

SESTERTERPENYL AND PHENYLIC TETRATERPENYLIC ESTERS FROM OLEO-RESIN OF *COMMIPHORA MYRRHA* (NEES) ENGL.

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ABSTRACT

Objective: Phytochemical investigation of the oleo-resin of *Commiphora myrrha* (Nees) Engl.

Methods: The structures of isolated phytoconstituent have been elucidated on the basis of spectral analysis and chemical reactions.

Results: The phytochemical investigation of oleo-resin of *C. myrrha* led to the isolation of one new sesterterpenic ester characterised as 2,10,14,18-tetramethyl-16-methylenehenecos-2,6(22),9-trien-3 β -olyl piperonylate (**3**) and one new phenylic tetraterpenylic ester formulated as 2,3,6-trimethylphenyl-9,13,18,22,26,30-hexamethylhenetriacont-8,12,16(Z),18-tetraen-15 β -olyl acetate (**5**) and along with three known esters identified as *n*-heptadecanyl capriate (**1**), *n*-dodecanyl myristate (**2**) and henetriacontanyl laurate (**4**).

Conclusion: The phytochemical investigation of oleo-resin of *C. myrrha* gave two new esters along with some known phytoconstituents. These new esters are isolated for the first time from this plant source.

Keywords: Myrrh, Oleo-resin, Phytochemical investigation, Phenylic tetraterpenylic esters, Sesterterpenic esters.

INTRODUCTION

Commiphora myrrha (Nees) Engl. (Burseraceae) is a small tree which grows in small sandy and rocky regions of Somalia, Sudan, Ethiopia, Kenya and Saudi Arabia. The schizogenous cavities of the stem and branches of this tree produce a scented oleo-resin which is known as myrrh. It is imported into India since long time and used in perfumery as food additive, fragrance, incense, antiseptic, astringent, stimulant, stomachic, and tonic and for embalming. It is an ingredient of toothpastes, mouthwashes and dentifrices. Myrrh tincture is useful in menstrual disorders and chlorosis. In China, it is prescribed to treat wounds, inflammation and menstrual pain due to blood stagnation [1]. Cadinenes, calamenes, triacont-1-ene [2], commiphoric acids, furanosesquiterpenoids [3-10], eudesmol and triterpenoids [11] and volatile oil [12] have been reported from the oleo-resin of *C. myrrha*. This paper describes the isolation and the characterization of the sesterterpenic ester and phenylic tetraterpenylic ester along with three known esters from the oleo-resin obtained from the Khari Baoli market of Delhi.

MATERIAL AND METHODS

General

The melting points were determined on a Perfit melting apparatus (Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Kong) spectrophotometer using KBr pellets; γ_{\max} values are given in cm^{-1} . ¹H and ¹³C NMR spectra were screened on Avance DRX 400, Bruker spectropin 400 and 100 MHz instruments (Karlsruhte, Germany) using TMS as an internal standard. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation, and spraying with ceric sulphate.

Plant material

The crude drug was procured from the local market of the Khari Baoli, Delhi. The sample was authenticated by Dr. H.B. Singh, Taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen of the sample (No. N/R/C/-06-07/803/120) was deposited in the NISCAIR, RHM Division, CSIR, New Delhi.

Extraction and isolation

The air dried oleo-resin (2.5 kg) was coarsely powdered and extracted with methanol at room temperature for one week. The extract was filtered and concentrated under reduced pressure to get 185 g (7.4 % yields) of dark brown mass. The concentrated extract of the oleo-resin was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) to form slurry. The slurry was air-dried and loaded on silica gel column (1.6 m \times 16 mm \times 2 mm) load in petroleum ether and then eluted successively with different solvents in increasing order of polarity in various combinations, such as petroleum ether, petroleum ether:chloroform (9:1, 3:1, 1:1, 1:3), chloroform, chloroform:methanol (19.9:0.1, 99:1, 97:3, 19:1, 93:7, 9:1, 17:3, 3:1, 3:2, 2:3) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of *C. myrrha* oleo-resin:

n-Heptadecanyl capriate (**1**)

Elution of the column with petroleum ether produced a light coloured sticky mass of **1**, recrystallized from methanol-acetone (9:1), 460 mg (0.0184% yield); R_f : 0.73 (petroleum ether:chloroform; 9: 1); m.p.: 76- 78°C; +ve ion FAB MS m/z (*rel. int.*): 410 [M]⁺ (C₂₇H₅₄O₂) (33.1), 171 (57.5), 155 (61.3).

n-Dodecanyl myristate (**2**)

Further elution of the column with petroleum ether gave a colourless amorphous powder of **2**, recrystallized from acetone-methanol (1:1), 1.41 g (0.0564% yield); R_f : 0.77 (chloroform-methanol; 97: 3); m.p.: 90- 92°C; +ve ion FAB MS m/z (*rel. int.*): 396 [M]⁺ (C₂₆H₅₂O₂) (15.6), 227 (33.1), 211 (26.3), 185 (26.7), 169 (73.5).

Myrrhasesterterpenyl piperonylate (**3**)

Elution of the column with petroleum ether:chloroform (1:1) furnished a yellow solid mass of **3**, recrystallized from acetone, 671 mg (0.026 % yield); R_f : 0.76 (chloroform : methanol, 97:3); m.p.: 74-75°C; UV λ_{\max} (MeOH): 225, 270, 360 nm (log ϵ 3.5, 2.9, 4.8); IR ν_{\max} (KBr): 2931, 2845, 1747, 1645, 1525, 1446, 1387, 1237, 1216, 1106, 1038, 961 cm^{-1} ; ¹H NMR (CDCl₃): δ 8.21 (1H, d, $J=2.5$ Hz, H-2'), 7.18 (1H, dd, $J=2.5, 8.6$ Hz, H-6'), 7.01 (1H, d, $J=8.6$ Hz, H-5'), 5.73 (1H, m, H-3), 5.52 (1H, m, H-9), 4.96 (2H, brs, H₂-22), 4.80 (1H, dd, $J=5.5, 8.3$ Hz, H-7 α), 3.42 (2H, brs, OCH₂O), 1.98 (3H, brs, Me-23), 1.72 (3H, brs, Me-

1), 1.70 (3H, brs, Me-21), 1.21 (3H, d, $J=6.5$ Hz, Me-24), 1.03 (3H, d, $J=6.2$ Hz, Me-25), 0.83 (3H, t, $J=6.1$ Hz, Me-20), 2.91-1.36 (22H, m, $10\times\text{CH}_2$, $2\times\text{CH}$); ^{13}C NMR (CDCl_3): Table 1; +ve ion FAB MS m/z (*rel. int.*): 510 [M]⁺ ($\text{C}_{33}\text{H}_{50}\text{O}_4$) (3.3), 341 (18.6), 301 (19.1), 287 (17.5), 209 (34.7), 169 (57.2).

Henetriacosanyl laurate (4)

Elution of the column with petroleum ether : chloroform (1:3) produced a colourless amorphous powder of **4**, recrystallized from methanol-acetone-diethyl ether (7:2:1), 1.76 g (0.0704% yield); R_f: 0.46 (chloroform-methanol; 17:3); m.p.: 82- 83°C; UV λ_{max} (MeOH): 213 nm (log ϵ 5.3); IR ν_{max} (KBr): 3020, 2927, 2857, 2359, 1726, 1605, 1522, 1432, 1215, 1044, 929, 760 cm^{-1} ; ^1H NMR (CDCl_3): δ 4.50 (2H, brs, H₂-1'), 1.98 (2H, brs, H₂-2), 1.66 (2H, m, CH₂), 1.51 (2H, m, CH₂), 1.29 (74H, brs, $37\times\text{CH}_2$), 0.81 (3H, t, $J=6.1$ Hz, Me-31'), 0.79 (3H, t, $J=6.0$ Hz, Me-12); +ve ion FAB MS m/z (*rel. int.*): 648 [M]⁺($\text{C}_{44}\text{H}_{88}\text{O}_2$) (37.3), 633 (12.2), 183 (100), 155 (21.7), 126 (25.8).

Commiphoratenyl acetate (5)

Elution of the column with chloroform gave a brown mass of **5**, recrystallized from methanol, 2.35 g (0.094% yield). R_f: 0.9 (chloroform); m.p.: 62-63°C; UV λ_{max} (MeOH): 222, 415, 462 nm (log ϵ 3.2, 5.6, 5.3); IR ν_{max} (KBr): 2928, 2855, 1739, 1649, 1525, 1445, 1379, 1218, 1149, 1087, 1025, 892 cm^{-1} ; ^1H NMR (CDCl_3): δ 7.19 (1H, d, $J=8.5$ Hz, H-4), 6.98 (1H, d, $J=8.5$, H-5), 5.82 (1H, dd, $J=5.5$, 10.8 Hz, H-17), 5.70 (1H, m, H-8), 5.47 (1H, dd, $J=9.9$, 10.8 Hz, H-16), 5.35 (1H, m, H-12), 5.27 (1H, m, H-19), 4.35 (1H, brm, $w_{1/2}=16.7$ Hz, H-15 α), 2.23 (3H, brs, Me-32), 2.19 (3H, brs, Me-33), 2.11 (3H, brs, Me-34), 1.96 (3H, brs, OAc), 1.88 (3H, brs, Me-35), 1.88 (3H, brs, Me-36), 1.66 (3H, brs, Me-37), 1.17 (3H, d, $J=6.7$ Hz, Me-39), 0.98 (3H, d, $J=6.5$ Hz, Me-38), 0.75 (3H, d, $J=7.5$ Hz, Me-40), 0.73 (3H, d, $J=7.2$ Hz, Me-31), 2.68 - 1.25 (27H, m, $12\times\text{CH}_2$, $3\times\text{CH}$); ^{13}C NMR (CDCl_3): Table 1; +ve ion FAB-MS m/z (*rel. int.*): 604 [M]⁺ ($\text{C}_{42}\text{H}_{68}\text{O}_2$) (5.2), 561 (12.1), 485 (25.3), 349 (15.9), 280 (11.3), 277 (7.5), 255 (95.8), 201 (15.8), 133 (21.9), 119 (63.7).

Table 1: ^{13}C NMR Spectral Data of Compound 3 and 5.

Position	3	5	Position	3	5
1.	22.45	146.87	25.	07.85	29.37
2.	136.73	145.60	26.	-	36.45
3.	121.92	135.46	27.	-	36.81
4.	49.37	123.25	28.	-	35.75
5.	40.31	122.06	29.	-	29.35
6.	137.21	135.30	30.	-	35.39
7.	77.21	49.87	31.	-	7.73
8.	47.85	123.11	32.	-	26.90
9.	122.46	137.53	33.	-	24.16
10.	137.55	44.82	34.	-	23.25
11.	47.46	45.57	35.	-	22.57
12.	34.32	121.85	36.	-	19.91
13.	26.52	137.03	37.	-	19.53
14.	38.69	46.52	38.	-	19.29
15.	28.99	71.11	39.	-	15.07
16.	29.11	112.64	40.	-	8.41
17.	28.95	113.81	1'	145.21	-
18.	35.30	136.09	2'	127.62	-
19.	24.10	119.89	3'	160.44	-
20.	15.03	42.72	4'	161.05	-
21.	23.45	32.84	5'	122.65	-
22.	107.14	35.11	6'	119.53	-
23.	20.10	32.66	7'	171.48	-
24.	19.87	31.83	OAc		20.62, 170.03

RESULTS AND DISCUSSION

Compounds **1**, **2** and **4** were the aliphatic esters characterized as *n*-heptadecanyl capriate, *n*-dodecanyl myristate and henetriacosanyl laurate, respectively.

Compound **3**, named as myrrha-sesterterpenyl piperonylate, was obtained as a yellow solid from the petroleum ether-chloroform eluants. Its IR spectrum showed the characteristic absorption bands for the ester group (1747 cm^{-1}), unsaturation (1645 cm^{-1}) and aromatic ring ($1525, 1038\text{ cm}^{-1}$). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **3** was determined at m/z 510 consistent to the molecular formula of sesterterpenyl ester $\text{C}_{33}\text{H}_{50}\text{O}_4$. The ion peaks arising at m/z 109 [$\text{C}_6 - \text{C}_7$ fission, C_8H_{13}]⁺, 287 [$\text{C}_7 - \text{C}_8$ fission, $\text{C}_9\text{H}_{14} - \text{OCO} - \text{C}_6\text{H}_3\text{OCH}_2\text{O}$]⁺, 301 [$\text{C}_8 - \text{C}_9$ fission, $\text{C}_{10}\text{H}_{16} - \text{OCO} - \text{C}_6\text{H}_3 - \text{OCH}_2\text{O}$]⁺ and 209 [$\text{M} - 301$]⁺, 169 [$\text{C}_{10} - \text{C}_{11}$ fission, $\text{C}_{12}\text{H}_{25}$]⁺ and 341 [$\text{M} - 169$]⁺ suggested the existence of the vinylic linkages at C₂, C₆₍₂₂₎ and C₉ positions and piperonylate function at C-7. The ^1H NMR spectrum of **3** displayed two one-proton doublets at δ 8.21 ($J=2.5$ Hz) and 7.01 ($J=8.6$ Hz) and a one-proton double doublet at δ 7.18 ($J=2.5, 8.6$ Hz) assigned to aromatic H-2', H-5' and H-6' protons respectively. Two one-proton multiplets at δ 5.73 and 5.52 and a two-proton broad singlet at δ 4.96 were ascribed vinylic H-3 and H-9 and H₂-22 protons, respectively. A one-proton doublet at δ 4.80 ($J=5.5, 8.3$ Hz) and a two-proton broad singlet at δ 3.42 were attributed to α -

oriented oxygenated H-7 and dioxomethylene protons, respectively. Three broad singlets at δ 1.98, 1.72 and 1.70, two doublets at δ 1.21 ($J=6.5$ Hz) and 1.03 ($J=6.2$ Hz) and a triplet at δ 0.83 ($J=6.1$ Hz), all integrated three-protons each, were accounted to methyl C-23, C-1 and C-21 protons attached to vinylic carbons, secondary C-24 and C-25 and primary C-20 methyl protons, respectively. The ^{13}C NMR spectrum of **3**, exhibited signals for aromatic and vinylic carbons between δ 161.05-107.14, ester carbon at δ 171.48 (C-7'), oxygenated methine carbon at δ 77.21 (C-7), dioxomethylene carbon at δ 103.16 and methyl carbons from δ 23.45 to 7.85. On the basis of the above discussion, the structure of **3** was formulated as 2,10,14,18-tetramethyl-16-methylenehenecos-2,6(22),9-trien-3 β -olyl piperonylate (Figure 1). This is a new sesterterpenic ester from the plant source.

Compound **5**, designated as commiphoratenyl acetate, was obtained as a dark brown mass from the chloroform eluants. Its IR spectrum exhibited characteristic absorption bands for ester group (1739 cm^{-1}), unsaturation (1649 cm^{-1}) and long aliphatic chain (762 cm^{-1}). Its molecular ion peak was determined at m/z 604 on the FAB mass and ^{13}C NMR spectra consistent with the molecular formula of a tetraterpenic ester $\text{C}_{42}\text{H}_{68}\text{O}_2$. The ion peaks arising at m/z 349, 255 [$\text{C}_{14} - \text{C}_{15}$, fission, $\text{C}_{19}\text{H}_{27}$]⁺, 561 [$\text{M} - \text{COCH}_3$]⁺ and 280 [$349 - \text{CH}_3\text{COO}$]⁺ indicated the presence of acetate group at C₁₅. The ion peaks generating at m/z 201 [$\text{C}_{11} - \text{C}_{12}$ fission, $\text{C}_{15}\text{H}_{21}$]⁺, 471, 133 [$\text{C}_7 - \text{C}_8$ fission]

and 485, 119 [C₁-C₇ fission]⁺ supported the presence of acetoxy function at C-15 and trimethyl substituted benzene ring at one terminal. The other important fragments arising at *m/z* 277 [C₁₅-C₁₆ fission, C₂₀H₃₇]⁺ and 251 [C₁₇-C₁₈ fission, C₁₈H₃₇]⁺ suggested location of the vinylic linkages at C-16 and C-18. The ¹H NMR spectrum of **5** exhibited two one-proton doublets at δ 7.19 and 6.98 (*J*=7.5 Hz each) assignable to ortho-coupled aromatic H-4 and H-5 protons, respectively. Two one-proton double doublets at δ 5.47 (*J*= 9.9, 10.8 Hz) and 5.82 (*J*=5.5,10.8 Hz) were ascribed to cis-oriented vinylic H-16 and H-17 protons, respectively. Three one-proton multiplets at δ 5.70, 5.35 and 5.27 were attributed to vinylic H-8, H-12 and H-19 protons, respectively. A one-proton broad multiplet at δ 4.35 (*w*_{1/2}=16.7 Hz) was ascribed to α-oriented oxygenated C-15 methine proton. Seven

three-proton broad signals at δ 2.23, 2.19, 2.11, 1.96, 1.88, 1.75 and 1.66 were accounted correspondingly to C-32, C-33 and C-34 methyl protons located on the aromatic ring, acetoxy protons and C-35, C-36 and C-37 methyl protons attached to vinylic carbons. Four three-proton doublets at δ 1.17 (*J*=6.7 Hz), 0.98 (*J*=6.5 Hz), 0.75 (*J*=7.5 Hz) and 0.73 (*J*=7.2 Hz) were associated with secondary C-39, C-38, C-40 and C-31 secondary methyl protons, respectively. The ¹³C NMR spectrum of **5** displayed important signals for ester carbon at δ 170.03 (Ac), aromatic and vinylic carbons between δ 152.44–112.64, and oxygenated methine carbon at δ 72.11 (C-15). On the basis of the above discussion, the structure of **5** was elucidated as 2,3,6-trimethylphenyl-9,13,18,22,26,30-hexamethylhentriacont-8,12,16(Z), 18-tetraen-15β-yl acetate (Figure 1). This is a new carotene ester.

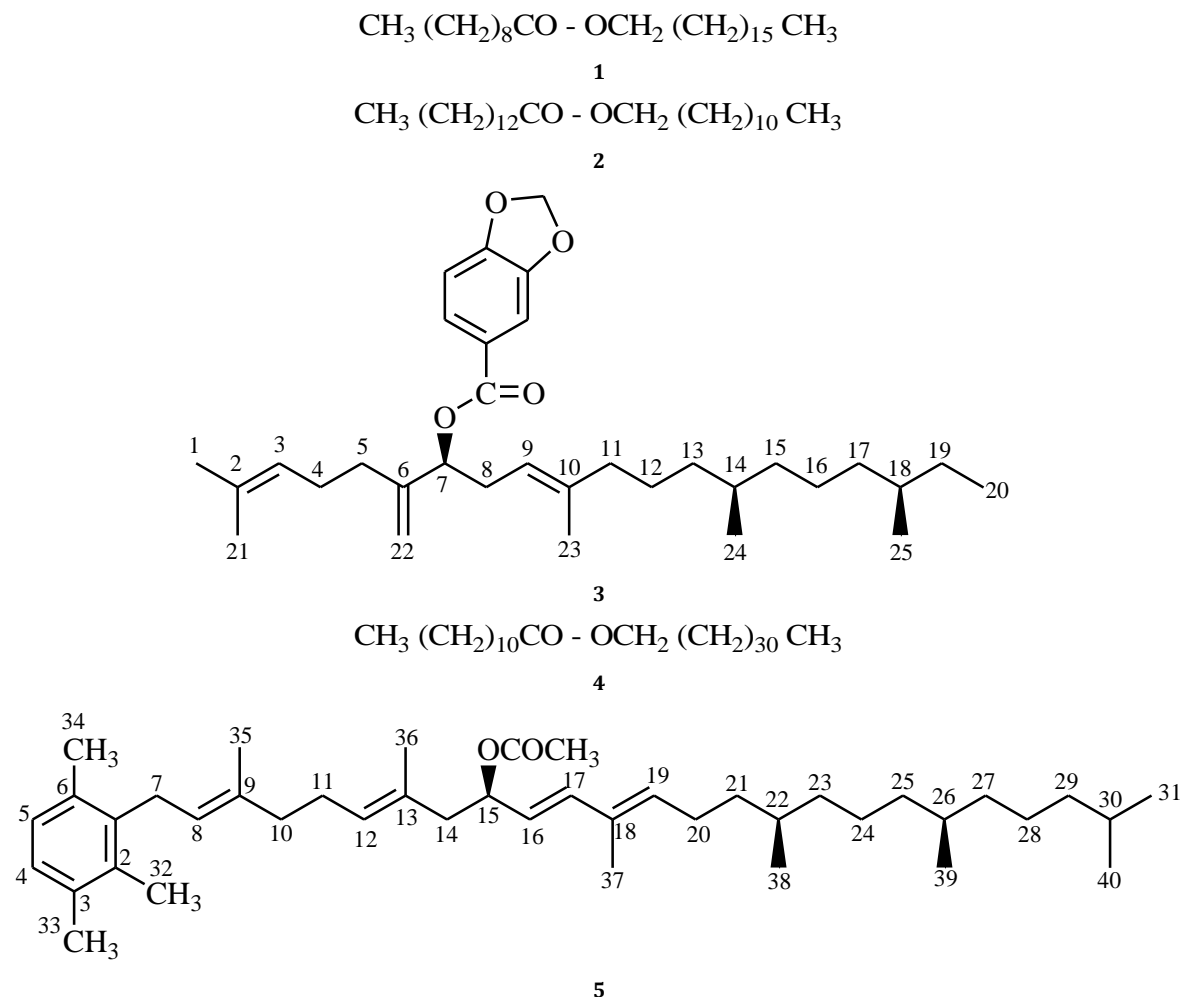


Fig. 1: The Structures of Compounds 1-5.

CONCLUSION

The phytochemical investigation of the oleo-resin of *C. myrrha* (Nees) Engl. gave to the isolation of one new sesterterpenic ester and one new phenylic tetraterpenylic ester along with three known esters. These compounds may be used as chromatographic markers and may also have some medicinal importance. This investigation will increase the existing knowledge about the phytoconstituents of *C. myrrha* and will also help to understand the complexity of phytoconstituents present in oleo-resin of *C. myrrha*.

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REFERENCES

1. Anonymous. **The Wealth of India**: A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials, National Institute of Science Communication and Information Resources, CSIR, New Delhi, 2004; 2(supp. 1): 162-163.
2. Ahmed F, Ali M, Singh O. New Compounds from *Commiphora myrrha* (Nees) Engl. **Pharmazie** 2006; 61(8): 728-731.
3. Zhu N, Kikuzaki H, Sheng S, Sang S, Rafi MM, Wang M, et al. Furanosesquiterpenoids of *Commiphora myrrha*. **J Nat Prod** 2001; 64(11): 1460-1462.
4. Dolara P, Corte B, Ghelardini C, Pugliese AN, Cerbai E, Menichetti S, Nastro AL. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. **Plant Med** 2000; 66: 356-358.

5. Dolara P, Moneti G, Pieraccini G, Romanelli N. Characterization of the action on central opioid receptors of furanoeudesma-1,3-diene, a sesquiterpene extracted from myrrh. **Phytother Res** 1996; 10: 81-83.
6. Maradufu A, Warthen JD. Furanosesquiterpenoids from *Commiphora myrrh* oil. **Plant Sci** 1988; 57(2): 181-184.
7. Brieskorn CH, Noble P. Three new furanogermacrenes from myrrh. **Tetrahedron Lett** 1980; 21(16): 1511-1514.
8. Brieskorn CH, Noble P. Furanosesquiterpenes from the essential oil of myrrh. **Phytochem** 1983; 22(5): 1207-1211.
9. Maradufu A. Furanosesquiterpenoids of *Commiphora erythraea* and *C. myrrh*. **Phytochem** 1982; 21(3): 677-680.
10. Shen T, Wan WZ, Wang XN, Yuan HQ, Ji M, Lon HX. A tetraterpenoid and sesquiterpenoids from the resinous exudates of *Commiphora myrrh*. **Helevitica Chemica Acta** 2009; 92(4): 645-652.
11. Mincione E, Lavarone C. Terpenes from Arabian *Commiphora myrra*. **Chimica e'Industria** 1972; 54(5): 424-425.
12. Brieskorn CH, Noble P. Two furanoeudesmanes from the essential oil of myrrh. **Phytochemistry** 1983; 22(1): 187-189.