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Research Article

Characterization of Antimicrobial Compounds from Abroma Augusta Leaf Extract

Mahfuja Majid[1]*, Farzana Pervin[2] , Sayontoni Projna[3] and Badrul Islam[4]*

[1]*Institute of Biological Sciences, University of Rajshahi, Bangladesh*

[2]*Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh*

[3]*Research Associate, Department of Dental Public Health, City Dental College and Hospital, Dhaka, Bangladesh*

[4]*Natural Products Research Division, BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Bangladesh*

***Corresponding Author:** Badrul Islam, Natural Products Research Division, BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Bangladesh.

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Abstract

Background: *Abroma augusta* is used extensively in traditional medicine, but little is known about its antimicrobial components.

Objective: The objective is to isolate, characterize and assess antimicrobial components from chloroform leaf extracts of *A. Agusta*.

Methods: The leaves were extracted using chloroform and then fractionated using column chromatography based on activity. IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy were used to provide structural information about the isolated molecules. Antimicrobial activity was evaluated using disc diffusion and MIC tests against pathogenic fungus and both Gram-positive and Gram-negative bacteria.

Results: Octacosanol and lupeol were the two chemicals that were purified. MIC values ranging from 32 to 128 µg/ml, depending on the test organisms, indicated that both exhibited antibacterial activity. Antifungal activity was also observed against *Candida albicans, Aspergillus niger, and Fusarium vasinfectum.*

Conclusion: *A. augusta's* ethnomedicinal use and promise as a source of natural antimicrobial agents are supported by the antimicrobial qualities of octacosanol and lupeol.

Keywords: *Abroma augusta*; Octacosanol; Lupeol; Antimicrobial Activity; Natural Products

Introduction

Natural products remain a cornerstone in the discovery of bioactive compounds, particularly in the search for new antimicrobial agents. The rise of multidrug-resistant pathogens poses a serious threat to global public health, creating an urgent need for novel antimicrobials with improved efficacy and safety profiles [1]. Plants are a valuable source of such compounds, as they produce diverse secondary metabolites with well-documented pharmacological properties [2].

Abroma augusta (L.) L.f. (family: Sterculiaceae), commonly known as Devil's cotton or Ulatkambal, is a perennial shrub widely distributed across tropical Asia, Africa, and Australia. In traditional medicine, various parts of the plant are used for gynecological disorders, including dysmenorrhea, amenorrhea, sterility, and as an abortifacient [3-5]. Ethnomedicinal practices also report its use in the treatment of diabetes, rheumatic pain, headache, and infectious diseases [6,7]. Pharmacological studies have confirmed its anti-inflammatory, analgesic, cytotoxic, and antimicrobial properties [8-11].

Although several studies have reported antimicrobial activity of crude extracts from *A. augusta*, very few investigations have focused on the isolation and structural characterization of the specific bioactive constituents responsible for these effects. Secondary metabolites such as triterpenoids, steroids, alkaloids, and long-chain alcohols are often implicated in antimicrobial action, yet clear identification from *A. augusta* remains limited.

In this study, we isolated and characterized two bioactive compounds - Octacosanol, a long-chain aliphatic alcohol, and Lupeol, a pentacyclic triterpenoid from chloroform extracts of *Alstonia augusta* leaves. Octacosanol has been documented in other plants for its hypolipidemic, neuroprotective, and antimicrobial properties [12,13]. Lupeol is recognized for its anti-inflammatory, anticancer, and antimicrobial effects [14-16]. Our results confirm the presence of these bioactive compounds in *A. augusta* and demonstrate their antimicrobial efficacy against Gram-positive and Gram-negative bacteria, as well as pathogenic fungi.

Materials and Methods

Preparation of plant materials for extraction: The fresh leaves have been collected from the Rajshahi University Campus and Meherchandi area near Rajshahi University. After drying, the leaves were powdered in a grinder.

Chemical extraction of the collected materials

In the present study chloroform (Merck/ Germany) was selected to extract the leaf dust of *A. augusta*. The ground dried materials were extracted with sufficient amount of chloroform (500g × 1500ml × 3 times followed by filtration through Whatman filter paper at 24 h interval in the same collection flask).The output extracts were poured in to glass vials and preserved in a refrigerator at 4ºC with proper labeling.

Screening for antimicrobial activity

Chloroform extract of the leaves were subjected for screening the activity against13 bacteria (5 Gram-positive bacteria) *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Sarcina lutea, Streptococcus -ß-haemolyticus* and (8 Gram-negative bacteria) *Salmonella typhi, Shigella dysenteriae,Shigella shiga , Shigella sonnei, Shigella boydii, Pseudomonas aeruginosa, Proteus sp.* and *Escherichia coli*at concentrations of 50 and 200 µg/disc along with a standard antibiotic, Ciprofloxacin 30µg/disc and seven pathogenic fungi *Fusarium vasinfectum*, *Aspergillus fumigatus* , *Aspergillus niger*, *Aspergillus flavus, Mucor sp.,Candida albicans* and *Penicillium notatum* at concentrations of 50 and 200 µg/disc along with a standard Nystatin, (50µg/disc).

Isolation of compounds

Isolation for the antimicrobial compounds from the leaf of *Abroma augusta* was done mainly by open column chromatography (OCC), while thin layer chromatography (TLC) was used as a preparative tool to ensure possible separation. For the first fractionation sephadex LH_{20} (Pharmacia) was used as the stationary phase and $CHCl_3$ and MeOH (1:1) was the eluent on a glass column of 2.5 × 32 cm for 500mg of the leaf extract. Elution time was adjusted to yield 1 ml/min. It gave 99 tubes, which were then spotted on TLC to run and reveal the compounds by reagent spray. Six fractions were made for tubes 1-12 (Fr. I), 13-30 (Fr. II), 31-49 (Fr. III), 50-65 (Fr. IV), 66-79 (Fr. V) and 80-99 (Fr. VI). Biological assay with fungi indicated Fr. iii for the presence of bioactive components there in and it was then subjected to fractionation. Selecting a solvent system by TLC, a slurry of cyclohexane and acetone (8:1) was applied on a glass column of 2×25 cm which was packed with silica gel (70-230 mesh, 40gm) (Sigma). The elution was kept similar to that of the previous one. This fractionation yielded 90 tubes and TLC was made for all of them to get six sub-fractions: tubes 1-9(Sfr. I), 10-20 (Sfr. II), 21-29 (Sfr. III), 30-50 (Sfr. IV), 51-68 (Sfr. V) and 69-90 (Sfr. VI). Biological assay with the test fungi indicated Sfr. IV for the presence of bioactive components and that was then subjected to fractionation. Again selecting a solvent system by TLC a slurry of cyclohexane and acetone (4:1) was applied on a glass column of 1.5 × 25 cm was packed with 20 gm silica gel (230-400 mesh, Sigma). The elution was kept similar to that of the previous one. The fraction yielded 88 tubes for 6 fractions for tubes 1-10 (Ssfr. I), 11-20 (Ssfr. II), 21-35 (Ssfr. III), 36-47 (Ssfr. IV), 48-68 (Ssfr. V) and 69-88(Ssfr. VI). Biological assay of these fractions against the test fungi indicated Ssfr. ii for the bioactive compound, which was a pure compound of 30 mg of white crystal powder and was named Compound A_1.

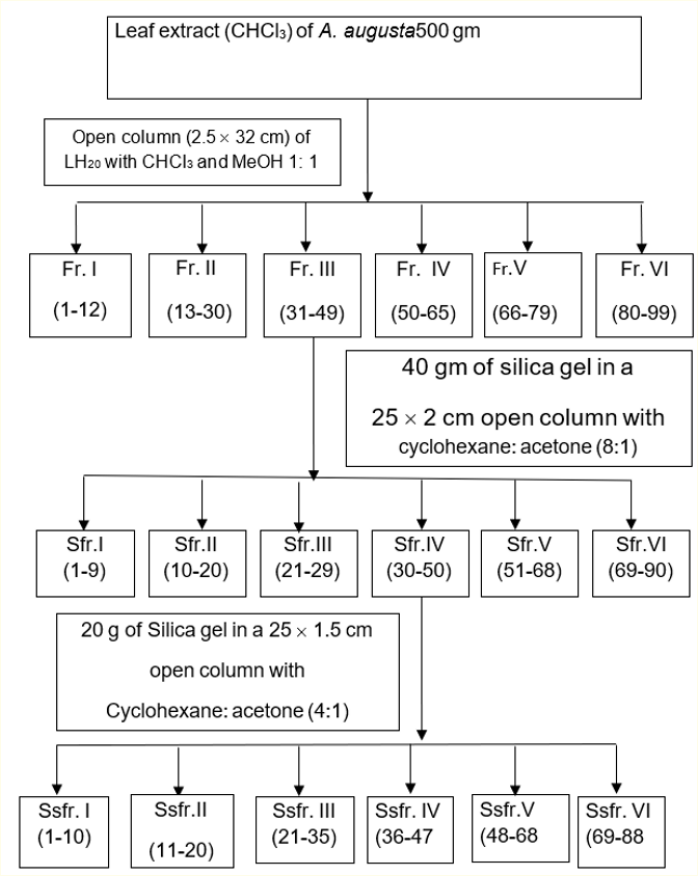

Figure 1: Isolation pathway of the compound A1 from the leaf of *A. augusta.*

Sephadex LH_{20} (Pharmacia) was used as the stationary phase and $CHCl_3$ and MeOH (1:1) was the eluent on a glass column of 2.5 × 32 cm for 500 mg of the leaf extract. Elution time was adjusted to yield 1 ml/min. which yielded 93tubes, which were then spotted on TLC to run and reveal the compounds by reagent spray. Six fractions were made for tubes 1-15(Fr. I), 16-30 (Fr. II), 31-47 (Fr. III), 48-60 (Fr. IV),61-75 (Fr. V) and 76-97(Fr.Vl). Biological assay with fungi indicated that the Fr. III contains bioactive components there in and it was then subjected to fractionation with solvent system n-Hexane: Acetone, (15:1) glass column of 2 × 25 cm packed with silica gel (70-230 mesh, 40 gm, Sigma). The elution was kept similar to that of the previous amount. This fractionation yielded 90 tubes and TLC was made for them to get six sub fractions: 1-10 (Sfr. I), 11-25 (Sfr. II), 26-39 (Sfr. III), 40-55 (Sfr. IV), 56-70 (Sfr. V) and 71-90 (Sfr. VI). Again biological assay with the test fungi indicated that the Sfr. IV Contains the presence of bioactive components and this was then subjected to fractionation selecting a solvent system by n-Hexane: Acetone, (9:1). A glass column of 1.5 × 25 cm was packed with 25gm silica gel (230-400 mesh, Sigma). The elution was kept all along same as used in the previous one. This fractionation yielded 90 tubes for 6 fractions for tubes 1-12 (Ssfr. I), 13-27 (Ssfr. II), 28-45(Ssfr. III), 46-65 (Ssfr. IV), 66-75 (Ssfr. V) and 76-90 (Ssfr. VI). Ssfr Il appeared to have a single compound and it was traced bioactive by antifungal activity test, while this purified compound was 30 mg in amount and was named Compound A_2 (Figure 2).

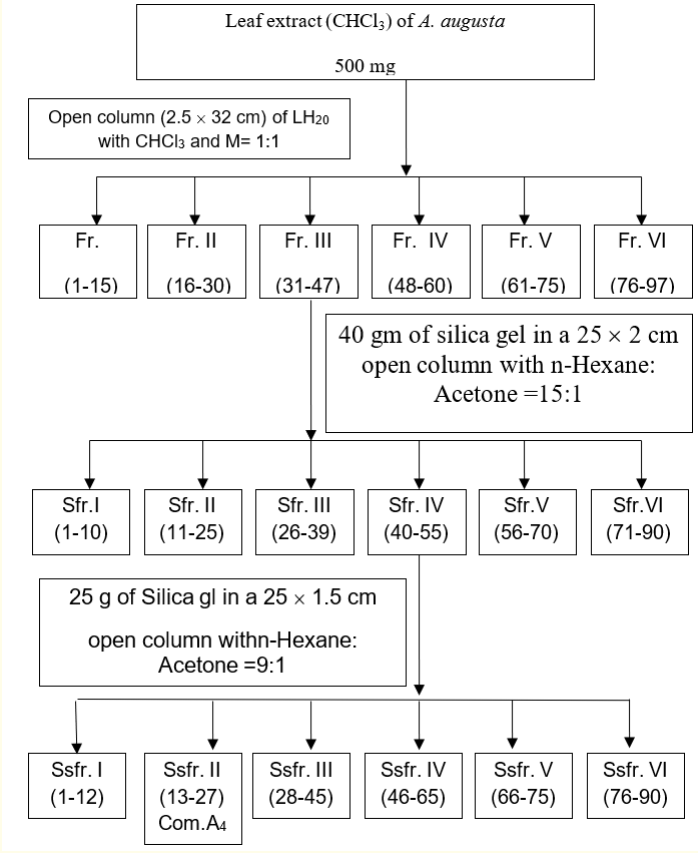

Figure 2: Isolation pathway of the compound A2 from the leaf of *A. augusta.*

Physical remarks of the purified compounds

The isolated compounds and their physical statures have been presented in (Table -). Compound A_1 and A2were isolated from the leaf extracts of *A.augusta*. However, all the ftwo compounds were subjected to NMR analysis, as well as their biological activity tests have been carried out.

Characterization of the leaf compounds through analyses of NMR and IR spectra

Compound 1

A. Purity analysis of compound 1

The purities of compound 1 were detected by TLC. The results (Plate 1) indicated compound 1 showed only one olive green spot along the chromatography plate. That is to say compound 1 should be a relative pure substance.

B. Structure Analysis of the Compound 1

- **Physical properties:** It was white crystalline solid, soluble in $CDCl_3$ and Rf value of the compound was 0.80 (4:1 ratio of Cyclohexane and Acetone).
- **Infrared (IR) Spectra:** Figure 8 shows the IR spectrum which is exhibited absorption band at 3400 cm-1, which is revealed the presence of hydroxyl (OH) group (stretch, H bonded). The IR spectrum shows absorptions at 2954.05, 2916.37, 2848.86 cm-1 due to presence of aliphatic C-H group.
- **1H-NMR Spectra (200 MHz, CDCl3):** Figure (3-4) shows spectrum at δH: 0.885 (3H, t, H-28), 1.276 (50H, br.s, H-3-H-27), 1.554 (2H, m, H-2), 3.647 (2H, t, H-1). The 1H-NMR data of compound A_1 (Table2) was revealed the presence of twenty seven (27) CH2 group at δH (3.647, 1.554, 1.274) ppm, at the position from 2 to 27. One methyl group (CH3) protons were shown peaks at δH: 0.885 ppm at the position of 28. The peak at point of 2.064 ppm was indicated the presence of hydroxyl group (OH) at the position of 1.

Solvent	Compound	Retention factor (R*f*)	Physical identity of the compounds	Colouration after Godin reagent spray
$CHCl_3$	A_1(Leaf)	0.80	White powder	Olive green
	A_2(Leaf)	0.50	Needle like	Greenish

Table 1: Compounds purified from *A. augusta* leafextracts.

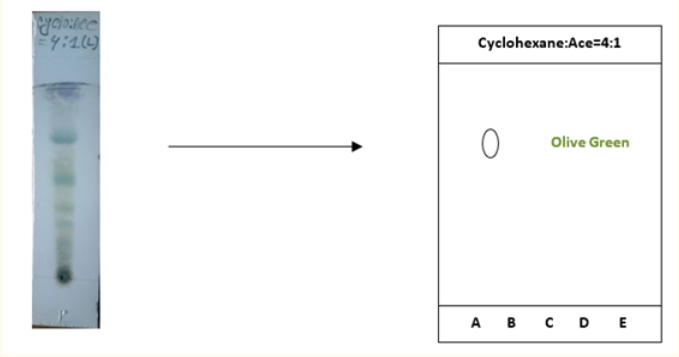

Plate 1: TLC analysis of compound 1 (Olive green).

Position	^{13}C NMR Peak δ in ppm	Types of carbon	^{1}H NMR Peak δ in ppm	Types of proton
1	63.126	CH_2	3.647(t); 2.064(s)	2H; OH
2	32.828	CH2	1.554(m)	2H
3	25.746	CH2	1.276(br.s)	2H
4	29.703	CH_2	1. 276(br.s)	2H
5	29.703	CH2	1. 276(br.s)	2H
6	29.703	CH2	1. 276(br.s)	2H
7	29.703	CH_2	1. 276(br.s)	2H
8	29.703	CH_2	1. 276(br.s)	2H
9	29.663	CH2	1.276(br.s)	2H
10	29.663	CH2	1. 276(br.s)	2H
11	29.663	CH_2	1.276(br.s)	2;H
12	29.663	CH	1.276(br.s)	2H
13	29.621	CH2	1.276(br.s)	2H
14	29.621	CH_2	1.276(br.s)	2H
15	29.621	CH2	1.276(br.s)	2H
16	29.621	CH2	1.276(br.s)	2H
17	29.607	CH2	1.276(br.s)	2H
18	29.607	CH2	1.276(br.s)	2H
19	29.607	CH2	1.276(br.s)	2H
20	29.607	CH2	1.274(br.s)	2H
21	29.441	CH2	1.274(br.s)	2H
22	29.441	CH2	1.274(br.s)	2H
23	29.365	CH2	1.274(br.s)	2H
24	29.365	CH2	1.276(br.s)	2H
25	30.365	CH2	1.276(br.s)	2H
26	31.933	CH2	1.276(br.s)	2H
27	21.695	CH2	1.276(br.s)	2H
28	14.112	CH3	1.884(t)	3H

Table 2: ^{13}C NMR and ^{1}H NMR spectral data of the compound A_1.

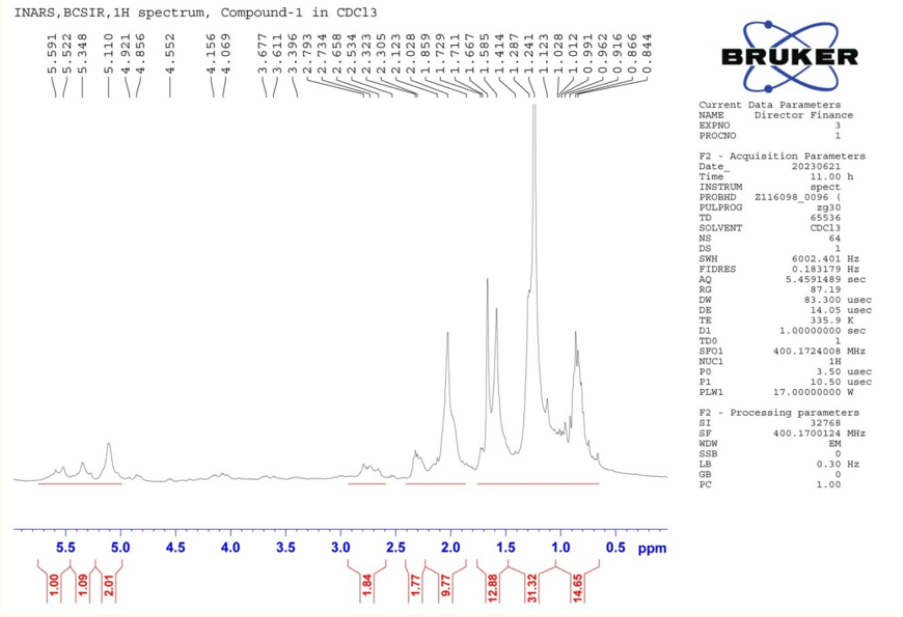

Figure 3: ^{1}H NMR spectrum of compound A_1 in $CDCl_3$ and TMS solutions (200MHz).

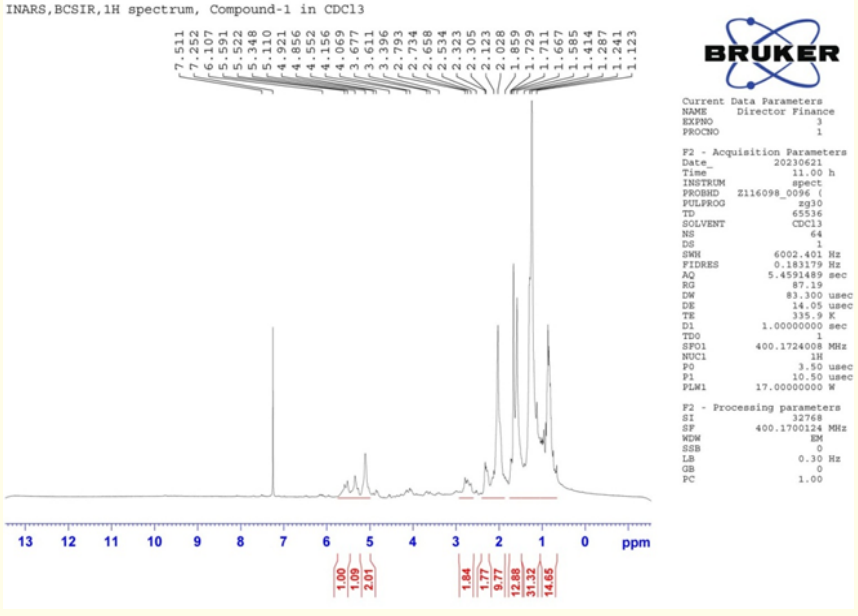

Figure 4: ^{1}H NMR spectrum of compound A_1 in $CDCl_3$ and TMS solutions (200MHz).

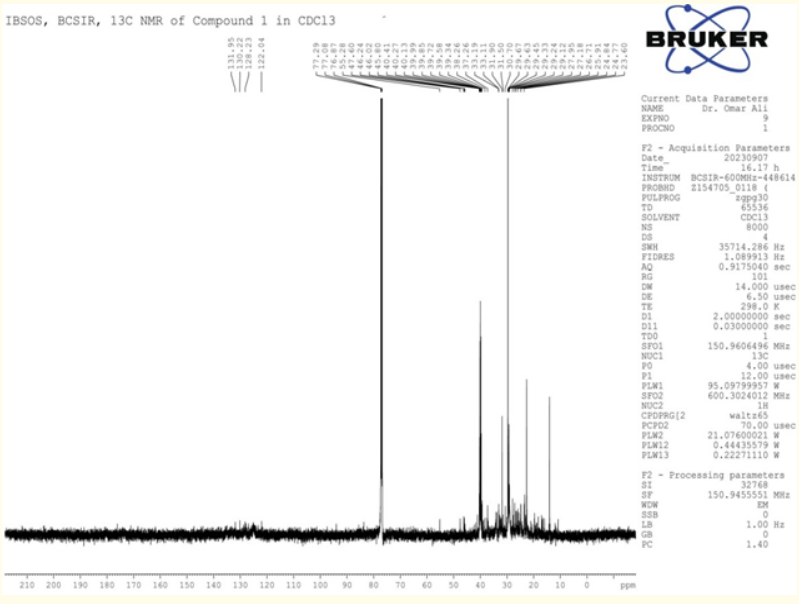

Figure 5: ^{13}C- NMR spectrum of compound A_1 in $CDCl_3$ and TMS solutions (200MHz).

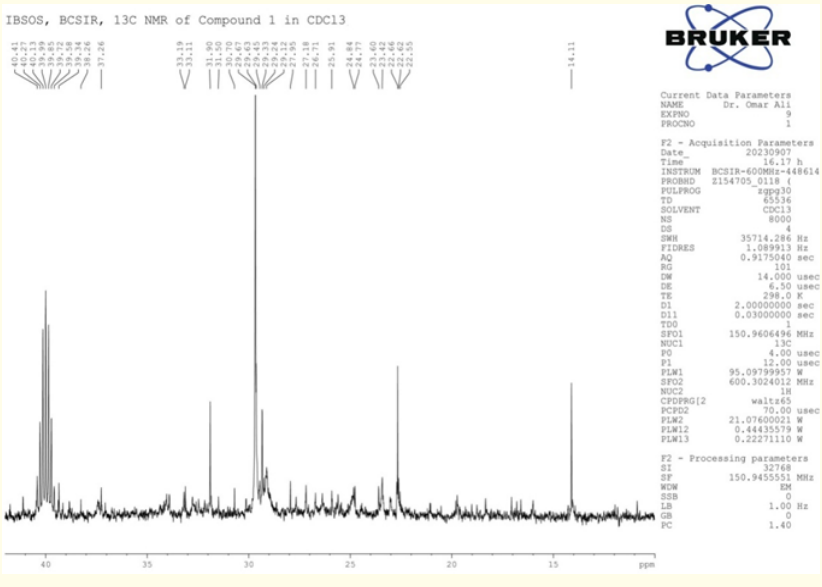

Figure 6: ^{13}C- NMR spectrum of compound A_1 in $CDCl_3$ and TMS solutions (200MHz).

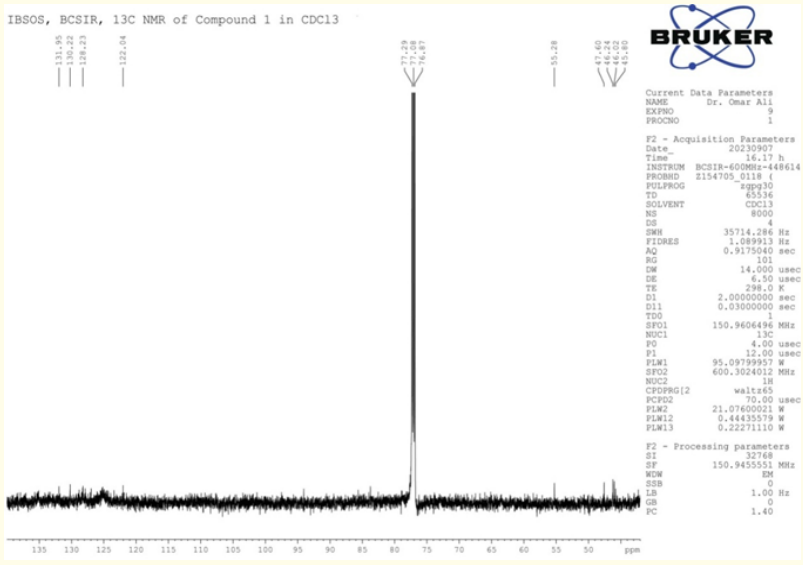

Figure 7: ^{13}C- NMR spectrum of compound A_1 in $CDCl_3$ and TMS solutions (200MHz).

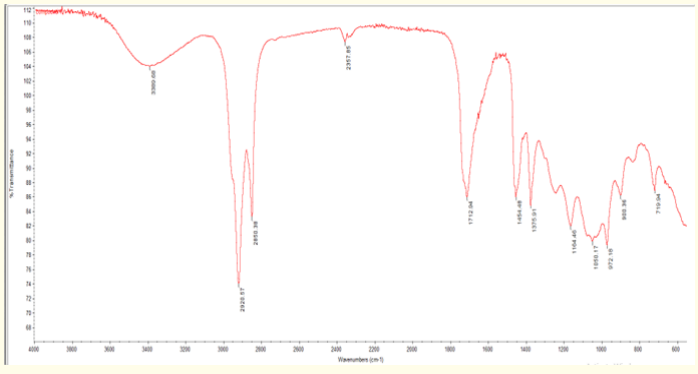

Figure 8: The infrared spectrum of compound 1.

- **13C-NMR Spectra (200 MHz, CDCl3):** From the analyses of different stages figure (5-7) shows spectrum at 13C NMR (CDCl3, 200 MHz) δC: 14.114 (CH3, C-28), 22.695 (CH2, C-27), 25.746 (CH2, C-3), 29.703 (CH2, C-4-C-25), 31.933 (CH2, C-26), 32.828 (CH2, C-2). The signals at δ 25.746 and 22.695 ppm shows the presence of two (CH2) group of aliphatic carbon chain in the molecule at theposition of 3 and 27 and the signals at δ 63.126 ppm indicated the presence of one (CH2) group due to hydroxyl (OH) group. In this case there are 22 more (CH2) group from 4 to 25 due to higher intensity peak at the point 29.621 ppm. The signals at δ 14.112 ppm demonstrated the presence of methyl (CH3) group in the molecule.

Structure of the compound A_1

Among the different analyses from the basis of given figure IR, 1H-NMR and 13C-NMR spectral data the name of the compound A_1 characterized as Octacosan-1-ol which is almost matched with the published data (Rastogi RP. *et al*, 1993). The structure of Octacosan-1-ol is given below

Compound 2

A. Purity analysis of compound 2

The purities of compound 2 were detected by TLC. The results (Plate 2) indicated compound 4 showed only one greenish spot along the chromatography plate. That is to say compound 4 should be a relative pure substance.

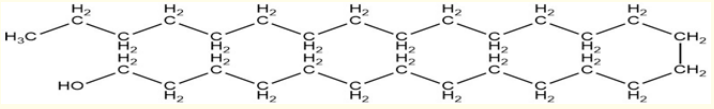

Figure 9: Octacosanol.

Characterization of compound2

Physical properties

It was greenish needle like solid, soluble in chloroform and Rf value of the compound was 0.50 (9:1 ratio of n-Hexane and Acetone).

Infrared (IR) Spectra

The figure 17 shows the IR spectrum which is exhibited absorption band at 3437.21 cm-1, which is revealed the presence of hydroxyl (OH) group (stretch, H bonded). The IR spectrum also shows absorptions at 2919.31, 2949.87 cm-1 due to presence of aliphatic CH group. There is a peak at 1670 cm-1 which indicates C=C (stretch).

1H-NMR Spectra (200 MHz, CDCl3)

The figure (10-11)shows the 1H-NMR data of compound A_4 (Table 3) which is revealed the presence of eleven CH_2 group at δ (1.655, 1.620, 1.643, 1.404, 1.488, 1.302, 1.001, 1.320, 1.954 and 1.338) ppm at the position of 1, 2, 4, 7, 11, 12, 15, 16, 21, 22 and 29. Six (CH) group protons are shown peaks at δ (3.210, 1.383, 1.276, 1.670, 0.930 and 2.369) ppm at the position 3, 5, 9, 13, 18 and 19. Seven methyl (CH_3) groups are shown peak at δ (0.973, 1.113, 0.844, 1.355, 0.950, 0.826 and 1.690) ppm at the position 23, 24, 25, 26, 27, 28 and 30. The peak at point of 2.032 ppm is indicated the presence of hydroxyl group (OH) at the position of 3

13C-NMR Spectra (200 MHz, CDCl3)

The figure (12-16) shows the down field signal at δ 158.104 ppm which is probably due to C=C at A.H-2, the signals at δ 25.908, 37.579, 41.338 and 158.104 ppm are indicated the presence of eight quaternary (C) group and the signals at δ 38.990, 28.002, 38.009, 33.708, 22.693, 27.152, 38.769, 35.796, 29.834 and 41.338 ppm indicated the presence of eleven (CH_2) group in the molecule.

Position	^{13}C NMR Peak δ in ppm	Types of carbon	^{1}H NMR Peak δ in ppm	Types of proton
1	38.990	CH2	1.655(m)	2H
2	28.002	CH2	1.620(m) ; 1.512(m)	2H
3	63.127	CHOH	3.210(dd); 2.032	1H; 1OH
4	38.009	CH2	1.643(m)	2H
5	55.543	CH	1.383(m)	1H
6	25.908	C	_	_
7	33.708	CH2	1.404(m)	2H
8	41.338	C	_	_
9	49.295	CH	1.276 (br.s)	1H
10	37.579	C	_	_
11	22.693	CH2	1.488(m)	2H
12	27.152	CH2	1.302(m)	2H
13	38.769	CH	1.670(m)	1H
14	41.338	C	_	_
15	38.769	CH2	1.001(s)	2H
16	35.796	CH2	1.320(s)	2H
17	41.338	C	_	_
18	49.295	CH	0.930(s)	1H
19	48.770	CH	2.369(t)	1H
20	158.104	C	_	_
21	29.834	CH2	1.954(d)	2H
22	41.338	CH2	1.338(m)	2H
23	15.428	CH3	0.973(s)	3H
24	15.428	CH3	1.113(s)	3H
25	17.507	CH3	0.844(s)	3H
26	15.454	CH3	1.355(m)	3H
27	15.454	CH3	0.950(s)	3H
28	18.806	CH3	0.826(s)	3H
29	116.887	CH2	5.550(q)	2H
30	21.320	CH3	1.690(m)	3H

Table 3: ^{13}C NMR and ^{1}H NMR spectral data of the compound A_2.

The signals at δ 55.543, 49.295, 38.769, 49.295 and 48.770 ppm indicated the presence of (CH) group in the molecule. The signals at δ 15.428, 15.454, 17.507, 18.806 and 21.320 ppm suggested the seven methyl group presences in the molecule. The peak at δ 63.127 ppm which is due to C-O bond (stretch).

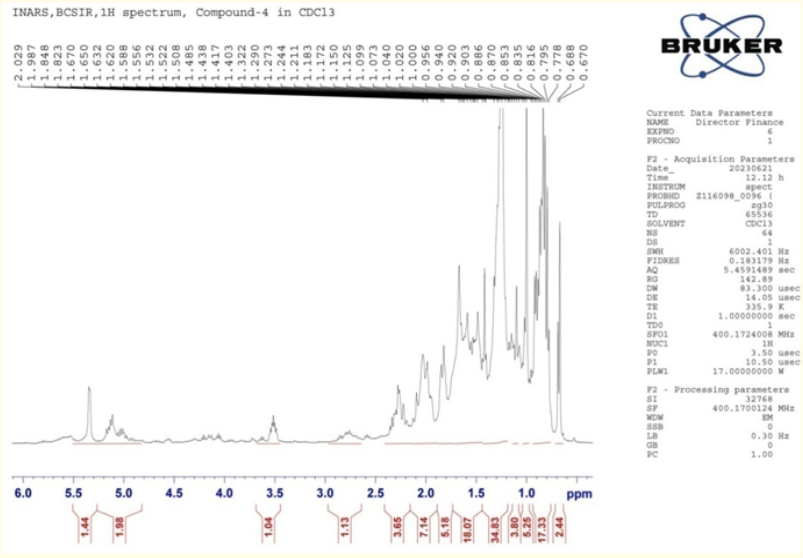

Figure 10: ^{1}H NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

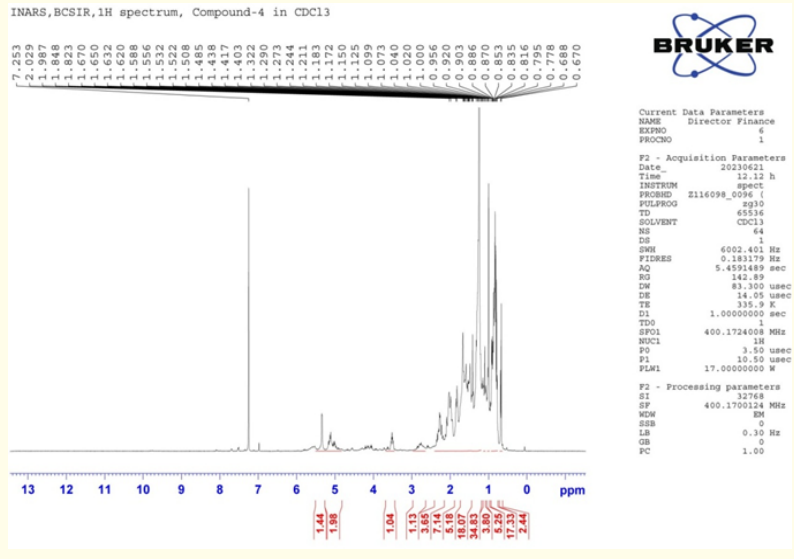

Figure 11: ^{1}H NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

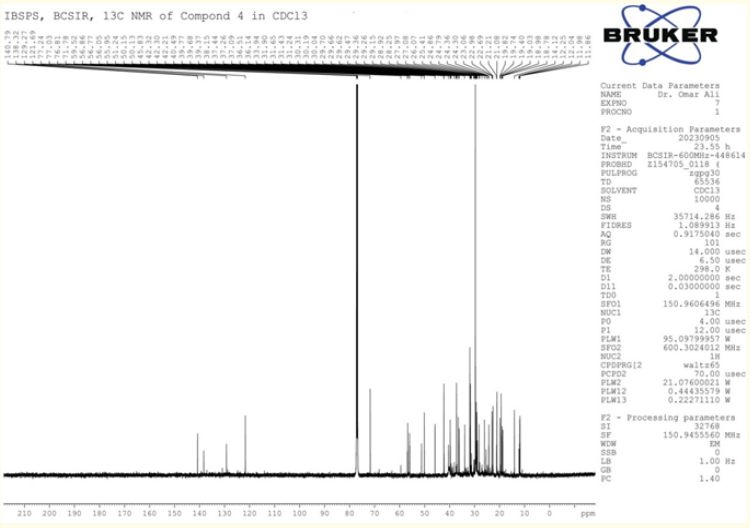

Figure 12: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

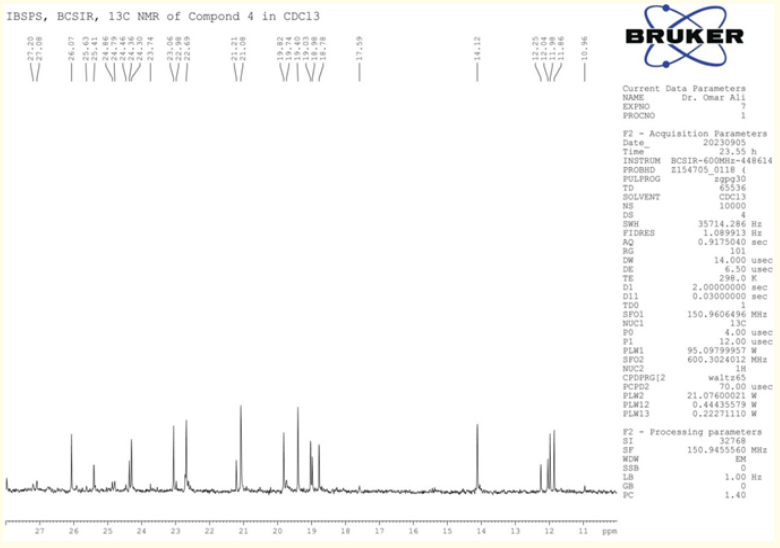

Figure 12: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

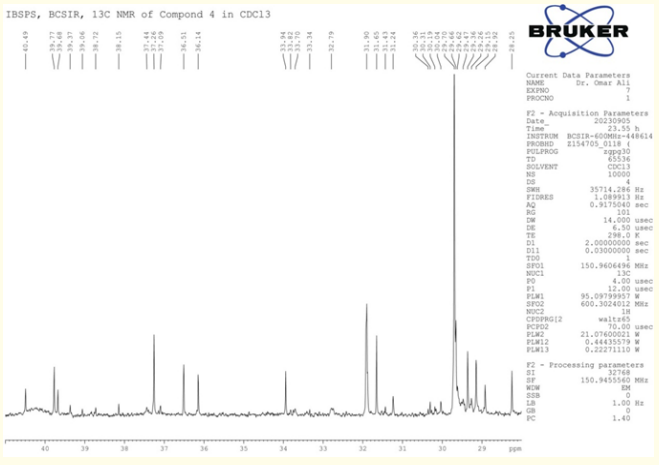

Figure 13: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

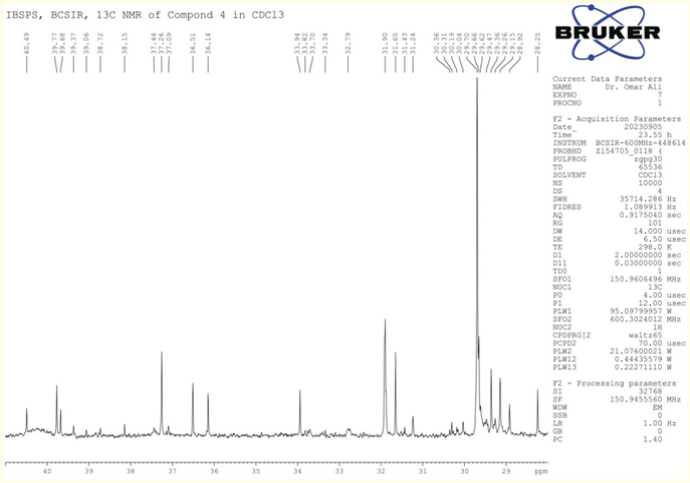

Figure 14: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

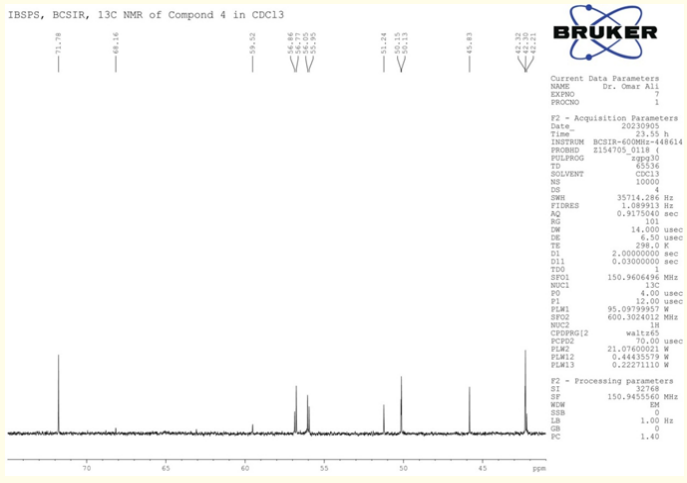

Figure 15: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

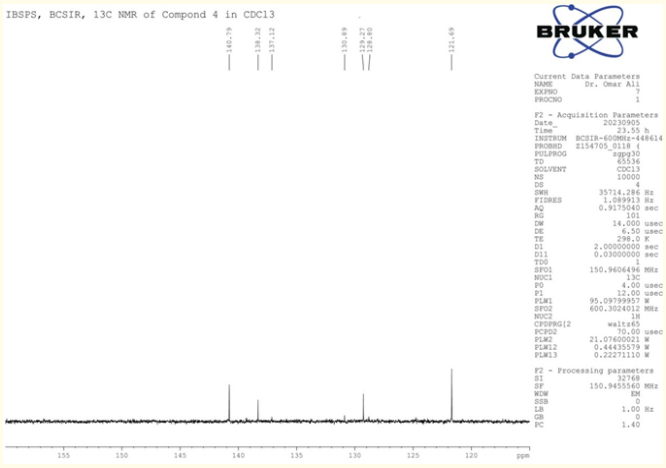

Figure 16: ^{13}C- NMR spectrum of compound A_2 in $CDCl_3$ and TMS solutions (200MHz).

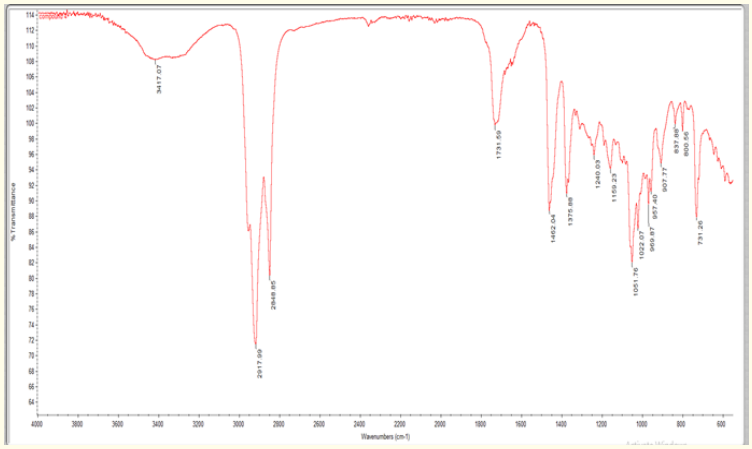

Figure 17: The infrared spectrum of compound 2.

Structure of the compound 2

From the different analyses of the isolated compound on the basis of IR, 1H-NMR and 13C-NMR spectral data the name of the compound A_4 as characterized icosahydro -3a, 5a, 5b, 7, 7, 11a-hexamethyl-1-(prop-1-en-2-yl)-1Hcyclopenta [α] chryse-9-ol or Lupeol which is almost matched with the published data (Jain p. *et* al, 2010 (expect some unwanted peak at δc (29.363, 33.107, 79.093) ppm and δH (1.373, 1.643, 2.079) due to the impurity either for sample or the solvent. The structure of the compound Lupeol is given below:

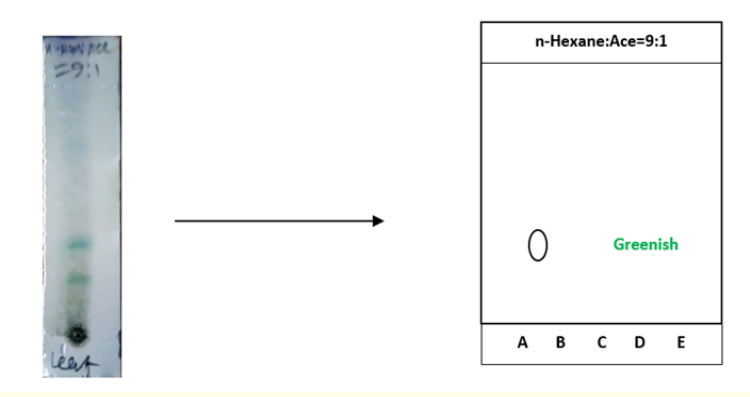

Plate 2: Compounds on point A Greenish is the compound 2.

Results and Discussion

Bioassay of the purified compounds

All the purified compounds of *A. augusta* isolated from the leaf were active against Gram positive and Gram negative bacteria and against the selected fungi; and the result is presented in (Table 4 and 7). The results of the Minimum Inhibitory Concentration (MIC) tests have been presented in Tables (5-6).

Antibacterial activity of the purified compounds

Among the test bacteria *S. aureus, B. megaterium, S.- ß -haemolyticus* , *S. typhi*, *S. dysenteriae*, *E. coli* and *K. pneumoni*were responsive to the $A_1$and A_4 compounds with the zones of inhibition given in the (Table 4) below in comparison to the inhibition by the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)		
	A_1	A_2	Ciprofloxacin
	200 µg/disc	200 µg/disc	30µg/disc
Gram positive bacteria.			
S. aureus	-	13	30
B. cereus	-	-	30
B. megaterium	14	12	30
B. subtilis	-	-	30
S. - ß -haemolyticus	13	12	30
Gram negative bacteria			
S. typhi	12	14	30
S. dysenteriae	16	16	30
S. sonnei	-	-	30
S. boydii	-	-	29
E. coli	14	18	30
P. aeruginosa	-	-	30
Proteus sp.	-	-	30
K. pneumoni	8	14	30

Table 4: Antibacterial activity of pure compounds A_1 and $A_2$of *A. augusta* and standard ciprofloxacin.

Minimum inhibitory concentrations (MICs) of the purified compound A1 against test bacteria:

Test tube No.	Nutrient broth medium added (ml)	Compound A_1 (µg/ml)	Inoculum added (µl)	*S. - ß -haemolyticus S*	*B. megaterium*	*S. dysenteriae*
1	1	512	10	-	-	-
2	1	256	10	-	-	-
3	1	128	10	-	-	-
4	1	64	10	-	-	+
5	1	32	10	+	-	+
6	1	16	10	+	+	+
7	1	8	10	+	+	+
8	1	4	10	+	+	+
9	1	2	10	+	+	+
10	1	1	10	+	+	+
Cm	1	0	0	-	-	-
Cs	1	512	0	-	-	-
Ci	1	0	10	+	+	+
Results of MIC values in (µg/ml)	64	32	128			

Table 5: Minimum inhibitory concentrations (MICs) of the purified compound A_1against test pathogenic bacteria.

Note: "+" = Growth "-" = No growth

The MIC values of the pure compound A_1were 128µg/ml against *S. dysenteriae*,64µg/ml against *S. - ß -haemolyticus* and32µg/ml against *B. megaterium*.

Minimum inhibitory concentrations (MICs) of the purified compound A2against test bacteria:

Test tube No.	Nutrient broth medium added (ml)	CompoundA_4 (µg/ml)	Inoculum added (µl)	*E. coli*	*S. aureus*	*S. dysenteriae*
1	1	512	10	-	-	-
2	1	256	10	-	-	-
3	1	128	10	-	-	-
4	1	64	10	-	-	+
5	1	32	10	+	-	+
6	1	16	10	+	+	+
7	1	8	10	+	+	+
8	1	4	10	+	+	+
9	1	2	10	+	+	+
10	1	1	10	+	+	+
Cm	1	0	0	-	-	-
Cs	1	512	0	-	-	-
Ci	1	0	10	+	+	+
Results of MIC values in (µg/ml)				128	32	64

Table 6: Minimum inhibitory concentrations (MICs) of the purified compound A_2against test pathogenic bacteria.

Note: "+" = Growth "-" = No growth

The MIC values of the pure compound A_2were 128 µg/ml against *E. coli*,64µg/ml against *S.dysenteriae* and32 µg/ml against *S. aureus.*

Antifungal activity of the purified compounds:

Among the test fungi *F. vasinfectum, A. niger*, *A. flavus, C. albicans* and *Mucor sp.* were responsive to the A_1 and A_4compounds with the zones of inhibition given in the (Table 7) below in comparison to the inhibition by the standard nystatin**.**

These results are similar with the results of Saikot., *et al.* (2012) which they demonstrated antimicrobial and cytotoxic activities of the crude extract of *Abroma augusta* [17].

Test Fungus	Diameter of zone of inhibition (in mm)		
	A_1	A_2	Nystatin 50µg/disc
	200 µg/disc	**200 µg/disc**	
F. vasinfectum	16	17	30
A. fumigatus	-	-	30
A. niger	15	16	30
A. flavus	17	15	30
C. albicans	20	16	30
P. notatum	-	-	30
Mucor sp.	15	16	30

Table 7: *In vitro* antifungal activity of compounds A_1nd A_2 of *A. augusta* and the standard nystatin.

In support of these findings screening results for cytotoxicity by many previous researchers done on an allied species of *A. augusta* were available. These findings support the ethanolic extracts of *Derris scandens* (Roxb.) Benth, along with other test extracts showed cytotoxicity (IC_{50} < 30 µg/ml) against lung and prostate cancer cell lines [13]. Another similar work was also available done on cytotoxicity. These tests showed LC_{50} of petroleum ether, chloroform and methanol extracts on *A. salina* Leach as 1.14, 1.1 and 54.9mg/l respectively.

These findings are in agreement with those of Uddin et al., 2012, who conducted a comparative study on the antibacterial, antifungal, and cytotoxic effects of different extracts of *Dillenia indica* Thunb and *Abroma augusta* Linn. In their study, the extracts were evaluated for brine shrimp lethality bioassay of the ethanolic and petroleum ether extracts of *Dillenia indica* Thunb and *Abroma augusta* Linn. were tested by following the procedure of Meyer where DMSO used as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality or not. For the extract, the number of nauplii died and percent mortality was counted. We have observed that the LC_{50} value of the ethanolic and petroleum ether extracts of *Dillenia indica* Thunb were 574.926 µg/ml and 334.283 µg/ml respectively whereas it was 380.875 µg/ml and 307.458 µg/ml respectively for ethanolic and petroleum ether extracts of *Abroma augusta* Linn [19].

The results showed that increasing concentrations of extracts increased the activities in all the microorganisms. The methanol extracts of plant parts showed significant antibacterial activity. The root wood was found to have maximum activity indices against *S. aureus* when tested by the disc diffusion method. The present results clearly demonstrated that chloroform, methanol, ethyl acetate and acetone extractives from various parts of *A. augusta* have significant antibacterial properties. The present data on the antibacterial activity of the experimental plant are supported by a number of recent works. The present findings also fit well with those of Rahmatullah., *et al*., (2010) which they found different activities against bacteria [9]. They explained that the seed oil of *A. augusta* Linn has the potential to be an antibacterial agent against different microorganisms. The oil was screened against various bacteria like *Corynebacterium diphtheria, Escherichia coli, Klebsiella pneumonia, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Staphylococcus aureus and Streptococcus pyogenes* for antibacterial activity. These findings also support the findings of Ahmad., *et al.* (2020) which they reported Ulatkambal (*Abroma augusta* L.): therapeutic uses and pharmacological studies-a review [20].

These results are in agreement with the results of Saikot., *et al.* (2012) which they also conducted research on the leaf extract of *Abroma augusta* used as antibacterial and cytotoxic activity and the results of the extract showed significant antibacterial activity tested on three gram positive (*Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus*) and four gram negative (*Escherichia coli, Shigella dysenteriae, Shigella sonnei and Salmonella typhi*) bacteria [17].

These results are more or less similar with the results of Kabir., *et al.* (2010) which they described antibacterial activity of *B. megaterium*, *B. subtilis, Staphylococcus aureus, Escherichia coli, Vibrio mimicus, Shigella boydii*. On the other hand, the bacteria *Bacillus cereus* and *S. typhi* were found to be resistant to it [19].

These results support the results of Rakesh *et al*. (2023) which they explained *Abroma augusta* (L.) f.: An ethno pharmacological review of its traditional uses and modern applications. They observed that acetone extract of leaves of *A. augusta* showed potent antimicrobial activity against both Gram negative and Gram-positive bacteria like *Bacillus subtilis*, *Bacillus megaratium, Staphylococcus aureus* and *Shigella sonnei* [22].

These results are in agreement with the results of Ivy., *et al.* (2021) which they described In-vitro determination of total phenolic and flavonoid content, and thrombolytic, antimicrobial and antioxidant activities of *Abroma augusta* Lnn. extract [23].

The current findings unequivocally illustrate the significant antifungal properties exhibited by all chloroform, methanol, ethyl acetate, and acetone extracts obtained from different parts of *A. augusta.*

The current results align closely with those of Saikot., *et al.* 2012, who elucidated the antimicrobial and cytotoxic activities of *A. augusta* Linn. leaf extract. Their study demonstrated robust antifungal activity against five fungi, including *A. flavus, A. niger, C. albicans, R. oryzae, and A. fumigatus* [17].

These findings corroborate the results of Kulsum et al., 2019, who investigated the antibacterial and antifungal activities of *A. augusta* L. seed extract. Their study indicated that the seed extracts of *A. augusta* exhibit potential as antifungal agents against *Rhyzopus sp*. and *F. lycopersici* [24].

These findings are consistent with those of Goswami., *et al*., 2023 [22], as described in their work "*Abroma augusta* (L.) L. f.: An ethno pharmacological review of its traditional uses and modern applications." In this study, the *A. augusta* extract exhibited positive antifungal activity, with inhibition zones ranging from 10 to 18 mm, while griseofulvin (1.0 g/disk) was used as the standard antifungal agent [19]. Additionally, the *Abroma augusta* ethyl acetate extract demonstrated high antifungal activity against *Candida albicans* (11 mm) [24].

These findings corroborate those of Zulfiker., *et al*., 2013, who conducted an investigation into the antioxidant and antimicrobial potential of chloroform and petroleum ether extracts of selected medicinal plants of Bangladesh [25]. In their study, the antifungal activities of the extracts were evaluated against two pathogenic fungi and compared with the standard Nystatin, with inhibition zones measured and expressed in millimeters (mm). Among the tested fungi, *Saccharomyces cerevisiae* exhibited the highest zone of inhibition (35 mm) for PLPE and the lowest zone of inhibition (8 mm) for AACE. These results are also consistent with those of Ahmad., *et al.* 2020, who discussed the therapeutic uses and pharmacological studies of Ulatkambal (*Abroma augusta* L.) [20].

These findings align with those of Uddin et al., 2012, who conducted a comparative study on the antibacterial, antifungal, and cytotoxic effects of different extracts of *Dillenia indica* Thunb and *Abroma augusta* Linn. In their study, the extracts were evaluated for antifungal activity against various fungi, with a standard disk of griseofulvin at 0.1 µg/disc used for comparison. However, the extracts exhibited limited antifungal activity against the tested organisms, as measured by the diameter of the zone of inhibition [19].

These findings closely resemble those of Kabir., *et al*., 2010, who investigated the antifungal activity against *Candida albicans* and *Aspergillus niger* [21].

These findings corroborate the research conducted by Khan., *et al*., 2003, which explored the biological and pharmacological properties of *A. augusta* Linn. seed oil [26]. The antifungal efficacy of the oil was assessed against various pathogens including human (*Trichophyton schoenleinii*, *Pseudallescheria boydii*, *Candida albicans, Aspergillus niger*), animal (*Microsporum canis*, *Trichophytonsimii*), and plant (*Fusarium solani* var. *lycopersici, Macrophomina phaseolina*) pathogens. Growth inhibition was evaluated by measuring linear growth (mm) and calculating inhibition (%) relative to a negative control. Results indicated moderate activity against human and animal pathogens, with the highest inhibition observed against *Trichophyton schoenleinii* (56%) and *Microsporum canis* (50%). Limited activity was observed against other fungi. This suggests the potential of *A. augusta* seed oil as an antifungal agent, particularly against *Trichophyton schoenleinii* and *Microsporum canis*. Further investigations at higher concentrations are warranted to explore its full antifungal potential, offering a promising avenue for safer fungal infection treatments, especially in immune compromised patients who often experience toxicities from conventional antifungal drugs due to high dosage and prolonged therapy [27].

These findings corroborate the conclusions drawn by Chowdhury., *et al*., 2019, in their review titled "Pharmacological Values and Phytochemical Properties of Devil's Cotton (Ulatkambal)" [28]. According to their review, the n-hexane extract derived from *A. augusta* seeds exhibited notable antifungal activity when evaluated using the agar tube dilution method.Antifungal activity of the oil was tested against *Trichophyton schoenleinii*, *Pseudallescheria boydii*, *Microsporum canis*, *Trichophytonsimii*(animal pathogens),*Candida albicans*, *Aspergilus niger*(humanpathogens), *Fusarium solani*, *Macrophomina phaseolina*(plant pathogen).The growth in the medium containing the oil was assessed by measuring the linear growth (in mm), while the growth inhibition (%) was calculated relative to the negative control. The findings suggested that the seed oil of ulatkambal exhibited moderate activity against both human and animal pathogens. However, there was no significant activity observed against the plant pathogens in the extract.The seed oil has the potential to be an antifungal against *Trichophyton schoenleinii* and *Microsporum canis*.

These result supports the results of Salau and Odeleye (2007) which they indicates that extract inhibited the growth of all the tested microorganisms with the exception of *Candida albicans* to various degrees. The extract showed strong antibacterial activity against *S. aureus* NCTC6571, *E. coli* NCTC 10418, *B. subtilis* NCTC8263, *Proteus mirabilis* NCIB 67, *P. aeruginosa* ATCC 10145(which is most times resistant to most antimicrobial agents). Their activity at 240 mg/ml is comparable to that of the control used (streptomycin at a concentration of mg/ml). *Candida albicans* was not sensitive to the extract. On the basis of the result obtained in this present investigation, we conclude that the chloroform extract of root woodhad significant *in vitro* antimicrobialactivity. The obtained results may provide a support tosome uses of the plant in traditional medicine. Furtherstudies are recommended to isolate the active components responsible for the antimicrobial activity [29].

These results also support the results of Rahmatullah., *et al.* (1981) which they screened the seed oil against various bacteria like *Corynebacterium diphtheria, Escherichia coli, Klebsiella pneumonia, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Staphylococcus aureus and Streptococcus pyogenes* for antibacterial activity [9].

Conclusion

In this study, two antimicrobial compounds, Octacosanol and Lupeol, were successfully isolated and structurally characterized from the chloroform extract of *Abroma augusta* leaves. Both compounds demonstrated inhibitory effects against a range of Gram-positive and Gram-negative bacteria as well as selected pathogenic fungi. Octacosanol was particularly effective against *Bacillus megaterium* and *Streptococcus β-haemolyticus*, while Lupeol showed notable activity against *Escherichia coli* and *Staphylococcus aureus*. MIC assays confirmed moderate potency (32-128 µg/ml), indicating that the purified compounds, although less active than standard drugs, contribute significantly to the antimicrobial profile of *A. augusta*. These findings validate the plant's ethnomedicinal applications in treating infectious diseases and highlight its potential as a natural source of antimicrobial agents. Further mechanistic and in vivo studies are recommended to assess therapeutic efficacy and possible synergistic effects with conventional antibiotics.

Future Perspectives

The present findings provide a foundation for further pharmacological investigations of *A. augusta*. Future studies should

- Evaluate synergistic effects of Octacosanol and Lupeol with conventional antibiotics to overcome microbial resistance.
- Explore mechanistic pathways of antimicrobial action through molecular docking, enzyme inhibition, and membrane interaction studies.
- Conduct in vivo studies to assess pharmacokinetics, bioavailability, and toxicity in animal models.
- Investigate other secondary metabolites from *A. augusta* that may act additively or synergistically with the identified compounds.
- Develop standardized formulations from the leaf extract for potential therapeutic or nutraceutical applications.

Such efforts could pave the way for the development of safe, plant-based antimicrobial agents in the fight against drug-resistant pathogens.

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