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This is a refereed journal and all articles are professionally screened and reviewed **ORIGINAL ARTICLE**

Phytochemical Study of the Biologically Active Fractions of the Oleo-gum-resins of *Boswellia carteri* and *Commiphora myrrha*

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ABSTRACT

The petroleum ether extract of the oleo-gum-resin of *Boswellia carteri* Bird., the chloroformic successive extract of the oleo-gum-resin of *Commiphora myrrha* Engl. as well as the prepared volatile contents from both resins showed significant anti-inflammatory activity in carragennan-induced edema in rats. Phytochemical investigation of the volatile fractions was carried out using GC/MS and revealed the presence of n-octyl acetate (45.41%) and sclarene (16.86 %) as major constituents in *Boswellia carteri* and γ-elemene (19.25 %) and Z-γ-bisabolene (18.22 %) as major constituents in *Commiphora myrrha* oleo-gum-resins. Investigation of the lipoidal matter from both oleo-gum-resins was done through GC/MS analysis of saponifiable and unsaponifiable matters prepared from both resins. The GC/MS analysis of the unsaponifiable matter (USM) of *Boswellia carteri* oleo-gum-resin resulted in the identification of 24-norursa-3,12-diene (24.45%) and incensole (22.55%) as major compounds whereas the GC/MS analysis of the unsaponifiable matter (USM) of the petroleum ether extract of the oleo-gum-resin of *Commiphora myrrha* resulted in the identification atractylone (20.26%) and 2-O-methyl-8,12-epoxygermacra-1(10)-4,7,11-tetraene (9.23%) as major compounds. The GC/MS analysis of the saponifiable matter of the petroleum ether extract of both oleo-gum-resins resulted in the identification of a number of fatty acids. The bioactive petroleum ether and chloroformic successive extracts of *B. carteri* and *C. myrrha* respectively were phytochemically studied using TLC, PTLC, CC and HPLC which led to the isolation of 3-acetyl-β-boswellic acid (1), β-boswellic acid (2), α-boswellic acid (3) and 15-Methyl-4-hexadecenoic acid(4) from the oleo-gum-resin of *B. c.* and Guggulsterol-Y from the oleo-gum-resin of *C.m.* which were identified using ^{1}H-NMR, ^{13}CNMR, DEPT, COSY, HMQC ,HMBC ,ESI/MS and LC/MS. The bioactive petroleum ether and chloroformic successive extracts of *B. carteri* and *C. myrrha* respectively were also tested for acute lethal toxicity where the former showed high safety margin therefore it was tested for chronic toxicity. The results of the chronic toxicity study showed that the ingestion of the non-polar bioactive extract of *B. carteri* oleo-gum-resin for a period of two months exerted no effect on the metabolism, liver and kidney functions of white male albino rats.

Key words: *Boswellia carteri*, *Commiphora myrrha*, anti-inflammatory activity, active constituents.

Introduction

Boswellia carteri Birdwood and *Commiphora myrrha* Engler oleo-gum-resins (incense and myrrh respectively) are ancient remedies known since the Ancient Egyptian time. The earliest recorded use of the oleo-gum-resin of *Boswellia* species is found on Egypt's Queen Hathsepsut's tomb. It was used as incense, then the charred resin was ground into a powder (called kohl) and used as eyeliner. In addition, they were used it in the embalming rituals, in the embalming medium and in mummification balms [32]. Both oleo-gum-resins has been two of four components constituting the Jerusalem Balsam which was an important remedy used in the Roman time for healing wounds of all kinds, bruises, and all skin disorders beside other systemic aliments. [23]. The oleo-gum-resin of *Boswellia* species has been used since a long time as a traditional remedy in Ayurvedic medicine in India for inflammatory diseases such as rheumatoid arthritis, ulcerative colitis and Crohn's disease [37].

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Myrrh is used today in Chinese medicine to treat wounds, relieve painful swelling, and to treat menstrual pain due to blood stagnation where it is called *mo yao* [18]. In India, myrrh is used for menstrual disorders, stomach complaints, wounds, ulcers and inflammation of the skin and mouth. Myrrh is used in folk medicine for the topical treatment of mild inflammations of the oral and pharyngeal mucosa. It is also used occasionally as a carminative for non-specific intestinal infections, as an expectorant and for stimulating the appetite and the flow of digestive juices [28].

The chemical composition of the oleo-gum-resins obtained from *Boswellia* and *Commiphora* species constitutes of volatile oils, gums and resinous matters. The resinous matter of *Boswellia* species oleo-gum-resin includes triterpenes with varying skeletons. The most dominant triterpenes belong to the ursane and oleanane skeletons [40] which are called boswellic acids to which the biological activity is attributed. Other triterpenes belong to lupane [41], tirucallane [4] and dammarane skeletons [11]. The resinous matter of *Commiphora* species contains triterpene of the ursane [35], lanostane [29], polypodane [15], dammarane, octanodammarane [29] and cycloartane types [33]. It also contains steroids referred to as guggulsterones [27]. These guggulsterones are responsible for the hypolipedimic activity of the oleo-gum-resins of *Commiphora* species. The volatile oils isolated from the oleo-gum-resins of *Boswellia* and *Commiphora* species showed varying percentages of mono, sesqui- and to a lesser extent diterpenes in addition to some long chain hydrocarbons [34,7].

Materials and Methods

Plant materials:

Samples of the dried oleo-gum-resin of *Boswellia carteri* Birdwood and *Commiphora myrrha* Engler Family Burseraceae were purchased from Haraz Egyptian herbal store, Cairo, Egypt.

Preparation of the successive extracts with selective organic solvents:

The powdered oleo-gum-resin of *Boswellia carteri* (500 gm) and of *Commiphora myrrha* (500 gm) were separately extracted in a continuous extraction apparatus (Soxhlet) until exhaustion with the following organic solvents in succession: petroleum ether (40-60 °C), diethyl ether, chloroform, methanol and 50% aqueous methanol. For each solvent, the extraction was continued till exhaustion. In each case, the solvent was stripped off by distillation under reduced pressure at a temperature below 40 °C and dried to constant weight in a vacuum dessiccator over anhydrous calcium chloride.

Preparation of volatile fractions:

The oleo-gum-resins of *Boswellia carteri* and *Commiphora myrrha* (500 gm each) were separately placed in round bottom flasks, covered with sufficient water and subjected to hydrodistillation in a modified Likens and Nickerson apparatus [12]**.** This allowed the distillation the volatile oil which was dehydrated over anhydrous sodium sulfate and analyzed using a Finngan SSQ 7000 gas chromatograph coupled with a mass spectrometer.

Preparation of unsaponifiable matter (USM) and fatty acids (FA):

Saponification of Petroleum Ether Extracts: (Tsuda et al., 1960):

Each of the petroleum ether extracts of the oleo-gum-resins of *Boswellia carteri* and *Commiphora myrrha* was refluxed separately for 6 hrs with 0.5 N alcoholic potassium hydroxide (60 mL) for saponification in a boiling water bath. The saponified extracts were concentrated to 1/3 their volumes. The cooled reaction mixtures were diluted with equal volumes of distilled water and exhaustively extracted with ether (negative test for sterols). The combined ethereal extracts were washed several times with water till free of alkalinity and dehydrated over anhydrous sodium sulfate. After evaporation of ether to dryness, the residues were kept for studying the USM. The alkaline aqueous solutions remaining after extraction of the USM were acidified with hydrochloric acid to liberate the fatty acids which were extracted several times with ether. The combined ethereal extracts were washed several times with distilled water till free of acidity, filtered over anhydrous sodium sulfate, and the filtrates were evaporated to dryness. The residues were kept for studying the fatty acid contents.

GC/MS conditions for the analysis of unsaponifiable matter:

The unsaponifiable matter from *Boswellia carteri* and *Commiphora myrrha* were analyzed using a Finngan SSQ 7000 gas chromatograph coupled with a mass spectrometer using the following conditions: Capillary column: DB-5 fused silica (5% phenyl methyl polysiloxane), 30 m length, 0.25 mm id and 0.25 μm thickness. Carrier Gas: Helium at 1 ml/min 13 psi, Oven Temperature: was programmed at 70-290°C at a rate of 4° C /min. Ion source temperature 180° C, Injector Temperature: 220°C. Ionization Energy: 70 eV. Volume Injected: 1μl.

Methylation of fatty acids:

The fatty acid fraction (0.5g) of each of the oleo-gum-resins under investigation was subjected to methylation by refluxing with 50 ml absolute methanol and 3 ml concentrated sulphuric acid for 2 hours. The methylated fatty acids were extracted with diethyl ether. After evaporation of the ether, the fatty acid methyl esters were introduced for GC analysis.

GC/MS Conditions for the analysis of fatty acid methyl esters:

The fatty acid methyl esters from *Boswellia carteri* and *Commiphora myrrha* were analyzed using a Finngan SSQ 7000 gas chromatograph coupled with a mass spectrometer using the following conditions: Capillary column: DB-WAX fused silica, 30 m length, 0.25 mm id and 0.25 µm thickness. Carrier Gas: Helium at 1 ml/min 13 psi, Oven Temperature: was programmed at 50-260°C at a rate of 4° C /min. Ion source temperature 180° C, Injector Temperature: 220°C. Ionization Energy: 70 eV. Volume Injected: 1µl.

General experimental procedures:

UV–visible spectrophotometer: Shimadzu UV 240 (PIN 204-5800) was used for recording UV spectra and measuring the absorbance in UV and visible range (UVPC). ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL-JNM-EX-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively). ESI-MS data was obtained with a JEOL JMS-700T mass spectrometer. Pre-coated silica gel 60 GF254 plates (E. Merck) were used for TLC. Solvent systems used for the development of chromatograms were (hexane: ethyl acetate) (8:2) v/v and (chloroform: methanol) (99:1) v/v and vanillin sulfruic acid as spray reagent was used for detection (Stahl, 1969). A reversed phase-HPLC: Cosmosil Packed Column $5C_{18}$-AR-II (10 x 250 mm, NACALAI TESQUE, INC.) was used in the chromatographic separation.

Anti-inflammatory Activity:

Animals:

White female albino rats of 150 g average body weight were used to test the anti-inflammatory activity of the previously prepared successive extracts (petroleum ether, ether, chloroform, methanolic and 50% aqueous methanolic) and the volatile oils prepared from both *Boswellia carteri* and *Commiphora myrrha* oleo-gum-resins.

Materials:

Carrageenan, type IV (Sigma, USA) was used for induction of acute inflammation in rats. One dose level (500 mg/kg rat body weight) was tried all over the study.

Design of the acute inflammation test:

Seventy eight rats were fasted for 16 hours before starting the experiment, and then divided into thirteen groups. One group served as control, six groups designated **(B)** were used as test groups for *Boswellia carteri* extracts and six groups designated **(C)** were used as test groups for *Commiphora myrrha* extracts and the last group served as the control group and was not given any treatment. After one hour of the oral administration, rats of all groups were injected into the subplanter surface of the right hind paw with 0.1 ml carrageenan (1% w/v in 0.9% sodium chloride) [16]. Paw thickness was measured using vernicrcaliper immediately before the injection of carrageenan and after 30 minutes, 1, 1.5, 2, 3, and 4 hours of carrageenan injection. The mean increase of the hind paw thickness of rats given the different resin extracts were compared with that of the control inflamed rats. Statistical analysis was carried out using student's t-test [19].

Acute Lethal Toxicity Test: (Balaze, 1970):

Male and female albino mice of 21 – 25 g body weight were fed with progressively increasing oral doses (1, 2, 4, 6, 8, 10 and12 g/kg mice body weight) of each of the non-polar extract of *Boswellia carteri* and the chloroformic successive extract of *Commiphora myrrha* oleo-gum-resins for testing acute lethal toxicity. The 24 hrs mortality counts among equal sized groups of lethally intoxicated mice (8 animals/ group) were estimated and the LD_{50} was calculated.

Chronic Toxicity Test: (Balaze, 1970):

White male albino rats of 125 g average body weight were divided into two groups' six rats each with the test group being fed with 500mg/kg rat body weight of the non-polar extract of *B. carteri* oleo-gum-resin daily for a period of 2 months whereas the control group was given no medication. The animals were kept individually in wire bottomed cages at room temperature of 25 ± 2°C. The initial and final body weights were recorded and asparate transaminase (AST), alanine transaminase (ALT) alkaline phosphatase (ALP) and serum creatinine were estimated in the collected blood samples at the end of the experiment. Statistical analysis of data was carried out according to t-student test.

Results:

The Results of the Anti-inflammatory Activity:

All of the extracts *Boswellia carteri* oleo-gum-resin showed inhibition of the induced inflammation with varying percentages although the potency tended to be in favor of the non-polar extracts. Yet even the aqueous extract showed inhibition with 36, 31, 28, 34, 27 and 19% at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively. The petroleum ether extract showed the highest anti-inflammatory activity with percentages of inhibition 68, 50, 59, 60, 45 and 30% at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively. Worth attention here is that the volatile oil showed high anti-inflammatory activity (68, 50, 35, 34, 37 and 13% at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively). The chloroformic extract of *Commiphora myrrha* oleo-gum-resin showed highest anti-inflammatory activity with percentages of inhibition 73, 62, 66, 72, 76 and 62% at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively. The ether and petroleum ether extracts and the volatile oil also showed high anti-inflammatory activities (table 1).

Table 1: Mean thickness (mm) of the hind paw of control and tested rats at different time intervals of carrageenan injection:

Groups		Time (hr)					
		0.5	1.0	1.5	2.0	3	4
Control	Mean	0.183	0.217	0.242	0.292	0.317	0.308
	SE+	0.017	0.017	0.015	0.015	0.021	0.020
	%Inhibition	-	-	-	-	-	-
Volatile oil of *B. carteri* (B)	Mean	0.058****	0.108****	0.158**	0.192****	0.200****	0.267
	±SE	0.008	0.008	0.008	0.015	0.022	0.021
	%Inhibition	68	50	35	34	37	13
Petroleum ether ext. of *B. carteri* (B)	Mean	0.058****	0.108****	0.100****	0.117****	0.175****	0.217***
	±SE	0.008	0.008	0	0.011	0.011	0.017
	%Inhibition	68	50	59	60	45	30
Ether ext. of *B. carteri* (B)	Mean	0.092****	0.142***	0.15****	0.192****	0.25*	0.25**
	±SE	0.015	0.015	0.013	0.015	0.018	0
	%Inhibition	50	53.8	38	34	21	19
Chloroformic ext. of *B. carteri* (B)	Mean	0.108****	0.158**	0.150****	0.150****	0.200****	0.283
	±SE	0.008	0.008	0.0	0.013	0.013	0.021
	%Inhibition	41	27	38	49	37	8
Alcoholic ext. of *B. carteri* (B)	Mean	0.117**	0.175	0.183*	0.233*	0.250*	0.233
	±SE	0.017	0.021	0.021	0.021	0.022	0.028
	%Inhibition	36	19	24	20	21	24
Aqueous ext. of *B. carteri* (B)	Mean	0.117***	0.150**	0.175*	0.192***	0.233*	0.250
	±SE	0.011	0.018	0.025	0.024	0.031	0.022
	%Inhibition	36	31	28	34	27	19
Volatile oil of *C. myrrha* (C)	Mean	0.058****	0.100****	0.100****	0.108****	0.142****	0.200****
	±SE	0.008	0.013	0.013	0.008	0.015	0.022
	%Inhibition	68	54	54	55	55	35
Petroleum ether ext. of *C. myrrha* (C)	Mean	0.067****	0.117****	0.117****	0.108****	0.125****	0.142****
	±SE	0.011	0.011	0.011	0.008	0.017	0.015
	%Inhibition	63	46	52	63	61	54
Ether ext. of *C. myrrha* (C)	Mean	0.075****	0.125****	0.100****	0.075****	0.108****	0.133****
	±SE	0.011	0.011	0	0.011	0.024	0.024
	%Inhibition	59	42	59	74	66	57
Chloroformic ext. of *C. myrrha* (C)	Mean	0.050****	0.083****	0.083****	0.083****	0.075****	0.117****
	±SE	0	0.011	0.011	0.011	0.017	0.021
	%Inhibition	73	62	66	72	76	62
Alcoholic ext. of *C. myrrha* (C)	Mean	0.083****	0.133***	0.142****	0.175****	0.217***	0.208***
	±SE	0.017	0.017	0.015	0.017	0.021	0.024
	%Inhibition	55	39	41	40	32	33
Aqueous ext. of *C. myrrha* (C)	Mean	0.150	0.233	0.233	0.258	0.258	0.283
	±SE	0	0.011	0.011	0.015	0.015	0.011
	%Inhibition	18	-7	3.7	12	19	8

Values significantly differ from control: *: p< 0.025, **: p< 0.010, ***: p<0.005, ****: p<0.001.

The Results of the Acute Lethal Toxicity Test:

The results of the acute lethal toxicity test showed that the non-polar extract of *B. carteri* oleo-gum-resin caused no mortality in any of the treated groups (table 2). The extract is safe up to 12 g/kg mice body weight which is equivalent to 93 g for a 70 kg human body weight after Paget and Barnes 1964. The chloroformic extract of *C. myrrha* oleo-gum-resin showed mortality with LD_{50} 7.5 g/kg mice body weight which is equivalent to 58.125 g for a 70 kg human body weight (table 3).

The Results of the Chroic Toxicity Test:

The results of the body weights and biochemical parameters for both the treated and the control groups showed no deviations which indicates that the ingestion of the non-polar bioactive extract of *B. carteri* oleo-gum-resin for a period of two months

exerted no effect on the metabolism, liver and kidney functions (table 4).

The Results of the Phytochemical Analysis:

The volatile oil prepared from *B. carteri* (2.2%) constituted mainly from n-octyl acetate (45.41%), sclarene (16.86 %), n-octanol (9.99 %) and incensole (5.86%).The oxygenated compounds constituted 67.65 % and included terpenoids (10.7 %), long chain aliphatic alcohols (9.99%), long chain aliphatic esters (45.49 %), long chain aliphatic acids (0.56%), aromatic ethers (0.68 %) and aromatic esters (0.23 %). The non oxygenated compounds constituted 31.06 % and included terpenoids (30.29 %) and long chain unsaturated hydrocarbons (0.77 %). The volatile oil of C. *myrrha* (0.94%) constituted mainly from γ-elemene (19.25 %), Z-γ-bisabolene (18.22 %) and δ-elemene (4.53%).The oxygenated compounds constituted 37 % and included sesquiterpenoids (4.74 %), aromatic ethers (26.86%), aromatic esters (0.18 %), alicyclic alcohols (4.32 %) and furanocumarins (0.9 %). The non oxygenated compounds constituted 47.58 % all of which were terpenoids. 1-methoxy-3,4,5,7-tetramethylnaphthalene was detected with a high percentage which is probably due to adulteration of the commercial resin.

Table 2: Acute lethal toxicity result of the bioactive non-polar extract of *B. carteri* oleo-gum-resin*:*

Group No.	Dose of extract (g/kg mice body weight)	Observed mortality	
		No. of dead/ tested	% of observed mortality
1	1	0/8	0
2	2	0/8	0
3	4	0/8	0
4	6	0/8	0
5	8	0/8	0
6	10	0/8	0
7	12	0/8	0

Table 3: Acute lethal toxicity result of the bioactive chloroformic extract of *C. myrrha* oleo-gum-resin*:*

Group No.	Dose (g/kg)	No. of mice/group	No. of dead mice	Z	D	Z.D
1	1	8	2	2	1000	2000
2	2	8	2	2.5	2000	5000
3	4	8	3	3	2000	6000
4	6	8	3	3	2000	6000
5	8	8	3	4.5	2000	9000
6	10	8	6	7	2000	14000
7	12	8	8	4	0	0

Z= Half the sum of dead mice from two successive doses.
D= the difference between the two successive doses.
Z.D= the product of Z and D.
Dm= the dose by which all the mice died.
n= Number of mice in each group.
LD_{50}= Dm-Z.D/n
LD_{50}= 7.5g/kg mice body weight.

Table 4: Body weights and biochemical parameters of the tested and control groups of the chronic toxicity test of the bioactive non-polar extract of *B. carteri* oleo-gum-resin:

Groups		Parameters						
		Initial body weight (g)	Final body weight (g)	Alkaline phosphatase (IU/L)	AST (U/L)	ALT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	Mean	149.25	187.25	10.617	62.38	15.13	33.89	0.715
	±SE	3.479	4.640	0.975	6.825	0.915	3.588	0.013
Non-polar ext of *B. carteri*	Mean	149	186.88	11.574	61.13	13.00	34.419	0.673
	±SE	3.312	5.595	0.728	7.872	1.753	2.079	0.018

GC/MS analysis of the unsaponifiable matter (USM) of *Boswellia carteri* and *Commiphora myrrha* oleo-gum-resins was carried out and the identification of the constituents was performed by comparison of the spectral fragmentation patterns with those of the available database libraries Wiley (Wiley Int.) USA, published data (Adams, 1995) and the published spectral data of the previously isolated compounds from the same and related species. Quantitative determination was carried out based on peak area integration. The analysis of the unsaponifiable matter (USM) of *Boswellia carteri* oleo-gum-resin resulted in the identification of seven terpenes: Incensole (22.55%) and verticilla-4(20),7,11-triene (4.08%) diterpenoids and 24-norursa-3,12-dien-11-one (17.13%), β-amyrin (2.93%), 24-norursa-3,12-diene which is the major compound (24.45%), 24-noroleana-3,9(11),12-triene (14.8%) and 24-Norursa-3,9(11),12-triene (2.98%) triterpenoids. These terpenoids were isolated and identified from the oleo-gum-resin of *Boswellia carteri* (Basar 2005). The GC/MS analysis of the unsaponifiable matter (USM) of the petroleum ether extract of the oleo-gum-resin of *Commiphora myrrha*

resulted in the identification of fourteen compounds including sesquiterpenes (40.47%): Atractylone (20.62%) and 2-O-methyl-8,12-epoxygermacra-1(10)-4,7,11-tetraene (9.23%), 2-Methoxy-8,12-epoxygermacra-1(10, 7, 11-trien-6-one (2.63%), aristolone (0.98%), 7-Epi-α-eudesmol (1.2%), γ-elemene (2.64%), germacrene B (2.03%) and δ-elemene (1.14%). It also contained triterpenes constituting 11.02%: Viminalol (1.64%), α-amyrin (1.39%), dammarenediol (1.04%) and ursan-12-en-3,11-dione (6.95%).

The GC/MS analysis of the methylated fatty acids of the petroleum ether extract of the oleo-gum-resin of *Boswellia carteri* resulted in the identification of two fatty acid methyl esters (methyl-24-methyl pentacosanoate (8.33%) and methyl-5,9-pentacosadienoate (1.66%)), one free fatty acid (trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrien-yl]cyclopropane carboxylic acid (15.01%)), one fatty alcohol (tetracos-15-en-1-ol (31.55%)) and four sterol acetates (5,6-epoxycholestan-3-yl acetate (1.58%), 23-acetoxy-9(11)-holostene-3-ol (1.54%), stigmastane-3,5-diol,3-acetate (0.79%) and 5-cholesta-7,14-diene-3,6-triol-3,6- diacetate (0.63%)). The analysis of the methylated fatty acids of the petroleum ether extract of the oleo-gum-resin of *Commiphora myrrha* resulted in the identification of twelve fatty acid methyl esters representing 74.83% and a single tricyclo-ketonic hydrocarbon 1-carbomethoxy-tricyclo[8.4.0]tetradec-2-en-12-one representing 20.19%. These included methyl palmitate (4.96%), tetracosanoate (4.46%), stearate (3.65%), octacosanoate (2.07%), 3-hydroxy-2,4,4-trimethylpentanoic methyl ester (3.98%), methyl oleate (4.1%), methyl-10,13,16-docosatrienoate (40.55%) which was the major compound and methyl-4,7,10,13-hexadecatetraenoate (6.43%). The high percentage of fatty acid methyl esters is a sign of adulteration or bad storage conditions which resulted in the degradation of high molecular weight compounds.

The phytochemical study of the biologically active extract of *B. carteri* using column chromatography, preperative TLC and HPLC led to the isolation and identification of four compounds which are 3-acetyl-β-boswellic acid, β-boswellic acid, α-boswellic acid and 15-methyl-4-hexadecenoic acid.

3-acetyl-β-boswellic acid (1):

White amorphous powder with an aromatic odor. It exhibited UV λmax (MeOH) at 208 nm. The ESI-MS showed a molecular ion peak $[M+1]^+$= 499 indicating a molecular weight of 498. ^{1}H-NMR analysis (399.65 MHz, CDCl3) is shown in table5.

β-boswellic acid (3α-Hydroxy-urs-12-en-24β-oic acid)(2):

White amorphous powder with an aromatic odor. UV λmax (MeOH) at 204 nm. ESI-MS showed a molecular ion peak [M-1] = 455 indicating a molecular weight of 456. ^{1}H-NMR (399.65 MHz, CDCl3) and ^{13}CNMR (100 MHz, CDCl3) analysis are illustrated in table 6.

Table 5: ^{1}H-NMR spectral data for compound B1:

C	δ^1H (δ, ppm)	*J* (Hz)	C	δ^1H (δ, ppm)	*J* (Hz)
1	1.57 m 1.59 m	- -	16	1.10 m	-
2	1.61 m 1.89 m	- -	17	-	-
3	5.21 br t	-	18	1.31 d	13.1
4	-	-	19	0.90 m	-
5	1.53 dd	13.1, 3	20	1.38 m	-
6	1.71 m	-	21	1.26 m	-
7	1.42 m	-	22	1.27 m 1.43 m	- -
8	-	-	23	1.29 s	-
9	1.60 m	-	24	-	-
10	-	-	25	0.90 s	-
11	1.93 m	-	26	1.04 s	-
12	4.82 br t	-	27	0.90 s	-
13	-	-	28	1.10 s	-
14	-	-	29	0.80 d	3.9
15	0.88 m	-	30	0.85 d	3.9
			*CH3CO	2.11 s	-

α-boswellic acid (3α-Hydroxy-olean-12-en-24β-oic acid)(3):

White amorphous powder with an aromatic odor. UV λmax (MeOH) at 222 nm. ESI-MS showed a molecular ion peak [M-1] = 455 indicating a molecular weight of 456. ^{1}H-NMR (399.65 MHz, CDCl3) and ^{13}CNMR (100 MHz, CDCl3) analysis are illustrated in table 6.

15-Methyl-4-hexadecenoic acid (4):

White amorphous powder with an aromatic odor. UV λmax (MeOH) at 222 nm. EI-MS of compound (4) showed a molecular ion peak $[M]^+$= 268 indicating a molecular weight of 268.^{1}H-NMR (399.65 MHz, CDCl3) analysis: 0.84 (6H, d, *J*= 8, H-16 & H-17), 1.24 (16H, m, H-7-H-14), 1.61 (1H, m, H-15), 1.60 (2H, m, H-3), 2.01 (2H, m, H-6), 2.34 (2H, t, *J*= 7.5, H-2), 5.33 (2H, m, H-4 & H-5).

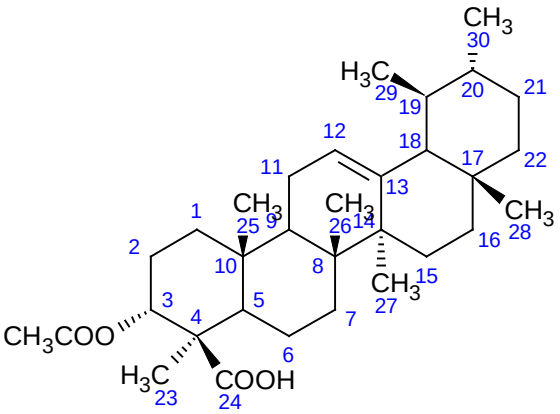
The phytochemical investigation of the bioactive extract of *C. myrrha* was done using column chromatography, HPLC and LC/MS analysis using defined ion targeting MS/MS technique. The peaks were monitored using a UV detector adjusted at 280 nm. By revising the fragmentation patterns of the compounds isolated from the oleo-gum-resin of *Commiphora myrrha* and related species, the fragmentation pattern of C1 was found to be more or less similar to Guggulsterol Y (m/z M^+ 434.3 C27H46O4) isolated from the oleo-gum-resin of *Commiphora wightii* [20]. The fragmentation of C3 showed ion peaks at m/z: 419.20 $[M+1]^+$ (35), 401.40 (19), 358.93 (100).

Table 6: NMR data of compounds (2) and (3):

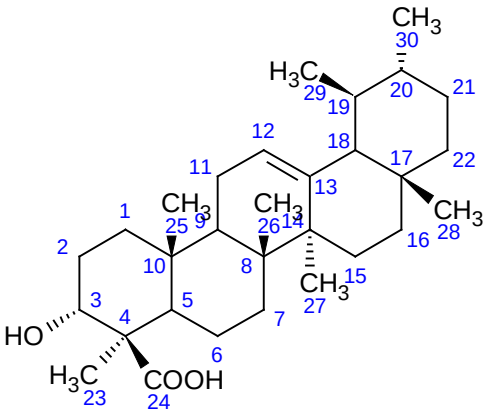
C	$\delta^{13}C^a$ (2)	$\delta^{13}C^a$ (3)	δ^1H (2)	Multiplicity (2)	*J* (Hz) (2)	δ^1H (3)	Multiplicity (3)	*J* (Hz) (3)
1	33.8	33.6	1.52	*m*		1.40	*m*	
			1.46	*m*		1.32	*m*	
2	23.4	26.2	1.96	*m*		2.01	*m*	
			1.66	*m*		1.59	*m*	
3	70.7	70.8	4.08	*br dd*		4.08	*t*	2.5
4	47.3	47.3	-			-		
5	49.1	49.0				1.49	*m*	
6	19.7	19.7	1.82	*m*		1.82	*m*	
			1.72	*m*		1.73	*m*	
7	33.1	32.8	1.51	*m*		1.52	*m*	
			1.46	*m*		1.35	*m*	
8	40.0	39.8	-			-		
9	46.8	46.8	1.60	*m*		1.66	*m*	
10	37.5	37.6	-			-		
11	23.5	23.7				1.88	*m*	
12	124.5	121.8	5.14	*dd*	3.4, 3.4	5.19	*dd*	3.4, 3.4
13	139.6	145.1	-			-		
14	42.3	41.9	-			-		
15	26.5	26.0	1.83	*m*		1.76	*m*	
			1.02	*m*		1.02	*m*	
16	28.1	26.9	2.00	*m*		2.00	*m*	
			1.01	*m*		0.81	*m*	
17	33.8	32.5	-			-		
18	59.2	47.3	1.51	*m*		1.96	*m*	
19	39.6	46.7	0.90	*m*		1.70 1.02	*m* *m*	
20	39.7	31.1	0.90	*m*		-	*m*	
21	31.3	34.7	1.38	*m*		1.33	*m*	
			1.26	*m*		1.23	*m*	
22	41.5	37.1	1.43	*m*		1.44	*m*	
			1.27	*m*		1.22	*m*	
23	24.1	24.1	1.35	*s*		1.35	*s*	
24	182.5	182.5	-			-		
25	13.3	13.1	0.90	*s*		0.89	*s*	
26	16.9	16.7	1.04	*s*		1.00	*s*	
27	23.2	25.9	1.10	*s*		1.15	*s*	
28	28.8	28.4	0.80	*s*		0.83	*s*	
29	17.4	33.3	0.80	*d*	5.5	0.87	*s*	
30	21.4	23.7	0.92	*d*	5.5	0.87	*s*	

[a]Assigned by the HMQC spectrum.

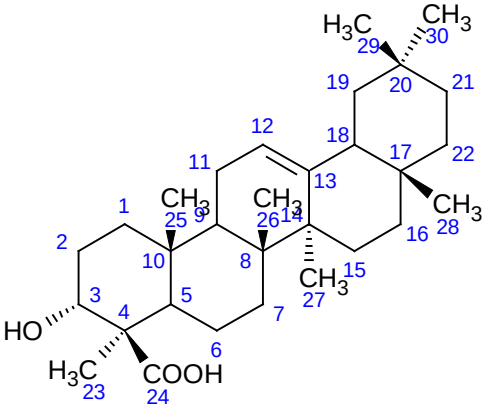
Adv. Environ. Biol., 7(9): 2573-2583, 2013



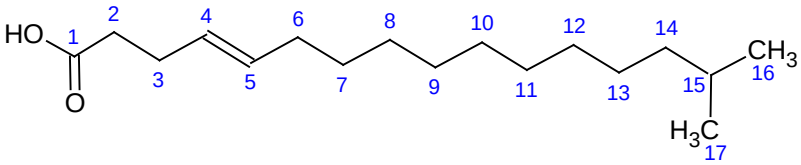
3α-Acetyl-urs-12-en-24β-oic acid
3-acetyl-β-boswellic acid (1)



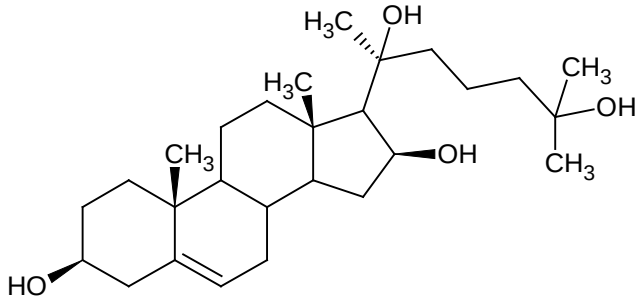
3α-Hydroxy-urs-12-en-24β-oic acid
(β-boswellic acid) (2)



3α-Hydroxy-olean-12-en-24α-oic acid
(α-boswellic acid) (3)



15-Methyl-4-hexadecenoic acid (4)



Guggulsterol-Y

Discussion:

The results of the acute anti-inflammatory assay revealed that the successive extracts of *Boswellia carteri* oleo-gum-resin showed inhibition of induced inflammation with varying percentages where the potency tended to be in favor of the non-polar extracts. The petroleum ether extract showed the maximum significant anti-inflammatory activity with percentages of inhibition 68, 50, 59, 60, 45 and 30% with significance $p<0.001$ at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively. The volatile oil of *B. carteri* oleo-gum-resin also showed high anti-inflammatory activity (68, 50, 35, 34, 37 and 13% at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively). It is the first time to report the anti-inflammatory activity for the volatile oil of *Boswellia carteri* oleo-gum-resin. In case of *Commiphora myrrha* oleo-gum-resin, the results of the acute anti-inflammatory test revealed that the chloroformic

extract showed the highest significant anti-inflammatory activity with percentages of inhibition 73, 62, 66, 72, 76 and 62% with significance $p<0.001$ at time intervals of 0.5, 1, 1.5, 2, 3 and 4 hours respectively. The ether and petroleum ether extracts and the volatile oil also showed high anti-inflammatory activities. Duwiejua *et al.* [10] reported that the aqueous extracts of the resins *C. incisa* and *C. mukul* showed significant activity in carrageenan-induced rat paw edema. This data is not matching with our results that showed nil or even negative activity for the aqueous extract. Tipton *et al.* 2005 stated that myrrh oil showed anti-inflammatory activity through a decrease in interleukin stimulated production of inflammatory cytokines.

The most biologically active successive extracts of the oleo-gum-resins of *B. carteri* and *C. myrrha* where phytochemically and toxicologically studied. The phytochemical study of the biologically active extract of *B. carteri* led to the isolation and identification of four compounds which are 3-acetyl-β-boswellic acid, β-boswellic acid, α-boswellic acid and 15-methyl-4-hexadecenoic acid. The investigation of the unsaponifiable matter (USM) of the petroleum ether extract of the oleo-gum-resin of *Boswellia carteri* resulted in the identification of seven terpenes: Incensole, verticilla-4(20),7,11-triene, 24-norursa-3,12-dien-11-one, β-amyrin, 24-norursa-3,12-diene, 24-noroleana-3,9(11), 12-triene and 24-Norursa-3,9(11),12-triene. Whereas the saponifiable matter of the petroleum ether extract of the oleo-gum-resin of *Boswellia carteri* resulted in the identification of two fatty acid methyl esters, one free fatty acid, one fatty alcohol and four sterol acetates. There was no previous record on the saponification of the lipoidal fraction of the oleo-gum-resin of *Boswellia* species and the identification of its constituents.

However the phytochemical study of the biologically active extract of *C. myrrh* led to the identification of guggulsterol Y. The investigation of the unsaponifiable matter (USM) of the petroleum ether extract of the oleo-gum-resin of *Commiphora myrrha* resulted in the identification of fourteen compounds including sesquiterpenes and triterpenes and the GC/MS analysis of the saponifiable matter resulted in the identification of twelve fatty acid methyl esters representing and a single tricyclo-ketonic hydrocarbon. Similarly, there was no previous record on the saponification of the lipoidal fraction of the oleo-gum-resin of *Commiphora* species and the identification of its constituents.

The results of the GC/MS analysis of the volatile oil of *Boswellia carteri* were commensurate with the revised literature to a great extent with some qualitative and quantitative variations. Alpha-pinene, limonene, cembrene and isocembrene were previously identified by Strappaghetti *et al*, [34] from the volatile oil of the oleo-gum-resin produced by *Boswellia frereana* collected from Somali. Also Ammar *et al*, [3] identified α-pinene and carvone among the constituents of the volatile oil of *Boswellia sacra* oleo-gum-resin from the South regions of the Sultanati of Oman. Mikhaeil *et al*, [21] identified octyl acetate (13.4%), o-methyl anisole, α-pinene, sclarene and n-octanol among the major components of the volatile oil of the oleo-gum-resin produced by *Boswellia carteri* Birdwood. These compounds were also identified here from the volatile oil of *Boswellia carteri* Abdel Wahab *et al*, [1] agreed with the high percentage of esters where she reported that the volatile oil of *B. sacra* from Somali contained 62.1% esters.

Concerning the volatile oil of *C. myrrha*, the results showed a high percentage of sesquiterpenes whereas the percentage of monoterpenes was so small. This agrees with the previously reported data by Brieskorn and Noble 1983, regarding the volatile oil of *Commiphora molmol*. They found that all the constituents of the oil were sesquiterpenes especially furanosesquiterpenes. β-elemene and epoxygermacrene were previously identified from the volatile oil of *Commiphora myrrha* by Morteza-Semnani and Saeedi [22]. Nasser *et al.* [24] identified δ-cadinene and α-muurolene from the volatile oil of *C. kua* and *C. myrrha.* These compounds were also identified here from the volatile oil of *C. myrrha*.

On the other hand, the results of the acute lethal toxicity test showed of the bioactive extract of *B. carteri* oleo-gum-resin caused no mortality in any of the treated groups and that the extract was safe up to 12 g/kg mice body weight. The long term ingestion of the non-polar extract of *B. carteri* oleo-gum-resin (a period of two months) caused no deviations on the metabolism, liver and kidney functions of the treated rats. Although the biological activities of the different extracts of *Boswellia* oleo-gum-resins were extensively studied, little was reported in literature concerning the assessment of its toxicological effects on short or long term use. Kimmatkar *et al.* 2003 noticed that *Boswellia serrata* extract was well tolerated except for minor gastrointestinal complains in a trial conducted on osteoarthritic patients.

The case for the bioactive extract of *C. myrrha* was different where it caused mortality in the acute lethal toxicity test with an LD_{50} 7.5 gm/kg mice body weight which is equivalent to 58.125 g for a 70 kg human body weight. Regarding the previous literature, there were two contradicting reports concerning the toxicological effects of *C. myrrha* oleo-gum-resin. The first was by Omer and Adam 1999**,** where they reported that the use of 1 or 5 g plant resin/kg/day caused grinding of teeth, salivation, soft feces, inappetence, jaundice, dyspnea, ataxia and recumbency in Nubian goat kids. They also reported that the enterohepatonephrotoxicity was accompanied by anemia, leucopenia, increases in serum ALP activity and concentrations of bilirubin, cholesterol, triglycerides and creatinine, and decrease

in total protein and albumin. The other report was by Rao *et al.* [31] who preformed acute and chronic oral toxicity studies on *Commiphora molmol* oleo-gum-resin in mice. The authors reported that no significant difference in mortality in acute or chronic treatment as compared to controls was observed. The results obtained from the acute lethal toxicity test for the chloroformic extract of *C. myrrha* came affirmative with the results by Omer and Adam [25]. The presence of high percentage of 1-methoxy-3,4,5,7-tetramethylnaphthalene, which is probably due to adulteration of commercial resin, could be the purpose of the observed toxicity. This is based on the reported toxicity of methylnaphthalenes [17].

Conclusion:

The significant anti-inflammatory activity of the successive extracts of *Boswellia carteri* oleo-gum-resin showed inhibition of the induced inflammation with varying percentages where the potency tended to be in favor of the non-polar extracts where the petroleum ether extract showed the maximum significant anti-inflammatory activity was attributed to the presence of boswellic acid and its active derivative acetyl-boswellic acid. In fact this was coinciding with the literature that correlated the anti-inflammatory activity of the oleo-gum-resins of *Boswellia* species to the triterpenes which are likely to be found in the petroleum ether extract. Besides, the presence of sterols in the non-polar fraction may also synergize the anti-inflammatory activity. The volatile oil of *B. carteri* oleo-gum-resin also showed high anti-inflammatory activity. It is the first time to report the anti-inflammatory activity for the volatile oil of *Boswellia carteri* oleo-gum-resin. This can give an explanation why some authors found the crude resin more potent than the individual boswellic acids.

The safety of the bioactive non-polar extract of *B. carteri* oleo-gum-resin was encouraging to start in the clinical trials after the approval of the local ethical committee for medical research in the National Research Center.

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