Chemistry and Immunomodulatory Activity of Frankincense Oil

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The yield of steam distillation of frankincense essential oil (3%); and its physicochemical constants were determined. Capillary GC/MS technique was used for the analysis of the oil. Several oil components were identified based upon comparison of their mass spectral data with those of reference compounds published in literature or stored in a computer library. The oil was found to contain monoterpenes (13.1%), sesquiterpenes (1%), and diterpenes (42.5%). The major components of the oil were duva-3,9,13-trien-1,5α-diol-1-acetate (21.4%), octyl acetate (13.4%), *o*-methyl anisole (7.6%), naphthalene decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2-pentenyl) (5.7%), thunbergol (4.1%), phenanthrene-7-ethenyl-1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl (4.1%), α-pinene (3.1%), sclarene (2.9%), 9-*cis*-retinal (2.8%), octyl formate (1.4%), verticiol (1.2%) decyl acetate (1.2%), *n*-octanol (1.1%). The chemical profile of the oil is considered as a chemotaxonomical marker that confirmed the botanical and geographical source of the resin. Biologically, the oil exhibited a strong immunostimulant activity (90% lymphocyte transformation) when assessed by a lymphocyte proliferation assay.

Key words: Frankincense Essential Oil, Immunomodulatory Activity

Introduction

Frankincense oleogum resin is known as Olibanum, Luban Dakar, Bakhor or Kendar (In Arabic), Salai Guggal (in ayurvedic medicine). It is obtained by incision of the bark of several species of *Boswellia*, Burseraceae (Wallis, 1967; Evans, 1996). The plant is native to India, Arabian Peninsula (Yemen, Sultanate of Oman), Red Sea region of North-East Africa (Somalia, Eritrea) (Dietrich, 1900; Leung and Foster, 1996; Maupetit, 1984; Tschrich and Stock, 1935). Several commercial brands of the resin are available; the Aden and Eritrean brands are usually regarded as derived from *Boswellia carterii* Birdw., and *B. frereana* Birdwood, respectively. Sudanese olibanum is obtained from *Boswellia papyrifera* Del. whereas Indian olibanum is derived mainly from *Boswellia serrata* Roxb. (Verghese, 1988). There has been a considerable work done on the composition of olibanum oil from different species and commercial brands of *Boswellia* (Abdel wahab *et al.*, 1987; Ammar *et al.*, 1994; Guenther, 1972;, Obermann, 1978; Strappaghetti *et al.*, 1982). The chemical profile of the oil can be used as a chemotaxonomical marker to distinguish between the different commercial varieties of frankincense. The composition of the oil differs according to the climate, harvest conditions, and geographical source (Yates and Wenninger, 1970). Accordingly, frankincense can be classified into country of origin by identifying certain constituents in the essential oil using GC/MS technique (Ammar *et al.*, 1994; Hayashi *et al.*, 1998; Strappaghetti *et al.*, 1982; Yates and Wenninger, 1970). The main constituent of Aden and Omani oil is α-pinene (43%) whereas, Eritrean, Turkish oils are rich in octyl acetate (52%). The Indian oil is rich in α-thujene (61%). The taxonomic origin of Omani, Aden oil is *B. frereana* Birdwood, while that of Eritrean and Turkish is *B. carterii* Birdw. The Indian brand comes from *B. serrata* Roxb (Hayashi *et al.*, 1998; Verghese, 1988). The essential oil of frankincense is one of the most commonly used oils in aromatherapy practice nowadays. The oil is very good for respiration, eases breathing and is therefore useful for asthma patients. It has a soothing action in colds, cough, bronchitis and laryngitis (Lawless, 1996). It has excellent actions on skin for removing scars and stretch marks (Ryman, 1997). Frankincense oil produced contraction of phrenic-nerve diaphragm muscle (skeletal muscle) and inhibition of

twitch response to nerve stimulation and exhibited a spasmogenic effect on smooth muscle *in vitro*. These effects may be due to post-junctional block of neuromuscular transmission and an action on the sarcoplasmic reticulum to increase intracellular calcium (Maria and Stephen, 1997). The oil exhibited anti-bacterial and antifungal activities (Abdel wahab *et al.*, 1987; Gangwal and Vardhan, 1995). Most of the previous reports investigated the anti-inflammatory, immunomodulatory, and anti-leukotriene activity of the resin and especially its major components, boswellic acid derivatives (Ammon *et al.*, 1991; Gupta *et al.*, 1997, 1998; Safayhi *et al.*, 1992, 1995; Sharma *et al.*, 1988; Singh and Atal, 1986), so it was deemed of interest to investigate the chemistry, and immunomodulatory activity of the essential oil.

Results and Discussion

The yield of volatile oil of Frankincense obtained by steam distillation of the finely powdered oleogum resin derived from *Boswellia carterii* Birdw. was 3%. The oil is pale yellow with agreeable balsamic slightly spicy and lemony odor. The oil possesses the following physicochemical constants: specific gravity (25 °C), 0.875; refractive index (20 °C), 1.446; optical rotation $[\alpha]_D^{20°}$: – 1.11 (1% solution in 95% ethanol); solubility 1:1 w/v in ethanol 95% and 1:2 w/v in ethanol 70%; acid value, 12.05; ester value and ester percentage (calculated as octyl acetate) are 127.46 and 39%, respectively; and the saponification value is 139.51.

The GC/MS chromatogram of the steam distilled oil revealed the presence of several components (Fig. 1; Table I) that were identified through comparison of the fragmentation patterns in the resulting mass spectra with those published in literature (Adams, 1989, 1995) and using NST mass spectral data base of the gas chromatograph computer.

The volatile oil contains a high proportion of esters (*ca.* 40.06%) of which duva-3,9,13-trien-1,5α-diol-1-acetate is the major component (21.35%), octyl acetate (13.39%), octyl formate (1.41%), bornyl acetate (0.09%), citronellyl acetate (0.74%), neryl acetate (0.48%), geranyl acetate (0.62%), hexyl *n*-hexanoate (0.09%), decyl acetate (1.16%), farnesyl acetate (E,E) (0.01%), benzyl benzoate (0.22%), and duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate (0.5%).

The oil contains several monoterpene hydrocarbons such as; β-pinene (0.25%), α-pinene (3.11%), isoterpinolene (0.04%), α-phellandrene (0.03%), β-phellandrene (0.19%), sabinene (0.22%), β-myrcene (0.2%), *d*-limonene (7.6%), and *cis*-ocimene (0.37%); monoterpene alcohols *viz.* β-citronellol (0.2%), *cis*-carveol (0.04%), isopinocampheol (0.12%), *trans*-terpin (0.46%); monoterpene ketones *viz.* carvone (0.26%), and piperitone (0.03%).

Unlike a previous analysis (Ammar *et al.*, 1994), that reported the absence of sesquiterpenoids in the essential oil of *Boswellia carterii* Birdwood; several sesquiterpenes were detected in the investigated oil *viz.* α-copaene (0.35%), δ-selinene (0.24%), maaliane (0.02%), viridiflorol (0.06%), α-muurolol (0.03%), β-bisabolene (0.15%), *cis*-calamenene (0.01%), spathulenol (0.03%), and *cis*-nerolidol (0.07%).

Several diterpenes were detected in the oil especially those of the cembranoid skeleton such as, the previously reported cembrene (0.27%), isocembrene (0.28%) (Ohloff, 1994), in addition, verticiol (1.22%), duva-4,8,13-trien-1,3α-diol (0.23%), thunbergol (4.07%), duva-3,9,13-trien-1,5α-diol (0.06%), duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate (0.5%), duva-3,9,13-trien-1,5α-diol-1-acetate (21.35%) are detected for the first time in olibanum essential oil. The presence of such high percent of cembranoid diterpenes in the essential oil of frankincense derived from *Boswellia carterii* Birdwood is reported for the first time. This rare and unique type of diterpenes has a 14-membered monocyclic ring and is characteristic for marine organisms such as soft corals (Rashid *et al.*, 2000) and also detected in tobacco (Eklund *et al.*, 1992). Diterpenes of other classes that were also detected for the first time in olibanum oil include, isophyllocladene (kaur-15-ene) (0.58%), phenanthrene-7-ethenyl-1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl (4.06%), beyerene (0.96%), sclarene (2.88%), and naphthalene decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2-pentenyl) (5.7%).

These results suggested that our sample of the frankincense oleogum resin is close to the Eritrean brand with octyl acetate being the major component (13.39%). The complete absence of verbenone and the low percent of α-pinene (3.11%) excluded the Aden variety while, lacking α-thujene precludes the Indian brand.

Lymphocyte proliferation (mitogenesis) assay involves study of a specific immune response. The as-

Table I. Results of GC/MS analysis of the essential oil of frankincense oleogum resin.

Peak scan #	Composition %	Retention Time (t_R) [min]	M^+ peak	Base peak	Major peaks (*m/z*)	Component	Adams (1995) DB-5*
302	0.25	5:46	136	93	40.3,55,67,79,107,121	β-pinene	386
361	3.11	6:05	136	93	41,53.2,67,77.1,91.1,105	α-pinene	319
410	0.04	6:30	134	93.1	40.3,53,67.2,91.1,121	isoterpinolene	602
424	0.03	6:37	136	93	40.3,53,65.2,77.2,91,119	α-phellandrene	435
500	0.19	7:15	136	93	41.2,53,69.2,77.1,91,121	β-phellandrene	482
519	0.22	7:25	136	93	41.2,53,69,83,121.1	sabinene	379
572	0.2	7:51	136	93	41.2,53,69.2,79,109	β-myrcene	408
664	0.18	8:37	122	122.1	41.2,51.1,77.1,91,107	*o*-methyl anisole	459
799	7.6	9:45	136	67	41,53,67,79,93,121	*d*-limonene	481
849	0.37	10:10	136	93.1	41,53,67,79,91,105,121,136	*cis*-ocimene	498
1065	1.08	11:59	130	41	55,69,83,97.2,112	n-octanol	564
1117 To 1418	1.41	13:40	158	56	42,70.1,84,93,121	octyl formate	–
1755 To 2051	13.39	18:56	172	43	41,56,61,70,84,112,116	octyl acetate	–
2064	0.2	20:21	156	41	55,60,69,73,84,95,105, 123, 138	β-citronellol	950
2077	0.04	20:27	152	41	55,59,67,69,81,84,109,119,134	*cis*-carveol	953
2098	0.26	20:38	150	82	41,54,67,79,93,108,137,150	carvone	984
2121	0.03	20:49	150	82	41,55,69,82,95,110,137	piperitone	1011
2155	0.56	21:06	152	152	44,52,66,77,91,109,123	2,6-dimethoxy toluene	–
2177	0.15	21:17	158	43	55,70,83,97,112,123,152	1-decanol	1063
2188	0.12	21:23	154	43	55,69,83,94,107,112,152	isopinocampheol	822
2206	0.09	21:32	196	43	55,69,80,95,108,121,136, 154	bornyl acetate	1099
2380	0.46	23:04	172	129	41,,43,55,60,83,87,97,101,115,129, 143	*trans*-terpin	–
2489	0.74	23:54	198	43	41,55,67,69,81,95,109,123,138	citronellyl acetate	1275
2529	0.48	24:14	196	41	43,53,69,80,93,107,121,136,154	neryl acetate	1303
2588	0.35	24:44	204	161	41,43,55,67,81,93,105,119,133,161	α-copaene	1334
2623	0.62	25:01	196	41	43,53,69,80,93,107,121,136,143	geranyl acetate	1352
2648	0.09	25:14	200	43	41,55,56,61,69,84,99,117	hexyl n-hexanoate	1352
2780	1.16	26:20	200	43	41,55,61,70,83,97,112,140,172	decyl acetate	1420
2908	0.24	27:24	204	43	41,55,60,67,79,91,93,105,119,129, 133,147,161,172,189	δ-selinene	–
2964	0.02	27:53	206	43	41,55,69,79,91,93,105,119,129,133, 147,161,189	maaliane	–
3029	0.06	28:25	222	43	41,55,67,71,81,95,105,109,119,121, 131,145,151,198,202	viridiflorol	1859
3042	0.03	28:32	222	43	55,67,79,81,91,93,105,119,133,135, 161,178,204	α- muurolol	1984
3132	0.01	29:17	264	41	55,69,81,93,107,119,121,137,148, 149,161,162,189	farnesyl acetate (E, E)	2419
3205	0.15	29:54	204	41	43,53,69,79,93,105,109,119,121,133, 147, 161,189	β-bisabolene	1667
3262	0.01	30:22	202	159	41,43,55,65,65,69,77,81,91,105,115, 129,131	*cis*-calamenene (1S)	1695
3286	0.03	30:34	220	43	41,55,67,69,79,83,91,93,105,119,131, 147,159,177,187,205	(–)-spathulenol	1825
3480	0.07	32:12	222	41	43,55,69,81,93,107,121,136,148,161, 189,206	*cis*-nerolidol	1724
4455–4466	0.22	40:21	212	105	43,51,65,77,91,112,145,167,194	benzyl benzoate	2245
4558–4671	1.11	41:37	270	43	43,55,60,73,83,105,129,159, 185,199,228	unknown	–
4729	0.27	42:38	272	159	41,43,55,69,91,105,119,133,145,159, 187,204,229	cembrene	2597
4795	0.28	43:12	272	159	41,43,55,69,81,91,105,119,133,145, 159,161,187,204,229,257	isocembrene	–
5000	0.58	44:54	272	43	41,55,69,81,91,93,95,105,107,119, 121,136,147,161,173,189,203,228, 257,272	isophyllocladene (kaur-15-ene)	2664

Table I (content).

Peak scan #	Composition %	Retention Time (t_R) [min]	M^+ peak	Base peak	Major peaks (*m/z*)	Component	Adams (1995) DB-5*
5064–5252	1.22	46:35	290	272	41,43,55,69,81,91,93,107,119,121,133, 147,161,173,187,201,229,257,288	verticiol	–
5305	4.06	47:27	272	93	41,55,68,81,93,107,121,133,147,161, 189,201,215,229,257,272	phenanthrene-7-ethenyl-1,2,3,4,4a,5,6,7, 8,9,10,10a-dodeca-hydro-1,1,4a,7-tetramethyl	–
5346	0.96	47:48	272	41	41,43,55,79,91,93,105,107,133,147, 159,174,186,201,215,227,255,272	beyerene	2587
5433–5565	2.88	49:00	272	257	41,55,67,79,91,93,107,121,133,147, 161,173,187,201,229,257,272	sclarene	2672
5615	5.7	50:03	272	272	41,55,67,79,91,107,119,121,147,161, 173,189,201,229,257,272	naphthalene decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2-pentenyl)	–
5631	2.82	50:11	284	95	41,55,67,79,91,105,119,134,147,151, 164,188,213,227,257,270,284	9-*cis*-retinal	–
5650	0.23	50:20	306	95	41,43,55,67,82,95,107,121,135,137, 149,159,177,189,213,227,256,288,304	duva-4,8,13-trien-1α,3α-diol	–
5691	4.07	50:41	290	177	41,43,55,67,81,91,109,121,133,147, 161,177,189,227,257,272,288	thunbergol	–
5774	0.06	51:59	306	43	41,55,69,81,93,107,121,135,147,159, 187,203,206,245,288	duva-3,9,13-trien-1,5α-diol	–
5889	0.5	52:20	346	43	41,55,67,81,93,107,121,133,147,161, 175,187,203,215,229,243,272,303	duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate	–
6270	21.35	55:31	348	43	41,55,71,81,93,107,125,136,151,156, 161,202,238,263,288,306,308,348	duva-3,9,13-trien-1,5α-diol-1-acetate	–

* Elution time in seconds according to Adams (1995).

say investigates the mitogenic effect of the crude drug on T-lymphocytes proliferation. Anti-proliferative activity on T-lymphocyte culture indicates immunosuppression while promotion of T-lymphocyte proliferative response means immunostimulation (Nores *et al.*, 1997; Puri *et al.*, 1993; Sairam *et al.*, 1997). The essential oil of frankincense (100 µl/ml) in DMSO induced a mitogenic response (90% lymphocyte proliferation) that is comparable to standard well known immunostimulants *e.g. Echinacea purpurea* aqueous extract (95%) and levamisole (85%) (Fig. 2).

In conclusion, based on the chemical profile of the oil, our sample of frankincense oleogum resin is of the Eritrean brand which is derived from *Boswellia carterii* Birdw. The oil demonstrated immunostimulant activity which is an added value to the previously reported anti-inflammatory, immunomodulatory, and anti-leukotriene activity of the resin. This encourages the use of *olibanum oleogum resin* in several immune disorders.

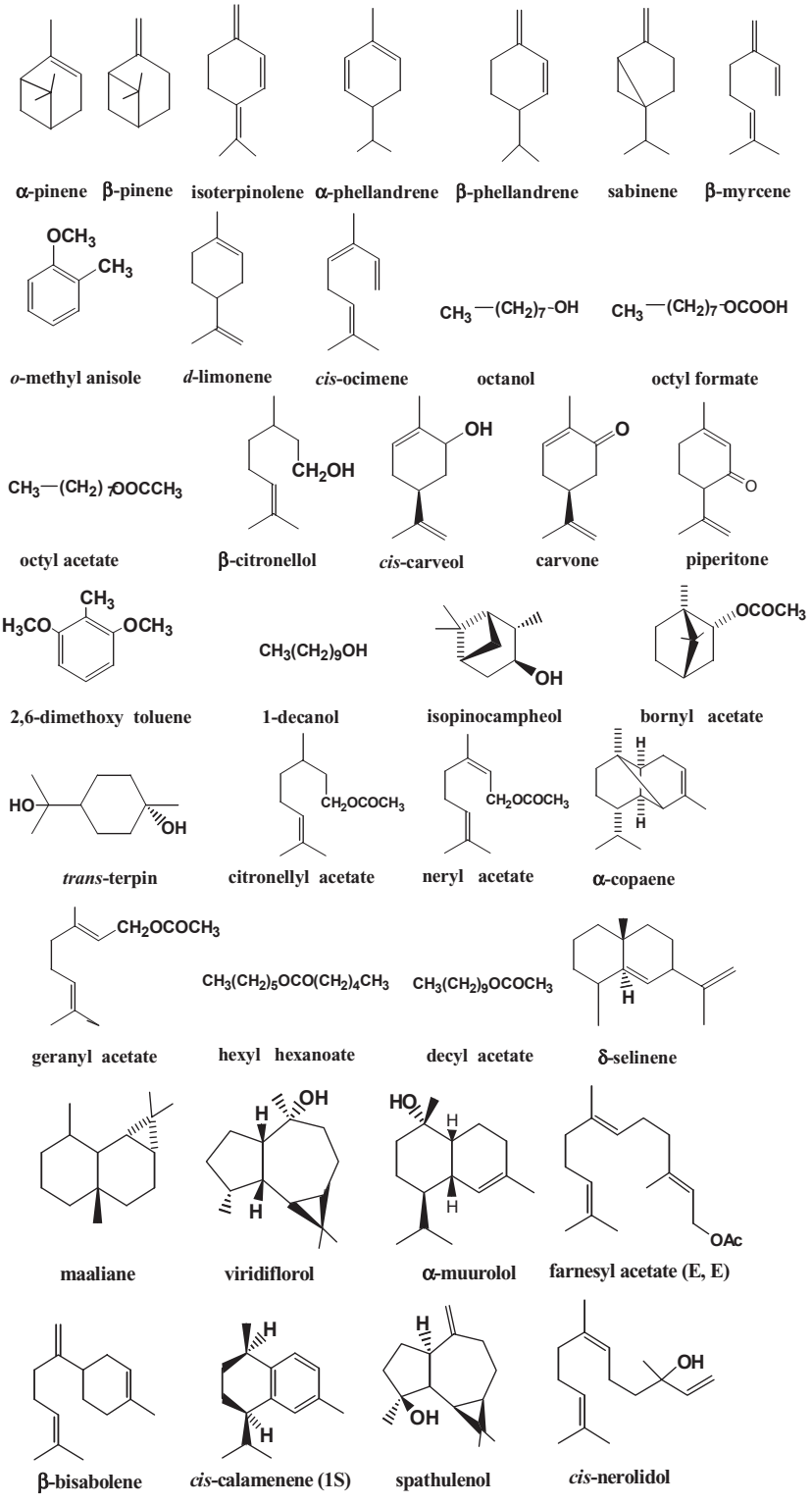
Experimental:

Plant material

Frankincense (oleogum resin of *Boswellia carterii* Birdwood, Burseraceae, was purchased from the local market of herbs and spices in Egypt. It was authenticated by comparison with a genuine sample kept in the drug museum of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Reagents for lymphocyte transformation assay

Heparinized peripheral venous blood was obtained from healthy volunteers at the blood bank of Mansoura University Hospital; Ficoll/Hypaque obtained from Amersham Pharmacia, Uppsala, Sweden; phytohaemagglutinin (PHA) obtained from Difco, Detroit, MI, USA; Hank's balanced salt solution (HBSS); foetal calf serum (FCS); glutamine; HEPES (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid)-buffer and RPMI-1640 me-


α-pinene
β-pinene
isoterpinolene
α-phellandrene
β-phellandrene
sabinene
β-myrcene
OCH3
CH3
o-methyl anisole
d-limonene
cis-ocimene
CH3—(CH2)7-OH
octanol
CH3—(CH2)7-OCOOH
octyl formate
CH3—(CH2)7OOCCH3
octyl acetate
CH2OH
β-citronellol
OH
cis-carveol
O
carvone
O
piperitone
CH3
H3CO
OCH3
2,6-dimethoxy toluene
CH3(CH2)9OH
1-decanol
OH
isopinocampheol
OCOCH3
bornyl acetate
HO
OH
trans-terpin
CH2OCOCH3
citronellyl acetate
CH2OCOCH3
neryl acetate
H
H
α-copaene
CH2OCOCH3
geranyl acetate
CH3(CH2)5OCO(CH2)4CH3
hexyl hexanoate
CH3(CH2)9OCOCH3
decyl acetate
H
δ-selinene
maaliane
H
OH
H
viridiflorol
HO
H
H
α-muurolol
OAc
farnesyl acetate (E, E)
β-bisabolene
H
H
cis-calamenene (1S)
H
OH
spathulenol
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cis-nerolidol

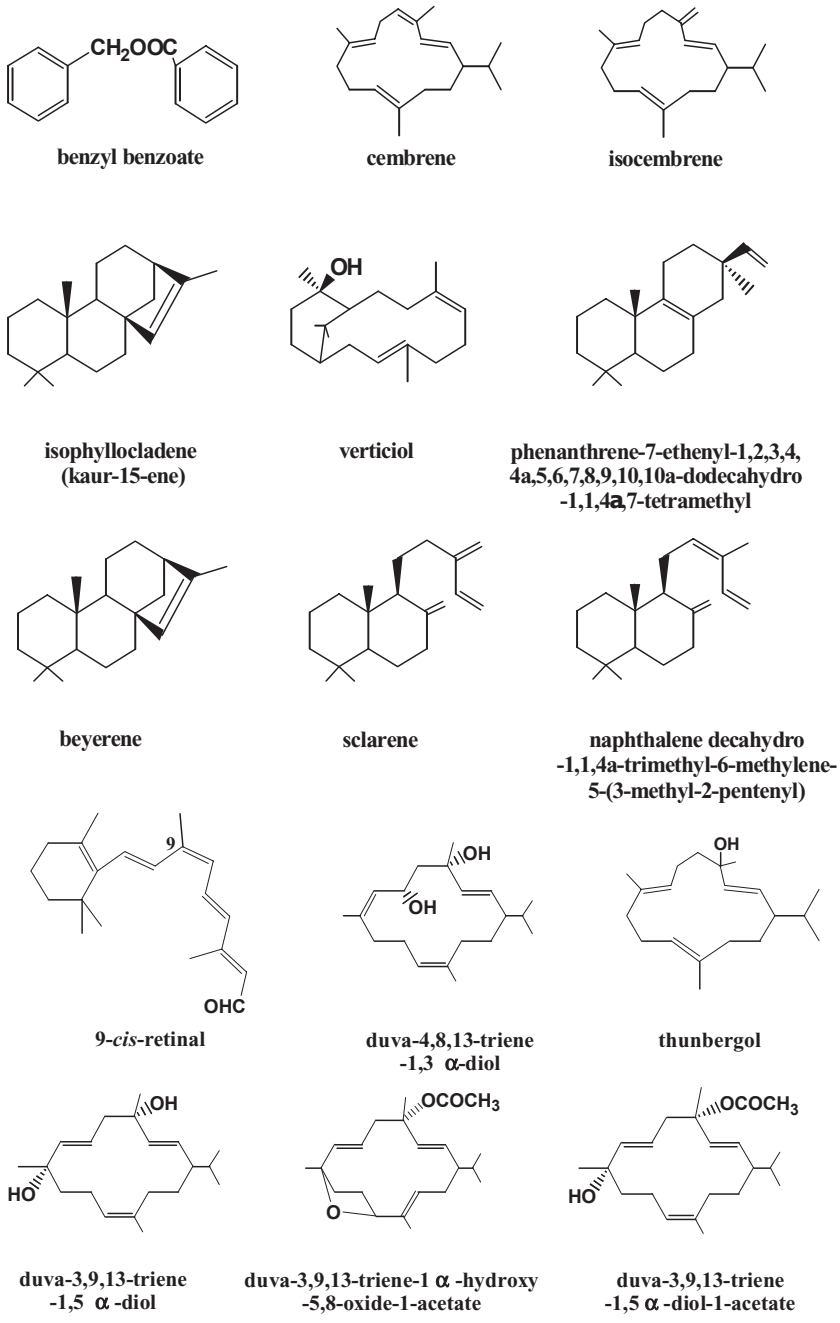

Fig. 1. Components of the essential oil of *Boswellia carterii* Birdwood.

dium obtained from Gibco BRL, Life Technologies, Pailsey, Scotland; crystalline penicillin G and streptomycin obtained from El-Nile Pharmaceutical Co., Cairo, Egypt.

Preparation of the volatile oil

Finely ground oleogum resin (500 g) was subjected to steam distillation using Clevenger's apparatus until complete exhaustion. The oil was col-

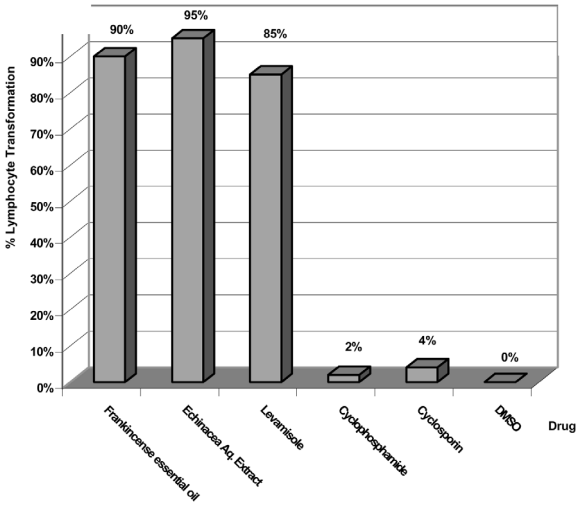

Fig. 2. Results of lympohocyte transformation assay of frankincense essential oil.

lected, dried over anhydrous sodium sulfate and kept in the refrigerator until analysis.

Determination of the physicochemical charateristics and constants

All physicochemical constants of the oil were determined according to BP 1998, optical rotation was determined using ADP 220 polarimeter (Bellingham and Stanely Ltd., Kent, England), refractive index using LEICA ABBE Mark II Refractometer (Leica Inc., Buffalo, NY, USA).

Capillary gas chromatography/ mass spectrometry (GC/MS) analysis

The GC/MS analysis of the oil was carried out at National Research Center, Dokki, Cairo, Egypt on GC/MS Finningan Mat SSQ 7000 with Digital DEC 3000 workstation fitted with a fused silica DB-5 (30 m × 0.25 mm ID, 5% phenyl methyl polysiloxane) capillary column with helium as carrier gas at a flow rate of 1.6 ml/min, column head pressure 13 Psi. The gas chromatograph was coupled to a mass selective detector (MS) at 70 eV in EI ionization mode. The sample was injected in 1 µl volume in splitless mode. The temperature was programmed initially at 60 °C for 3 min, then increased with a rate of 3 °C/min up to 250 °C.

Assessment of the immunomodulatory activity of the oil: lymphocyte blast transformation (mitogensis) assay

The lymphocyte blast transformation (mitogensis) or proliferation assay was applied. The assay was adapted as a test for cell-mediated immunity (Stites, 1987). The cell-mediated immune response was determined in the peripheral blood lymphocytes (PBL) in response to mitogenic stimulation using either phytohaemagglutinin (PHA) or concanavalin A (Con A) as mitogens that stimulate human T and B cells but T-cells more vigorously.

A) Separation of peripheral blood lymphocytes (PBL)

Lymphocytes were separated from peripheral human venous blood by Ficoll/Hypaque gradient technique (Boyum, 1976). For each sample, 5 ml of heparinized blood was diluted with equal volume of Hank's balanced salt solution (HBSS) in a sterile plastic centrifuge tube. 6 ml of diluted blood were carefully overlaid on 4 ml Ficoll/Hypaque solution gradient without allowing the solution to become mixed by keeping the pipette against the tube wall 5–10 mm above the fluid meniscus. The tube was centrifuged at 1200 rpm at room temperature. The lymphocytes were localized as a whitish layer on the upper meniscus of the gradient solution. Using a fine pasteur pipette, the zone containing lymphocytes was taken and washed twice in HBSS (10 min at 1200 rpm). The residue is a buffy coat of polymorphnuclear leucocytes (PMNLs).

B) Lymphocyte transformation assay

The viable lymphocytes were adjusted to a concentration of 2×10^6 cells/ml in RPMI-1640 medium supplemented with 600 µl penicillin, 0.1 ml streptomycin, 1% glutamine, 25% HEPES (N-2-Hydroxyethylpiperazine-N′–2-ethanesulfonic acid)-buffer, and 20% foetal calf serum (FCS). The lymphocytes were plated into 96-well tissue culture plates (or Ependorf tubes). 100 µl of the volatile oil solution in DMF (100 µl/ml) and 20 µg of the mitogen (PHA) were added to each well. Cell cultures were incubated at 37 °C in 5% CO_2 atmosphere for 72 h, during which the mitogen produces its maximal effect on DNA synthesis. After culture,

cell films were stained by Giemsa stain and average count of percentage of transformed (proliferated) blasts was determined. Aqueous *Echinaceae purpurea* extract (Immulone®) and levamisole (Ketrax®) were used as positive control (standard immunostimulant) while cyclophosphamide (Endoxan®) and cyclosporin (Sandimmune®) were used as negative control (standard immunosuppressant) 100 μg/ml of each drug in DMSO.

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