

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease



journal homepage: www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(14)60609-5

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Composition of the non-polar extracts and antimicrobial activity of *Chorisia insignis* HBK. leaves

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PEER REVIEW

Peer reviewer

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Comments

The article is valuable, and the comment is scientific and fair.

Reda Sayed Mohammed, Department of Pharmacognosy, National Research Centre, 12622, Dokki, Cairo, Egypt.

Comments

This is a valuable study in which the authors isolated and identified the constituents of the non polar fraction and the article contains the spectroscopic data which lead to identification of these compounds. Details on Page 479

ABSTRACT

Objective: To investigate the chemical constituents of the petroleum ether extract and the ether fraction of the 70% ethanol extract of *Chorisia insignis* HBK. leaves, as well as screen its antimicrobial activity.

Methods: Different chromatographic methods were applied to investigate the non–polar extracts and the diffusion assay method was applied to study the antimicrobial activity.

Results: A total of 50 compounds from the unsaponifiable matter and 20 fatty acid methyl esters were identified from the petroleum ether extract by GC/MS analysis. *n*-Hentriacontane, *n*-tritriacontane, stigmastanol, 3-methoxy-5, 6-dihydrostigmasterol, 7,8-dihydroergosterol, 4-methylcholesterol, cholestanol, multiflorenol, cholest-5-en-3-one, cholest-6-one, 5,6-dihydroergosterol, stigmasterol, dihydroalbigenin and 11-methyl- $\Delta^{5,7,9,15,17,23}$ -triacont-hex-ene were isolated from the petroleum ether extract. Methyl heptacosanoate and quinic acid ester of rhamnose were isolated from the ether fraction of the 70% ethanol extract. Antimicrobial activity of the total alcohol extract and the successive fractions showed that the ether and the ethyl acetate fractions have potent antibacterial activity against *Bacillus subtilis* and *Bacillus cereus*. **Conclusions:** The ether and the ethyl acetate fractions could be used in pharmaceutical

formulations as antibacterial agents against *Bacillus subtilis* and *Bacillus cereus*, and further clinical trials should be performed in order to support the above investigations.

KEYWORDS

Antimicrobial activity, *Chorisia insignis*, Fatty acid, GC/MS, Methyl heptacosanoate, Quinic acid ester of rhamnose

biological activities of the plant.

1. Introduction

No. 2/5/12).

Chorisia insignis (C. insignis) HBK., known as white floss silk tree, belongs to family Bombacaceae. It is native to South America, Peru, Brazil and Argentina^[1,2].

Chorisia was named in honor of the botanical artist and traveler Ludwig I. Choris (1795–1828, 19th century)^[1]. *C. insignis* is mainly cultivated for its ornamental brilliant flowers. It is also cultivated for the silky white fibre (or floss) that is obtained from the ripened fruits. This floss has been used to form cushions and vests which explain the common name of this tree, "floss silk tree"^[1].

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Foundation Project: Supported by the National Reasearch Centre, Cairo, Egypt (Grant

t three flavonoids were isolated and it was found that the plant possesses significant anti-inflammatory, antihyperglycemic, antioxidant and hepatoprotective activities^[4]. It was also found that the polar extracts contain several phenolic compounds and that the plant has

Phytochemical investigations of *C. insignis* remained confined to the isolation of one flavonoid (rhoifolin

glycoside) from fresh leaves^[3]. Thus, it was deemed of

interest to investigate the phytoconstituents as well as the

pharmacological actions of the leaves were investigated,

In our previous work, the flavonoid content and the

Received 10 May 2014 Received in revised form 19 May, 2nd revised form 23 May, 3rd revised form 30 May 2014 Accepted 21 Jun 2014

Available online 10 Jul 2014

Article history:

significant cytotoxic activity against larynx carcinoma^[5]. The polysaccharide content of the leaves and stems were studied^[6]. Moreover, El Alfy *et al.* confirmed the identity and the purity of *C. insignis* through studying its macro and micromorphological features, determination of its pharmacopoeial constants, DNA fingerprinting as well as protein electrophoresis to monitor the seed storage protein and determination of the total protein and amino acids contents^[7].

The present work was carried out to study the chemical constituents of the petroleum ether extract and the ether fraction of the 70% ethanol extract and the antimicrobial activity of *C. insignis* leaves cultivated in Egypt.

2. Materials and methods

2.1. Plant material

Samples of the leaves of *C. insignis* were collected from National Research Centre (NRC) garden, Dokki, Cairo, Egypt in June, and were kindly authenticated by Dr. Mohamed Gibali, senior botanist and by Agricultural Engineer Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex-director of Orman Botanical Garden, Giza, Egypt. A voucher specimen (No. 23569) is kept at NRC Herbarium. Samples of the plant under investigation were separately air-dried, powdered and kept in tightly closed amber coloured glass containers.

2.2. Solvents

Petroleum ether (40–60 °C), diethyl ether, chloroform, ethyl acetate and ethanol were used in the present study. All solvents used were of analytical grade (RFCL Limited, New Delhi, India).

2.3. Adsorbents for chromatography

• Silica gel 60 F254 for thin layer chromatography (TLC) (Fluka Chemie AG, Switzerland).

• Silica gel 60 for column chromatography (CC) (E. Merck, Darmstadt, Germany).

• Sheets of Whatman filter paper No. 1 for paper chromatography (PC) (Whatman Ltd., Maidstone, England).

• Sheets of Whatman filter paper No. 3 for paper partition chromatography.

• Sephadex LH-20 for CC (Fluka Chemie AG, Switzerland).

2.4. Solvent systems (v/v)

S1: benzene: ethyl acetate	(9:1)
S2: n -BuOH: acetic acid: H_2O	(4:1:5)
S3: acetic acid: H_2O	(15:85)
S4: MeOH	(100%)

2.5. Tested microorganisms (MO)

Bacteria, fungi and yeast strains were kindly obtained from the Microbial Chemistry Department, NRC, Egypt. *Bacillus subtilis* (M1), *Bacillus cereus* (M2), *Staphylococcus* aureus (M3) and Streptococcus pyogenes (M4) were the Grampositive bacterial strains used, while Escherichia coli (M5) was the Gram-negative strain. Also, four fungi Aspergillus niger (M6), Fusarium oxysporum (M7), Botrytis allii (M8) and Trichoderma viride (M9), and one yeast Saccharomyces cervisiae (M10) were used.

2.6. Culture media

All the chemicals used in the preparation of the media were of the analytical grade. Distilled water was used. Routine sterilization was carried out by autoclaving for 20 min at 15 psi (121 °C). The following media including Lauria– Bertani medium^[8], potato dextrose agar growth medium^[9], and yeast extract peptone dextrose medium^[10] were used.

2.7. Standard drugs

Ampicillin (ADWIC, El Nasr Pharmaceutical Chemicals Co.) was used as a standard antibacterial drug, while clotrimazole (Locasten, Alexandria Company for Pharmaceuticals) was used as a standard antifungal drug.

2.8. Apparatus

• Mass spectrometer: Finnigan Model 3200 Mass spectrometer at 70 eV.

• Gas chromatograph coupled with a mass spectrometer (GC/MS): Finnigan Mat SSQ 7000, Digital DEC EL, 70 eV for GC/MS analysis of unsaponifiable matter and fatty acid methyl esters.

• UV-visible spectrophotometer: UV-vis double beam UVD-3500 spectrophotometer, Labomed, Inc.

- Electrospray ionization mass spectrometer (ESI-MS): Thermo Finnigan (ion trap).
- NMR: Joel ECA 500 (¹H–NMR at 500 MHz).

2.9. Experimental

2.9.1. Preparation of crude extracts

2.9.1.1. Successive extracts

A total of 550 g of air-dried powdered leaves were exhaustively defatted using petroleum ether (40-60 °C) (E1) in a Soxhlet apparatus. The defatted powder was refluxed with 70% ethanol till complete exhaustion. The combined ethanol extract was evaporated to dryness to give 145 g, then suspended in water (600 mL) and partitioned successively with ether (E2) (10×100 mL) followed by chloroform (E3) (15×100 mL), ethyl acetate (E4) (15×100 mL) and *n*-butanol (E5) (12×100 mL). The solvents were evaporated to dryness under reduced pressure at 40 °C.

2.9.1.2. Total alcohol extract (E6)

About 100 g of air-dried powdered leaves were exhaustively extracted by refluxing with 95% ethanol. The combined extract was evaporated under reduced pressure at 40 °C to give 14 g total alcohol extract.

2.9.2. Saponification of E1

A total of 0.5 g of the residue of E1 was saponified

according to the method described by Tsuda *et al.* to give 0.30 g unsaponifiable matter (UNSAP) and 0.08 g fatty acids^[11]. Preparation of the fatty acid methyl esters (FAME) was carried out according to Finar^[12].

GC/MS analysis for UNSAP and FAME were performed using capillary column of fused silica, 30 m length, 0.32 mm inner diameter and 0.25 mm thickness, and helium at 1 mL/min, 13 psi as the carrier gas. The ion source temperature adjusted at 180 °C and the ionization voltage 70 eV using MS detector. DB–WAX was the stationary phase for FAME and DB–5 for UNSAP; the temperature programming was 50–260 °C at a rate of 4 °C/min and 70– 290 °C at a rate of 4 °C/min respectively. Identification of the constituents was carried out by comparison of their spectral fragmentation patterns with those of the available database libraries: Wiley, USA and National Institute of Standards and Technology and/or published data^[13,14].

2.9.3. Isolation and identification of the major constituents of E1

A total of 15 g of E1 was applied on 300 g silica gel (150 mm×5 mm) column using petroleum ether (40–60 °C) then increasing the polarity by adding ether gradually till 100% ether then chloroform then methanol. Fractions were screened by TLC silica gel using S1. Compound T1 was obtained from 100% petroleum ether fraction, compounds T2–T4 from 80% CHCl₃/Ether fraction, compounds T5–T9 from 90% CHCl₃/Ether fraction and compounds T10–T12 from 2% MeOH/CHCl₃ fraction. All compounds were isolated from the corresponding fraction by preparative TLC using S1 as the developing system.

2.9.4. Investigation of fraction E2

Fraction E2 was subjected to PC examination using Whatman No. 1 sheets with S2 and S3 for developing. The chromatograms were examined under UV light before and after exposure to ammonia vapour or spraying with AlCl₃ solution; two spots were detected. About 8 g of E2 were chromatographed by paper partition chromatography using Whatman No. 3 sheets and developed using S3 to give compound A1 and band A21, the latter purified on column Sephadex LH-20 using S4 as eluant to give compound A2.

2.9.5. Antimicrobial activity

The antimicrobial test was carried out according to the diffusion assay method^[15].

3. Results

3.1. GC/MS analysis

Analysis of UNSAP of E1 (Table 1) resulted in the identification of 50 compounds constituting 95.81% of the total peak area. The non-oxygenated compounds constitute 37.97% while the oxygenated constitute 57.84%. n-Triacontane (21.45%) was the major constituent. The oxygenated compounds classified into eight classes; ketones (27.31%) and the major constituent was 4-hydroxy-4-methyl-2-pentanone (14.65%), followed

by hydroxylated compounds (19.83%) and its major constituent was butylated hydroxytoluene (16.52%), then terpenoids (9.11%) and the major constituent was Δ^{12} –lupan–3–ol (8.49%), anhydrides (0.56%), ether compounds (0.37%), steroidal compounds (0.36%), aldehydes (0.19%) and finally miscellaneous compounds (0.11%).

Table 1

GC/MS analysis of the unsaponifiable matter of the petroleum ether extract of *C. insignis* leaves.

44.4 116 4.3 1.4.65 C_H_{10} Cyclohexanone 5.78 98 5.3 5.99 C_H_{10} Cyclohexanone 7.77 18 4.3 5.99 C_H_{10} Yamethyl cyclopentanone 7.57 188 7.3 0.88 $C_{m}H_{10}$ Yamethyl cyclopentanone 7.57 188 7.3 0.58 C_mH_{10} Particle enterplane 12.97 154 4.3 0.42 C_mH_{10} Particle enterplane 12.97 154 7.3 0.10 C_H_{20} Particle enterplane 12.97 154 7.3 0.10 C_H_{20} Particle enterplane 12.97 154 7.3 0.10 C_H_{20} Particle enterplane 12.03 12.03 1.01 C_mH_{20} Particle enterplane Particle enterplane 12.10 1.13 C_mH_{20} Particle enterplane Particle enterplane 22.18 20 1.5 C_mH_{20} Pariterideeente Pari	Rt	MWt	BP	Area (%)	MF	Compound	
5.8 98 4.8 C, H _u O Cyclohexanone 5.79 98 4.3 5.90 C, H _u O 2-methyl cyclopentanone 7.13 154 4.3 0.42 C _m H _u O Yomogi alcohol (2,5,5-trimethyl-3,6-heptadien-2-ol) 7.14 154 7.3 0.83 0.42 C _m H _u O Tespenth-2-en-1-ol 1.15 156 7.1 0.43 C _m H _u O Oispenth-2-en-1-ol 1.15.1 156 7.1 1.41 C _m H _u O Oispenth-2-en-1-ol 1.15.1 1.15 0.15 C _m H _u n-tridecane 1.16.1 0.15 C _m H _u O Propylhomologue of β-ionone 2.13 127 1.13 C _m H ₂ O Propylhomologue of β-ionone 2.14 10 0.15 C _m H ₂ O Protylhomologue of β-ionone 2.14 10.13 C _m H ₂ O Protylhomologue of β-ionone 2.14 10 0.13 C _m H ₂ O Protyloarmacol 2.14 10 0.13 C _m H ₂ O Protyloarmacol <	4.64	116	43	14.65	$C_6H_{12}O_2$	4-hydroxy-4-methyl-2-pentanone	
5.79 98 4.3 5.99 $C_{u}H_{u0}$ Yomogi alcohol (2,5,5-trimethyl-3,6-heptadien-2-ol) 7.13 154 6.3 $C_{u}H_{u}$ Vanioal alcohol (2,5,5-trimethyl-3,6-heptadien-2-ol) 7.57 158 73 0.58 $C_{u}H_{u}$ 2.8-dimethyl nonane 11.25 156 43 0.49 $C_{u}H_{u}$ 2.8-dimethyl nonane 12.97 154 43 0.49 $C_{u}H_{u}$ n-tridecane 14.16 170 57 1.24 $C_{u}H_{u}$ n-tridecane 14.15 184 57 0.15 $C_{u}H_{u}$ n-tridecane 20.53 192 43 1.06 $C_{u}H_{u}$ n-tridecane 21.8 220 235 1.632 $C_{u}H_{u}$ Notpi homologue of β -ionone 21.8 220 73 0.20 $C_{u}H_{u}$ Notpi homologue of β -ionone 21.8 220 73 0.20 $C_{u}H_{u}$ Notpi homologue of β -ionone 21.8 240 70 0.18 C_uH_u	5.58	98	55	4.48	$C_6H_{10}O$	Cyclohexanone	
7.13 154 4.3 0.42 $C_u H_u O$ Yomogi alcohol (2,5,5-trimethyl-3,6-heptadiem-2-ol) 7.57 158 7.3 0.58 $C_u H_u O$ Inalool tetrahydride 151 154 4.3 0.44 $C_u H_u$ 2-s-dimethyl nonane 1297 154 4.3 0.42 $C_u H_u$ 0 Sinone 1247 154 7.3 0.10 $C_u H_u$ n-dodecane 14.16 170 57 0.15 $C_u H_u$ n-tetradecane 17.00 188 7.0 0.10 $C_u H_u$ n-tetradecane 21.83 236 1.0 $C_u H_u$ n-tetradecane 21.84 230 7.0 0.20 $C_u H_u$ n-tetradecane 21.83 236 1.0 0.20 $C_u H_u$ n-tetradecane 21.83 236 1.0 0.20 $C_u H_u$ n-tetradecane 21.84 20.0 1.0 0.20 $C_u H_u$ n-tetradecane 21.85 7.0	5.79	98	43	5.99	C6H10	2-methyl cyclopentanone	
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14.50184570.15C. ₁ H ₂ An-tridecane14.73144730.10C,H ₂ O2-methoxy-2.4.4-trimethyl pentane17.001887.0.13C,H ₂ Oβ-ionone20.662341770.13C,H ₂ OProyl homologue of β-ionone21.8120002516.52C, ₁ H ₂ OMethyl bulnesol23.83236410.18C, ₁ H ₂ OMethyl bulnesol24.33230730.20C, ₁ H ₃ OMethyl bulnesol24.37240570.20C, ₁ H ₃ OMethyl bulnesol24.38240570.20C, ₁ H ₃ OMethyl bulnesol25.0870.23Unknown26.89240570.45C, ₁ H ₃ OMethyl bulnesol27.61223160.13C, ₁ H ₃ OHydrxy-dehydrochamazulene27.83288430.49C, ₁ H ₃ ONethylaceane27.83288430.49C, ₁ H ₃ ONethylaceane27.83288430.49C, ₁ H ₃ ONethylaceane27.81290.61C, ₁ H ₃ ONethylaceane27.812910.13C, ₁ H ₃ ONethylaceane27.81290.62C, ₁ H ₃ ONethylaceane27.81290.61C, ₁ H ₃ ONethylaceane27.8130510.13C, ₁ H ₃ ONethylaceane27.81310.31C, ₁ H ₃ ONeth	14.16	170	57	1.24	$C_{12}H_{26}$	<i>n</i> -dodecane	
14.73144730.10 C_uH_uO 2-methoxy-2,4,4-trimethyl pentane17.00198570.15 C_uH_uO n -tetradecane20.35192431.06 C_uH_uO Propyl homologue of β-ionone21.812020516.52 C_uH_uO Butylated hydroxytoluene23.38236410.18 C_uH_uO Butylated hydroxytoluene23.38230730.20 C_uH_uO Butylated hydroxytoluene24.37240570.20 C_uH_uO 21.0.12-trimethyl-7-methoxy tridecane24.70256570.23Unknown26.89240570.43 C_uH_uO Hydroxy-dehydrochamazulene27.65730.24 C_uH_uO Hydroxy-dehydrochamazulene27.66224410.13 C_uH_uO Hydroxy-dehydrochamazulene27.68730.23 C_uH_uO Neophytadiene31.01268570.29 C_uH_uO Neophytadiene31.30254410.13 C_uH_uO Ioknown31.31254410.33 C_uH_uO Ioknown31.3224430.31Unknown31.33530.51 C_uH_uO Ioknown34.83430.13Unknown35.66242990.31 C_uH_uO 36.62310570.25 C_uH_uO Ioknown36.62310570.25 C_uH_uO Ioknown<	14.50	184	57	0.15	$C_{13}H_{28}$	<i>n</i> -tridecane	
17.00 198 57 0.15 C _μ H ₀ 0 <i>n</i> -tetradecane 20.35 129 43 1.06 C _μ H ₂ O Propyl homologue of β-ionone 22.18 200 16.52 C _μ H ₂ O Bettylated hydroxytoluene 22.38 26 41 0.18 C _μ H ₂ O Methyl bulnesol 24.33 200 73 0.20 C _μ H ₃ O Methyl bulnesol 24.37 205 77 0.20 C _μ H ₃ O 2.10.12-trimethyl-7-methoxy tridecane 25.08 70 0.54 C _μ H ₃ O n-heptadecane 6.10.14-trimethyl-7-methoxy tridecane 27.65 202 41 0.13 C _μ H ₃ O n-cotadcane 6.10.14-trimethyl-2-pentadecanone (Hexahydrofarnesyl actor) 29.00 254 57 0.29 C _μ H ₃ O p-ionadcane 6.10.14-trimethyl-12-pentadecanone (Hexahydrofarnesyl actor) 31.01 268 57 0.29 C _μ H ₄ O isophytadiane 3.10.14-trimethyl-2 31.30 254 41 0.33 C _μ H ₄ O isophytadiane 3.10.14-trimethyl-2 31.31 262 19 0.31	14.73	144	73	0.10	$C_9H_{20}O$	2-methoxy-2,4,4-trimethyl pentane	
20.35 192 43 1.06 $C_{u}H_{u}O$ β-ionone 20.66 234 177 0.13 $C_{u}H_{u}O$ Butylated hydroxytoluene 23.38 236 41 0.18 $C_{u}H_{u}O$ Methyl bulnesol 23.34 320 57 0.20 $C_{v}H_{u}O$ Dehydrofarnesol 24.57 240 57 0.20 $C_{v}H_{u}O$ 2.10,12-trimethyl-7-methoxy tridecane 25.68 73 0.23 Uhknown octadecane 27.63 202 41 0.13 $C_{u}H_{u}O$ Hydroxy-dehydrochamazulene 29.83 268 43 0.49 $C_{u}H_{u}O$ Acctone octadecane 31.01 254 41 0.13 $C_{u}H_{u}O$ Hydroxy-dehydrochamazulene 31.02 254 11 0.33 $C_{u}H_{u}O$ octadecane 31.01 254 41 0.13 $C_{u}H_{u}O$ Hydroxy-dehydrofarnesol 31.02 254 41 0.13 $C_{u}H_{u}O$ H	17.00	198	57	0.15	$C_{14}H_{3}0$	<i>n</i> -tetradecane	
20.66 234 177 0.13 C _u H _a O Propyl homologue of β-ionone 22.18 20 05 16.52 C _u H _a O Butylated hydroxytoluene 23.38 236 41 0.18 C _u H _a O Dehydrofarnesol 24.33 200 73 0.20 C _u H _a O 2.10.12-trimethyl-7-methoxy tridecane 24.70 256 57 0.23 Unknown	20.35	192	43	1.06	C13H20	β-ionone	
22.18 22.0 20.5 16.52 $C_u H_u O$ Butylated hydroxytoluene 23.38 236 41 0.18 $C_u H_u O$ Methyl bulnesol 24.33 220 73 0.20 $C_u H_u O$ Dehydrofarnesol 24.70 256 77 0.23 $C_u H_u O$ 2.10,12-trimethyl-7-methoxy tridecane 25.89 70 0.54 $C_u H_u O$ Hydroxy-dehydrochamazulene 26.89 240 57 0.61 $C_u H_u O$ Hydroxy-dehydrochamazulene 27.65 202 41 0.13 $C_u H_u O$ Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_u H_u O$ n -nonadecane 31.01 268 57 0.29 $C_u H_u O$ Hydroxy-dehydrofarnesol 31.30 254 41 0.13 $C_u H_u O$ Hydroxy-dehydrofarnesol 32.62 214 99 0.21 $C_u H_u O$ Hydroxy-dehydrofarnesol 31.30 254 41 0.13 $C_u H_u O$	20.66	234	177	0.13	$C_{16}H_{26}O$	Propyl homologue of β-ionone	
23.38 236 41 0.18 $C_{u}H_{u}O$ Methyl bulnesol 24.33 20 73 0.20 $C_{11}H_{u}$ Dehydrofarnesol 24.57 240 57 0.20 $C_{11}H_{u}$ 3-Methylhexadecane 24.70 256 57 0.27 $C_{11}H_{u}$ 21,012-trimethyl-7-methoxy tridecane 25.08 70 0.54 $C_{11}H_{u}$ n-heptadecane 27.65 202 41 0.13 $C_{12}H_{u}$ Hydroxy-dehydrochamazulene 27.65 77 0.51 $C_{12}H_{u}$ Hydroxy-dehydrochamazulene Anotatecane 27.68 43 0.49 $C_{11}H_{u}$ Hydroxy-dehydrochamazulene Anotatecane 30.30 278 82 1.49 $C_{10}H_{u}$ Neophytadiene Anotatecane 31.01 268 71 0.36 $C_{21}H_{u}$ Hexanoic anhydride (Caproic anhydride) 32.25 43 0.54 Unknown Unknown 35.66 269 71 0.35 C_{12}H_{u}<	22.18	220	205	16.52	$C_{15}H_{24}O$	Butylated hydroxytoluene	
24.33 220 73 0.20 $C_{13}H_{40}$ Dehydrofarnesol 24.57 240 57 0.20 $C_{17}H_{80}$ 3-Methylhexadecane 24.70 256 57 0.27 $C_{17}H_{80}$ 2.10.12-trimethyl-7-methoxy tridecane 25.08 73 0.54 $C_{17}H_{80}$ $n-heptadecane$ 25.765 202 41 0.13 $C_{44}H_{40}$ Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_{18}H_{80}$ n -octadecane 31.01 268 57 0.29 $C_{90}H_{80}$ Neophytadiene 31.01 254 41 0.13 $C_{16}H_{20}$ Heophytadiene 31.30 254 43 0.54 Unknown Unknown 31.32 254 43 0.54 Unknown 31.52 264 99 0.21 $C_{27}H_{20}$ Heptanoic anhydride (Caproic anhydride) 31.52 256 0.71 0.86 $C_{29}H_{20}$ Heptanoic anhydride	23.38	236	41	0.18	$\mathrm{C_{16}H_{28}O}$	Methyl bulnesol	
24.57 240 57 0.20 $C_{17}H_{36}$ 3-Methylhexadecane 24.70 256 57 0.27 $C_{17}H_{36}$ 21,0,12-trimethyl-7-methoxy tridecane 25.08 73 0.23 Unknown 26.89 200 57 0.54 $C_{17}H_{36}$ H_{10} Hydroxy-dehydrochamazulene 27.65 202 41 0.13 $C_{14}H_{30}$ Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_{18}H_{36}$ n -octadecane 30.36 278 82 1.49 $C_{33}H_{36}$ Neophytadiene 31.10 268 57 0.29 $C_{17}H_{30}$ Kophytol 32.26 214 9 0.21 $C_{12}H_{30}$ Hexanoic anhydride (Caproic anhydride) 32.25 43 0.54 Unknown Unknown 34.83 43 0.13 Unknown Unknown 35.06 242 97 0.13 $C_{14}H_{30}$ 3-Hexadecenyl acetate 36.62 3	24.33	220	73	0.20	$C_{15}H_{24}O$	Dehydrofarnesol	
24.70 256 57 0.27 $C_y H_y O$ 2,10,12-trimethyl-7-methoxy tridecane 25.08 73 0.23 Unknown 26.89 240 57 0.54 $C_y H_y O$ $H_y droxy-dehydrochamazulene 27.65 202 41 0.13 C_{tx} H_y O H_y droxy-dehydrochamazulene 29.00 254 57 0.61 C_{tx} H_y O H_y droxy-dehydrochamazulene 29.83 268 43 0.49 C_{tx} H_y O A_{total trimethyl-2-pentadecanone (Hexahydrofarnesyl acetone) 30.36 278 82 1.49 C_{2y} H_y O n-nonadecane 31.10 268 57 0.29 C_{12} H_y O Hexanoic anhydride (Caproic anhydride) 32.25 41 0.13 C_{tx} H_y O Hexanoic anhydride (Caproic anhydride) 32.62 214 99 0.21 C_{ty} H_y O Hexanoic anhydride 32.62 214 99 0.25 C_{xH_y O} Hexanoic anhydride 32.62 214 99 0.25 $	24.57	240	57	0.20	$C_{17}H_{36}$	3-Methylhexadecane	
25.08 73 0.23 Unknown 26.89 240 57 0.54 $C_{rr}H_{so}$ n-heptadecane 27.65 202 41 0.13 $C_{u}H_{u0}$ Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_{u}H_{so}$ $n-octadecane$ 29.01 268 43 0.49 $C_{u}H_{so}$ $hophytalicne$ 30.30 278 82 1.49 $C_{20}H_{so}$ $n-nonadecane$ 31.01 268 57 0.29 $C_{u}H_{so}$ Neophytalicne 31.02 254 41 0.13 $C_{u}H_{so}$ Neophytadicane 31.02 254 41 0.13 $C_{u}H_{so}$ Hexanoic anhydride (Caproic anhydride) 32.92 43 0.54 Unknown Unknown 32.95 43 0.54 Unknown 35.06 242 99 0.35 $C_{11}H_{so}$ Heptanoic anhydride (Caproic anhydride) 35.66 255 0.19 $C_{u}H_{so}$ Jelexadecenyl acetate 36.62 310 57 0.25 <	24.70	256	57	0.27	C17H36O	2,10,12-trimethyl-7-methoxy tridecane	
26.89 240 57 0.54 C_r,H_s <i>n</i> -heptadecane 27.65 202 41 0.13 $C_{ts}H_{st}$ 0 Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_{ts}H_{st}$ 0 n -octadecane 29.83 268 43 0.49 $C_{ts}H_{st}$ 0 $hoptadecane$ 30.30 278 82 1.49 $C_{2s}H_{st}$ 0 Neophytadiene 31.01 268 57 0.29 $C_{ts}H_{st}$ 0 Neophytadiene 31.30 254 41 0.13 $C_{ts}H_{st}$ 0 Jophytol 32.62 214 99 0.21 $C_{ts}H_{st}$ 0 Jophytol 32.92 43 0.54 Unknown Unknown 34.83 43 0.13 Unknown 35.66 282 97 0.13 $C_{ts}H_{st}$ 0 3-Hexadecenja 36.62 105 7.1 0.56 C_{22}H_{st}0 Hytoi 36.62 310 57 0.25 $C_{ts}H_{st}$ 0 Heptanoic anhydride 35.66 282 97 0.13 </td <td>25.08</td> <td></td> <td>73</td> <td>0.23</td> <td></td> <td>Unknown</td>	25.08		73	0.23		Unknown	
27.65 202 41 0.13 $C_{u}H_{u0}$ Hydroxy-dehydrochamazulene 29.00 254 57 0.61 $C_{u}H_{u0}$ n -octadecane 29.83 268 43 0.49 $C_{u}H_{u0}$ h -octadecane 30.36 278 82 1.49 $C_{u}H_{u0}$ Neophytadiene 31.01 268 57 0.29 $C_{10}H_{u0}$ n -nonadecane 31.30 254 41 0.13 $C_{u1}H_{u0}$ (Sychexadecanolide) 31.43 254 41 0.30 $C_{21}H_{u0}$ Hexanoic anhydride (Caproic anhydride) 32.205 43 0.54 Unknown Unknown 34.83 43 0.13 Unknown 35.06 242 99 0.35 $C_{14}H_{u0}$ Heptanoic anhydride 36.22 210 57 0.25 $C_{22}H_{u0}$ Heptanoic anhydride 36.24 90 0.35 $C_{14}H_{u0}$ Heptanoic anhydride 36.25 0.19 $C_{14}H_{u0}$	26.89	240	57	0.54	$C_{17}H_{36}$	<i>n</i> -heptadecane	
29.00 254 57 0.61 $C_{18}H_{36}$ <i>n</i> -octadecane 29.83 268 43 0.49 $C_{18}H_{36}$ $6_{10,14-trimethyl-2-pentadecanone (Hexahydrofamesyl actone)$ 30.36 278 82 1.49 $C_{38}H_{36}$ Neophytadiene 31.01 268 57 0.29 $C_{18}H_{30}$ 0 Cyclohexadecanolide 31.30 254 41 0.13 $C_{19}H_{30}$ 0 Sophytol 32.62 214 99 0.21 $C_{12}H_{20}$ 0, Hexanoic anhydride (Caproic anhydride) 32.55 43 0.54 Unknown 34.83 43 0.13 Unknown 35.56 296 71 0.86 $C_{39}H_{30}$ 0 Phytol 36.10 266 55 0.19 $C_{18}H_{30}$ 0 3-Octadecenal 36.22 97 0.13 $C_{18}H_{30}$ 2 -Hexadocenyl acetate 36.26 282 97 0.11 $C_{28}H_{30}$ 0 1-Octoaceane 38.86 308 41 0.11	27.65	202	41	0.13	$\mathrm{C_{14}H_{18}O}$	Hydroxy-dehydrochamazulene	
29.83 268 43 0.49 $C_{13}H_{30}$ $6_{10,14-trimethyl-2-pentadecanone (Hexahydrofarnesyl actor) 30.36 278 82 1.49 C_{33}H_{30} Neophytadiene 31.01 268 57 0.29 C_{19}H_{30} n-nonadecane 31.02 254 41 0.13 C_{10}H_{30} Cyclohexadecanolide 31.20 254 41 0.13 C_{10}H_{30} Cyclohexadecanolide 31.20 254 41 0.13 C_{10}H_{20} Hexanoic anhydride (Caproic anhydride) 32.25 43 0.54 Unknown Unknown 35.36 242 99 0.35 C_{14}H_{30} Heptanoic anhydride 36.62 310 57 0.25 C_{29}H_{30} 13-Octadecenal 36.62 308 41 0.11 C_{38}H_{30} 13-Octadecenal 36.62 308 41 0.11 C_{39}H_{30} 14-Decane 38.6 436 97 0.11 C_{38}H_{30} <$	29.00	254	57	0.61	$C_{18}H_{38}$	<i>n</i> -octadecane	
acctone)30.36278821.49 $C_{39}H_{38}$ Neophytadiene31.00268570.29 $C_{19}H_{30}$ 0Cyclohexadecanolide31.30254410.13 $C_{19}H_{30}$ 0Kyohytol31.92296710.30 $C_{29}H_{30}$ 0Isophytol32.62214990.21 $C_{12}H_{20}$ 0Hexanoic anhydride (Caproic anhydride)32.95430.54Unknown34.83430.13Unknown35.06242990.35 $C_{14}H_{30}$ 0Heytanoic anhydride35.64266550.19 $C_{18}H_{30}$ 0Hoton36.62310570.25 $C_{22}H_{48}$ 0Phytol36.62310570.25 $C_{22}H_{48}$ n -docosane38.08308410.11 $C_{29}H_{30}$ 0Ethyl iso-allocholate41.33336710.89 $C_{31}H_{40}$ 0Ethyl iso-allocholate41.33366710.89 $C_{31}H_{40}$ 2.2,4,4-tetramethyl heptacosane41.33366710.89 $C_{31}H_{40}$ 2.1,13-labddien-8,15-diol41.344225721.45 $C_{39}H_{30}$ 0Heytanotane45.59428700.23 $C_{31}H_{40}$ Heytanotane45.59428700.23 $C_{31}H_{40}$ Heytanotane45.59428700.23 $C_{31}H_{40}$ Heytanotane45.5942870 <td< td=""><td>29.83</td><td>268</td><td>43</td><td>0.49</td><td>C₁₈H₃₆O</td><td>${\small 6,10,14-trimethyl-2-pentade canone~(Hexahydrofarnesyl}$</td></td<>	29.83	268	43	0.49	C ₁₈ H ₃₆ O	${\small 6,10,14-trimethyl-2-pentade canone~(Hexahydrofarnesyl}$	
30.36 278 82 1.49 $C_{sp}H_{sp}$ Neophytadiene 31.01 268 57 0.29 $C_{sp}H_{sp}$ O ₂ Cyclohexadecanolide 31.30 254 41 0.13 $C_{u_2}H_{sp}O_2$ Cyclohexadecanolide 31.92 296 71 0.30 $C_{2s}H_{sp}O_3$ Hexanoic anhydride (Caproic anhydride) 32.62 214 99 0.21 $C_{12}H_{2r}O_3$ Hexanoic anhydride (Caproic anhydride) 32.95 43 0.54 Unknown 35.06 242 99 0.35 $C_{14}H_{sh}O_3$ Heptanoic anhydride 35.66 266 55 0.19 $C_{14}H_{sh}O_3$ Heptanoic anhydride 36.10 266 55 0.19 $C_{14}H_{sh}O_3$ Heptanoic anhydride 36.22 29 0.13 $C_{04}H_{sh}O_3$ J-Hexadecenyl acetate 36.26 282 97 0.13 $C_{24}H_{sh}O_3$ Labd-13E-8,15-diol 38.86 308 41 0.11 $C_{28}H_{sh}O_2$ Labd-13E-8,15-diol 41.33 436 71 0.89 $C_{31}H$					~ **	acetone)	
31.01 268 57 0.29 $C_{ya}H_{av}$ n=nonadecane 31.30 254 41 0.13 $C_{ya}H_{av}O_2$ Cyclohexadecanolide 31.30 254 41 0.13 $C_{ya}H_{av}O_2$ Isophytol 32.62 214 99 0.21 $C_{12}H_{22}O_3$ Hexanoic anhydride (Caproic anhydride) 32.95 43 0.54 Unknown 34.83 43 0.13 Unknown 35.56 296 71 0.86 $C_{23}H_{av}O$ Heptanoic anhydride 36.61 266 55 0.19 $C_{14}H_{30}O_3$ Heptanoic anhydride 36.62 310 57 0.25 $C_{22}H_{av}$ n=docosane 38.08 308 41 0.11 $C_{29}H_{30}O_2$ Labd=13E=8,15=diol 38.84 36 97 0.11 $C_{28}H_{40}O_2$ Labd=13E=8,15=diol 41.33 436 71 0.89 $C_{31}H_{40}$ 2,2,4,4=tetramethyl heptacosane 41.33 436 71 0.89 $C_{31}H_{40}$ 2,1,13=labddien=8,15=diol 41.	30.36	278	82	1.49	C ₂₀ H ₃₈	Neophytadiene	
31.30 254 41 0.13 $C_{u_1}H_{u_2}O_3$ Cyclohexadecanolide 31.92 296 71 0.30 $C_{u_3}H_{u_2}O_3$ Isophytol 32.62 214 99 0.21 $C_{13}H_{22}O_3$ Hexanoic anhydride (Caproic anhydride) 32.95 43 0.54 Unknown 34.83 43 0.13 Unknown 35.06 242 99 0.35 $C_{11}H_{u_3}O_3$ Heptanoic anhydride 35.61 266 55 0.19 $C_{u_1}H_{u_3}O_3$ -Hexadecenyl acetate 36.62 282 97 0.13 $C_{u_1}H_{u_3}O_3$ -Hexadecenyl acetate 36.62 308 41 0.11 $C_{u_3}H_{u_6}$ n-docosane 38.84 36 97 0.11 $C_{u_3}H_{u_6}$ Ethyl iso-allocholate 41.03 346 71 0.16 $C_{u_3}H_{u_6}$ 2.2.4.4-tetramethyl heptacosane 41.33 436 71 0.89 $C_{11}H_{u_6}$ 2.1,13-labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09	31.01	268	57	0.29	C ₁₉ H ₄₀	<i>n</i> -nonadecane	
31.92 296 71 0.30 $C_{33}H_{30}O$ Isophytol 32.62 214 99 0.21 $C_{13}H_{20}O$, Hexanoic anhydride (Caproic anhydride) 32.95 43 0.54 Unknown 34.83 43 0.13 Unknown 35.06 242 99 0.35 $C_{14}H_{30}O$, Heptanoic anhydride 35.66 242 99 0.35 $C_{14}H_{30}O$, Phytol 36.10 266 55 0.19 $C_{14}H_{30}O$, 3-Octadecenal 36.26 282 97 0.13 $C_{14}H_{30}O$, 2-Hexadecenyl acetate 36.62 308 41 0.11 $C_{23}H_{30}O$, Eabd-13E-8,15-diol 38.86 368 97 0.11 $C_{23}H_{30}O$, 2-4,4-4etramethyl heptacosane 41.33 436 71 0.16 $C_{33}H_{30}O$, 2-4,4-4etramethyl heptacosane 41.33 436 71 0.89 $C_{31}H_{44}O$, 2-4,4-4etramethyl heptacosane 41.34 96 0.49 Unknown Unknown 43.09 396 41 0.15 $C_{23}H_{45}$ n-triacontane 44.3.0<	31.30	254	41	0.13	C ₁₆ H ₃₀ O ₂	Cyclohexadecanolide	
52.62 214 99 0.21 $C_{12}H_{22}0_{3}$ Hexanoic anhydride (Caproic anhydride) 32.95 43 0.54 Unknown 32.95 43 0.13 Unknown 35.06 242 99 0.35 $C_{12}H_{28}0_{3}$ Heptanoic anhydride 35.06 242 99 0.35 $C_{14}H_{26}0_{3}$ Heptanoic anhydride 36.10 266 55 0.19 $C_{18}H_{34}0_{3}$ 3-Octadecenal 36.26 282 97 0.13 $C_{12}H_{34}0_{3}$ -Hexadecenyl acetate 36.62 308 41 0.11 $C_{28}H_{30}0_{3}$ Labd-13E-8,15-diol 38.86 368 97 0.11 $C_{38}H_{30}0_{3}$ Al,13-labddien-8,15-diol 41.33 436 71 0.16 $C_{38}H_{30}0_{3}$ Al,13-labddien-8,15-diol 41.33 436 71 0.89 $C_{31}H_{44}0_{3}$ Al,13-labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 43.09 396 41 0.49 $C_{29}H_{44}0_{3}$ n-triacontane	31.92	296	71	0.30	C ₂₀ H ₄₀ O	Isophytol	
32.95 43 0.54 Unknown 34.83 43 0.13 Unknown 35.66 242 99 0.35 $C_{11}H_{36}O_3$ Heptanoic anhydride 35.66 242 99 0.35 $C_{11}H_{36}O_3$ Heptanoic anhydride 35.66 242 99 0.35 $C_{11}H_{36}O_3$ 13 -Octadecenal 36.10 266 55 0.19 $C_{18}H_{34}O_3$ 3 -Hexadecenyl acetate 36.62 310 57 0.25 $C_{22}H_{36}$ n -docosane 38.08 308 41 0.11 $C_{38}H_{36}O_3$ $Labd-13E$ - $8,15$ -diol 41.33 436 71 0.16 $C_{38}H_{36}O_3$ $2,2$ -dimethyl hexacosane 41.33 436 71 0.16 $C_{38}H_{36}O_3$ $2,1,13$ -labddien- $8,15$ -diol 41.33 436 71 0.89 $C_{31}H_{46}O_3$ $1-demethyl$ squalene 41.43 422 57 21.45 $C_{38}H_{30}O_3$ $1-demethyl$ squalene 41.55 410 0.9	32.62	214	99	0.21	$C_{12}H_{22}O_3$	Hexanoic anhydride (Caproic anhydride)	
34.8.3 43 0.13 Unknown 35.06 242 99 0.35 $C_{14}H_{26}O_3$ Heptanoic anhydride 35.66 242 99 0.35 $C_{14}H_{26}O_3$ Heptanoic anhydride 35.66 266 71 0.86 $C_{23}H_{30}O$ Phytol 36.10 266 55 0.19 $C_{18}H_{34}O_3$ 3-Octadecenal 36.62 310 57 0.25 $C_{22}H_{46}$ n-docosane 38.08 308 41 0.11 $C_{38}H_{36}O_2$ Labd=13E=8,15-diol 38.08 366 71 0.16 $C_{38}H_{38}O_2$ 2,2-dimethyl hexacosane 41.33 436 71 0.16 $C_{38}H_{38}O_2$ 2,1,13-labddien=8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 $C_{28}H_{48}$ n-triacontane 44.30 422 57 21.45 $C_{38}H_{30}$ Pehydrolupan=3-ol 46.18 410 69 0.31 $C_{38}H_{40}$ Pehydrolupan=3-ol 47.31	32.95		43	0.54		Unknown	
55.06 242 99 0.35 $C_{u_1}H_{u_2}0_1$, Heptanoic anhydride 355.6 296 71 0.86 $C_{u_3}H_{u_3}0_1$ 13-Octadecenal 36.10 266 55 0.19 $C_{u_3}H_{u_3}0_2$ 3-Hexadecenyl acetate 36.26 282 97 0.13 $C_{u_3}H_{u_3}0_2$ 3-Hexadecenyl acetate 36.62 310 57 0.25 $C_{22}H_{u_4}$ n-docosane 38.08 41 0.11 $C_{u_3}H_{u_3}0_2$ Labd-13E-8,15-diol 38.86 436 97 0.11 $C_{u_3}H_{u_4}0_2$ Ethyl iso-allocholate 40.00 94 71 0.16 $C_{u_3}H_{u_4}0_2$ 2,4,4-tetramethyl heptacosane 41.33 436 71 0.89 $C_{u_3}H_{u_4}0_2$ 2,1,13-labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 $C_{u_3}H_{u_2}$ n-triacontane 44.30 422 57 21.45 $C_{u_3}H_{u_2}$ pelveloupan-3-ol 45.59 428 70 0.23 $C_{u_3}H_{u_2}$ <td>34.83</td> <td></td> <td>43</td> <td>0.13</td> <td>. .</td> <td>Unknown</td>	34.83		43	0.13	. .	Unknown	
55.56 296 71 0.86 $C_{38}H_{30}O$ Phytol 36.10 266 55 0.19 $C_{18}H_{34}O_{2}$ 3-Octadecenal 36.12 262 97 0.13 $C_{18}H_{34}O_{2}$ 3-Hexadecenyl acetate 36.22 310 57 0.25 $C_{22}H_{46}$ n -docosane 38.08 308 41 0.11 $C_{38}H_{34}O_{2}$ Labd-13E-8,15-diol 38.86 436 97 0.11 $C_{38}H_{40}$ 2,2-dimethyl hexacosane 41.03 436 71 0.16 $C_{38}H_{41}O_{2}$ 2,2-dimethyl hexacosane 41.57 306 41 0.15 $C_{39}H_{41}O_{2}$ 2,1,13-labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 $C_{39}H_{41}$ n-triacontane 44.30 422 57 21.45 $C_{39}H_{42}$ n-triacontane 47.31 424 207 8.47 $C_{39}H_{41}$ 1-pmethyl hracontane 47.31 424 207 8.47 $C_{39}H_{41}$	35.06	242	99	0.35	$C_{14}H_{26}O_3$	Heptanoic anhydride	
36.10 266 55 0.19 $C_{u_1}H_{u_1}O$ 13-Octadecenal 36.26 282 97 0.13 $C_{u_1}H_{u_1}O$ 3-Hexadecenyl acetate 36.26 310 57 0.25 $C_{22}H_{u_0}$ n-docosane 38.08 308 41 0.11 $C_{29}H_{u_0}O_{s}$ Labd-13E-8,15-diol 38.08 308 41 0.11 $C_{29}H_{u_0}O_{s}$ Ethyl iso-allocholate 40.00 394 71 0.16 $C_{28}H_{ss}O_{2}$ 2,2-dimethyl hexacosane 41.33 436 71 0.16 $C_{29}H_{ss}O_{2}$ $\Delta 1_1$ -labddien-8,15-diol 41.57 306 41 0.15 $C_{29}H_{ss}O_{2}$ $\Delta 1_1$ -labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 $C_{29}H_{ss}$ n-triacontane 44.30 422 57 21.45 $C_{39}H_{sO}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{29}H_{sO}$ Δ^{12} -lupan-3-ol 47.31 424 207 8.47	35.56	296	71	0.86	C ₂₀ H ₄₀ O	Phytol	
50.20 28.2 97 0.13 $C_{u_0}H_{u_0}U_2$ 3-Hexadecenyl acetate 36.62 310 57 0.25 $C_{u_2}H_{u_0}$ n-docosane 38.08 308 41 0.11 $C_{u_0}H_{u_0}O_2$ Labd-13E-8,15-diol 38.08 308 41 0.11 $C_{u_0}H_{u_0}O_2$ Ethyl iso-allocholate 40.00 394 71 0.16 $C_{u_0}H_{u_0}O_2$ Althole Hacosane 41.33 436 71 0.16 $C_{u_0}H_{u_0}O_2$ Althole Hacosane 41.37 306 41 0.15 $C_{u_0}H_{u_0}O_2$ Althole Hacosane 41.57 306 41 0.15 $C_{u_0}H_{u_0}O_2$ Althole Hacosane 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 C ₂₀ H_{u_0}D Pehydrolupan-3-ol 44.30 422 57 21.45 $C_{u_0}H_{u_0}D Squalene 47.31 424 207 8.47 C_{u_0}H_{u_0}D Alti-1upan-3-ol 47.11 424 207 8.47 C_{u_0}H_{u_0}D$	36.10	266	55	0.19	$C_{18}H_{34}O$	13-Octadecenal	
36.0.2 310 57 0.25 $C_{23}H_{46}$ n -docosane 38.08 308 41 0.11 $C_{39}H_{36}O_{2}$ Labd-13E-8,15-diol 38.08 436 97 0.11 $C_{38}H_{36}O_{2}$ Ethyl iso-allocholate 40.00 394 71 0.16 $C_{38}H_{38}$ 2,2-dimethyl hexacosane 41.33 436 71 0.89 $C_{31}H_{44}$ 2,2,4,4-tetramethyl heptacosane 41.57 306 41 0.15 $C_{39}H_{34}O_{2}$ $\Delta 1,13$ -labddien-8,15-diol 42.84 96 0.49 Unknown 43.09 396 41 0.49 $C_{39}H_{45}$ n-triacontane 44.30 422 57 21.45 $C_{39}H_{35}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39}H_{30}$ Squalene 47.31 424 207 8.47 C_{39}H_{46} 15-methyl triacontane 47.91 380 133 0.23 $C_{39}H_{45}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{39}H_{46}$ 1	36.26	282	97	0.13	$C_{18}H_{34}O_2$	3-Hexadecenyl acetate	
38.06 308 41 0.11 $C_{33}H_{34}U_{3}$ Labou-13E-8,15-chol 38.86 436 97 0.11 $C_{33}H_{44}O_{3}$ Ethyl iso-allocholate 40.00 394 71 0.16 $C_{33}H_{38}$ 2,2-dimethyl hexacosane 41.33 436 71 0.89 $C_{31}H_{44}$ 2,2,4,4-tetramethyl heptacosane 41.57 306 41 0.15 $C_{39}H_{34}O_2$ $\Delta 1,13$ -labddien-8,15-diol 42.84 96 0.49 Unknown 43.09 396 41 0.49 $C_{39}H_{36}O$ 44.30 422 57 21.45 $C_{39}H_{35}O$ Dehydrolupan-3-ol 45.59 428 70 0.23 $C_{39}H_{30}O$ Squalene 47.31 424 207 8.47 $C_{39}H_{30}O$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{64}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{32}H_{44}O$ 4-methyl- $\Delta^{1,1,3}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{30}O$ 9-lupa	30.62	310	57	0.25	C_22H ₄₆	n-docosane	
55.80 450 97 0.11 $C_{23}H_{44}U_5$ ELHYI ISO-AllOCHOLATE 40.00 394 71 0.16 $C_{23}H_{38}$ 2,2-dimethyl hexacosane 41.33 436 71 0.16 $C_{23}H_{38}$ 2,2-dimethyl hexacosane 41.37 306 41 0.15 $C_{29}H_{34}O_2$ $\Delta 1,13$ -labddien-8,15-diol 42.84 96 0.49 Unknown Unknown 43.09 396 41 0.49 $C_{29}H_{48}$ 10-demethyl squalene 44.30 422 57 21.45 $C_{39}H_{42}$ n-triacontane 45.59 428 70 0.23 $C_{39}H_{30}$ Squalene 47.31 424 207 8.47 $C_{39}H_{40}$ Δ^{12} -lupan-3-ol 47.13 424 207 8.47 $C_{39}H_{41}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{39}H_{41}$ 4-methyl- $\Delta^{1,1,3}$ -cholesttriene 48.08 70 0.11 $C_{39}H_{42}$ 0 Pehydrolupan-3-ol 51.77 42 07 0.28 C_{39	38.08	308	41	0.11	$C_{20}H_{36}O_2$	Labu-13E-8,15-0101	
40.00 394 71 0.16 $C_{33}H_{35}$ 2,2-cumethyl hexacosane 41.33 436 71 0.89 $C_{31}H_{4i}$ 2,2,4,4-tetramethyl heptacosane 41.57 306 41 0.15 $C_{39}H_{4i}$ 2,2,4,4-tetramethyl heptacosane 41.57 306 41 0.15 $C_{39}H_{4i}$ 2,2,4,4-tetramethyl heptacosane 42.84 96 0.49 Unknown 43.09 396 41 0.49 $C_{39}H_{4i}$ 10-demethyl squalene 44.30 422 57 21.45 $C_{39}H_{4i}$ n-triacontane 45.59 428 70 0.23 $C_{39}H_{30}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39}H_{30}$ Squalene 47.31 424 207 8.47 $C_{39}H_{4i}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{23}H_{4i}$ 4-methyl- $\Delta^{1,1,3-}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{30}$ 9-lupanol 51.77 450 57 0.25 $C_{32}H_{4i}$	38.86	436	97	0.11	C_26H44O5	EINYI ISO-Allocholate	
$4_{1.55}$ 4_{350} 7_{11} 0.59 $C_{31}H_{44}$ $2.22,4.4$ -retrainentyl neptacosane 41.57 306 41 0.15 $C_{39}H_{34}O_2$ $\Delta 1,13$ -labddien-8,15-diol 42.84 96 0.49 Unknown 43.09 396 41 0.49 $C_{39}H_{45}$ 44.30 422 57 21.45 $C_{39}H_{45}$ n -triacontane 45.59 428 70 0.23 $C_{39}H_{50}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39}H_{30}$ $\Delta 1^{12}$ -lupan-3-ol 47.31 424 207 8.47 $C_{39}H_{46}$ 15 -methyl triacontane 47.91 380 133 0.23 $C_{38}H_{41}$ 4 -methyl- $\Delta^{1,13-}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{30}$ 6 -lupanol 51.77 450 57 0.28 $C_{38}H_{30}$ 6 -lupanol 51.77 450 57 0.35 $C_{28}H_{46}$ $2,2$ -dimethyl triacontane 53.97	40.00	394	71	0.16	C 11	2,2-unnethyl hexacosane	
41.37 505 41 0.13 $C_{23}n_{34}V_2$ 241_{15} -habduen-8,15-dioi 42.84 96 0.49 Unknown 43.09 396 41 0.49 $C_{29}H_{48}$ 10-demethyl squalene 44.30 422 57 21.45 $C_{39}H_{42}$ n -triacontane 45.59 428 70 0.23 $C_{39}H_{52}0$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39}H_{30}$ Squalene 47.31 424 207 8.47 $C_{39}H_{40}$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{64}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{32}H_{43}$ 4-methyl- $\Delta^{1,1,3}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{30}$ 9-lupanol 51.77 450 57 0.25 $C_{23}H_{60}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{27}H_{60}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown	41.55	430	/1	0.89	C U O	A1 12 labddion - 8 15 dial	
$z_{2.0^4}$ z_{00} 0.49 $C_{29}H_{48}$ 10 -demethyl squalene 43.09 396 41 0.49 $C_{29}H_{48}$ 10 -demethyl squalene 44.30 422 57 21.45 $C_{39}H_{40}$ n -triacontane 45.59 428 70 0.23 $C_{39}H_{50}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39}H_{40}$ Δ^{12} -lupan-3-ol 47.31 424 207 8.47 $C_{39}H_{40}$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{64}$ 15 -methyl triacontane 47.91 380 133 0.23 $C_{28}H_{44}$ 4 -methyl- $\Delta^{1,3-5}$ -cholesttriene 48.44 426 207 0.28 $C_{38}H_{30}O$ -1 upanol 51.77 450 57 0.35 $C_{28}H_{66}$ $2,2$ -dimethyl triacontane 53.97 370 124 0.13 $C_{28}H_{60}$ 3^{2} -cholestene 54.53 87 0.42 Unknown 10 10 10 <	41.57	306	41	0.15	$C_{20}H_{34}O_2$	1,15-iabddien-8,15-dioi	
43.05 570 41 0.49 $C_{39} R_{48}$ 10-tennethyl squalette 44.30 422 57 21.45 $C_{39} R_{42}$ n-triacontane 45.59 428 70 0.23 $C_{39} R_{50}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{39} R_{50}$ Squalene 47.31 424 207 8.47 $C_{39} R_{40}$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31} R_{44}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{28} R_{44}$ 4-methyl- $\Delta^{1,3,5}$ -cholesttriene 48.08 428 70 0.11 $C_{39} R_{50}$ Dehydrolupan-3-ol isomer 48.41 426 207 0.28 $C_{39} R_{50}$ 3-lupanol 51.77 450 57 0.35 $C_{32} R_{46}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{29} R_{46}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown Max 14	42.64	206	90	0.49	C U	10_domethyl scuelene	
H-1.30 42.2 37 21.4.3 $C_{3p}H_{52}$ <i>n</i> =tracontative 45.59 42.8 70 0.23 $C_{3p}H_{52}$ Dehydrolupan-3-ol 46.18 410 69 0.31 $C_{3p}H_{50}$ Squalene 47.31 424 207 8.47 $C_{3p}H_{40}$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{64}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{28}H_{44}$ 4-methyl- $\Delta^{1,3,5}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{520}$ Dehydrolupan-3-ol isomer 48.41 426 207 0.28 $C_{39}H_{50}$ 3-lupanol 51.77 450 57 0.35 $C_{22}H_{60}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{27}H_{60}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown 1 1	45.09	422	41	21.45	C H	n-triacontane	
45.15 42.6 7.5 6.2.5 $C_{39}H_{25}O$ beingdituitpaire3-or 46.18 410 69 0.31 $C_{39}H_{30}$ Squalene 47.31 424 207 8.47 $C_{39}H_{30}O$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{64}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{38}H_{44}$ 4-methyl- $\Delta^{1,3,3}$ -cholesttriene 48.08 428 70 0.11 $C_{38}H_{32}O$ Dehydrolupan-3-ol isomer 48.41 426 207 0.28 $C_{38}H_{30}O$ 3-lupanol 51.77 450 57 0.35 $C_{32}H_{66}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{28}H_{60}$ Δ^{33} -cholestene 54.53 87 0.42 Unknown	45 50	422	70	0.23	CHO	Dehvdrolupan-3-ol	
410 69 6.31 $C_{39}H_{30}$ Squarene 47.31 424 207 8.47 $C_{39}H_{48}O$ Δ^{12} -lupan-3-ol 47.58 436 57 0.27 $C_{31}H_{44}$ 15-methyl triacontane 47.91 380 133 0.23 $C_{28}H_{44}$ 4-methyl- $\Delta^{1,3.5}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{32}O$ Dehydrolupan-3-ol isomer 48.41 426 207 0.28 $C_{39}H_{30}O$ 3-lupanol 51.77 450 57 0.35 $C_{32}H_{66}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{27}H_{66}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown	46 19	428	60	0.25	C H	Scuelono	
$7_{1,3}$ $2_{2,4}$ $2_{3,1}$ $C_{3,1}$ $C_{3,1}$ L_3 <td< td=""><td>40.18</td><td>410</td><td>207</td><td>8.47</td><td>CHO</td><td>Δ^{12}-lupan-3-ol</td></td<>	40.18	410	207	8.47	CHO	Δ^{12} -lupan-3-ol	
75 45 57 627 $C_{31}\Gamma_{64}$ 13-methyl tracontaine 47.91 380 133 0.23 $C_{23}H_{44}$ 4-methyl- $\Delta^{1.3.5}$ -cholesttriene 48.08 428 70 0.11 $C_{39}H_{52}0$ Dehydrolupan-3-ol isomer 48.41 426 207 0.28 $C_{39}H_{50}0$ 3-lupanol 51.77 450 57 0.35 $C_{27}H_{66}$ $2,2$ -dimethyl triacontane 53.97 370 124 0.13 $C_{27}H_{66}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown 10 10 10	47.51	424	57	0.47	C H	15_methyl triacontane	
48.08 428 70 0.11 $C_{39}H_{32}O$ Dehydrolupan-3-ol isomer 48.08 428 70 0.28 $C_{39}H_{32}O$ Dehydrolupan-3-ol isomer 48.14 426 207 0.28 $C_{39}H_{30}O$ 3-lupanol 51.77 450 57 0.35 $C_{27}H_{66}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{27}H_{66}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown	47.01	380	133	0.27	C H	4 -methyl- $\Lambda^{1,3,5}$ -cholesttriene	
48.41 426 207 0.28 $C_{39}H_{30}O$ 3-lupanol 51.77 450 57 0.35 $C_{22}H_{66}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{22}H_{46}$ Δ^{23} -cholestene 54.53 87 0.42 Unknown	18 09	429	70	0.25	CHO	Debydrolupan_3_ol isomer	
51.77 450 57 0.35 $C_{23}H_{46}$ 2,2-dimethyl triacontane 53.97 370 124 0.13 $C_{22}H_{46}$ Δ^{25} -cholestene 54.53 87 0.42 Unknown	48 /1	428	207	0.28	C H O	3-lunanol	
53.97 370 124 0.13 $C_{22}H_{46}$ Δ^{25} -cholestene 54.53 87 0.42 Unknown	51 77	420	57	0.26	C H	2 2-dimethyl triacontane	
54.53 87 0.42 Unknown	53.07	370	124	0.35	C H	Λ^{25} -cholestene	
	54.53	570	87	0.42	27**46	Unknown	

Rt: retention time, MWt: molecular weight, BP: base peak, MF: molecular formula.

Analysis of FAME (Table 2) resulted in the identification

Table 2

GC/MS analysis of the	e fatty acid	methyl ester of	C. insignis leaves.
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Rt	MWt	BP	Area (%)	MF	Compound
6.50	220	205	19.87	$C_{14}H_{20}O_2$	Methyl-2-(3',3'-dimethyl-1'-butyn-1'-yl)-1-cyclohexene carboxylate
8.15	220	41	0.16	$C_{14}H_{20}O_2$	$Methyl-2-(4'-methylene-1'-buten-1'-yl)-1-cyclohexene\ carboxylate$
11.24	242	74	0.67	$C_{15}H_{30}O_2$	Methyl tetradecanoate (Methyl myristate)
15.63	256	74	0.02	$C_{16}H_{32}O_2$	Methyl pentadecanoate
15.93	270	74	11.05	$C_{17}H_{34}O_2$	Methyl hexadecanoate (Methyl palmitate)
18.27	270	74	0.02	$C_{17}H_{34}O_2$	Methyl-14-methyl penadecanoate
18.49	284	74	0.42	$C_{18}H_{36}O_2$	Methyl-14-methyl hexadecanoate
19.77	294	67	2.14	$C_{19}H_{34}O_2$	Methyl-9,12-octadecadienoate
19.91	296	55	5.11	$C_{19}H_{36}O_2$	Methyl-9-octadecenoate (Methyl oleate)
20.77	298	74	7.53	$C_{19}H_{38}O_2$	Methyl octadecanoate (Methyl stearate)
21.19	312	55	0.02	$C_{19}H_{36}O_{3}$	Methyl-4-hydroxy-9-octadecenoate
21.41	324	55	0.49	$C_{21}H_{40}O_2$	Methyl-3-methyl-8-nonadecenoate
25.18	326	74	7.74	$C_{21}H_{42}O_2$	Methyl eicosanoate (Arachidic acid methyl ester)
26.91	338	43	0.39	$C_{22}H_{42}O_2$	Methyl-11-heneicosenoate
28.88	354	74	4.16	$C_{23}H_{46}O_2$	Methyl docosanoate (Methyl behenate)
29.72	354	71	0.06	$C_{23}H_{46}O_2$	Methyl-19,19-dimethyl eicosanoate
30.44	378	207	0.07	$C_{25}H_{46}O_2$	Methyl-19,21-dimethyl-15,19-docosadienoate
30.94	378	41	0.35	$C_{25}H_{46}O_2$	Methyl-17,23-tetracosadienoate
32.20	380	55	28.22	$C_{25}H_{48}O_2$	Methyl-22-tetracosenoate
33.68	406	43	2.32	$C_{27}H_{50}O_2$	Methyl-16,22-hexacosadienoate

Rt: retention time, MWt: molecular weight, BP: base peak, MF: molecular formula.

of 20 compounds constituting 90.81%. The unsaturated FA constitute 59.14% while the saturated constitute 31.67%. Methyl-22-tetracosenoate (28.22%) was the major monounsaturated FA, followed by a polyunsaturated compound, methyl-2-(3',3'-dimethyl-1'-butyn-1'-yl)-1- cyclohexene carboxylate (19.87%), and a saturated compound, methyl palmitate (11.05%). It was the first time to study the lipoidal matter of *C. insignis* leaves.

3.2. Isolation and identification of the major constituents of *E1*

Upon chromatographing E1 on silica gel column, the following compounds were isolated and identified for the first time from the plant (Figure 1).

T1: white crystals (500 mg), appeared as one spot in TLC (R_j = 0.98 S1). Two peaks (P1 and P2) appeared in the mass spectrum. On running the mass of each peak, P1 and P2 showed a molecular ion peak (MIP) at m/z 436 [M⁺, 8%] and m/z 464 [M⁺, 5.8%] calculated for the molecular formula (MF) C₃₁H₆₄ and C₃₃H₆₈, respectively. Both compounds have mass fragmentation pattern of typical straight chain alkane with the base peak (BP) at m/z 57. Therefore, P1 and P2 were identified as *n*-hentriacontane and *n*-tritriacontane, respectively.

T2: steroid (2 mg) appeared as one spot in TLC (R_{f} =0.37 S1). Two peaks (P3 and P4) appeared in the mass spectrum. On running the mass of each peak, P3 showed MIP at m/z 416 [M⁺, 30.5%], BP at m/z 415 [M⁺-H, 100%] calculated for MF C₂₉H₅₂O, in addition to the following characteristic peaks at 401 [M⁺-CH₃, 17%], 373 [M⁺-43, 7.5%], 303 [M⁺-113, 19%], 288 [M⁺-113-CH₃, 12%], 279 [M⁺-H-CH₃-85-CH₃-H₂O-3H, 30%] and 212 [34.5%]. Therefore, P3 was identified as 5,6-dihydrositosterol (stigmastanol).

P4 showed MIP at m/z 428 [M⁺, 4%], BP at m/z 59 ($^{\circ}CH_2CH_2OCH_3$) calculated for MF C₃₀H₅₂O, in addition to the following characteristic peaks at 413 [M⁺-CH₃, 9%], 398 [M⁺-2CH₃, 7.5%], 239 [M⁺-side chain-CH₃OH-CH₃-3H, 55%], 111 [31.5%], 83 [111-

 $2CH_2$, 35.5%] and 57 [58%]. Therefore, P4 was identified as 3-methoxy-5, 6-dihydrostigmasterol.

Compound T3: (1 mg) showed MIP at m/z 398 [M^+ , 6%], BP at m/z 69 (side chain-43-CH) calculated for MF C₂₈H₄₆O and the following characteristic peaks at 381 [M^+ -OH, 7%], 256 [M^+ -side chain-OH, 21%], 225 [M^+ -side chain-2CH₃-H₂O, 14%] and 95 [side chain-2CH₃, 24%]. Therefore, T3 was identified as 7,8-dihydroergosterol.

Compound T4: (4 mg) showed MIP at m/z 400 [M⁺, indistinct], BP at m/z 83 calculated for MF $C_{28}H_{48}O$. In addition, the following characteristic peaks at 385 [M⁺-CH₃, 20.5%], 382 [M⁺-H₂O, 16.5%], 323 [M⁺-OH-4CH₃, 19%] and 266 [M⁺-H₂O-3CH₃-43-2CH₂, 41%] were observed. Therefore, T4 was identified as 4-methylcholesterol.

Compound T5: (2.4 mg) showed MIP at m/z 388 [M⁺, 17.54%], BP at m/z 293 [M⁺-H₂O-CH₃-2H-CH₃(CH)CH₃CH₂-3H, 100%] calculated for MF C₂₇H₄₈O, in addition to the characteristic peaks at 373 [M⁺-CH₃, 17.64%], 355 [M⁺-H₂O-CH₃, 14.25%], 352 [M⁺-H₂O-CH₃-3H, 43.88%], 303 [M⁺-85, 19.39%], 275 [M⁺-side chain, 31.31%], 245 [M⁺-side chain-2CH₃, 46.37%] and 227 [M⁺side chain-2CH₃-H₂O, 32.59%]. From the above data, T5 was identified as cholestanol.

Compound T6: (3.1 mg) showed MIP at m/z 426 [M^* , 33.19%], BP at m/z 257 calculated for MF C₃₀H₅₀O and the characteristic peaks at 411 [M^* -CH₃, 19.56%], 396 [M^* -2CH₃, 21.47%] and 288 [M^* -H₂O-8CH₃, 13.80%]. From the above data and by comparison with published data^[16], T6 was identified as multiflorenol.

Compound T7: (1.5 mg) showed MIP at m/z 384 [M^* , indistinct], BP at m/z 285 [M^* -(CH₂)3CH(CH₃)2-CH₂, 100%] calculated for MF C₂₇H₄₄O and the peaks at 342 [M^* -CH₂=C=O, 47.46%], 341 [M^* -43, 47.46%] and 284 [M^* -43-3CH₂-CH₃, 59.5%]. From the above data, T7 was identified as cholest-5-en-3-one.

Compound T8: (5 mg) showed MIP at m/z 386 [M⁺, indistinct], BP at m/z 279 [M⁺-4CH₂-2CH₃-18-3H, 100%] calculated for MF $C_{27}H_{46}O$, in addition, the following characteristic peaks at 341 [M⁺-3CH₃, 11%], 329 [M⁺-(CH₃)2CH-CH₂, 9%] and 273 [M⁺-side



Figure 1. Structure of the compounds isolated from the petroleum ether extract of *C. insignis* leaves.

chain, 21%] were observed. Therefore, T8 was identified as cholest-6-one.

Compound T9: (1.3 mg) showed MIP at m/z 398 [M⁺, indistinct], BP at m/z 239 [M⁺-side chain-H₂O-CH₃-H, 100%] calculated for MF $C_{28}H_{46}O$, and the following characteristic peaks 273 [M⁺-side chain, 16%], 243 [M⁺-side chain-2CH₃, 54.5%] and 225 [M⁺-side chain-2CH₃-H₂O, 47%] were observed. From the above, T9 was identified as 5,6-dihydroergosterol.

Compound T10: (2 mg) showed MIP at m/z 412 [M^+ , 21.5%], BP at m/z 57 calculated for MF $C_{29}H_{48}O$, and the following characteristic peaks 397 [M^+ -CH₃, 7.5%], 369 [M^+ -43, 16%], 340 [M^+ -43-CH₂CH₃, 6%], 327 [M^+ -85, 4.5%], 279 [M^+ -H₂O-2CH₃-85, 12.5%], 258 [M^+ -side chain-CH₃, 14.5%] and 111 [49%] were observed. From the above data, T10 was identified as stigmasterol.

Compound T11: (1 mg) showed MIP at m/z 430 [M⁺, 25%], BP at m/z 69 calculated for MF $C_{29}H_{50}O_2$ and the characteristic peaks at 322 [M⁺-6CH₃-H₂O, 43.52%], 280 [M⁺-7CH₃-OH-2CH₂, 64.45%] and 167 [98.64%]. From the above data and by comparison with published data^[17], T11 was identified as dihydroalbigenin.

Compound T12: (1.7 mg) showed MIP at m/z 424 [M⁺, 18%], BP at m/z 57 calculated for MF $C_{31}H_{52}$ and the characteristic peaks at 341 [M⁺-83, 15%], 111 [M⁺-83-122-108, 18%] and 83 [M⁺-341, 73%]. Therefore, T12 was expected to be 11-methyl- $\Delta^{5,7,9,15,17,23}$ -triacont-hex-ene.

Fraction E2 was subjected to PC investigation and two compounds were isolated and identified for the first time from the plant (Figure 2).



Figure 2. Structure of the compounds isolated from the ether fraction of the 70% ethanol extract of *C. insignis* leaves.

A1: methyl heptacosanoate, A2: quinic acid ester of rhamnose.

3.3. Investigation of fraction E2

Compound A1 isolated as yellow amorphous powder (45 mg), R_j = 0.82 and 0 in S2 and S3, respectively. It appeared as a yellow spot (2 mg) under UV light unchanged on exposure to ammonia vapour or spraying with AlCl₃. The UV spectral data in MeOH showed one main band at 279 which did not give shift by addition of NaOMe. The EI-MS spectrum showed MIP at m/z 424 [M⁺, 86.20%], BP at m/z 74 calculated for MF C₂₈H₅₆O₂. The mass fragmentation pattern was typical FAME with the characteristic peaks at 143 [M⁺-CH₃-19CH₂, 18.68%], 87 [M⁺-CH₃-23CH₂, 70.90%], and 71 [M⁺-COOCH₃-21CH₂, 28.32%]. The structure was confirmed by ¹H-NMR spectrum which exhibited a singlet at δ 3.12 assigned to the acetoxy group,



Figure 3. Antimicrobial activity of the total alcohol and petroleum ether extracts and fractions of the 70% ethanol extract of *C. insignis* leaves. Data are expressed as mean±SE.

a singlet at δ 2.47 assigned to the CH₂ group α to COOCH₃, a singlet at δ 2.11 assigned to the CH₂ group β to COOCH₃, a multiplet at δ 1.18 assigned to the methylene groups of C4–C26 and a multiplet at δ 0.80 assigned to the terminal methyl group. Based on the previous discussion, A1 was identified as methyl heptacosanoate.

Compound A2 isolated as white amorphous powder (10 mg), R_{f} = 0.78 and 0.52 in S2 and S3, respectively. It appeared as a blue fluorescent spot under UV light unchanged on exposure to ammonia vapour or spraying with AlCl₃. UV spectral data in MeOH showed one main band at 271. The 'H-NMR spectrum exhibited signals for three oxymethine protons at δ 4.10 (m, 2H) and 3.64 (m, 1H) and two sets of methylene protons at 8 1.16 (d, J=2.5 Hz, 2H) and 1.24 (d, J=2.5 Hz, 2H) assigned to H-2 and H-6, suggesting a quinic acid moiety in the molecule. An anomeric proton signal at δ 5.40 (d, J=2.5 Hz, 1H) together with a broad singlet of three protons at δ 0.85 for Me-6' indicating the presence of rhamnose. The structure was confirmed by determination of positive electrospray ionization mass spectrometry (ESI-MS): m/z 339 $[M^{+}_{+}H]$ (which is the molecular weight of quinic acid ester of rhamnose+H). Therefore, A2 was identified as quinic acid ester of rhamnose.

3.4. Antimicrobial screening

Reviewing current literature, nothing was reported concerning the antimicrobial activity of *C. insignis*. E6 and the successive fractions were tested against representative M0; results are shown in Figure 3.

There was no significant difference between the effect of E2 and the standard antibiotic, ampicillin, on M1 and M2. It showed high activity against both bacteria. It had moderate activities on M3, M4, M5 and M7. E3 showed moderate activities against M4, M7, M8 and M9. The E4 showed high activity on M1 and M2. It had moderate activities on M3, M4, M6 and M7. All the extracts were inactive against M10. E1, E5 and E6 were inactive against all M0.

4. Discussion

It was reported that the ether and ethyl acetate extracts of several plants possess antimicrobial activities against different MO, for example, the ether extract of Artemisia nilagirica leaf showed antibacterial activity against one or more of 12 tested bacterial strains^[18]. Extracts of Ficus racemosa Linn. leaves produced significant antibacterial potential against M1, M3, M5, Bacillus pumilis and Pseudomonas aeruginosa^[19]. The ether extract of the lichen Cladonia foliacea showed antimicrobial activities against nine bacteria and fungi^[20]. The diethyl ether extract of Plantago major L. displayed activity on Escherichia coli^[21]. Quinic acid derivatives isolated from Ageratina adenophora Spreng showed antibacterial activity toward M3, Bacillus thuringiensis, M5, Salmonella enterica and Shigella dysenteria^[22].

In addition, several flavonoids were reported to possess antimicrobial activity^[23]. For instance, the ethyl acetate extract of *Bryophyllum pinnatum* Lank. and its isolated kampferol derivatives have interesting antimicrobial properties^[24]. The ethyl acetate extract of *Marsilea quadrifolia* showed excellent antibacterial activities against 5 Gram–positive and 11 Gram– negative human pathogenic bacteria^[25]. Previously, El Sawi *et al.* found that the ethyl acetate extract of *C. insignis* contains flavonoids and specially kampferol derivatives which may be responsible for the antimicrobial activity^[5].

From this study it can be concluded that the ether and the ethyl acetate fractions have potent antibacterial activity against *Bacillus subtilis* and *Bacillus cereus*, so they could be used in pharmaceutical formulations. Further clinical trials should be performed in order to support the above investigations and to facilitate their pharmaceutical formulations.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was supported by National Research Centre, Cairo, Egypt (Grant No. 2/5/12).

Comments

Background

The literature reported the importance of *C. insignis* plant which were found to exhibit variable biological activities and contain different constituents as reported previously by the author, so it is very important to test for its antimicrobial activity.

Research frontiers

This study was carried out to complete the chemical and biological investigation of *C. insignis* and to add another importance of the plant as antimicrobial. The chemical constituents of the petroleum ether extract and the ether fraction of the 70% ethanol extract were studied and their activities as antimicrobial were screened.

Related reports

The plant was reported to possess different biological activities as published before by the authors. The study completes the research on the plant as antimicrobial which is not reported before.

Innovations & breakthroughs

The authors studied in this work the antimicrobial activity of *C. insignis* which was not studied before and it appeared that two fractions of the plant possess significant activity against two microorganisms. Also, this is the first report for the isolation and identification of the chemical constituents of the non-polar fractions of the plant.

Applications

It is interesting to know that fractionation of inactive antimicrobial extract lead to different fractions which possess variable antimicrobial activities, the synergism and the antagonism properties appear in this study in the total ethanol extract which is inactive against all MO while the ether and the ethyl acetate fractions have potent antibacterial activity specially against *Bacillus subtilis* and *Bacillus cereus*.

Peer review

This is a valuable study in which the authors isolated and identified the constituents of the non polar fraction and the article contains the spectroscopic data which lead to identification of these compounds.

References

- Huxley A. The new royal horticultural society dictionary of gardening. London: Macmillan Press; 1992.
- Barwick M. Tropical and subtropical trees: an encyclopedia guide. Portland, Oregon: Timber Press; 2004.
- [3] Coussio JD. Isolation of rhoifolin from *Chorisia* species (Bombacaceae). *Experientia* 1964; 20(10): 562.
- [4] El Alfy S, El Sawi S, Sleem A, Moawad D. Investigation of

flavonoidal content and biological activities of *Chorisia insignis* HBK. leaves. *Aust J Basic Appl Sci* 2010; **4**(6): 1334–1348.

- [5] El Sawi S, Moawad D, El Alfy S. Activity of *Chorisia insignis* HBK. against larynx carcinoma and chemical investigation of its polar extracts. *J Appl Sci Res* 2012; 8(11): 5564–5571.
- [6] El Sawi S, Moawad D, El Alfy S. Phytochemical screening and qualitative analysis of the polysaccharide contents of *Chorisia* insignis. J Herbs Spices Med Plants 2013; 19(4): 313–320.
- [7] El Alfy T, El Sawi S, Abd El Tawab S, Moawad D. Pharmacognostical study of *Chorisia insignis* HBK. grown in Egypt. *Bull Fac Pharm Cairo Univ* 2012; **50**(1): 17–39.
- [8] Green MR, Sambrook J. *Molecular cloning*. New York: Cold Spring Harbor Laboratory; 2012.
- [9] Rao NS. Soil micro-organisms and plant growth. Lebanon: Science Publishers Inc.; 1995.
- [10] Dillon JR, Nasim A, Nestmann ER. Recombinant DNA methodology. New York: John Wiley and Sons; 1985. p. 127.
- [11] Tsuda K, Sakai K, Tanabe K, Kishida Y. Isolation of 22dehydrocholestrol from *Hypnea japonica*. J Am Chem Soc 1960; 82: 1442-1443.
- [12] Finar IL. Organic chemistry. London: Longmans Green & Co.; 1967, p. 212.
- [13] Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. New York: Allured Publishing Corporation; 2007.
- [14] Jennings W, Shibamato T. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. New York: Academic Press; 1980.
- [15] Hammond SM, Lambert PA. Antibiotic and antimicrobial activity action. London: Edward Arnold Publishers Ltd; 1978.
- [16] Sengupta P, Khastgir HN. Terpenoids and related compounds-III: bauerenol and multiflorenol from *Gelonium multiflorum* A. Juss. The structure of multiflorenol. *Tetrahedron* 1963; **19**: 123–132.
- [17] Barua AK, Raman SP. Triterpenoids-XII: the constitution of albigenin-a new triterpene from *Albizia lebbeck* benth. *Tetrahedron* 1962; 18: 155-159.
- [18] Ahameethunisa AR, Hopper W. Antibacterial activity of Artemisia nilagirica leaf extracts against clinical and phytopathogenic bacteria. BMC Complement Altern Med 2010; 10: 6.
- [19] Mandal SC, Saha BP, Pal M. Studies on antibacterial activity of *Ficus racemosa* Linn. leaf extract. *Phytother Res* 2000; 14(4): 278– 280.
- [20] Yilmaz M, Türk AO, Tay T, Kivanç M. The antimicrobial activity of extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin, and fumarprotocetraric acid constituents. *Z Naturforsch C* 2004; **59**(3-4): 249-254.
- [21] Najib A, Alam G, Halidin M. Isolation and identification of antibacterial compound from the diethyl ether extract of *Plantago major L. Pharmacogn J* 2012; 4(31): 59-62.
- [22] Zhang M, Liu WX, Zheng MF, Xu QL, Wan FH, Wang J, et al. Bioactive quinic acid derivatives from Ageratina adenophora. Molecules 2013; 18(11): 14096-14104.
- [23] Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005; 26(5): 343–356.
- [24] Tatsimo SJ, Tamokou Jde D, Havyarimana L, Csupor D, Forgo P, Hohmann J, et al. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. *BMC Res Notes* 2012; 5: 158.
- [25] Ripa FA, Nahar L, Haque M, Islam Md M. Antibacterial, cytotoxic and antioxidant activity of crude extract of *Marsilea quadrifolia*. *Eur J Sci Res* 2009; **33**(1): 123–129.