment, skin diseases etc. as well as the bark and seeds are used as fish poisons [14,15]. A research work isolated a compound named betulin from *A. alba* which possesses moderate antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* [16]. Another report has indicated the existence of antimicrobial activity in the tissue extracts of this plant against several pathogenic microbes [17]. In view of the potential of this mangrove species for application in human health areas, further studies involving its products are highly desirable. We detected significant antimicrobial activity in the wood tissue extract of this plant in a very preliminary investigation done earlier [18]. The aim of this study has been to investigate this antimicrobial activity in a more detailed manner. In course of the present study, we have identified and isolated a new previously unreported phytochemical with antimicrobial activities from the wood tissue of *A. alba* and named it Albain 1 for easy reference.

2. MATERIALS AND METHODS

2.1 Plant Materials

Wood tissues of *Avicennia alba* Blume were collected from Sundarban estuary regions of West Bengal state, India. The dried specimen of this plant, after proper identification, was deposited in the Herbarium of Botany department, Visva-Bharati University, India.

2.2 Preparation of Plant Extracts

Plant wood tissues were rinsed thoroughly in water and dried at room temperature for several

weeks. Then the samples were crushed into fine powder using an electric grinder. Organic solvents of varying polarity were used for plant extract preparations. The dried powder (20 g) was extracted separately with hexane, benzene, chloroform, methanol (1: 10 w/v) for three days with occasional shaking at room temperature. Extracts were then filtered with Whatman No. 1 filter paper and concentrated and dried in a rotary evaporator. The dried extracts were stored at +4°C for further use [14].

2.3 Tested Microorganisms

The antimicrobial activity of *Avicennia alba* was evaluated by using seven Gram-positive bacterial strains viz., *Bacillus subtilis* (MTCC 121), *Bacillus coagulans* (lab isolate), *Bacillus cereus* (ATCC 11778), *Bacillus polymyxa* (NCTC 4747), *Bacillus pumilus* (ATCC 14884), *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 29737); seven Gram-negative bacterial strains viz., *Escherichia coli* (MTCC 484), *Proteus vulgaris* (MTCC 426), *Bordetella bronchiseptica* (ATCC 4617), *Providencia spp.* (lab isolate), *Pseudomonas aeruginosa* (ATCC 25619), *Shigella sonnei* (NK 4010), *Salmonella F 14669*; two fungal strains: *Candida albican* (MTCC 183) and *Microsporium gypseum* (MTCC 4523). For fungal strains, spores were used for the antimicrobial assays.

2.4 Thin Layer Chromatography (TLC) and TLC-bioautography of Plant Extracts

TLC and TLC-bioautography methods have been carried out as described by Gupta et al. [19] and Simlai et al. [20].

2.5 Phytochemical Nature of the Active Constituents

The phytochemical nature of the bioactive constituents of wood extracts of *A. alba* were partially identified by spraying the TLC plates with specific spraying reagents for phenols, flavonoids, tannins, alkaloids, sterols and steroids etc [21,22].

2.5.1 Test for phenols

1 N Folin-Ciocalteu reagent was sprayed on TLC plate and heated for 10 m at 80°C for the identification of phenolic constituents. Blue colored spots indicate presence of phenolic constituents in plant extract [21].

2.5.2 Test for flavonoids

1% ethanolic solution of aluminium chloride (AlCl_3) was used as a spraying reagent on TLC plate to detect the presence of Flavonoids. TLC plate was observed under ultraviolet light at 366 nm. In this staining technique, fluorescent yellow spot gives positive indication for flavonoids [21].

2.5.3 Test for tannins

For tannin detection TLC plates were sprayed with alcoholic FeCl_3 (5% w/v) solution. Visible brownish grey colored spots confirm the presence of Tannin on TLC plate [23].

2.5.4 Test for alkaloids

Dragendorff's reagent was sprayed on TLC plates for the detection of alkaloids in plant sample. Plate was visualized under both visible and ultraviolet light at 254 nm. Orange colored spots demonstrate the presence of alkaloid on TLC plate [21].

2.5.5 Test for sterols and steroids

For sterols and steroids, 15 ml 85% phosphoric acid was diluted to 100 ml with methanol and then sprayed on TLC plate. Plate was heated at 120°C for 15-30 min. All compounds of this class fluoresce in long wave UV light. Larger amounts of substance (sterols and steroids) yield spots which are visible in daylight [21].

After the application of spraying reagents, the colors of the developed spots were noted and their Retention factor (Rf) values were calculated using the formula:

$$\text{Retention Factor (Rf)} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}} [24].$$

2.6 Structural Characterization of Isolated Bioactive Fraction

The purity of the isolated fraction was evaluated using high performance liquid chromatography (HPLC; Shimadzu Prominence system) using a C-18 column (Phenomenex Luna, 4.6 × 250 mm, 5 μm or Enable 10 × 250 mm, 10 μm) at a flow rate of 1 mL/min. The mobile phase consisted of acetonitrile and water with a gradient elution programme from 100% water to 100% acetonitrile in 30 min. Partial structural characterization of the isolated fraction was

carried out using analytical techniques like HRMS (Micromas Q- Tof micro TM), FTIR and ^1H NMR. The Fourier transform infrared (FTIR) analysis of this sample (1 mg) was recorded on a Spectrum 100 FTIR spectrometer (Perkin Elmer; Model Spectrum 100, USA) by using potassium bromide (KBr) disc technique. The FTIR spectra were recorded within the range of 450 – 4000 cm^{-1} . ^1H Nuclear magnetic resonance (NMR) was recorded on a Bruker spectrometer (400 MHz; Magnet System Ascend ULH, Germany). HRMS, FTIR, ^1H NMR etc. methods have been used for the determination of preliminary chemical structure of Albain 1 described in this manuscript. These are routine methods frequently used in structural studies of small chemical compounds.

2.7 Antimicrobial Activity

2.7.1 MIC determination

The minimum inhibitory concentration (MIC) of the isolated fraction Albain 1 against pathogenic microorganisms was determined by microdilution method using 96-well flat-bottom micro-test plates in sterile environment [20]. In this assay, 100 μl of fresh culture media containing different concentrations of isolated Albain 1 was inoculated with test microorganisms (approx 5×10^4 number of CFU of bacteria or fungal spores). The incubation period of inoculated plates was 8 h for bacteria and 42 h for fungal spores. After the end of the incubation period, 20 μl tetrazolium salt solution (5 mg of 3-(4, 5-dimethyl-2thiazolyl)-2.5-diphenyl tetrazolium bromide in 1ml PBS) was added into each well and allowed to again incubate for 30 min and 2 h for bacteria and fungi respectively. After the incubation, purple colored formazan crystals were formed in the wells. These formazan crystals were solubilised by adding 100 μl of acidic isopropanol into each well. Then those micro-test plates were read at 570 nm test wavelength and 630 nm reference wavelength on a Spectramax M3 Multifunctional spectrofluorometer equipped with SoftmaxPro software. The lowest concentration of Albain 1, which showed equal or lower absorbance than its corresponding control (well received no microorganism) was considered as Minimum Inhibitory Concentration (MIC). Each experiment was carried out in duplicate using appropriate controls. Positive controls of this assay were ampicillin for bacterial cultures and fluconazole for fungal spores (data not shown). Dimethylsulfoxide (DMSO) was used as a

negative control and a solubilising agent of Albain 1 / antibiotic.

3. RESULTS AND DISCUSSION

3.1 TLC-bioautography of Crude Wood Extract of *A. alba*

We have used TLC and bioautography to identify and subsequently purify the phytochemical Albain 1 having dominant antimicrobial activity from the wood tissue extracts of *A. alba*. TLC is a commonly used technique for separation of natural substances based on their molecular weight and polarity. This technique has wide applications in the determination and identification of various constituents present in biological and chemical samples. In TLC-bioautography method, constituents of a biological sample or crude extract are first separated by standard TLC technique followed by layering of live bacterial cultures on the TLC plate itself [19]. If any of the separated components on the TLC plate has growth inhibitory property for the particular species of bacteria, the growth of the layered bacteria on the plate where the active component is present, is inhibited, thus revealing / identifying the antimicrobial nature of that particular phytochemical component in the crude extract. The bioautography plate can be stained with various vital-staining procedures – we used widely acknowledged MTT assay [20,25]. Thus, initial determination and analysis of antibacterial activity in the wood extracts of *A. alba* has been carried out employing TLC followed by TLC-bioautography method. One such TLC is shown in Fig. 1 in which hexane, benzene, chloroform and methanol extracts of wood tissue of *A. alba* (Fig. 1: Lanes A,B,C and D) were run after which separated phytochemical components were visualised under UV light. However, this TLC plate cannot identify which of the many spots/bands have antimicrobial activities. This question was answered by subjecting the same plate to TLC-bioautography using Gram-positive bacterium *B. subtilis* (Fig. 1: Lanes E, F, G, H). The study suggests that chloroform extract of *A. alba* wood tissue has strong growth limiting antimicrobial activities against gram positive bacteria (Fig. 1: Lane G) compared to other test extracts (Fig. 1: Lanes E, F and H). The phytochemical responsible for one of these strong activities shown in Lane G (Fig. 1: Panel B) was named Albain 1 for easy reference. No growth inhibitory activity by the

tissue extracts has been detected in case of the Gram-negative bacteria tested such as *E. coli* or *P. vulgaris* (data not shown). The variation in susceptibility to external agents between Gram-positive and Gram-negative bacteria have been already reported earlier where Gram negative bacteria have been found to exhibit more resistance due to the presence of outer lipopolysaccharide layer [26]. The analysis has been confirmed by another confirmatory TLC test with greater resolving length (Fig. 2). The chromatograms are shown to contain distinct bands when visualized under UV light at 366 nm (Fig. 2: Lane A) and 254 nm (Fig. 2: Lane B). Some of these bands in the wood chloroform extract have shown antibacterial properties when subjected to bioautography against *B. subtilis* and *B. coagulans* (Fig. 2: Lanes C and D respectively at Rf 0.939, 0.872, 0.734). Clear white zones of inhibitions obtained on the TLC plates suggest the presence of active substances in these regions which inhibited the growth of test organisms *Bacillus subtilis* and *Bacillus coagulans* (Fig. 2: Lanes C, D). The white zones in TLC plates (Fig. 1: Panel B; marked with white arrows and Fig. 2: Lanes C and D) indicate strong killing of test microbes in response to challenges by components present in *A. alba* tissue extracts which were separated by TLC in these lanes. As mentioned in the Figs. 1 and 2, strong antimicrobial activities observed at Rf 0.872 has been named as Albain 1 for easy reference and studied further.

3.2 Phytochemical Nature of Active Constituents

To identify the nature of the bioactive constituents present at the growth inhibitory zones on the TLC-bioautography plates (Fig. 2), colour reactions with different spraying reagents specific for phenolics, flavonoids, tannins, alkaloids, sterols and steroids etc. have been done. The TLC replicate of wood chloroform extract sprayed with phenolic specific colouring reagent have been found to develop few bluish spots. This suggests presence of phenolic compounds at these regions (Fig. 2: Lane E shown with an arrow). But the Rf position of this signal for phenolics is clearly different from the compounds exhibiting antimicrobial properties as shown in Fig. 2, Lanes C and D. On the other hand, sterols and steroids specific reagent has demonstrated positive signals for steroids and triterpenoids at Rf 0.939, 0.872, 0.734 (Fig. 2: Lane F) which are identical to the regions showing antimicrobial activity on TLC-bioautography plates (Fig. 2: Lanes C and D). No signal for flavonoids, tannins and alkaloids has been observed in the wood tissues of *A. alba* by this experiment (data not shown). Therefore, our observations indicate that the phytochemical constituents including Albain 1 in wood-chloroform extract of *A. alba* having major antimicrobial properties are most probably sterols /steroids or triterpenoids (Rf 0.939, 0.872 and 0.734) in nature.

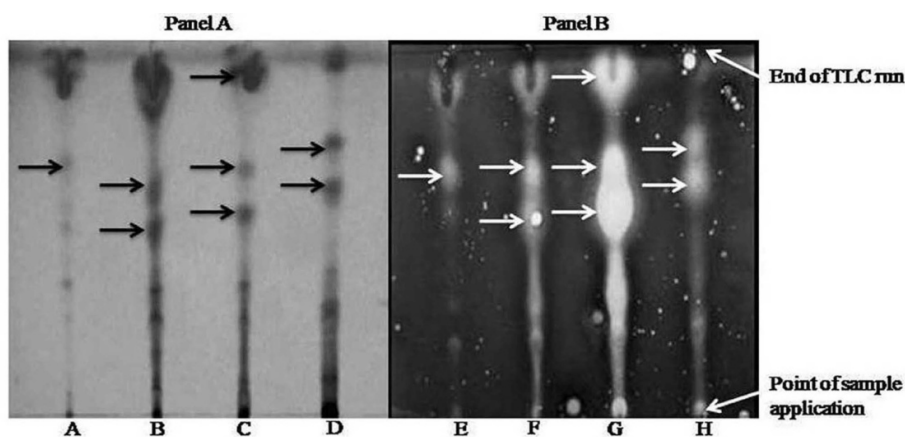


Fig. 1. TLC/ TLC- bioautography of wood tissue of *A. alba*

Panel A - TLC of hexane extract (Lane A), benzene extract (Lane B), chloroform extract (Lane C) and methanol extract (Lane D) of wood tissue of *A. alba*. Panel B - Bioautography of hexane extract (Lane E), benzene extract (Lane F), chloroform extract (Lanes G) and methanol extract (Lane H) of *Avicennia alba* wood tissues against test microbe *Bacillus subtilis*. The white zones in Panel B (marked with white arrows) indicate strong killing of test microbes in response to challenges by components present in *A. alba* tissue extracts which were separated by TLC in these lanes. The phytochemical responsible for one of these strong activities shown in Lane G (third arrow) was named Albain 1 for easy reference

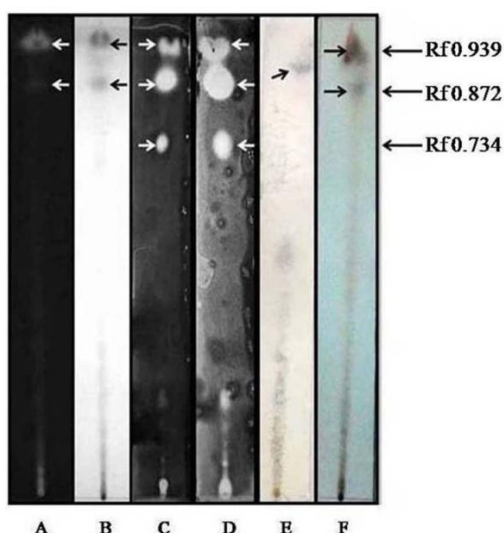


Fig. 2. Preliminary determination of chemical nature of the antimicrobial components in the wood extracts of *A. alba* by TLC (Lanes A and B), TLC-bioautography (Lanes C and D) and staining (Lanes E and F)

Lane A was visualized under long wave UV light (366 nm); lane B under short wave UV light (254 nm).

Arrows indicate phytochemical components of wood extracts. Lanes C and D are bioautography plates with *B. subtilis* and *B. coagulans*. White arrows indicate antibacterial activity on bioautography plates. TLC plate in Lane E was sprayed with F.C. reagent and visualized under visible light for detection of phenolics. Lane F was sprayed with sterols and steroids specific reagent. The phytochemical at Rf = 0.872 which was responsible for the strong antimicrobial activity on TLC plate (run in BEA system) was named Albain 1 for easy reference

3.3 Isolation of Albain 1

Preparative TLC plate has been used for the isolation of Albain 1 with antibacterial properties using BEA as the mobile phase (Fig. 3). After separation of total wood extract of *A. alba* on standard 20 cm long TLC plates, silica between Rf 0.611 and Rf 0.950, (Fig. 3: Panel A) was scraped out, extracted with methanol and analysed by analytical TLC. Fig. 3, Panel B analytical TLC shows that this fraction extracted with methanol from silica contains several spots, which on analysis by bioautography against *Bacillus subtilis* (Fig. 3: Panel C), reveals that some of them including Albain 1 have strong antimicrobial activities. For separation and further isolation of the Albain 1 bioactive band from the remaining other components, the methanol extracted fraction mentioned above was again separated on a longer preparative TLC (56.5 cm

× 16.5 cm) plate and the band corresponding to Albain 1 at specific Rf of 0.872 was scraped off, extracted with methanol and again tested by analytical TLC. Panel D shows this fraction, extracted second time with methanol from preparative long TLC plate, as a single spot of Albain 1 at Rf 0.872 and a single clean major bioautography signal with *Bacillus coagulans* (Panel E) due to its antimicrobial effect.

3.4 Structural Characterization of Albain 1

The isolated phytochemical Albain 1 was subjected to HPLC analysis to determine its level of purity. The data obtained from HPLC analysis has indicated the presence of a major peak at retention time 20.729 min (Fig. 4) at an acetonitrile concentration of 82%. There have been a few minor components in the isolated sample but overall purity of isolated Albain 1 appeared to be suitable for further chemical analysis with regard to its structure. The organic functional groups analysis of this active fraction was carried out by infrared (IR) spectroscopy and the FTIR spectral data is shown in Table 1. The appearance of band at 3450 cm^{-1} indicates the presence of hydroxyl group ($-\text{OH}$) and the signal at 1600 cm^{-1} also suggestive of the presence of carboxylic group and unsaturation in this compound. The $^1\text{H NMR}$ study of this compound, dissolved in deuterated chloroform (CDCl_3), has been carried out at 400 MHz. The $^1\text{H NMR}$ data was analyzed with their probable assignments in Table 2. The chemical shifts indicated by signals around δ 0.75, 0.81, 0.85, 0.95, 0.96 suggest the presence of tertiary methyl group in this compound. Appearance of one proton signal at δ 1.68 indicates the presence of $>\text{C}-\text{CH}_3$ group while the presence of an olefinic proton in this compound is indicated by another signal at δ 4.72. The HRMS analysis and the mass spectra obtained in the procedure by electron ionization technique helped to interpret the molecular formula of this compound. The molecular formula for this compound has been assigned to $\text{C}_{30}\text{H}_{48}\text{O}_4$ based on the molecular ion peak at m/z 472 and also other significant ion peaks at m/z 207, 220, 235, 264. On the basis of this spectral data it may be assumed that the most probable structure of this compound is a derivative of betulinic acid. We arrived at this conclusion after a detail study of the mass spectral analysis of the fragmented molecule which is shown in Fig. 5a. The chemical shift values are shown in Fig 5b which also depicts the probable structure of the compound. Fragmentation of a molecule in a mass spectral analysis is a useful technique;

the fragments of a molecule show a unique pattern in the mass spectrum which is helpful in determining the tentative structure of that unknown compound. In Fig. 5a the mass fragmentation of the unknown compound has been given. In another, Fig. 5b the structure of the unknown compound has been numbered and

chemical shift values have also been mentioned. On the basis of these analyses it has been indicated that the most probable molecular formula for this unknown compound has been assigned to $C_{30}H_{48}O_4$. For the sake of easy reference we have addressed this compound as Albain 1.

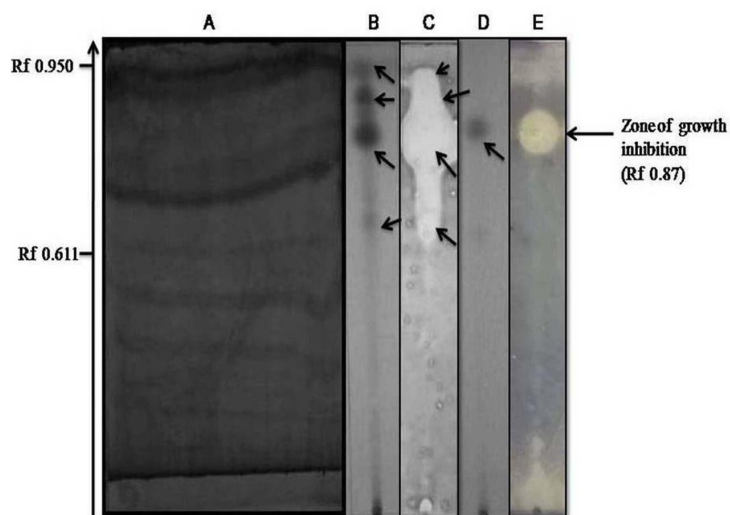


Fig. 3. Isolation of Albain 1

Panel A: Preparative TLC of wood chloroform extract of *Avicennia alba* was done using mobile phase BEA (18: 2: 0.2). Silica from Rf 0.611 to Rf 0.950 was then scraped off the TLC plate, extracted and analysed further by analytical TLC as shown in Panel B. Panel C: TLC-bioautography of the same TLC plate shown in Panel B against test microbe *Bacillus subtilis*. Panel D: TLC of re-isolated major bioactive band at Rf 0.872 from Panel C. Panel E: TLC-bioautography of the isolated bioactive band in Panel D against test organism *Bacillus coagulans*. Panel B and D were visualized under UV 254 nm. The white zones observed on the analytical TLC plate after bioautography in Panel C and E are due to the killing of test microbes by the phytochemicals with antibacterial activities separated on the TLC plate. Direction of sample run on TLC plates is shown by arrow

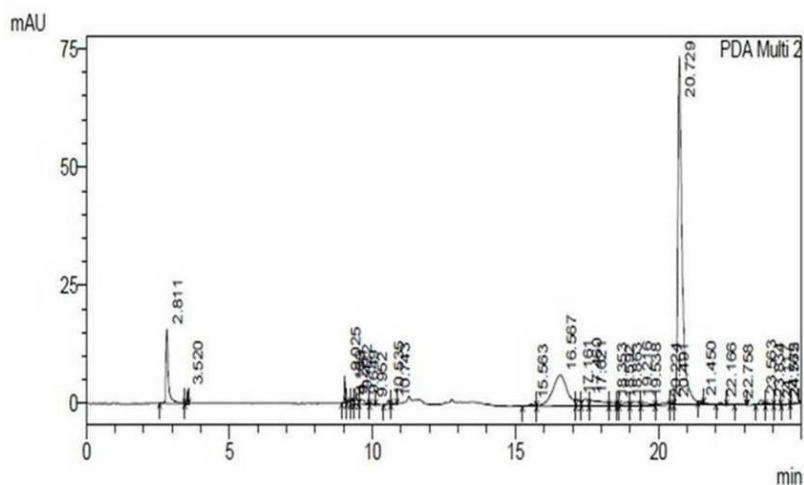


Fig. 4. Analytical HPLC (C-18, 5 μ m, 4.6 \times 250 mm) chromatogram of Albain 1 fraction isolated from the wood of *A. alba*

Spectral data was recorded at 254 nm

3.5 Antimicrobial Activity of Purified Albain 1

The antimicrobial activity of the isolated wood fraction has been evaluated by the micro-dilution method (MTT microtitre plate assay). MIC value determination of this fraction has been carried out by spectrophotometrically reading the intensity of the purple coloured formazan crystals. These are produced due to the reduction of MTT in the presence of mitochondrial dehydrogenase of viable bacterial

and fungal cell [25,27]. Albain 1 exhibited strong antibacterial (MIC: 125 µg/ml) activity against *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus pumilus* (Table 3) which are known to be pathogenic in nature. *B. cereus* has been reported as a causative agent of food borne illness and also known to cause ocular and skin infection whereas *B. pumilus* causes cutaneous infections, food poisoning etc. [28,29,30]. This assay also confirmed that the concentrations of the isolated fraction are unable to effect the growth of the remaining tested microorganisms.

Table 1. Infrared absorption spectrum of the isolated compound Albain 1

Characteristic absorption band cm^{-1}	Probable assignment
3450	OH
1600	>C=O, Unsaturation

Table 2. ^1H NMR proton signal of the compound in CDCl_3 at 400 MHz

Chemical shift (δ)	No. of protons	Probable assignment
0.75	3	Tertiary methyl
0.81	3	Tertiary methyl
0.85	3	Tertiary methyl
0.95	3	Tertiary methyl
0.96	3	Tertiary methyl
1.68	3	>C-CH ₃
4.72	2	Olefinic unsaturation
2.18, 2.20	2	>C=CH ₂
3.20, 3.60	1	-O-H

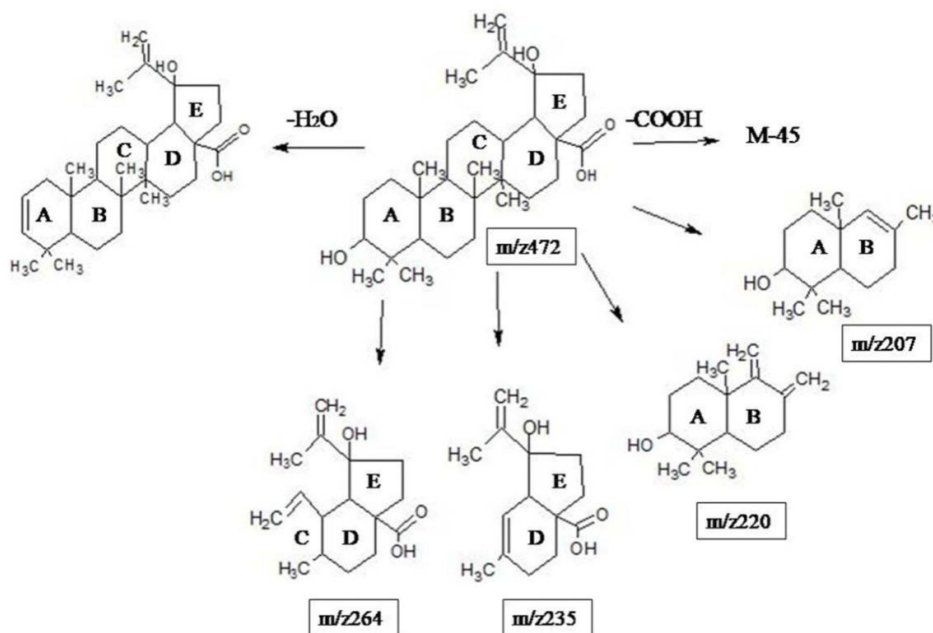


Fig. 5a. Fragmentation of the isolated unknown compound in a mass spectral analysis

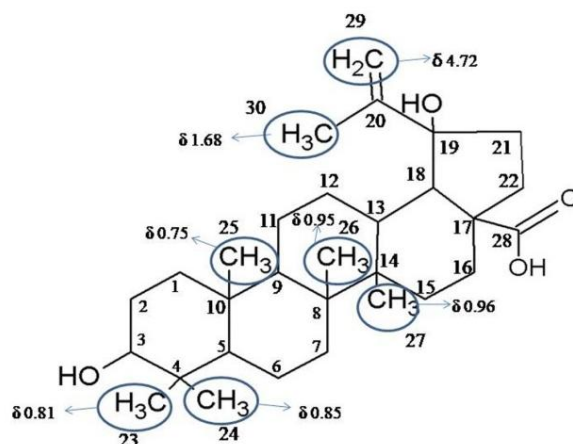


Fig. 5b. Probable structure of the isolated compound ($C_{30}H_{48}O_4$) indicating 1H NMR data

Table 3. Antimicrobial activity (MIC) of Albain 1 from *A. alba*

Microorganisms	Albain 1 MIC value ($\mu g/ml$)
Bacteria	
<i>Bacillus cereus</i> (ATCC 11778)	125
<i>Bacillus polymyxa</i> (NCTC 4747)	125
<i>Bacillus pumilus</i> (ATCC 14884)	125
<i>Staphylococcus aureus</i> (ATCC 29737)	>500
<i>Bordetella bronchiseptica</i> (ATCC 4617)	>500
<i>Micrococcus luteus</i> (ATCC 10240)	>500
<i>Providencia</i> spp. (lab isolate)	>500
<i>Pseudomonas aeruginosa</i> (ATCC 25619)	>500
<i>Salmonella</i> F 14669	>500
<i>Shigella sonnei</i> (NK 4010)	>500
Fungi	
<i>Candida albican</i> (MTCC 183)	>500
<i>Microsporium gypseum</i> (MTCC 4523)	>500

4. CONCLUSION

Mangrove plants are natural treasures of many useful bioactive compounds [15]. To explore these natural wonders for the well-beings of humans, scientific researches are going on all over the world. Previously reported research work on *Avicennia alba* discovered antimicrobial activity of this plant extracts against several pathogenic strains [11,17] of bacteria and fungi. However, they carried out these studies only in the crude extracts of this plant tissue. Our experiments extended these studies further by identifying and investigating the antimicrobial profiles of different phytochemical compounds in the tissue extracts of this plant followed by isolation of one of the most promising antimicrobial components among these phytochemicals and determined its

chemical nature as a triterpene. Named Albain 1, this triterpene has exhibited strong antimicrobial activities against some non pathogenic and pathogenic Gram-positive bacteria such as *B. subtilis*, *B. coagulans*, *B. pumilas*, *B. polymyxa*, *B. cereus* etc. Although we have also determined the preliminary chemical structure of Albain 1, the chemical groups in its molecule that are responsible for the observed antimicrobial activities have not been determined and will be dealt with in future communications. Thus, the findings from this current study suggest that the Albain 1 has remarkable antimicrobial activity and may be considered further to reveal its prospects in pharmaceutical industries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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