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Phytochemical and Biological Evaluation of *Ambrosia Maritima.*

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ABSTRACT

This article presents the phytochemical and biological evaluation of *A. maritima* L. (family *Asteraceae*), an important medicinal plant. The phytochemical investigation resulted in the separation and identification of the sesquiterpene lactone ambrosin **1**, damsin **2**, β-sitosterol **3**, and stigmasterol **4**, in addition to the identification of the volatile constituents of petroleum ether, methylene chloride and ethyl acetate fractions by GC/MS analysis. Structures of separated compounds were elucidated by spectral analysis. Additionally, antimicrobial activity, antioxidant activity and cytotoxicity assay of different fractions of *A. maritima* were studied. The antimicrobial activity of petroleum ether and butanol fractions indicated their broad effect, where both of them were active against 10 out of 12 tested species (83.33%). The activity index of butanol extract against *Shigella* spp. and *Bacillus subtilis* using standard antibiotics streptomycin and kanamycin was found to be 100% and 87.5%, respectively. The activity index of petroleum ether extract against *Shigella* spp. using standard antibiotic streptomycin was found to be 91.66%. *Staphylococcus epidermis*, a Gram positive bacterial species that was not investigated previously for its antimicrobial susceptibility, was inhibited by butanol, pet. ether, ethyl acetate extracts. The inhibition zones were found to be 9, 7, and 7 mm, respectively. The antioxidant activity, by both ABTS and DPPH methods, was found to be butanol fraction > ethyl acetate fraction > petroleum ether fraction > methylene chloride fraction. The cytotoxicity activity of the ethyl acetate fraction against HePG2 was strong, while that of butanol as well as petroleum ether fractions were moderate, and methylene chloride fraction was weak. The cytotoxicity activity of the butanol and ethyl acetate fractions against MCF-7was strong, while the activity of petroleum ether fraction was moderate and methylene chloride fraction was weak.

Keywords: Asteraceae, *Ambrosia maritima*, sesquiterpene lactone, β-sitosterol, stigmasterol.

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INTRODUCTION

Ambrosia maritima L`Her (Asteraceae) is known locally as "Damsissa". It is a widely distributed weed especially near water catchment. The species was found in Sudan and Egypt. In Egypt, it grows in Nile bank, south Sinia, Alkharga Oasis and Alwady Algaded [1, 2].

Traditionally, the decoction of the whole plant is used to cure gastrointestinal disturbance, abdominal pain, kidney inflammation and renal colic, whereas the leaves are used for diabetes and blood pressure. In addition, its curative properties extend to include molluscicidal, antimalarial and antitumor activities [3, 4].

The phytochemical investigation of *A. maritima* revealed the presence of pseudoguaianolide sesquiterpene lactones ambrosin, damsin, damsinic acid, neoambrosin, hymenin [7, 6, 3] and 11β-hydroxy-1-chloro-11,1-dihydrohymenin [10].

RESULTS AND DISCUSSION

Phytochemical evaluation

The separation of plant material extract of *A. maritima* afforded some known natural products, including ambrosin **1,** damsin **2** [7, 6, 3, 5], β-sitosterol [8] and stigmasterol [9]. The structures of the separated compounds were proven by 1-D and 2-DNMR data, GC/MS analysis which agreed with the corresponding one from the literature.

Many chromatographic separation trials did not succeed to separate the extracts, as complicated fatty material mixtures were present. Consequently, samples of petroleum ether and methylene chloride extracts, as well as the less polar fraction of ethyl acetate extract were analyzed by GC/MS. A sample from petroleum ether extract afforded by method A 18 compounds, representing 52.06% from the sample, with n-eicosane (10.49%), n-heneicosane (9.30%) and n-tricosane (7.33%) being the major components.

A sample from methylene chloride extract afforded by method A 14 compounds, representing 31.06% from the sample, with 2,6,10-trimethylhexadecane (11.16%), 2,6,10,14–Tetramethylpentadecane (2.56%) and 2-methylhexadecane (2.05%) being the major components.

A sample from the less polar fraction of ethyl acetate extract afforded by method A 16 compounds, representing 47.03% from the sample, with n-docosane (21.63%), n-tetracosane (6.89%) and n-pentacosane (3.92%) being the major components.

As the method A failed to separate sesquiterpene lactones, another sample of petroleum ether and methylene chloride were analyzed by method B, that was described by [7, 6] to afford sesquiterpene lactones. A sample from petroleum ether gave 29 compounds, representing 65.67% from the sample, with the sesquiterpene lactone ambrosin (4.44%) and n-tridecane (6.10%),n-heptadecane (5.30%) and n-eicosane (5.02%) being the major components.

A sample from methylene chloride extract afforded by method B 15 compounds, representing 84.35% from the sample, with ambrosin (27.05%), damsin (24.60%) and n-tetracosane (11.48%) being the major components.

Antimicrobial activity assessment (disc diffusion assay)

The antimicrobial potentials of petroleum ether, methylene chloride, ethyl acetate and butanol extractsof *A. maritima* were examined by disc diffusion assay method, using twelve pathogenic microbial species; *Enterobacter aerogenes*, *Erwinia spp.*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Shigella* spp, *Proteus vulgaris* and *Proteus vulgaris*, Gram negative species, and *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Bacillus subtilis*, Gram positive species, and *Candida albicans*, pathogenic fungal species. The data were presented in Table 1 and Fig. 1

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This study was carried out with an objective to investigate the antibacterial and antifungal potentials of aerial parts extracts of *A. maritima*. The butanol, petroleum ether, ethyl acetate and methylene chloride extracts showed remarkable inhibitions of the bacterial growth against the tested organisms. The butanol and petroleum ether extracts exhibited the highest activities, i.e., they were able to inhibit (83.33 %) of microorganisms of interest (Fig.1). On the other hand, the extract of ethyl acetate inhibited 50% of the tested organisms and extract of methylene chloride inhibited only 33.33% of the tested organisms.

The activity index of butanol extract against *Shigella* spp. and *Bacillus subtilis* using standard antibiotics streptomycin and kanamycin was found to be 100% and 87.5%, respectively. The activity index of petroleum ether extract against *Shigella* spp. using standard antibiotic streptomycin was found to be 91.66%. *Staphylococcus epidermis*, a Gram positive bacterial species that was not investigated previously for its antimicrobial susceptibility, was inhibited by butanol, petroleum ether, and ethyl acetate extracts. The inhibition zones were found to be 9, 7, and 7 mm, respectively.

Table 1: the inhibition zone in mm and activity index% of extracts of *A.maritima* compared to standard antibiotics

Microorganism	Standard Antibiotic/ Inhibition zone (mm)	Am1		Am2		Am3		Am4	
		Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%
Enterobacteraerogenes	kanamycin/ 20	8	40.00	0	0	0	0	8	40.00
Bacillus subtilis	kanamycin/ 20	13	65.00	13	65.00	9	45.00	17.5	87.50
Erwinia spp.	streptomycin/ 38	7	18.42	7	18.42	7	18.42	7	18.42
Escherichia coli	ampicillin/ 14	7	50.00	7	50.00	0	0	7	50.00
Klebsiellapneumoniae	ampicillin/ 27	8	29.62	0	0	0	0	9	33.33
Pseudomonas aeruginosa	tobramycin/ 12	8	66.66	0	0	0	0	11	91.66
Shigella spp.	streptomycin/ 12	11	91.66	0	0	0	0	12	100
Proteus vulgaris	ampicillin/ 16	0	0	0	0	0	0	0	0
Staphylococcus aureus	ampicillin/ 16	7	43.75	0	0	8	50.00	7	43.75
Staphylococcus epidermis	-	7	-	0	-	7	-	9	-
Streptococcus pyogenes	ampicillin/ 25	0	0	0	0	0	0	0	0
Candida albicans	clotrimazole/ 15	11	73.33	12	80.00	7	46.66	11	73.33

Am1= petroleum ether extract; Am2=methylene chloride extract; Am3= ethyl acetate extract; Am4 =butanol extract.

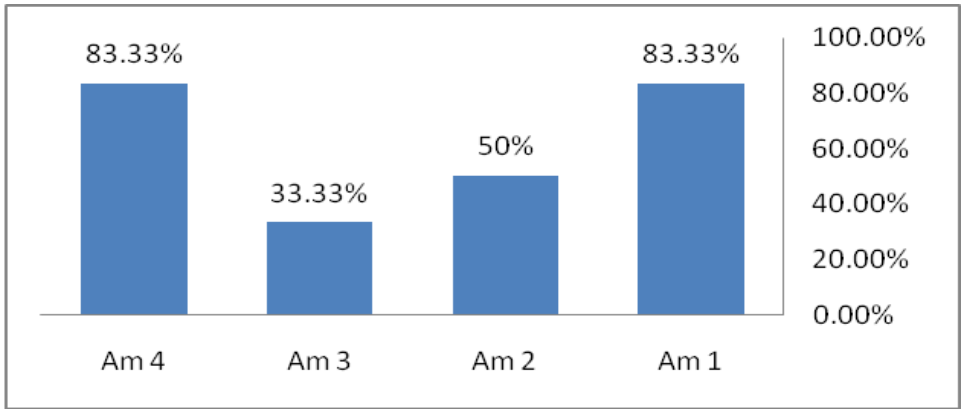

Figure 1: % of affected microorganisms by extracts of *A. maritima* (Am1-Am4)

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Antioxidant activity assessment

Free radical scavenging method (DPPH)

The free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used for detection of the antioxidant activity of the plant extracts. The concentration of an antioxidant needed to lower the initial DPPH concentration by 50% (IC50) is a parameter used to measure the antioxidant activity [11].The lower the IC50, the higher the antioxidant scavenging activity. Butanol extract had the highest scavenging activity. The free radical scavenging activity of the extracts and standard decreased in the following order: ascorbic acid, Am4, Am 3, Am 1 and Am 2 (Table 2 and Fig. 3).

Table 2: IC50 values of the extracts of *A. maritima* by DPPH, compared to ascorbic acid

Extracts	IC50
petroleum ether (Am 1)	5.17
Am2) (methlyene chloride	9.97
Etheyl actate (Am3)	4.98
Butanol (Am 4)	3.42
ascorbic acid	3.11

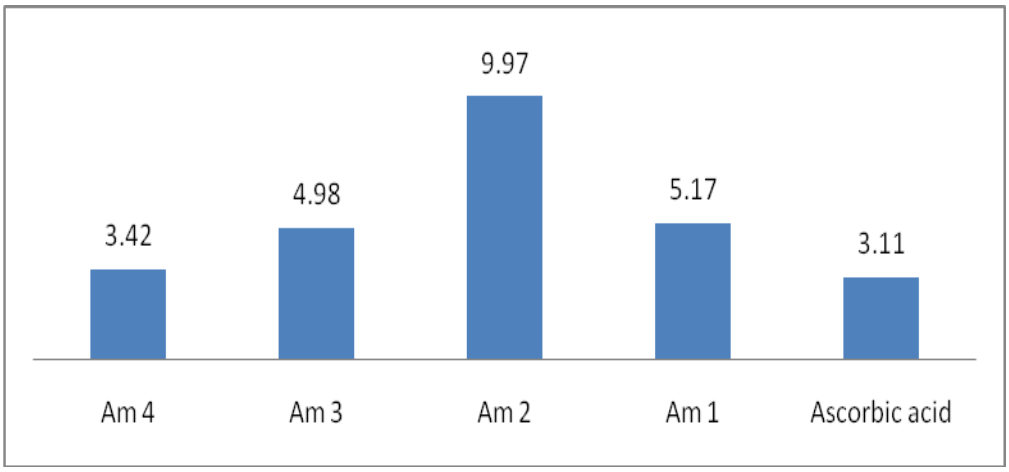

Figure 2: IC50 values of the extracts of *A. maritima* by DPPH, compared to ascorbic acid

Free radical scavenging method (ABTS)

The free radicals of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) is used for detection of the antioxidant activity of the plant extracts. The reduction in color intensity was expressed as inhibition percentage [12]. The lower the (% inhibition), the higher the antioxidant scavenging activity. Butanol extract had the highest scavenging activity. The scavenging effect of the extracts and ascorbic acid decreased in the following order: ascorbic acid, Am 4, Am 3, Am 1 and Am 2 (Table 3 and Fig. 3).

Table 3: The Absorbance and the free radical inhibition % of the extracts of *A. maritima* compared to ascorbic acid

Extracts	Absorbance of samples	%inhibition
Control of ABTS	0.505	0%
Ascorbic acid	0.060	88.1%
Am 1	0.413	18.2%
Am 2	0.472	6.5%
Am 3	0.265	47.5%
Am 4	0.091	82.0%

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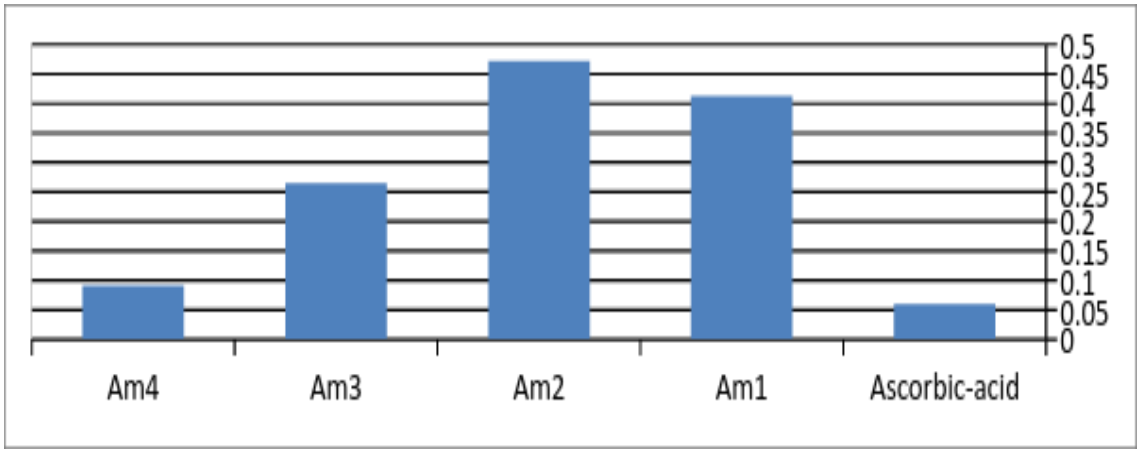

Figure 3: The Absorbance at 734 nm of the extracts of *A. maritima* compared to ascorbic acid

Cytotoxicity assessment of *A. maritima* extracts, against human tumor cells HePG2 and MCF-7

The results (Table 4) indicated that the in vitro cytotoxicity IC50 (µg/ml) to HePG2 was strong for Am 3, moderate for both Am 4 and Am 1, and weak for Am 2. The in vitro cytotoxicity IC50 (µg/ml) to MCF-7 was strong for both Am 4 and Am 3, moderate for Am 1 and weak for Am 2.

Table 4: In vitro IC50 of *A. maritima* extracts against HePG2 and HePG2 cells compared to 5-fluorouracil

Extracts	In vitro cytotoxicity IC50 (µg/ml)	
	HePG2	MCF-7
5-fluorouracil	6.6±0.24	4.7±0.11
Am 1	40.3±2.06	35.0±1.97
Am 2	70.3±3.11	78.5±3.60
Am 3	11.7±0.68	20.3±0.98
Am 4	29.8±1.35	16.7±1.12

IC50 (µg/ml): 1 – 10 (very strong), 11 – 20 (strong), 21 – 50 (moderate), 51 – 100 (weak), above 100 (non-Cytotoxic)

EXPERIMENTAL

^{1}H NMR

Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet. Data were analyzed using Topspin 3.1 Software.

GC/MS analysis

Mothed A: The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251 mm, 0.1 mm film thickness).For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 150°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C /min (hold 4 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system at National Research Center, Dokki, Cairo.

Mothed B: Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric column PAS-5ms (30 m x 0.32 mm x0.25 um film thickness). Samples were injected under the following condition: Helium was used as carrier gas at approximately 1 ml /min, pulse dsplitless mode. The solvent delay was 3 min. and the injection

size was 1.0 µl. The mass spectrophotometric detector was operated in electron impact ionization mode an ionizing energy of 70 eV. scanning from *m/z* 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained at 1250 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C / min. and 10 min. hold at 280°C the detector and injector temperature were set at 280 and 250°C, respectively. Wiley and Nist 05 mass spectral data base was used in the identification of the separated peaks, at the Central Laboratory of the Ministry of Agriculture, Al Bhooth, Cairo.

Material and reagents: PTLC were performed on silica gel (Kieselgel 60, GF 254) of 0.25 mm thickness; petroleum ether (60-80), diethyl ether, hexane, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from Adwic Company; The cell lines HePG-2, hepatocellular carcinoma (liver) and MCF-7, mammary gland (breast) were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt; The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

Plant material

A. maritima was collected from Alkharga Oasis, Alwady Algaded on March, 2014. The plant species was identified by Dr. Maha M. ELShamy, Botany Department, Faculty of Science, Mansoura University, according Egypt flora books [13, 14, 15]. A herbarium specimen was deposited within a special collection of Dr. Maha M. ELShamy in the herbarium of Botany Department, Faculty of Science, Cairo University, Egypt.

Processing of the plant material

The aerial parts were dried in an oven at 40°C for 24 hour and been grinded to give 300 g of dried powder material, which was extracted by a soxhlet extractor using different solvents; petroleum ether (60-80°C), methylene chloride, ethyl acetate and butanol, respectively to obtain four fractions; petroleum ether fraction (18.361 g, 6.120% w/w), methylene chloride fraction (3.070g, 1.023% w/w) and ethyl acetate fraction (1.676 g, 0.558% w/w).

The petroleum ether fraction (18.361 g) was defatted through saponification by dissolving in a small amount of butanol and addition of 5% NaOH then extraction by hexane, followed by methylene chloride to give two successive fractions (2.222 g, 0.524 g, respectively). Hexane fraction (132 mg of which) gave by TLC (silica gel GF 254, benzene / ethyl acetate, 41 : 9) β-sitosterol **3**, and stigmasterol **4**, in a mixture (7 : 8, 8 mg, R_f 0.3026). Methylene chloride fraction (60 mg of which) gave by TLC (silica gel GF 254, benzene / ethyl acetate, 41 : 9) ambrosin **1**, damsin **2** (6 : 6 mg, R_f 0.1875 : 0.3214) respectively.

The ethyl acetate fraction gave (1.676 g) was extracted by methylene chloride using a separatory funnel to give methylene chloride extract (Am 31, 0.455 g, 27.147% w/w). The butanol fraction was extracted by butanol using a separatory funnel to give butanol extract (Am 4, 2.309 g, 76.9% w/w).

GC/MS analysis of petroleum ether fraction (Am1): A sample of petroleum ether extract (Am 1) was analyzed using GC/MS analysis technique to afford by Method A: n-pentadecane (R_t 5.53 min., 0.48 %), n-hexadecane (R_t 6.29 min., 0.53%), n-heptadecane (R_t 6.33 min., 0.92%), n-octadecane (R_t 7.78 min., 0.43%), n-nonadecane (R_t 12.89 min., 7.27%), n-eicosane (R_t 15.21 min., 10.49%), n-heneicosane (R_t 17.42 min., 9.30%), 2-methyl-heneicosane (R_t 18.20 min., 0.25%), n-docosane (R_t 18.71 min., 0.47%), n-tricosane (R_t 21.63 min., 7.33%), n-tetracosane (R_t 23.62 min., 5.69%), n-pentacosane (R_t 24.78 min., 0.30%), n-hexacosane (R_t 27.38 min., 2.63%), n-heptacosane (R_t 29.17 min., 1.65%), n-octacosane (R_t 30.89 min., 0.92%), n-nonacosane (R_t 32.57 min., 0.84%) n- tricosane (R_t 34.19 min., 0.30%), n-hentriacosane (R_t 35.77 min., 0.68%).

A sample of petroleum ether extract (Am 1) was analyzed using GC/MS analysis technique to afford by Method B: n-dodecane (R_t 11.42 min., 2.47%), 2,6-dimethylundecane (R_t 11.68 min., 0.37%), 4,6-dimethyldodecane (R_t 12.83 min., 1.76%), n-tridecane (R_t 13.41 min., 6.10%), n-tetradecane (R_t 14.56 min., 0.95%), 2-methyltridecane (R_t 15.33 min., 1.56%), 2-methylpentadecane(R_t 16.36 min., 0.47%), n-pentadecane (R_t 17.09 min., 1.09%), β-bisabloene (R_t 17.29 min., 4.10%), n-hexadecane (R_t 18.65 min., 3.19%), n-heptadecane (R_t 20.25 min., 5.30%), 4,6,8-trimethyl-2-propylazulene (R_t 21.21 min., 0.32%), n-octadecane (R_t

21.68 min., 2.11%), n-nonadecane (R_t 23.05 min., 3.48%), hexadecanoic acid (R_t 24.04 min., 0.69%), n-eicosane (R_t 24.35 min., 5.02%), n-heneicosane (R_t 25.59 min., 4.44%), (E)-3,7,11,15-tetramethylhexadec-2-en-1-ol (R_t 25.76 min., 0.65%), ambrosin (R_t 26.28 min., 1.18%), n-docosane (R_t 26.79 min., 4.55%), n-tricosane (R_t 27.90 min., 4.20%), damsin (R_t 28.11 min., 24.60%), n-tetracosane (R_t 28.98 min., 1.98%), n-pentacosane (R_t 29.98 min., 2.76%), n-hexacosane (R_t 30.49 min., 0.73%), n-heptacosane, (R_t 32.00 min., 2.42%), n-octacosane, (R_t 33.20 min., 1.62%), n-nonacosane, (R_t 34.64 min., 2.22%), n-triacontane (R_t 36.30 min., 0.67%), n-heneitriacontane (R_t 38.42 min., 1.54%).

GC/MS analysis of methylene chloride fraction (Am 2): A sample of methylene chloride extract (Am 2) fraction was analyzed using GC/MS analysis technique to afford by Method A:n-tridecane(R_t 22.03min., 1.09%),n-tetradecane(R_t25.36min., 5.25%), 2-methyltridecane(R_t 24.09min., 0.35%), 2,6,10–trimethyldodecane(R_t 24.50min., 0.56%), 2,6,10-trimethyltridecane(R_t 27.25min., 1.60%), 2,6,10,14–tetramethylpentadecane(R_t 34.35min., 2.56%), 2,6,10-trimethylhexadecane(R_t 34.25min., 11.16%), 6-methyloctadecane(R_t 33.07min., 0.76%), 2-methyltetradecane (R_t 27.25min., 1.49%), 2,6,10,14–tetramethylheptadecane(R_t 32.65min., 1.47%),2,6,10–trimethyltetradecane(R_t 27.38min., 0.36%), 2-methylhexadecane(R_t 32.53min., 2.05%), 1-nonadecene (R_t35.50min., 0.31%), (*E*)-heptadeca-9-enoic acid (R_t 32.53min., 2.05%).

A sample of methylene chloride extract (Am 2) fraction was analyzed using GC/MS analysis technique to afford by Method B: β-bisabloene (R_t 17.07 min., 3.75%), hexadecanoic acid (R_t 23.65 min., 5.59%), n-heneicosane (R_t 25.26 min., 0.90%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (R_t 25.49 min., 1.52%), 9,12,15-octadecatrienoic acid (R_t 25.91 min.,3.97%), ambrosin (R_t 26.14 min., 27.05%), n-docosane (R_t 26.45 min., 1.30%), n-tricosane (R_t 27.60 min., 4.97%), n-tetracosane (R_t 28.70 min., 11.48%), n-pentacosane (R_t 29.75min., 6.76%), hexacosane (R_t 30.76 min., 5.72%), n-heptacosane (R_t 31.84 min., 4.98%), n-octacosane (R_t 33.07 min., 4.49%).

GC/MS analysis of ethyl acetate fraction (Am 3): A sample of methylene chloride extract (Am 3) fraction was analyzed using GC/MS analysis technique to afford by Method A: n-eicosane (R_t 15.06 min., 2.71%), n-docosane (R_t 17.32 min., 21.63%), (*E*)-nonadeca-9-enoic acid (R_t 18.01 min., 0.80%), n-tricosane (R_t 18.24 min., 0.30%), n-tetracosane (R_t 18.87 min., 6.89%), 2-methyltriacosane (R_t 19.35 min., 1.08%), n-pentacosane (R_t 21.71 min., 0.22%), n-pentacosane (R_t 25.49 min., 3.92%), n-hexacosane (R_t 27.35 min., 3.69%) n-heptacosane (R_t 28.47 min., 0.29%), n-octacosane (R_t 30.89 min., 2.06%), n-nonacosane (R_t 32.57 min., 1.32%), n-triacontane (R_t 34.20 min., 0.47%), n-heneitriacontane (R_t 35.77 min., 0.54%), n-dotriacontane (R_t 37.34 min., 0.31%).

Table 5: MS data of compounds identified by GC/MS analyses (m/z [identity] (rel.int. %))

Name. of Compound	MS Data: m/z [identity] (rel. abund. %)
n-dodecane	170[M^+](6.89),151(0.86),141[$C_{10}H_{21}$]$^+$(1.72),127 [C_9H_{19}]$^+$ (3.44),112 [C_8H_{16}]$^+$(4.31),98 [C_7H_{14}]$^+$(8.62),85 [C_6H_{13}]$^+$ (44.82),71 [C_5H_{11}]$^+$(68.98), 57 [C_4H_9]$^+$(100).
2,6-dimethylundecane	184[M^+](0.83),169 [$C_{12}H_{25}$]$^+$(1.66),162(1.66),155 [$C_{11}H_{23}$]$^+$ (1.66),148(3.33),141 [$C_{10}H_{21}$]$^+$(3.33),133(7.5),120(13.33) ,112 [C_8H_{16}]$^+$(8.33),105(3.33),98[C_7H_{14}]$^+$(13.33),85 [C_6H_{13}]$^+$(16.66),78(1.66),71 [C_5H_{11}]$^+$(53.33),64(1.66), 57 [C_4H_9]$^+$(100),50(166).
4,6-dimethyldodecane	198[M^+](1.66),189(1.66),180(3.33),165(8.33),155 [$C_{11}H_{23}$]$^+$(6.66),141 [$C_{10}H_{21}$]$^+$(10),131(16.66),122(3.33),113 [C_8H_{17}]$^+$(18.33),98 [C_7H_{14}]$^+$(10.0),85 [C_6H_{13}]$^+$(23.33),71[C_5H_{11}]$^+$(86.66), 57 [C_4H_9]$^+$(100).
n-tridecane	184[M^+](10),174(1.66),155 [$C_{11}H_{23}$]$^+$(3.33),142 [$C_{10}H_{21}$]$^+$(6.5),127 [C_9H_{19}]$^+$(6.66),115(18.33),99 [C_7H_{15}]$^+$(13.33),85 [C_6H_{13}]$^+$ (60),71 [C_5H_{11}]$^+$(76.66),57 [C_4H_9]$^+$(100).
n-tetradecane	198[M^+](1.66),174(3.33),156 [$C_{11}H_{23}$]$^+$(5.0),141[$C_{10}H_{21}$]$^+$(16.66),127 [C_9H_{19}] (10),113 [C_8H_{17}]$^+$(10),99 [C_7H_{13}]$^+$ (16.66),85 [C_6H_{13}]$^+$ (66.66), 71 [C_5H_{11}]$^+$(83.33), 57 [C_4H_9]$^+$(100).
2-methyltridecane	198[M^+](0.5),183 [$C_{13}H_{27}$]$^+$(0.5), 155 [$C_{11}H_{22}$]$^+$(3), 113 [C_8H_{17}]$^+$(3),99 [C_7H_{13}]$^+$(8),85[C_6H_{13}]$^+$ (18), 71[C_5H_{11}]$^+$(40), 57 [C_4H_9]$^+$(64).
2-methylpentadecane	226[M^+](1.66),211[$C_{15}H_{31}$]$^+$(3.33), 197[$C_{14}H_{29}$]$^+$ (5),183[$C_{13}H_{27}$]$^+$ (6.66),169 [$C_{12}H_{25}$]$^+$(13.33),155 [$C_{11}H_{23}$]$^+$(26.66),141 [$C_{10}H_{21}$]$^+$(43),127 [C_9H_{19}]$^+$(15), 113 [C_8H_{17}]$^+$(21.66),99 [C_7H_{15}]$^+$ (26.66) ,85 [C_6H_{13}]$^+$(66.66),71 [C_5H_{11}]$^+$(96.66), 57 [C_4H_9]$^+$(100).
beta-bisabloene	204[M^+](44.82),189 [$C_{14}H_{21}$]$^+$(17.24), 176(8.62),161 [$C_{12}H_{17}$]$^+$(68.96),147 [$C_{11}H_{15}$]$^+$(10.34),133(20.68),109(62.06),93[C_7H_9]$^+$(100) ,69(72.41),55(20.68).

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4,6,8-trimethyl-2-propylazulene	212[M^+](1.66),198 [$C_{15}H_{17}$]$^+$(61.66),184 [$C_{14}H_{16}$]$^+$(90),165 (50),141[$C_{11}H_9$]$^+$(30),119 [$C_{11}H_9$]$^+$(76.66),105[C_8H_9]$^+$ (36.66),91 [C_7H_7]$^+$(100), 71 [C_5H_{11}]$^+$(66.66), 57 [C_4H_9]$^+$(83.33).
n-hexadecanoic acid	256[M^+](60.0),238 [$C_{16}H_{30}O$] (16.66), 212 [$C_{15}H_{32}$]$^+$ (93.33),193(61.66),165(43.33),145(26.66),129(66.66),111 (46.66), 85 [C_6H_{13}]$^+$(66.66), 57 [C_4H_9]$^+$(100).
(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	296[M^+](1.66),288(3.33),268(6.66), 252(10),226 [$C_{17}H_{33}O$]$^+$(26.66), 207 [$C_{15}H_{27}$]$^+$(20),191(16.66),165 [$C_{12}H_{21}$]$^+$ (20), 141 [$C_{11}H_9$]$^+$(10),123 [C_9H_{15}]$^+$(43.33),97 [C_7H_{13}]$^+$(46.66), 71 [C_5H_{11}]$^+$(100), 55 [C_4H_7]$^+$(43.33).
damsin	248 [M^+] (0.96), 233 [$C_{14}H_{17}O_3$]$^+$(100), 207 [$C_{12}H_{15}O_3$]$^+$(7.69), 190 [$C_{11}H_{10}O_3$]$^+$(5.76) , 163 [$C_9H_7O_3$]$^+$(7.69), 145 (5.76) , 123[$C_7H_7O_2$]$^+$(23.07), 97 [$C_5H_5O_2$]$^+$(19.23), 79 (7.69), 59 [$C_2H_3O_2$]$^+$(7.69), 41(11.53).
ambrosin	24 6 [M^+] (69.4),217 [$C_{13}H_{13}O_3$]$^+$(36.1),189 [$C_{11}H_9O_3$]$^+$(44.4) , 161[$C_9H_8O_3$]$^+$(50), 145 [$C_8H_6O_3$]$^+$ (50),123[$C_7H_7O_2$]$^+$(47.2), 107 [C6H_3O_2]$^+$ (13.6),91(52.7),67 [C_5H_5]$^+$ (47.2),51[C_4H_3] (33.3).
2,6,10 –trimethyldodecane	212[M^+](0.5),183(1)[$C_{13}H_{27}$]$^+$,141(1)[$C_{10}H_{21}$]$^+$,127(6) [C_9H_{19}]$^+$,97(7)[C_7H_{13}]$^+$,85(22)[C_6H_{13}]$^+$, 71(63)[C_5H_{11}]$^+$, 57 (100)[C_4H_9]$^+$.
2,6,10 -trimethyltridecane	226[M^+](0.5),197[$C_{14}H_{29}$]$^+$(0.5),169 [$C_{12}H_{25}$]$^+$(1), 141 [$C_{10}H_{21}$]$^+$(3),127 [C9H19]$^+$(6),99 [C_7H_{15}]$^+$(7),85[C_6H_{13}]$^+$(24), 71 [C_5H_{11}]$^+$(44), 57 [C_4H_9]$^+$ (100).
2,6,10,14 –tetramethylpentadecane	268[M^+](0.5),240 [$C_{17}H_{36}$]$^+$(0.5),211 [$C_{15}H_{31}$]$^+$(0.5), 183 [$C_{13}H_{27}$]$^+$ (0.5),155 [$C_{11}H_{23}$]$^+$(1), 127 [C9H19]$^+$(6),99 [C_7H_{15}]$^+$(7),85 [C_6H_{13}]$^+$(24), 71 [C_5H_{11}]$^+$(44), 57 [C_4H_9]$^+$(100).
2,6,10 -trimethylhexadecane	268[M^+](0.5),225 [$C_{16}H_{33}$]$^+$(0.5), 183 [$C_{13}H_{27}$]$^+$ (3),155 [$C_{11}H_{23}$]$^+$(2), 127 [C9H19]$^+$(3),113 [C_8H_{17}]$^+$(10), 85[C_6H_{13}]$^+$(22), 71 [C_5H_{11}]$^+$(60), 57 [C_4H_9]$^+$ (100).
6-methyloctadecane	268[M^+](0.5),266(0.5),239 [$C_{17}H_{35}$]$^+$(0.5), 211 [$C_{15}H_{31}$]$^+$(0.5), 169 [$C_{12}H_{25}$]$^+$(1), 141 [$C_{10}H_{21}$]$^+$(2), 99 [C_7H_{15}]$^+$(6),85 [C_6H_{13}]$^+$(18), 71[C_5H_{11}]$^+$(28), 57 [C_4H_9]$^+$(56).
2,6,10,14 –tetramethylheptadecane	296[M^+](0.5),282 [$C_{20}H_{42}$]$^+$(0.5),254 [$C_{18}H_{38}$]$^+$(0.5), 211 [$C_{15}H_{31}$]$^+$ (0.5), 169 [$C_{12}H_{25}$]$^+$(2),127 [C9H19]$^+$(2), 98 [C_7H_{14}]$^+$(8),85[C_6H_{13}]$^+$(18), 71 [C_5H_{11}]$^+$(36), 57 [C_4H_9]$^+$(100).
2-methylhexadecane	284[M^+](0.5),216(0.5), 169 [$C_{12}H_{25}$]$^+$(3), 141[$C_{10}H_{21}$]$^+$(2), 113 [C_8H_{17}]$^+$(6), 85 [C_6H_{13}]$^+$(14),71 [C_5H_{11}]$^+$(36), 57 [C_4H_9]$^+$(100).
nonadecane1-ene	266[M^+](0.5),263(0.5), 238 [$C_{17}H_{34}$]$^+$(0.5),196 [$C_{14}H_{28}$]$^+$(2),165 (4), 141 [$C_{10}H_{21}$]$^+$(4),111(8),97(12),83 [C_6H_{11}]$^+$ (48),69 (33),55(68).
(*E*)-nonadeca-9-enoic acid	298[M^+](33.33),255 [$C_{16}H_{31}O_2$]$^+$(15.68),241 [$C_{15}H_{29}O_2$] $^+$(5.68),199 [$C_{12}H_{23}O_2$]$^+$(11.76),165(4.41),143 [$C_8H_{15}O_2$]$^+$ (20.58),126(6.37),97(30.39),74 [$C_3H_6O_2$]$^+$(100), 57 [C_4H_9]$^+$(34.31).
3,7,11,15-tetramethyl-2-hexadecen-1-ol	296[M^+](0.83),295(0.83),281 [$C_{19}H_{37}O$](0.83),267[$C_{18}H_{35}O$](0.83),253[$C_{17}H_{33}O$](0.83),236[$C_{17}H_{32}$](0.83),211(0.83), 196(0.83),179[$C_{13}H_{23}$](0.83),165[$C_{12}H_{21}$](0.83),151 [$C_{11}H_{19}$] (3.33),137 [$C_{10}H_{17}$] (3.33),123[C_9H_{15}] (30),109 [C_8H_{13}] (13.33),95 [C_7H_{11}](18.33),71(100),57(36.66).
9,12,15-octadecatrienoic acid	278[M^+](3.33),263 [$C_{17}H_{27}O_2$]$^+$(3.33),249 [$C_{16}H_{25}O_2$]$^+$(3.33), 222 [$C_{14}H_{22}O_2$]$^+$(8.33),207 [$C_{14}H_{23}O$] (3.33),193 [$C_{13}H_{23}O$] (1.66),165 [$C_{11}H_{17}O$]$^+$(3.33),149(10),121(16.66),95(45),79 [C_6H_7]$^+$(100),55 [C_4H_7]$^+$(46.66).
2-methyltriacosane	338[M^+](2.45),308 [$C_{22}H_{44}$]$^+$(9.80),278(5.88),251 [$C_{18}H_{35}$]$^+$(4.90), 224 [$C_{16}H_{32}$]$^+$(4.90), 195 [$C_{14}H_{27}$]$^+$(8.82), 154 [$C_{11}H_{22}$]$^+$(8.82),139 [$C_{10}H_{19}$]$^+$(21.56),125 [C_9H_{17}]$^+$(33.33),111 [C_8H_5]$^+$(41.17),96(32.35),83 [C_6H_{11}]$^+$(100),69 [C_5H_9]$^+$(47.05),55 [C_4H_7]$^+$(54.90).
n-pentadecane	212 [M^+] (3.26),208(3.26),183 [$C_{13}H_{27}$]$^+$(6.52),168 [$C_{12}H_{26}$]$^+$(13.04),141 [$C_{10}H_{21}$]$^+$(17.39),113 [C_8H_{17}]$^+$(15.17),99 [C_7H_{15}]$^+$(30.4),85[C_6H_{13}]$^+$(50),70 [C_5H_{12}]$^+$(13.02),57 [C_4H_9]$^+$(100).
n-hexadecane	226 [M^+](10.8),197 [$C_{14}H_{29}$]$^+$(5.4),170 [$C_{12}H_{26}$]$^+$ (34.7), 155 [$C_{11}H_{23}$]$^+$(28.2),113 [C_8H_{17}]$^+$(21.7),84 [C_6H_{12}]$^+$(23.9),71[C_5H_{10}]$^+$(67.3),57 [C_4H_9]$^+$(1 00).
n-heptadecane	240[M^+](2.6),227 (43.4),193(2.17),169 [$C_{12}H_{25}$]$^+$(10.8), 143 (5.43),125 [C_9H_{17}]$^+$(13),96(15.2),85 [C_6H_{13}]$^+$(80.4),69(32.6), 57 [C_4H_9]$^+$(100).
n-octadecane	254[M^+](3),249(4.3),233(10),202(8), 183 [$C_{13}H_{27}$]$^+$(30), 168 [$C_{12}H_{26}$]$^+$(26),111(41),91(100).
n-nonadecane	268[M^+](21.7),238 [$C_{17}H_{34}$]$^+$(1.6),211 [$C_{15}H_{31}$]$^+$(4.3),183 [$C_{13}H_{27}$]$^+$(8.6),155 [$C_{11}H_{23}$]$^+$(1.08),126 [C_9H_{18}]$^+$(7.6),99 [C_7H_{15}]$^+$(41.3),85 [C_6H_{13}]$^+$(0.91),70 [C_5H_{10}]$^+$(21.7), 57 [C_4H_9]$^+$(100).
n-eicosane	282[M^+](10.8),253 [$C_{18}H_{37}$]$^+$(2.71),225 [$C_{16}H_{33}$]$^+$(3.26),197 [$C_{14}H_{29}$]$^+$(4.3),169 [$C_{12}H_{25}$]$^+$(4.3),140 [$C_{10}H_{20}$]$^+$(8.69),112 [C_8H_{12}]$^+$(10.8),85 [C_6H_{13}]$^+$(22.8), 71 [C_5H_{11}]$^+$(21.7), 57 [C_4H_9]$^+$(100).
n-heneicosane	296[M^+](13.0),281 [$C_{20}H_{41}$]$^+$(0.21), 253 [$C_{18}H_{37}$]$^+$(0.21),225 [$C_{16}H_{33}$]$^+$(5.43),198 [$C_{14}H_{30}$]$^+$(1.0),169 [$C_{12}H_{24}$] (4.3) $^+$,141 [$C_{10}H_{21}$]$^+$(2.71), 127[C_9H_{19}]$^+$(15.2),112 [C_8H_{12}]$^+$(2.71),85 [C_6H_{13}]$^+$(13.0), 71 [C_5H_{11}]$^+$(100), 57 [C_4H_9]$^+$(20.6).
2-methylheneicosane	310[M^+](4.34),294 [$C_{21}H_{42}$]$^+$(4.89),236(8.69), 197[$C_{14}H_{29}$]$^+$(13.0), 183 [$C_{13}H_{27}$]$^+$(19.5), 155 [$C_{11}H_{23}$]$^+$(28.2),126 [C_9H_{18}]$^+$(19.5),97(34.7) ,85 [C_6H_{13}]$^+$(89.1),71 [C_5H_{11}]$^+$(100), 57 [C_4H_9]$^+$(78.2).
n-docosane	310[M^+](4.34),266 [$C_{19}H_{38}$]$^+$(10.86), 225 [$C_{16}H_{33}$]$^+$(5.43),197 [$C_{14}H_{29}$]$^+$(6.52),169 [$C_{12}H_{24}$]$^+$(8.69), 154 [$C_{11}H_{22}$]$^+$(2.6),141 [$C_{10}H_{21}$]$^+$(8.69),99 [C_7H_{15}]$^+$(26.08), 85 [C_6H_{13}]$^+$(21.7), 71 [C_5H_{11}]$^+$(48.91), 57 [C_4H_9]$^+$(100).
n-tricosane	324[M^+] (10.86),294 [$C_{21}H_{44}$]$^+$(0.21),254 [$C_{16}H_{33}$]$^+$(1.08),225(3.26),182 [$C_{13}H_{26}$]$^+$(3.26),154[$C_{11}H_{23}$]$^+$ (7.6), 127[C_9H_{19}]$^+$(10.08),113 [C_8H_{17}]$^+$(17.39),85 [C_6H_{13}]$^+$(71.73), 71 [C_5H_{11}]$^+$(91.3), 57 [C_4H_9]$^+$(100).
n-tetracosane	338[M^+] (15.21),295 [$C_{21}H_{43}$]$^+$(2.17),253 [$C_{18}H_{37}$]$^+$(4.34),225 [$C_{16}H_{33}$]$^+$(4.34),197 [$C_{14}H_{29}$]$^+$(6.52),168 [$C_{12}H_{24}$]$^+$(4.34),155 [$C_{11}H_{23}$]$^+$(10.86),97[C_7H_{13}]$^+$(17.39),69 [C_5H_9]$^+$(20.65), 57 [C_4H_9]$^+$(100).
n-pentacosane	352[M^+] (6.52),337 [$C_{24}H_{49}$]$^+$(10.8),309 [$C_{22}H_{45}$]$^+$(15.21),295 [$C_{21}H_{43}$]$^+$(3.26),267 [$C_{19}H_{39}$]$^+$(5.43),253

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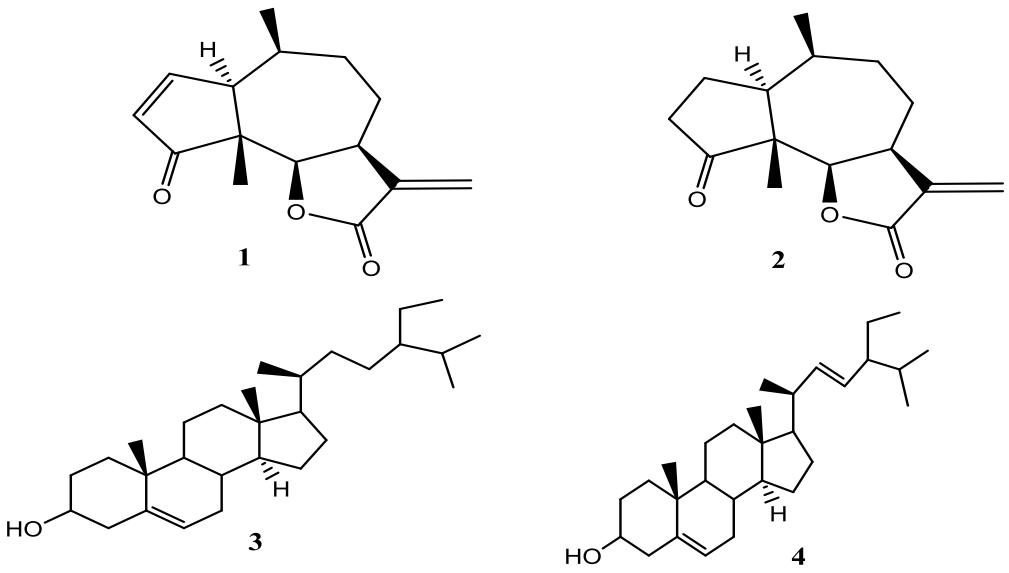
	$[C_{18}H_{37}]^+$(13),197 $[C_{14}H_{29}]^+$(23.91),169 $[C_{12}H_{25}]^+$(15.21),141 $[C_{10}H_{21}]^+$(17.39),99 $[C_7H_{15}]^+$(42.34),85 $[C_6H_{13}]^+$(72.82), 71 $[C_5H_{11}]^+$(91.3), 57 $[C_4H_9]^+$(100).
n-hexacosane	366$[M^+]$ (0.54),309 $[C_{22}H_{45}]^+$(4.34),281 $[C_{20}H_{41}]^+$(6.52),252 $[C_{18}H_{36}]^+$(3.26),211$[C_{15}H_{31}]^+$(5.43),182 $[C_{13}H_{26}]^+$(4.34), 169 $[C_{12}H_{25}]^+$(15.21),141 $[C_{10}H_{21}]^+$(6.52),97 $[C_7H_{13}]^+$(15.21), 85 $[C_6H_{13}]^+$ (86.95), 57 $[C_4H_9]^+$(100).
n-heptacosane	380$[M^+]$ (0.54),337 $[C_{24}H_{49}]^+$(1.08),295 $[C_{21}H_{43}]^+$(2.17),253 $[C_{18}H_{37}]^+$(2.17),224 $[C_{16}H_{32}]^+$(2.17),196$[C_{14}H_{28}]^+$(2.17),155 $[C_{11}H_{23}]^+$(1.08),98 $[C_7H_{14}]^+$(6.52),69 $[C_5H_{11}]^+$(19.56), 57 $[C_4H_9]^+$(100).
n-octacosane	394 $[M^+]$ (1.08),267 $[C_{19}H_{39}]^+$(3.26),239 $[C_{17}H_{35}]^+$(0.54),210$[C_{15}H_{30}]^+$(3.26),183 $[C_{13}H_{27}]^+$(4.34),155 $[C_{11}H_{23}]^+$(4.34),113 $[C_8H_{15}]^+$(5.43),83 $[C_6H_{11}]^+$(11.95),71 $[C_6H_{11}]^+$(91.3), 57 $[C_4H_9]^+$(100).
n-nonacosane	408 $[M^+]$ (6.52),379 $[C_{27}H_{55}]^+$(1.08),337 $[C_{24}H_{49}]^+$(1.63),310$[C_{22}H_{46}]^+$(1.08),281 $[C_{20}H_{41}]^+$(2.17),253 $[C_{18}H_{37}]^+$(5.43),211$[C_{15}H_{31}]^+$(8.69),169 $[C_{12}H_{25}]^+$(13.04),140 $[C_{10}H_{20}]^+$(5.43),113 $[C_8H_{17}]^+$(15.21),99 $[C_7H_{15}]^+$(36.95),85 $[C_6H_{13}]^+$(100),71 $[C_5H_{11}]^+$(16.3), 57 $[C_4H_9]^+$(13.04).
n-triacontane	422 $[M^+]$ (8.69),379 $[C_{27}H_{55}]^+$(8.69),337 $[C_{24}H_{49}]^+$(6.52),308$[C_{22}H_{44}]^+$(6.56),295 $[C_{21}H_{43}]^+$(10.86),280 $[C_{20}H_{40}]^+$(6.52), 267 $[C_{19}H_{39}]^+$(15.21),239 $[C_{17}H_{35}]^+$(15.21),210 $[C_{15}H_{30}]^+$(8.69), 183 $[C_{13}H_{27}]^+$(13.04),168 $[C_{12}H_{24}]^+$(6.56),155 $[C_{11}H_{23}]^+$(16.3), 141 $[C_{10}H_{20}]^+$(34.78),126(22.82),99)$[C_7H_{15}]^+$(22.82), 57 $[C_4H_9]^+$(100).
n-heneitriacontane	436$[M^+]$(9.78),407 $[C_{29}H5_9]^+$(2.17),379 $[C_{27}H_{55}]^+$(4.34),337$[C_{24}H_{49}]^+$(5.43),323 $[C_{23}H_{47}]^+$(1.08),309 $[C_{22}H_{45}]^+$(5.45),281$[C_{20}H_{41}]^+$(6.52),267 $[C_{19}H_{39}]^+$(2.17),239 $[C_{17}H_{35}]^+$(6.52),210$[C_{15}H_{30}]^+$(2.17),183$[C_{13}H_{27}]^+$(14.13),154 $[C_{11}H_{22}]^+$(2.17),126 $[C_9H_{18}]^+$(13.0),99 $[C_7H_{15}]^+$ (52.17),83(17.39),69 $[C_5H_9]^+$(42.39), 57 $[C_4H_9]^+$(19.56).
n-dotriacontane	450$[M^+]$(8.69),435 $[C_{31}H_{63}]$ (3.26), 407 $[C_{29}H5_9]^+$(6.52), 365$[C_{26}H_{53}]^+$(8.69),327(41.30),294 $[C_{21}H_{42}]^+$(13.04),280 $[C_{20}H_{40}]^+$(8.69), 267 $[C_{19}H_{39}]^+$(13.04),239 $[C_{17}H_{35}]$(8.69),197 $[C_{17}H_{35}]^+$(13.04),169 $[C_{14}H_{29}]^+$(10.86), 141 $[C_{10}H_{20}]^+$(17.39), 111 $[C_8H_{15}]^+$(23.91), 98 $[C_7H_{14}]^+$(11.95), 85 $[C_6H_{13}]^+$(100), 55 $[C_4H_7]^+$(32.60).



Microbial susceptibility (disc diffusion assay)

The antimicrobial activity of the extracts produced by the extraction of *A. maritima* with different solvents, dissolved in DMSO were estimated by filter paper disc method [17] using inoculums containing 10^6 bacterial or10^8 yeast cells / ml to spread on nutrient agar and Sabouraud agar plates, respectively . The sterilized filter paper discs (Whatman no.1, 6mm in diameter) were saturated with extracts obtained for all plants and another set of filter paper discs were soaked in DMSO and served as controls. The discs were placed on the surface of agar plates seeded with the test organisms. The plates were incubated at 37^0C for bacteria and at 30^0C for yeast. Diameters of inhibition zone (mm) were measured after 18-24 hours for bacteria and 24-48 hours for yeast [18]. The % activity index for the complex was calculated by the formula as under:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diametre)}}{\text{Zone of inhibition by standard (diametre)}} \times 100$$

Evaluation of antioxidant activity of the plant extracts

Free radical scavenging method (DPPH)

The effect of the extracts on DPPH radical was estimated [16]. 5 dilutions of each sample were prepared in butanol. Concentrations ranged from 20 mg to1 mg per ml for less active extracts and from 1.5 mg to (0.01 or less) mg per ml for more active extracts.

An aliquot of 1 ml of the prepared concentrations of the tested extracts was added to 1 ml of DPPH$^{\cdot}$ (0.135 mM). Absorbance was measured at 517 nm after 30 minutes incubation at exclusion of light. A solution free of the sample was used as a blank and contained instead of the sample butanol. The percentage of remaining DPPH$^{\cdot}$ of each tested concentration at the steady state was calculated as follows: % DPPH$^{\cdot}$ remaining = % DPPH$^{\cdot}$ sample/% DPPH$^{\cdot}$blank X 100

These values were plotted against mg of plant extractor show the amount of antioxidant necessary to decrease the initial DPPH$^{\cdot}$ concentration by 50% (IC50). Ascorbic acid was used as references.

Antioxidant Assays by ABTS

Antioxidant activity screening assay ABTS method: For each of the investigated samples (2 ml) of ABTS solution (60 µM) was added to 3 ml MnO_2suspension (25mg/ml), in aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to approx. ca. 0.5. Then, 50 µl of (2 mµ) solution of the tested sample in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L–ascorbic acid was used as standard antioxidant (Positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of tested samples. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only [19, 20]. The free radical inhibition percentage of each sample was calculated as follows:

ABTS (%inhibition) =Abs (control) – Abs (test) / Abs (control) X 100

The Cytotoxicity assay test

MTT assay [21, 22]: The cell lines were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37^{0}C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0x104 cells/well at 37^{0}C for 48 h under 5% CO_2[23]. After incubation the cells were treated with different concentration of extracts and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide in volume of 100 µl is added into each well to dissolve the purple formazan formed. The absorbance was recorded at 570 nm using a plate reader (EXL 800). 5-Fluorouracil was used as a standard anticancer drug for comparison. The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

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