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ANTI-HERPES ACTIVITY OF ISOLATED COMPOUNDS FROM FRANKINCENSE

F. A. Badria[1]*, M. Abu-Karam[2], B. R. Mikhaeil[1], G. T. Maatooq[1] and M. M. Amer[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy,
Mansoura University, Mansoura 35516 (Egypt)
[2]Department of Medicinal Chemistry, College of Pharmacy,
University of Minnesota, MN 55455 (USA)

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ABSTRACT

The antiviral and cytotoxicity bioassay-guided fractionation of the oleogum resin of frankincense (*Boswellia carterii* Bird wood) resulted in the isolation and identification of 9 compounds; palmitic acid and eight triterpenoids belonging to lupane, ursane, oleanane, and tirucallane skeleta were isolated form the resin. These triterpenoids are lupeol, β-boswellic acid, 11-keto-β-boswellic acid, acetyl β-boswellic acid, acetyl 11-keto-β-boswellic acid, acetyl-α-boswellic acid, 3-oxo-tirucallic acid, and 3-hydroxy-tirucallic acid. The structures of the isolated compounds were deduced based on spectroscopic evidences. The total acid mixture showed the highest activity among all compounds against *Herpes simplex* type I virus and was able to reduce the number of the plaques by 100 % with a minimum antiviral concentration at 20 μg/ml and followed by acetyl-11- keto-β-Boswellic acid (75% inhibition at 20 μg/ml), β-Boswellic and total alcoholic extract (50% inhibition at 40 μg/ml), acetyl-β-Boswellic and 11- keto-β-Boswellic (75% inhibition at 80 μg/ml), 3-hydroxy-tirucallic acid, 3-oxo-tirucallic acid, acetyl-α- boswellic acid, and total volatile oil (50% inhibition at 80 μg/ml). On the other hand, gum, palmitic acid and lupeol reduced the number of plaques by 25% at relatively high concentrations. The antiviral and cytotoxicity assay of the isolated compounds proved that the total extract retained more activity than that of any of the purified compounds.

Keywords: Anti-Herpes activity, Frankincense oleogum resin and triterpenoids.

INTRODUCTION

Frankincense oleogum resin is obtained by incision of the bark of several species of *Boswellia*, Burseraceae. It is known as Olibanum, Luban Dakar, Bakhor or Kendar (In Arabic), and Salai Guggal (In Ayurvedic medicine) (Evans, 1996). The plant is native to India, Arabian Peninsula (Yemen, Sultanate of Oman), Red Sea region of North-East Africa (Somalia, Eritrea) (Maupetit, 1984). *Boswellia* resin and its individual components has shown various biological activities; including anti-inflammatory activity (Sharma *et al.*, 1989); leukotriene biosynthesis-inhibitory activity (Ammon *et al.*, 1991; Gupta *et al.*, 2001); and anti-tumor activity (Shao *et al.*, 1998). Recently, (Badria et al., 2002) proved the clinical efficacy of boswellia-curcumin preparation for treatment of knee osteoarthritis. Frankincense oleogum resin is a complex mixture containing a series of mono-, sesqui-, di-, and triterpenoids (El-Khadem *et al.*, 1972; Pradhy and Bhattacharyya, 1978). Both the alcoholic extract of Salai Guggal (AESG) and boswellic acids (a mixture of triterpenoids obtained from the oleogum resin *Boswellia serrata*), influenced both cellular and humoral immune responses in rats and mice (Sharma *et al.*, 1988 and 1996). The defatted alcoholic extract of *Boswellia serrata* caused almost total inhibition of the classical complement pathway of the immune system (Wagner, 1989) while β-boswellic acid demonstrated a marked inhibitory effect on both classical and alternate complement systems (Knaus and Wagner, 1996). Recently, The extracts of *Boswellia serrata* gum resin and its constituents, the boswellic acids (BAs), activated the mitogen-activated protein kinase (MAPK) p42 and (MAPK) p38 in isolated human polymorphonuclear leukocytes (PMNLs) (Altmann *et al.*, 2002). In view of such activities of the oleogum resin and its components, we initiated a bioassay-guided fractionation to monitor the antiviral and cytotoxicity activity of the frankincense resin obtained from *Boswellia carterii* Birdwood.

MATERIAL AND METHODS

1. Materials for chromatographic Study

Silica gel $G60F_{254}$ for TLC (E-Merck, Germany), silica gel for column chromatography (70-230 mesh) (E-Merck, Germany), reversed phase silica ($RP\text{-}C_{18}$) for column chromatography (E-Merck, Germany), precoated silica gel GF_{254} plates, aluminium and plastic sheets for TLC (E-Merck and Machery-Nagel, Germany), precoated reversed phase silica plates for TLC (E-Merck, Germany).

2. General Instrumentation

UV spectra were recorded in MeOH using a Shimadzu 1601-PC UV/Visible spectrophotometer, IR spectra were recorded on Buck model 500 Infra red spectrophotometer, NMR spectra were recorded using Bruker AM-300 spectrometer, Drx-400, and Varian Mercury-300 spectrometer using $CDCl_3$, and DMSO-d_6 as solvents and TMS as internal standard. Chemical shifts (δ) are expressed in ppm. DEPT, APT, COSY, HMQC, and HMBC experiments were conducted under standard conditions. EI/MS were performed using a Finningan Mat SSQ 7000 mass spectrometer with a Digital DEC 3000 workstation.

3. Antiviral and cytotoxicity assay:

a. Sample preparation

Samples were prepared for assay by dissolving in 50 µl of DMSO and diluting aliquots into sterile culture medium. These solutions were subdiluted in sterile medium and the two solutions used as stocks to test samples at 2-200 µg/ml in triplicate in the wells of micotiter plates.

b. Virus used in assay

The compounds were tested for antiviral activity against *Herpes simplex* type 1 (HS-1) grown on *Vero African* green monkey kidney cells.

c. Culture

Herpes simplex type I (HS-I) was the gift of Dr. R.G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. Virus stocks were prepared as aliquots of culture medium from Vero cells infected at multiplicity of 1 virion per 10 cells and cultured 3 days. They were stored at - 80 °C. Working stocks were prepared by titering virus by serial dilution in culture medium and assayed in triplicate on Vero monolayers in the wells of microtiter trays. Virus suspensions that gave about 30 plaques per well were stored at 4°C until used. *Vero African* green monkey kidney cells were purchased from Viromed Laboratories, Minnetonka, MN, and grown in Dulbeccois modified Eagle's medium supplemented with 10 % (v/v) calf serum (HyClone Laboratories, Ogden, UT), 60 mg/ml Penicillin G and 100 mg/ml streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 15 % (v/v) CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Vero stocks were maintained at 34°C in culture flasks filled with medium supplemented with 1 % (v/v) calf serum. Subcultures for virus titration or antiviral screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton Dickinson Labware, Lincolin Park, NJ) by suspending Vero cells in medium following trypsin-EDTA treatment, counting the suspension with a hemocyto-meter, diluting in medium containing 10% calf serum to 2 x 104 cells per 200 ml cultur, aliquoting into each well of a tray and culturing until confluent.

d. Procedure

Microtiter trays with confluent monolayer cultures of Vero cells were inverted, the medium shaken out and replaced with serial dilutions of sterile extracts in triplicate in 100ul medium followed by titered virus in 100 ul medium containing 10 % (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were not treated with compounds or not treated with virus. The trays were cultured for 66 hours. The trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7 % (v/v) formaldehyde in saline for at least 20 minutes. The fixed cells were rinsed with water, and examined visually. Antiviral activity is identified as confluent, relatively unaltered monolayers of stained Vero cells treated with HS-1. Cytotoxicity was estimated as the concentration that caused approximately 50 % loss of the monolayer present around the plaques caused by HS-1.

4. Plant material

The oleogum resin of *Boswellia carterii* Birdwood (Bursearceae) was purchased from the local herbal stores in Mansoura on March 1999. It was authenticated by comparison with a genuine sample kept in the Drug Museum of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo.

Extraction

The finely ground oleogum resin (500 grams) was extracted with methylene chloride (5 liters). The extract was concentrated under reduced pressure to yield 330 gms of semisolid oleoresin.

Isolation and Identification

The total extract (15 gms) was applied onto the top of a glass column (120 x 5 cm) previously packed with silica gel (300 g) in petroleum ether (b.p. 60-80 °C). The extract was gradiently eluted with petroleum ether containing increasing proportions of ethyl acetate. The effluent was collected in 250-ml fractions. Each fraction was concentrated, *in vacuo*. Fractions (51-70) gave white waxy crystals (400 mg) which were further purified by column chromatography (60 x 1.5 cm) using (20 g) silica gel. Elution was adopted using petroleum ether (b.p. 60-80 °C) containing gradually increasing proportions of ethyl acetate. Sub-fractions (8-10) afforded 329 mg of 1. Fractions (71-100) were subjected to reversed phase Rp-C_{18} flash column chromatography using isocratic elution with methanol-water (8:2 v/v) to afford 2 (11.9 mg). Fractions (101-130) were further purified by preparative TLC using petroleum ether-ethyl acetate (9:1 v/v) as solvent system affording 30 mg of 3. Fractions (131-160) were further purified by reversed phase Rp-C_{18} flash column chromatography using methanol-water (95:5 v/v) to afford 4 (86 mg). Upon crystallization, fractions (161-190) gave 5 (14 mg). Fractions (191-220), (221-250), and (251-280) were separately purified by reversed phase Rp-C_{18} flash column chromatography adopting isocratic elution with methanol-water (9:1 v/v), to afford 6 (52 mg), 7 (130 mg), and 8 (12.1 mg), respectively. Fractions (311-340) were purified in the same manner but using methanol-water (80:20 v/v) as solvent system to afford 9 (28 mg).

Compound 1; hexadecanoic acid; palmitic acid

$C_{16}H_{32}O_2$; white waxy crystals; mp 57-62 °C; UV λ_{max} (MeOH) nm, 202.5; IR (KBr) υ_{max} cm^{-1}: 723, 940, 1297, 1414, 1464, 1701 (C=O), 2851, 2955, and 3321 (O-H); EI/MS *m/z* (rel. int.): 256 (56.9) $[M]^+$, 228 (15.5), 213 (19.4), 185 (22.7), 157 (17.5), 129 (58.9), 97 (39.2), 83 (44.6), 73 (94.7), and 60 $[C_2H_4O_2]^+$ (100); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.83 (3 H, *t*, H-16), 1.23 (24 H, *m*, H- 4-15), 1.58 (2 H, *m*, H-3), and 2.30 (2 H, *t*, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$, δ in ppm): 14.2 (C-16), 22.7 (C-15), 24.7 (C-3), 29.1 (C-4), 29.3 (C-5), 29.4 (C-6), 29.6 (C-7), 29.7 (C-8 to C-11), 31.9 (C-14), 34.0 (C-2), and 179.6 (C-1).

Compound 2; lup-20(29)-en-3β-ol; lupeol.

$C_{30}H_{50}O$; short fine colorless needles; mp 214-217 °C; UV λmax nm (MeOH), 203.5; IR υmax cm^{-1}: 1534, 1618 (C=C), 2366, 2970, and 3490 cm^{-1} (O-H); EI/MS m/z (rel. int.): 426 (61.7) $[M]^+$, 411 (32.1) $[M-CH_3]^+$, 393 (15.7) $[M-CH_3-H_2O]^+$, 316 (13.0), 274 (12.1), 229 (10.6), 218 (100), 189 (90.6), 175 (29.4), 135 (54.1), 95 (61.6), and 69 (57.9); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, J = Hz): 0.79 (3H, *s*, H-25), 0.94 (3H, *s*, H-27), 0.96 (3H, *s*, H-24), 1.04 (3H, *s*, H-26), 1.29 (3H, *s*, H-28), 1.33 (3H, *s*, H-23), 1.68 (3H, *s*, H-30), and 3.42 (1H, *dd*, 19.5, 3.3, H-3), 4.57, 4.68 (2H, *d*, 2, 2, H-29). The ^{13}C-NMR data of compounds 2-9 are shown in Table 1.

Compound 3; 3α-acetoxy-urs-12-ene-24β-oic acid; acetyl-β-boswellic acid

$C_{32}H_{50}O_4$; colorless needles; mp 250-252 °C; UV λ_{max} (MeOH) nm, 206; IR (KBr) υ_{max} cm^{-1}: 1275, 1376, 1456, 1618 (C=C), 1702 (-COOH), 1727 (CH_3CO-), 2965, and 3400 (O-H); EI/MS *m/z* (rel. int.): 498 (4.5) $[M]^+$, 438 (0.7), 394 (0.42), 379 (0.17), 255 (3.0), 203 (17.3), 175 (5.1), and 119 (6.0); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.80 (3H, *d*, 12.5, H-29), 0.85 (3H, *d*, 13, H-30), 0.90 (3H, *s*, H-25), 0.90 (3H, *s*, H-27),1.02 (2H, *dd*, 16.0, 2.0, H-16), 1.04 (3H, *s*, H-26), 1.12 (3H, *s*, H-28), 1.24 (3H, *s*, H-23), 1.28 (2H, *dd*, 10.2, 2.0, H-21), 1.31 (1H, *d*, 13.6, H-18), 1.49 (1H, *dd*, 13.0, 1.3, H-5), 2.09 (3H, *s*, H-3, Ac), 5.15 (1H, *br t*, H-3), and 5.30 (1H, *br t*, H-12).

Compound 4; 3α-acetoxy-olean-12-ene-24β-oic acid; acetyl-α-boswellic acid

$C_{32}H_{50}O_4$; colorless needles; mp 247-250°C; UV λ_{max} (MeOH) nm, 204.5; IR (KBr) υ_{max} cm^{-1}: 1275, 1376, 1456, 1618 (C=C), 1708 (-COOH), 1727 (CH_3CO-), 2965, and 3450 (O-H); EI/MS *m/z* (rel. int.): 498 (0.55) $[M]^+$, 423 (0.59), 343 (0.03), 280 (0.16), 255 (2.57), 218 (100), 203 (22.5), 189 (8.9), 133 (6.4), and 43 (3.6); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.80 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 0.87 (3H, *s*, H-29), 0.90 (3H, *s*, H-25), 1.04 (3H, *s*, H-26), 1.11 (3H, *s*, H-30), 1.49 (1H, *dd*, 13.0, 1.3, H-5), 2.09 (3H, *s*, H-3 Ac), 5.14 (1H, *br t*, H-3), and 5.20 (1H, *t*, H-12).

Compound 5; 3-keto-tirucall-8, 24-diene-21-β-oic acid; 3-oxotirucallic acid

$C_{30}H_{46}O_3$; colorless needles; mp 263-265 °C; UV λ_{max} (MeOH) nm, 206; IR (KBr) υ_{max} cm^{-1}: 1193, 1347, 1420, 1448, 1620 (C=C), 1708 (C=O), 1710 (-COOH), 2954, and 3450 (O-H); EI/MS *m/z* (rel. int.): 454 (64.1) $[M]^+$, 439 (100) $[M-CH_3]^+$, 421 (14.3), 393 (17.2), 311 (4.6), 297 (23.7), 243 (5.8), 173 (7.3), 159 (10.7), 119 (14.0), and 95 (12.6); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.83 (3H, *s,* H-18), 0.91 (3H, *s,* H-19), 1.04 (3H, *s,* H-30), 1.05 (3H, *s,* H-28), 1.09 (3H, *s,* H-29), 1.29 (1H, *dd*, 9.0, 6.3, H-5), 1.54 (2H, *dd*, 9.0, 6.3, H-16), 1.59 (3H, *s,* H-27), 1.68 (3H, *s,* H-26), 2.3 (1H, *dd*, 12.5, 5.1, H-17), and 2.5 (2H, *ddd*, 15.5, 10.8, 4.0, H-2).

Table 1: Cytotoxicity and Antiviral Results of the Isolated compounds from *Boswellia carterii* Birdwood

Extract, or Compound	Cytotoxicity µg/ml (CD50)	% Reduction of plaques (µg/ml)*
Total alcoholic extract	80 µg/ml	50% at 40 µg/ml
Gum	> 200 µg/ml	25% at 200 µg/ml
Volatile oil	> 200 µg/ml	50% at 80 µg/ml
Total acid extract	~ 120 µg/ml	100% at 20 µg/ml
1 (*palmitic acid*)	> 200 µg/ml	25% at 200 µg/ml
2 (*lupeol*)	~ 160 µg/ml	25% at 80 µg/ml
3 (*acetyl-β- boswellic acid*)	~ 160 µg/ml	75% at 80 µg/ml
4 (*acetyl-α- boswellic acid*)	> 200 µg/ml	50% at 80 µg/ml
5 (*3-oxo-tirucallic acid*)	> 200 µg/ml	50% at 80 µg/ml
6 (*acetyl-11-keto-β-boswellic acid*)	~ 30 µg/ml	75% at 20 µg/ml
7 (*β-boswellic acid*)	~ 60 µg/ml	50% at 40 µg/ml
8 (*3-hydroxy-tirucallic acid*)	> 200 µg/ml	50% at 80 µg/ml
9 (*11-keto-β-boswellic acid*)	~ 120 µg/ml	75% at 80 µg/ml

* Concentrations range 2- 200 µg/ml

Compound 6; 3α-acetoxy-urs-12-ene-11-keto-24β-oic acid, acetyl-11-keto-β-boswellic acid (AKBA).

$C_{32}H_{48}O_5$; colorless needles; mp 274-276 °C; UV λ_{max} (MeOH) nm, 250; IR (KBr) υ_{max} cm^{-1}: 1238, 1274, 1379, 1457, 1622 (C=C), 1658 (α, β-unsaturated C=O), 1706 (-COOH), 1728 (CH_3COO-), 2864, 2970, and 3350 (O-H); EI/MS *m/z* (rel. int.): 512 (0.01) $[M]^+$, 408 (14.5), 393 (5.9), 353 (2.7), 273 (41.8), 232 (77.7), 189 (12.1), 175 (14.3), 161 (27.9), 119 (35.1), 105 (49.5), 91 (37.5), 55 (49.8), and 43 (100); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 6.3, H-29), 0.80 (3H, *d*, 7.4, H-30), 0.93 (3H, *s*, H-28), 1.01 (2H, *dd*, 11.8, 3.0, H-21), 1.12 (3H, *s*, H-25), 1.17 (3H, *s*, H-26), 1.21 (3H, *s*, H-23), 1.33 (3H, *s*, H-27), 1.37 (1H, *d*, 12.3, H-18), 1.38 (1H, *dd*, 12.0, 2.0, H-5), 2.07 (3H, *s*, H-3 Ac), 2.39 (1H, *s*, H-9), 5.28 (1H, *t*, H-3), and 5.54 (1H, *s*, H-12).

Compound 7; 3α-hydroxy-urs-12-ene-24β-oic acid; β-boswellic acid.

$C_{32}H_{48}O_5$; colorless needles; mp 226-228 °C; UV λ_{max} nm (MeOH), 204; IR (KBr) υ_{max} cm^{-1}: 1367, 1456, 1629 (C=C), 1708 (-COOH), 2918, and 3400 (O-H); EI/MS *m/z* (rel. int.): 456 (5.2) $[M]^+$, 441 (1.6) $[M\text{-}CH_3]^+$, 379 (0.1), 326 (0.2), 293 (0.1), 238 (13.2), 218 (100), 203 (24.8), 159 (8.3), 133 (15.5), 119 (23.0), 95 (18.7), 69 (18.0), and 57 (15.9); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 5.67, H-29), 0.79 (3H, *d*, 3.25, H-30), 0.81 (2H, *d*, 12.3, H-15), 0.89 (3H, *s*, H-25), 0.91 (3H, *s*, H-27), 1.02 (2H, *dd*, 16.0, 2.0, H-16), 1.03 (3H, *s*, H-26), 1.33, (1H, *d*, 8.9, H-18), 1.34 (3H, *s*, H-23), 1.48 (1H, *dd*, 11.8, 3.1, H-5), 1.68 (2H, *dd*, 14.1, 3.0, H-6), 2.22 (2H, *dd*, 14.0, 4.0, H-2), 4.08 (1H, *t*, H-3), and 5.14 (1H, *t*, H-12).

Compound 8; 3α-hydroxy-tirucall-8, 24-diene-21-β-oic acid; 3β-hydroxytirucallic acid.

$C_{30}H_{48}O_3$; colorless long very fine needles; mp 258-260 °C; UV λ_{max} nm (MeOH), 206; IR (KBr) υ_{max} cm^{-1}: 1466, 1622 (C=C), 1702 (-COOH), 2942, and 3416 (O-H); EI/MS *m/z* (rel. int.): 456 (67.9) $[M]^+$, 441 (85.6) $[M\text{-}CH_3]^+$, 423 (93.3), 395 (10.9), 341 (7.4), 299 (12.9), 281 (32.9), 187 (70.9), 119 (65.2), and 82 (100); ^{1}H-NMR (400 MHz, $CDCl_3$, ä in ppm, *J* = Hz): 0.74 (3H, *s*, H-19), 0.82 (3H, *s*, H-28), 0.87 (3H, *s*, H-30), 0.93 (3H, *s*, H-18), 0.99 (3H, *s*, H-29), 1.11 (1H, *dd*, 11.8, 2.0, H-5), 1.2 (2H, *dd*, 9.9, 3.1, H-1), 1.37 (2H, *dd*, 12.2, 6.8, H-16), 1.53 (2H, *dd*, 14.4, 7.3, H-7), 1.58 (3H, *s*, H-27), 1.67 (3H, *s*, H-26), 1.75 (2H, *dd*, 12.8, 3.0, H-12), 2.27 (1H, *dd*, 12.1, 3.5, H-17), and 3.23 (1H, *dd*, 11.5, 4.4, H-3).

Compound 9; 3α-hydroxy-urs-12-ene-11-keto-24β-oic acid; 11-keto-β-boswellic acid.

$C_{32}H_{48}O_5$; colorless needles; mp 195-197 °C; UV λ_{max} nm (MeOH), 249.5; IR υ_{max} cm^{-1}: 1235, 1457, 1625, 1669 (á,â-unsturated C=O), 1708 (-COOH), 2921, and 3455 (O-H); EI/MS *m/z* (rel. int.): 470 (1.81) $[M]^+$, 425 (3.1), 408 (5.6), 287 (4.9), 273 (54.4), 232 (100), 189 (12.6), 175 (14.3), 148 (13.9), 135 (33.7), 119 (25.9), 105 (41.2), 69 (34.8), and 55 (74.9); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 6.3, H-29), 0.79 (3H, *d*, 8.6, H-30), 0.93 (3H, *s*, H-28), 1.12 (3H, *s*, H-25), 1.17 (3H, *s*, H-26), 1.30 (3H, *s*, H-27), 1.33 (3H, *s*, H-23), 1.46 (1H, *dd*, 13.0, 3.0, H-5), 1.53 (1H, *d*, 11.2, H-18), 2.42 (1H, *s*, H-9), 2.49 (2H, *dd*, 13.0, 1.2, H-1), 4.07 (1H, *t*, H-3), and 5.54 (1H, *s*, H-12).

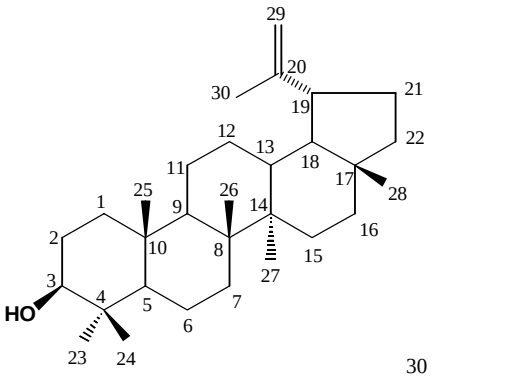
RESULTS AND DISCUSSION

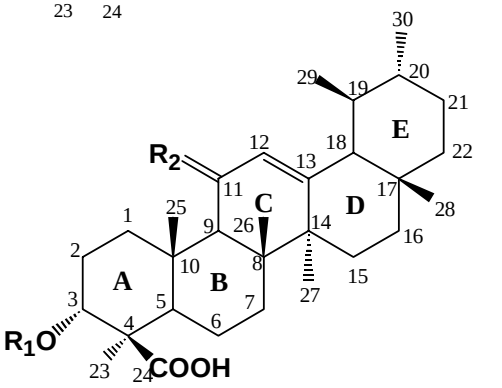
The isolated compounds (Fig. 1) were tested for their possible antiviral and cytotoxicity activity. An improved plaque-reduction assay for antiviral activity was used to test the isolated compounds (Abou-Karam and Shier 1990). The compounds were tested against (HS-I) grown on *Vero* African green monkey kidney cells. Each compound exhibits some cytotoxicity. The total acid mixture showed the highest activity among all compounds and was able to reduce the number of the plaques by 100 % with a minimum antiviral concentration at 20 μg/ml and followed by acetyl-11- keto-β-Boswellic acid (75% inhibition at 20 μg/ml), β-Boswellic and total alcoholic extract (50% inhibition at 40 μg/ml), acetyl-b-Boswellic and 11- keto-β-Boswellic (75% inhibition at 80 μg/ml), 3-hydroxy-tirucallic acid, 3-oxo-tirucallic acid, acetyl-α-boswellic acid, and total volatile oil (50% inhibition at 80 μg/ml). On the other hand, gum, palmitic acid and lupeol reduced the number of plaques by 25% at relatively high concentrations.

The individual components of the resin elicited mild to moderate antiviral activity. The results revealed that the total extract exhibited a better activity than that of any of the isolated pure compounds. This may be partly attributed to the synergistic effect among the different components of the total extract. Interestingly, the total acid fraction proved to be less toxic and more potent (100% at 20 μg/ml) than that of the total alcohol extract (50% at 40 μg/ml).

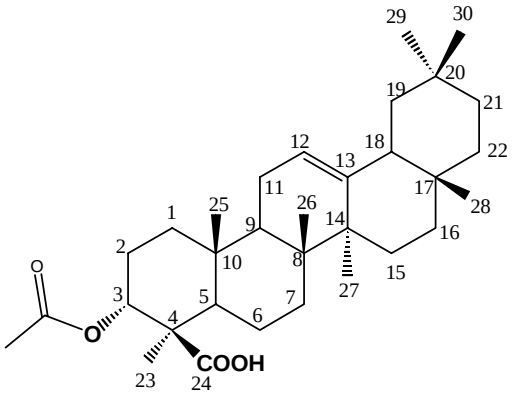
The spectral data of compound 1 indicated that it is palmitic acid, based on the EI/MS molecular ion peak at m/z 256 confirming the molecular formula of $C_{16}H_{32}O_2$. The IR spectrum showed absorption bands at 1701 cm^{-1} (C = O), and 3321 cm^{-1} (O-H). The ^{13}C-NMR spectrum showed the presence of 16 signals, the multiplicities of which were determined using APT experiment, that revealed the presence of one methyl signal at δ 14.2 that could be assigned to a terminal methyl (C-16), and fourteen methylene signals, one quaternary carbon signal. The ^{13}C-NMR signal at ä 22.7, 24.7, 31.9, and 34.0 could be assigned to methylene carbons (C-15), (C-3), (C-14), and (C-2), respectively. The remaining methylene carbon signals from 29.1 to 29.7 ppm could be assigned to carbons from (C-4) to (C-13). The remaining quaternary carbon signal at δ 179.6 could be assigned to the carboxylic acid function (C-1). ^{1}H-NMR of compound 1 showed the following signals, one methyl triplet at δ 0.83 (3H, *t*) which could be assigned to terminal methyl group (H-16). A multiplet at δ 1.23 ppm that could be assigned to protons (H-4) to (H-15). A multiplet at δ 1.6 ppm (2H, *m*) which could be assigned to methylene protons (H-3). A triplet at δ 2.3 ppm (2H, *t*) which could be assigned to methylene protons (H-2). The EI/MS mass spectrum confirmed the above assignments showing a base peak at m/z 60 that resulted from Mclafferty rearrangement (Silverstein *et al.*, 1991). The rest of the EI/MS spectrum consists of a homologous series of hydrocarbon clusters at intervals 14 mass units (-CH_2 group) due to gradual breakdown of the hydrocarbon side chain. Other characteristic fragments are those of $[CH_3(CH_2)_4]^+$ at m/z 71, and $[(CH_2)_2\ COOH]^+$ at m/z 73. The above mentioned data, co-chromatography with a series of fatty acids and previously reported literature data (Pouchert and Behnke, 1993) made us to figure out compound 1 to be the long-chain fatty acid, *n-hexadecanoic acid* (*palmitic acid*). To the best of our knowledge, this is the first report of isolation of palmitic acid from the genus *Boswellia*.

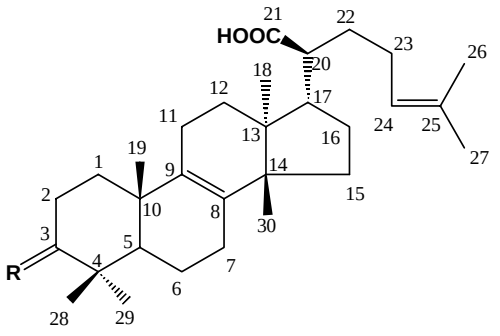
The ^{13}C-NMR spectrum of compound 2, showed the presence of 30 carbons, the multiplicities of which were determined using an APT experiment, that revealed the presence of seven methyl, six methine, eleven methylene, and six quaternary carbon signals. Compound 2 showed an EI/MS molecular ion peak at m/z 426 suggesting a molecular formula of $C_{30}H_{50}O$. More evidences came from the ^{13}C-NMR signals at δ 150.9 (C-20), and 109.5 (C-29), as well as ^{1}H-NMR signals for two vinylic protons at 4.57, and 4.68 ppm (1H, *d*, *J*=2 each), the ^{13}C-NMR signal for an oxygenated carbinylic carbon signal at 76.5 that was assigned to C-3. All the above evidences; suggested the presence of a lupane triterpene skeleton. The 3-hydroxyl group is β-configurated





	R_1	R_2
3	Ac	2 H
6	Ac	O
7	H	2 H
9	H	O





	R
5	O
8	â-OH, á-H

Fig. 1. The Anti-Herpes compounds isolated from the oleoresin of *Boswellia carterii* Birdwood

as reflected by the broad peak half-height width $W_{1/2}$ (12 Hz) of axially oriented H-3 signal at 3.42 ppm (1H, *dd*, *J* =19.5, 3.3) (Mahato and Kundu, 1994). Such axial orientation of H-3 is corroborated by the presence of the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction. From the above data, and through comparison with literature data (Reynolds *et al*., 1986); it was concluded that compound 2 is *lup-20(29)-en-3β-ol* (*lupeol*), that has been previously isolated from the closely related species *Boswellia frereana* Birdwood (Fattorusso *et al*., 1985).

The ^{13}C-NMR spectra of compound 3 showed the presence of 32 signals, the multiplicities of which were determined, by DEPT experiment, into seven methine, eight methyl, nine methylene, and eight quaternary carbons. The EI/MS molecular ion peak at m/z 498 suggests a molecular formula of $C_{32}H_{50}O_4$ thus giving a double bond equivalent of 8, five of which were assigned to the 5 rings of a pentacyclic triterpene skeleton. Two equivalents were assigned to an acetyl carbonyl (δ 170.5), and a carboxylic acid carbonyl (δ 183.1). The last equivalent was assigned to a double bond that was consistent with that of Δ^{12}-ursane skeleton based on its ^{13}C-NMR resonances, δ 124.9 (C-12), and 139.9 (C-13).

The appearance of a vinylic proton at δ 5.30 (H-12) as a broad triplet, appearance of H-18 as a doublet at δ 1.31 (*J* = 13.6), in addition to the appearance of a two methyl groups signals, each as a doublet at δ 0.8, and 0.85 assigned for 29, and 30 positions, respectively, unambiguously confirmed the presence of Δ^{12}-ursane skeleton. The location of the carboxylic acid group was found to be at 24-position based on the downfield shift of C-4 resonating at δ 47.6 ppm relative to similar compounds cited in literature (Mahato and Kundu, 1994). The ^{13}C-NMR signal for an oxygenated methine carbon signal at 73.6 was assigned to C-3 on both biosynthetic (Kojima and Ogura, 1989) and analogy grounds (Pradhy and Bhattacharyya, 1978), as well as HMQC correlations. The latter revealed crosspeaks between methyl protons (H-23) resonating at δ 1.24 (3H, s) and the oxygenated carbinylic carbon (C-3) at δ 73.6, quaternary carbon (C-4) at δ 47.1, and methine carbon (C-5) at δ 51.0. In addition, HMBC showed strong contour between the carboxyl carbon (C-24) at δ 183.1 and methyl protons (H_3-23) at δ 1.24. These established connectivities supported the substitution pattern of ring A.

The interaction crosspeaks between the vinylic proton (H-12) at δ 5.15 (1H, *t*), and methine carbon (C-9) at ä 47.2, methyl carbon (C-27) at δ 21.7 (weak $^4J_{CH}$ interaction); between proton (H-9) at δ 1.61 (1H, *m*) and both the (C-11) at δ 23.8, and the methyl carbon (C-26) at δ 17.3 confirmed the substitution pattern of rings B,C. The interaction crosspeaks between the methyl protons (H_3-27) at δ 0.90, and the quaternary carbons (C-8) at δ 40.2 ppm, as well as, (C-13) at δ 139.9, and (C-14) at δ 40.4 confirmed the substitution pattern of ring D. The presence of a 3-acetate group is evident from the downfield shift of *ca.* 2-3 ppm of (C-3), the upfield shift of *ca.* 3 ppm of (C-2), and the upfield shift of *ca* 1 ppm of (C-4) relative to non-acetylated compounds *viz.* 7 and 9 (Mahato and Kundu, 1994). The 3-acetoxyl group was found to be á-configurated, as reflected by the narrow peak half-height width $W_{1/2}$ (5 Hz) of the equatorially-oriented H-3 signal at 5.3 ppm (1H, *t*) (Siddiqui *et al*., 1989). Such equatorial orientation of H-3 is corroborated by the absence of the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction, and this also was confirmed by the most recent X-ray crystallographic analysis (Rajnikant *et al*., 2001). Assignment of other atoms was made by comparison with other separated compounds, referring to reported compilation data of a variety of similar compounds (Mahato and Kundu, 1994). The above evidences revealed that compound 3 is *acetyl*-β-*boswellic acid*.

EI/MS of compound 6 showed [M^+] peak at m/z 512, suggesting the molecular formula; $C_{32}H_{48}O_5$. The analysis of all the spectral data for compound 6 indicated its similarity to compound 3, but it contains an additional α,β-unsaturated oxo function, an enone system, that was confirmed from IR spectrum where a carbonyl group absorption band was observed at 1658 cm^{-1}, and by the appearance of a ^{13}C-NMR carbonyl signal at 199.6 ppm. The location of this oxo group was concluded to be at 11-position, since H-12 proton signal was downfield shifted from δ 5.3 to 5.54, and appeared as singlet, rather than broad triplet. Furthermore, ^{13}C-NMR signals of (C-9), (C-12), (C-13) were downfield shifted from δ 47.2, 124.9, and 139.9 to δ 60.7, 130.8, and 165.3, respectively. Further structure connectivities, and assignments by HMQC, HMBC, and COSY spectra confirmed that compound 6 is *acetyl-11-keto-β-boswellic acid*.

The spectral data for compound 7 was found to be similar to that of compound 3, but lacking the 3-acetate function. This was proved through the absence of ^{13}C-NMR signals at δ 21.7, and 170.5, as well as, ^{1}H-NMR signal at δ 2.07, and the

appearance of the 2 ppm upfield shifted hydroxylated carbinylic carbon (C-3) signal at δ 71.8, relative to that in compound 3. EI/MS spectrum of compound 7 showed a molecular ion peak $[M]^+$ at m/z 456 suggesting a molecular formula of $C_{30}H_{48}O_3$, confirming the absence of a 3-acetate group. Therefore, compound 7 was concluded to be *β-boswellic acid*.

EI/MS of compound 9 showed a $[M^+]$ peak at m/z 470 suggesting a molecular formula of $C_{30}H_{46}O_4$. It was found to be the 11-keto derivative of compound 7, as deduced from the appearance of carbonyl carbon signal at δ 199.8 (C-11), and based on a similar arguments as mentioned under compound 6. Therefore, it was confirmed that compound 9 is *11-keto-β-boswellic acid*.

The EI/MS of compound 4 showed a molecular ion peak at m/z 498 suggesting a molecular formula of $C_{32}H_{50}O_4$. The spectral data of 4 are more or less similar to that of compound 3, but with few differences. Firstly, the two ^{1}H-NMR doublets for methyls at 29, and 30 positions are absent, and appeared as singlets at δ 0.87, and 1.11, respectively; referring to the possible presence of an oleanane skeleton. This was corroborated by the upfield shift of C-12 signals to ä 122.2, and downfield shift of C-13 to δ 145.5 (27). Accordingly, compound 4 has been identified as *acetoxy-olean-12-ene-24β-oic acid* known as *acetyl-α-boswellic acid*.

The analysis of the spectral data of compound 5, concluded its structure as *3-keto-tirucall-8,24-diene-21-β-oic acid*, known as *3-oxo-tirucallic acid*. The ^{13}C-NMR spectral data of 5 showed the presence of 30 carbon signals, the multiplicities of which were determined using an APT experiment, that revealed the presence of seven methyl, ten methylene, four methine, and nine quaternary carbon signals. The EI/MS molecular ion peak at m/z 454 suggests a molecular formula of $C_{30}H_{46}O_3$ thus giving a DBE of 8, four of which were assigned to the 4 rings of the tetracyclic tirucallane triterpene skeleton. Two of the remaining 4 double bond equivalents were assigned to 2 double bonds, C_8-C_9 double bond that is evident from the two quaternary ^{13}C-NMR signals at ä 134.4 (C-8), and 132.2 (C-9), and C_{24}-C_{25} double bond reflected by the ^{13}C-NMR signals at ä 123.5 (C-24) and 132.7 (C-25), the two remaining DBEs were assigned to 2 carbonyl groups, the ketonic function (C-3) at δ 216.9, and the carboxyl carbonyl (C-21) at ä 182.4. The carboxylic group (C-21) is linked to (C-20) indicated by the 10-12 ppm downfield shift of that carbon relative to that closely related compounds lacking C-21 carboxyl (Benosman *et al*., 1995; Puripattanavong *et al*., 2000). The ketonic function is definitely (C-3) as revealed by the 7 ppm downfield shift of (C-2) in comparison with similar compounds with hydroxyl group at (C-3) such as compound 7. Assignment of other atoms was made by comparison with literature data (Benosman *et al*., 1995; Puripattanavong *et al*., 2000). EI/MS fragments corroborated the above assignments showing a molecular ion peak $[M]^+$ at m/z 454 corresponding for the molecular formula $C_{30}H_{46}O_3$. The base peak at m/z 439 resulted from the loss of one methyl group. Subsequent loss of a water molecule resulted in the peak at m/z 421. Fragment at m/z 257 resulted from the retro-Diels Alder (RDA) fragmentation characteristic of several triterpenoids (Budzikiewicz *et al*., 1964), followed by decarboxylation.

Compound 8 was analyzed for $C_{30}H_{48}O_3$ from EI/MS spectrum. It was found to be the 3-hydroxy analogue of compound 5. This was confirmed by appearance of ^{13}C-NMR signal at δ 79.4 ppm that was assigned to an oxygenated methine carbon (C-3). The 3-hydroxyl group is β-configurated reflected by the wide peak half-height width $W_{1/2}$ (16 Hz) of the axially oriented H-3 signal at 3.23 ppm (1H, *dd, J*= 11.5 and 4.4) (Siddiqui *et al*., 1989). Such axial orientation of H-3 is corroborated by the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction. The aforementioned data suggested that compound 8 is *3-hydroxy-tirucall-8,24-diene-21-β-oic acid* known as *3-hydroxy-tirucallic acid*.

In conclusion, eight triterpenoids, in addition to a fatty acid isolated for the first time from the resin, were isolated from the oleogum resin of *Boswellia carterii* Birdwood. Compounds 3-9 have been previously isolated from the oleogum resin of *Boswellia carterii* Birdwood (El-Khadem *et al*., 1972) and *Boswellia serrata* Roxb. (Pradhy and Bhattacharyya, 1978; Mahajan *et al*., 1995). All the isolated compounds exhibited antiviral activity as reflected by antiviral assay. Interestingly, it was found that the antiviral activity of the total extract (100 % at 20 µg/ml) is much greater than that of the individual components; accordingly it is advisable to use the total acid extract of the oleogum resin in herbal preparations intended to be used as antiviral. These results suggested that frankincense could be used as a potential agent for treatment of *Herpes simplex* type I infections.

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