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Comparative phytochemical and pharmacological study of antitussive and antimicrobial effects of boswellia and thyme essential oils

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ABSTRACT

Essential oils are commonly used in herbal cough mixtures as antitussive and antimicrobial preparations, for instance Thyme oil is used in many cough preparations in the Egyptian market and also Boswellia oil is traditionally used as an antitussive. The aim of this study is to compare the antitussive and antimicrobial activity of essential oils of Boswellia carterii and Thymus vulgaris referring to their chemical components which were studied by using different methods of analysis (UV, HPTLC, HPLC, GC and GC/MS). HPLC technique was used for the first time for analysis of Boswellia oil. Results showed that the principal component of Boswellia oil was octyl acetate (35.1%), while the major constituent of Thyme oil was thymol (51%). Both oils were effective as antitussives but Thyme oil was more efficient (89.3%) than Boswellia oil (59%) and also as antimicrobial. It could be concluded that Thyme and Boswellia oils are effective as antitussives but less with Boswellia oil which could serve as an adjuvant in herbal cough mixture but cannot replace Thyme oil.

Key words: *Thyme* oil, *Boswellia* oil, HPLC, HPTLC fingerprint, antitussive, antimicrobial.

INTRODUCTION

Cough is a protective physiologic reflex that augments mucociliary clearance of airway secretions [1]. A significant fraction of non responders to treatment of cough are due to inadequate dosage or duration of treatment, so the treatment regimens become insufficient[2]. That's why prevention of cough before its occurrence is very important by either avoidance of precipitating cause or treatment of underlying condition.

Since the use of herbs has been long considered to be an honored approach to strengthening the body and treating disease, therefore several herbs can be used for prevention and even treatment of cough as Thyme (*Thymus vulgaris* L.) [3] and Licorice (*Glycyrrhiza glabra*) [4]. Also several oils were used in herbal cough mixtures in the Egyptian market such as Eucalyptus, Fennel, Peppermint, Lemon and Anise in addition to Thyme oils.

Frankincense (Boswellia) resin is obtained from trees of genus *Boswellia* (family Burseraceae). There are numerous species and varieties of frankincense trees, including *Boswellia serrata* in India, *Boswellia carterii* in East Africa and China, *Boswellia frereana* in Somalia, and *Boswellia sacra* in Arabia. Each species is producing a slightly different type of resin. Differences in soil and climate create more diversity in the type of resin, even within the same species [5]. The main constituent of Aden and Omani type of oil is α-pinene (43%) whereas; Eritrean and Turkish oils are rich in octyl acetate (52%). The Indian oil is rich in α-thujene (61%). The taxonomic origin of

Omani and Aden oils is *Boswellia frereana* Birdwood, while that of Eritrean and Turkish is *Boswellia carterii* Birdwood. The Indian brand comes from *Boswellia serrata* Roxb [6].

Boswellia carterii Birdwood essential oil analyzed by GC/MS revealed the presence of α-pinene, myrcene, limonene and α-cedrene (15.1, 8.2, 18.2 and 6.1 % respectively) [7]. It was revealed that the hydrodistillate of *Boswellia carterii* resin was pale yellow oil with average yield of 2.44 % (v/w) dry weight[8]. The GC/MS investigation led to the identification of forty chemical constituents. The major constituents were identified in the order of verticiol (14.48 %), isobutylcyclopentane (12.25%), n-octyl acetate (9.20%) and 9-oxabicyclo-[6.1.0]-non-3-yne (9.12%). In this work we made UV finger print for Boswellia oil, HPTLC analysis and a new method of HPLC.

Analysis of *Thyme* oil by GC/MS revealed the presence of *p*-cymene (8.41%), γ-terpinene (30.90%) and thymol (47.59%) [9], while the HPLC method revealed that the concentrations of thymol and carvacrol in essential oil were (41.2% and 4.3%) respectively [10].

The resins of Boswellia carterii and Boswellia serrata have been used for the treatment of rheumatoid arthritis and other inflammatory diseases [11]. The anti-inflammatory, immune-modulatory and anti-leukotriene activity of the resin and especially its major components, boswellic acid derivatives were previously reported by [12][13]. Boswellia resin volatile oil can discriminate bladder cancer cells and normal urothelial cells in culture. The oil suppresses cell survival and induces apoptosis in cultured bladder cancer cells [14]. Boswellia oil also found to have an ameliorating effect on bronchial asthma symptoms such as cough, it also relieves bronchitis and laryngitis [15]. The oil exhibited also antibacterial and antifungal activities [16].

The antitussive activity of Thyme oil is reported to be related to the presence of thymol and carvacrol phenolic compound [17]. In vitro studies have shown that thyme oil has antifungal activity against selected strains of fungi, including Cryptocomlus neoformans, Aspergillus, Saprolegnia, and Zygorhynchus species [18][19]. The antibacterial activity of Thyme oil against Salmonella typhimurium, Staphylocomlus aureus, Escherichia coli and a number of other bacterial species was also reported [20][21].

In this study the UV fingerprint of Boswellia oil , HPTLC analysis and a new method of HPLC were carried out as a new report. This work also aimed to evaluate in a comparative study the antitussive and antimicrobial activities of Boswellia and Thyme oils referring to their chemical constituents using different spectral techniques of analysis in order to correlate between their effects and their chemical constituents.

MATERIALS AND METHODS

Plant material

Boswellia (oleogum resin of *Boswellia carterii* Birdwood, Burseraceae) was purchased from the local market of herbs and spices in Egypt. Thyme oil was purchased from Medizen Company, Egypt and assigned purity of 99%.

Chemicals and reagent

Anisaldehyde (Merck, Germany) reagent was used for visualization. *p*-Anisaldehyde sulphuric acid reagent was prepared according to [22]. The solvents used (toluene, ethyl acetate, methanol, sulphuric acid and glacial acetic acid) were obtained from Adwic, Egypt. The de-ionized water was obtained from NODCAR, Egypt. HPLC grade acetonitrile, *n*-hexane and methanol were obtained from Merck, Germany. Also diphenhydramine hydrochloride (DH) and terbutaline were obtained from Chemical Industries Development, (CID), Egypt and diethyl ether and citric acid were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Diagnostic kits

Kits for determination of ALT (alanine aminotransferase), AST (aspartate aminotransferase), urea and creatinine, were purchased from Biodiagnostic company, Egypt.

Test organisms

The microorganisms: *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylocomlus aureus* 43300, *Staphylocomlus aureus* 3242, *Enterocomlus faecalis*, *Sarcina lutea*, *Bacillus subtilis*, *E.coli* 25928, *E.coli* 5087 and *E.coli* bl21 were supplied from the Microbiology Department, Beni -Suef University, Egypt. They were checked for purity, identity and regenerated to obtain active microorganisms; the culture glycerol stocks were stored in - 80 °C and reactivated on a media suitable for each microorganism.

Animals

Wistar albino rats, weighing 150 g were used. The animals were obtained from the animal house colony of the National Organization for Drug Control and Research (NODCAR), Egypt. The animals were housed in standard metal cages in an air conditioned room at 22 ± 3°C, 55 ± 5% humidity and provided with standard laboratory diet and water *ad libitum*. All experimental procedures were conducted according to the recommendations of Ethics Committee of the National Research Centre with approval certificate registration Number:15-128 .

Sample preparation

Isolation of Boswellia essential oil

Oleogum resin (500 g) was subjected to steam distillation using Clevenger's apparatus until complete exhaustion. The oil was collected, dried over anhydrous sodium sulfate.

Preparation of Boswellia and Thyme oil

The essential oils of Boswellia and thyme, 10 mg each, were added separately into a volumetric flask and diluted to 100 ml with methanol (100 µg/ml) for UV fingerprint HPTLC and HPLC analysis and with 100 ml *n*-hexane for GC and GC/MS analysis (supplementary file).

Phytochemical study

Determination of the physicochemical characters

All physicochemical constants of each oil were determined according to [23], the optical rotation was determined using ADP 220 polarimeter (Bellingham and Stanely Ltd., Kent, England) and refractive index using LEICA ABBE Mark II Refractometer (A. KAÜSS, OPTRONIC, AR2008, Germany).

UV Spectrophotometric (UV) conditions

SPECORD 210 PLUS UV/Visible spectrophotometer (Analytik Jena AG, Germany) with a pair of matched quartz cells of 1 cm width, the UV/Visible spectrophotometer scan of oils from 200-400nm using methanol as blank solution was carried out.

High performance thin layer chromatography (HPTLC) Conditions

Each sample was seperately spotted in the form of bands of 6mm width with a Camag micro liter syringe on precoated silica gel ready-made aluminum plate 60 F_{254} (20 cm × 10 cm with 0.2 mm thickness; E. Merck, Darmstad, Germany) using a Camag Linomat V (CAMAG, Muttenz, Switzerland). A constant application rate of 150 nL/s was employed with a space between two bands of 5 mm. The slit dimension was kept at 6mm × 0.3 mm, and 20 mm/s scanning speed was employed. These parameters were kept constant throughout the analysis of samples. The mobile phase used, consisted of toluene / ethyl acetate (9.3: 0.7 v/v). Plates were developed in ascending order with a CAMAG twin trough glass tank which was pre-saturated with the mobile phase for 15 min. The length of each run was 8 cm. TLC runs were performed under laboratory conditions (Temp: 25 ± 2°C and % RH: 60 ± 5). The plates were then air dried. Densitometric analysis was performed at 560 nm with a Camag TLC scanner III operated by Win CATS software (Version 1.2.0). The source of radiation utilized was deuterium and Tungsten lamp. The composition of the mobile phase for TLC was optimized using different solvents of varying polarity and good resolution was achieved using toluene/ ethyl acetate (9.3: 0.7 v/v) as mobile phase. Spraying with anisaldehyde/ sulphuric acid using CAMAG automatic sprayer, the scanning selected wavelength was 560 nm.

Gas chromatography (GC) conditions (supplementary file).

Hewlett–Packard 6890 series GC equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector (split–split less) was used. A polar HP-5 (Crosslinked 5% Phenyl Methyl Siloxane) HP INNO wax (cross linked PEG) (30m X 0.32mm X 0.5um), Carrier gas: Nitrogen 40cm/sec, 11.8 Psi (50°C), Flow: Constant flow 1ml/min, injector: Split mode (100:1), 0.5µl injection volume inlet temp. 220°C, oven temperature program: initial temp: 120°C, initial time: 3min, rate: 20°C/min, final temp: 300°C, final time: 5 min, Detector: FID 275°C. The detector air flow was 200 ml/min and hydrogen flow was 20 ml/min (10% of air flow).

Mass Spectrometry (GC/MS) conditions (supplementary file).

Gas chromatograph HP 5890 series II plus interfaced (equipped) with a HP 5989B quadrupole mass spectrometer (Hewlett Packard, Palo Alto, USA) with electron impact (EI) ion source of ionization energy of 70 eV. The system was run from a computer with the Hewlett-Packard MS Chemstation software, version B.02.05, Searched library: Wiley 275. LIB. The MS interface (transfer line) was heated at 280°C, the MS ion source to 250°C, the quadrupole (selective mass detector) to 150°C, and the injector (split ratio 60:1) at 225°C. A HP- 5 MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The sample was injected in 1 µl volume in splitless mode. The temperature was programmed initially at 60 °C for 3min, then increased with a rate of 3 °C /min up to 250 °C.

High performance liquid chromatographic (HPLC) conditions for analysis of Boswellia oil
Agilent HPLC (USA) 1260 infinity and consisted of a quaternary pump and UV detector equipped with sampler TML, under computer control was used. All analyzed compounds were separated on a lichrospher C18 (10µm) column (250 x 4.6mm) I.D. Merck, Darmstadt, Germany. The mobile phase consisted of methanol (100%) and UV detection at 254 nm.

High performance liquid chromatographic (HPLC) conditions for analysis of Thyme oil
Following the same conditions under Boswellia oil except the mobile phase consisted of water/ acetonitrile (60: 40 v/v), and UV detection at 274 nm.

Antimicrobial activity
Minimum inhibitory Concentration (MIC)
Essential oils of Thyme and Boswellia were tested and their minimal inhibitory concentration (MIC) was determined as the lowest concentration of oils inhibiting visible growth of each organism [24] for each tested bacteria. They were grown on nutrient broth medium for 12 hrs, after that 100µl of 10^6 cell/ ml were spotted on each plate supplement with varying concentrations of the essential oils. The plates were incubated at 37°C for 24 hrs with the exception of Candida at 30°C.

Pharmacological activity
In vivo biological studies were conducted to determine some pharmacological activities of Boswellia oil and Thyme oil from point of view safety and antitussive effects. By using the dose of Thyme oil (0.5cc/kg) according to [25], it was found to produce marked hypnotic effect. So we used a fraction of it (0.2 and 0.1 cc/kg) in the present study.

Acute toxicity study of Boswellia oil was done according to[26], who reported that in the typical protocol for acute toxicity study if just one dose level at 5g/kg is not lethal no longer requirement for determination of an LD50 value. Since 1000 gm of Boswelia powder yielded 8 cc of Boswellia oil therefore 5 gm of Boswellia yielded 0.04cc of oil. As 0.04cc is too small to accurately quantified therefore 0.36 cc of olive oil was added to complete the volume to 0.4 cc/kg for each rat. The control group received the 0.2cc/kg of olive oil. No mortality was recorded 24 hours later. After 15 days blood was obtained from all groups of rats after being lightly anaesthetized with ether by puncturing retro-orbital plexus [27], the blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 minutes before centrifugation to avoid hemolysis. Then blood samples were centrifuged for 15 minutes at 2500, rpm the clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube to use for determination serum levels IU/L of: Alanine aminotransferase (ALT) (SGPT), Asparate aminotransferse, (AST) (SGOT) according to [28], Urea according to [29] and Creatinine according to [30].

Histopathological study
Animals were sacrificed, the thoracic cavities opened livers rapidly and carefully excised and all attached vessels and ligaments were trimmed off. Abdominal cavities were opened and the kidneys removed. The removed livers and kidneys were washed with cold saline, dried with filter papers and weighed, then put into jars containing 10% formalin as a fixative and kept for histopathological examination. Liver and kidney slides were prepared and stained with hematoxylin and eosin (H& E) staining [31].

Antitussive effect of Boswellia oil and Thyme oil
Based on results of acute toxicity study which revealed that no mortalities had occurred among rats receiving 0.04cc of Boswellia oil equivalent to 5 gm of Boswellia powder, the selected doses of Boswellia oil for the efficacy study were 1/5 and 1/10 of 0.04 ml /kg of Boswellia oil i.e. (0.008 and 0.004 cc/kg) which was completed to 0.4 cc/kg by Olive oil.

Citric Acid was used to induce an animal model of cough according to [32], to investigate the antitussive potential of Boswellia and Thyme oils.

Male Wistar albino rats were treated orally by intragastric gavage with the recommended doses of oils and DH+terbutaline. After 15 minutes, they were individually placed in a closed plexi glass chamber (20 × 10 × 10 cm) and exposed to citric acid inhalation (0.1 g/ml) for 7 min. The cough reflexes were counted for the following 5 min and compared with those of the control group of animals.

Animals were classified into the eight groups (8 rats each):
Group 1: Negative control received 0.2ml/ kg olive oil and weren't exposed to citric acid.
Group 2: Positive control exposed to citric acid inhalation (0.1 g/ml) for 7 min [32].
Group 3: Positive control received 0.2ml/ kg olive oil and was exposed to citric acid inhalation (0.1 g/ml) for 7 min.

Group 4: Reference group received DH in a dose 1.26 +terbutaline 0.13 mg/kg (dose calculated by Paget's table [33].

Groups 5-8: Received Boswellia oil in doses of 0.004 and 0.008 cc/kg and Thyme oil in doses of 0.1 and 0.2cc/kg

Statistical analysis
Comparison between means was carried out using one tailed *t*-test for acute toxicity study, and one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test for efficacy study. $p<0.05$ was considered as being significant in all types of statistical tests. Graph pad prism software (version 6) was used to carry out all statistical tests.

RESULTS AND DISCUSSION

Physicochemical characters
Boswellia carterii oil yielded 1.5 %. The oil was pale yellow with agreeable balsamic slightly spicy and lemony odor. It possesses the following physicochemical constants: specific gravity (25°C), 0.875; refractive index (20°C), 1.446; optical rotation [α] 200 D : D 1.31 (1% solution in methanol); solubility 1:1 w/v in ethanol 95% and 1:2 w/v in ethanol 70%. While thyme oil was clear, yellow or very dark reddish-brown with a characteristic, aromatic, spicy odour and possesses the following physicochemical constants: specific gravity (25°C), 0.935; refractive index (20°C), 1.505; optical rotation [α] 200 D: D 1.52 (1% solution in methanol); solubility 1:2 w/v in ethanol 80%; miscible with ethanol and with light petroleum.

Phytochemical analysis
GC, HPLC, HPTLC and UV analysis
Octyl acetate peak in *Boswellia* oil was observed at the retention time 4.9 min in GC chromatogram and at 3.2 min in HPLC chromatogram also with R_f 0.89 in HPTLC chromatogram and with max 230 nm in UV spectrum. Whereas thymol peak in Thyme oil was observed at the retention time 5.1 min in GC chromatogram and at 5 min in HPLC chromatogram also with R_f 0.56 in HPTLC chromatogram and with max 275 nm in UV spectrum as shown in (Figure 2, 3, 4 and 5). Quantization results showed octyl acetate in Boswellia oil by HPLC (29.9%), GC (45.5%) and HPTLC (22.9%) as shown in (Table 1) and thymol peak in Thyme oil by HPLC (81%), GC (52.5%) and HPTLC (53%) as shown in (Table 2).

GC/MS analysis (could be as supplementary file).
Results of GC/MS analysis of Boswellia oil revealed variety in the detected compounds. It showed the presence of octyl acetate (35.1%), 1-octanol (21.5%), (-)-limonene (13.1%), β- ocimene (5.1%), α- pinene (5.1%), 6-octen-1-ol (3.1%), *p*-cymene (1.4%), (+)-α-Thujene (1.7%), incensole acetate (1.9%), 1,6 octadiene3-ol (1.5%), (-)-linalool (1.4%), bornyl acetate (1.2%), phenanthrene (1.2%), cembrene A (0.9%) camphene (0.5%), β-pinene (0.7%), β-myrcene (0.7%) hexylacetate (0.3%), terpinene -4-ol (0.5%), 9,10 anthracenediol (0.5%) and 12-hydroxy-9-octadecenoic acid (0.7%) as shown in (Table 3, Fig 1). The results were in agreement with the data reported by [34], who revealed that octyl acetate (39.9%) was the main constituent, followed by 1-octanol (11.9%). It was found that limonene (33.5%) and (E)-β-ocimene (32.2%) were the predominant compounds[35]. It was revealed that octanol acetate was the main volatile (45.2%), followed by phyllocladene (13.2%) and incensole acetate (13%)[36]. Duva-3,9,13-trien-1,5a-diol-1-acetate was identified as the main volatile (21.4%), followed by octyl acetate (13.4%)[37]. The main components identified in *B. carterii* oil were; α-pinene, myrcene, limonene and α-cedrene (15.1, 8.2, 18.2 and 6.1 % respectively) [7].

GC/MS analysis of Thyme oil revealed the presence of thymol (51%), carvacrol (2.8%), camphor (6.5%), borneol (4.9%), limonene (1.3%), 1,8-cineol (8.4%), β-myrcene (3.2%), linalool (5.5%), β- pinene (4.2%), α- pinene (4.9%), camphene (1.8%), γ-terpinene (1.7%), terpinene -4-ol (1.2%), p-cymene (1.19%) α-phellandrene (0.38%), β-ocimene (0.21%) and isoborneol (0.59%) as shown in (Table 3, Fig 1). It was revealed that the basic components of the studied Thyme oil were thymol (41.33%), *p*-cymene (18.08%), and γ-terpinene (13.12%)[38]. Thyme oil consists of 10% - 64% thymol and 10% - 56% *p*-cymene[39].

Antimicrobial activity
MIC of the oils ranged from more than 3 and less than 800 µg/ml. This study revealed that Thyme oil showed maximum activity with MIC values more than 3 µg/ml followed by Boswellia oil with MIC values ranging from more than 3 to less than 800µg/ml against all the tested microorganisms as shown in (Table 4).

It is clear that the antimicrobial activity of oils can't be attributed to one single component, but to the synergistic activity of several constituents. It was revealed by [7], that *B. carterii* resin oil demonstrated the highest degree of

activity against the methicillin-resistant *S. aureus* (MRSA) ATML 43866 and against *P. aeruginosa* ATML 9027; while *B. rivae* resin oil showed the lowest MIC value against *E. coli* ATML 25922.

Thyme essential oil strongly inhibited the growth of the clinical resistant strains of *Staphylocomlus*, *Enterocomlus*, *Escherichia*, and *Pseudomonas* genus, so it can protect and treat various human infections as revealed by [40].

Table (1) Quantization results of Boswellia oil using GC, HPLC and HPTLC

	GC		HPLC		HPTLC	
Spot no.	Retention time	percentage	Retention time	percentage	Retention time	percentage
1	1.8	5.4%	3.2	29.9%	0.63	3.1%
2	2.8	15.5%	4.3	23%	0.76	13%
3	3	5%	7.2	10%	0.84	21%
4	3.4	12.5%	8.7	19%	0.91	12%
5	4.4	45.5%	8.9	5%	0.98	22.9%

Table (2) Quantization results of Thyme oil using GC, HPLC and HPTLC

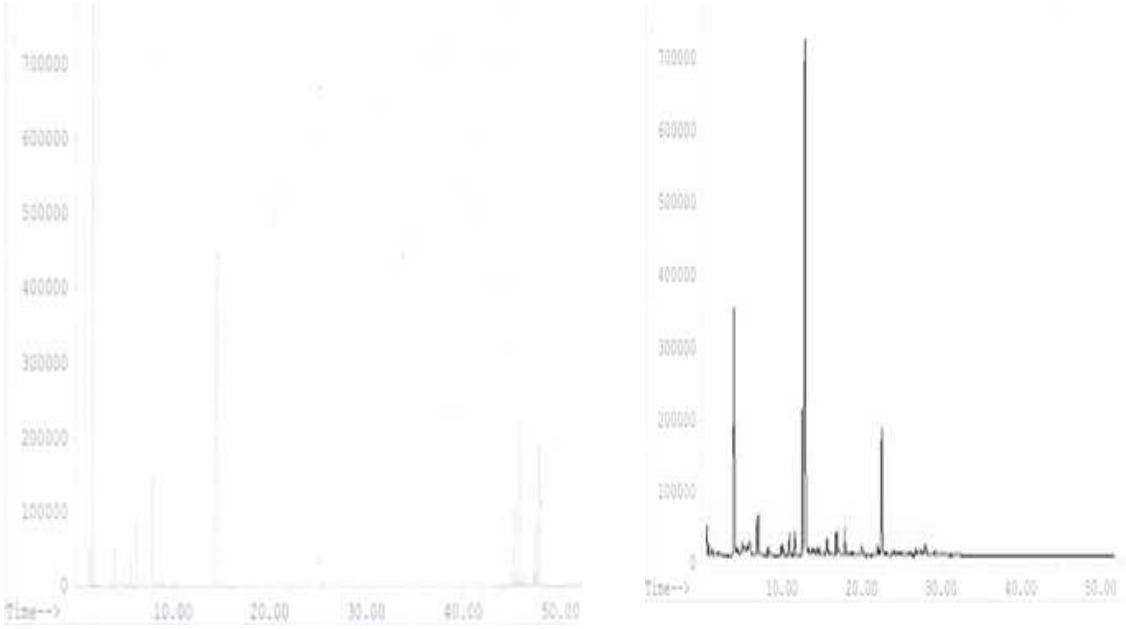
	GC		HPLC		HPTLC	
Spot no.	Retention time	percentage	Retention time	percentage	Retention time	percentage
1	2.05	5.3%	3.5	2.1%	0.24	17%
2	3.05	19.3%	4.5	9.8%	0.52	2.5%
3	3.3	3%	5.1	81%	0.56	53%
4	3.7	3%	7.4	5%	0.87	4.5%
5	5.1	52.5%	8.01	3.1%		

Table (3) GC /MS analysis of Boswellia and Thyme oils

RT	Identified compounds	Percentage (%)		Molecular weight
		Boswellia oil	Thyme oil	
5.4	α- Pinene	5.1	4.9	136
5.9	Camphene	0.5	1.8	136
6.4	β-Pinene	0.7	4.2	136
6.9	β-myrcene	0.7	3.2	136
7.5	*P*-Cymene	1.4	1.19	134
7.9	Hexylacetate	0.3	-	144
8	α-Phellandrene	-	0.38	136
8.4	(-)-Limonene	5.1	0.2	136
9.03	β- Ocimene	13.1	1.3	136
9.5	Camphor	-	6.5	152
10.8	1-Octanol	21.5	-	130
9.3	1,8-cineol	-	8.4	154
9.8	Borneol	-	4.9	154
12.1	Phenanthrene	1.2	-	178
14.8	Octyl acetate	35.1	-	172
15.1	Terpinene -4-ol	0.5	1.2	136
15.6	9,10 Anthracenediol	0.5	-	210
15.9	6-Octen-1-ol	3.1	-	156
11.2	Linalool	1.4	5.5	154
13.2	Isoborneol	-	0.59	154
17.9	Bornyl acetate	1.2	-	196
19	1,6 Octadiene3-ol	1.5	-	154
19.8	(+)-α-Thujene	1.7	-	136
15.4	Thymol	-	51	150
15.8	Carvacrol	-	2.8	150
25.1	γ-terpinene	-	1.7	136
37	12-hydroxy-9-octadecenoic acid	0.7	-	298
37.2	Cembrene A	0.9	-	272
38.3	Incensole acetate	1.9	-	348
Total identified %			99.7	

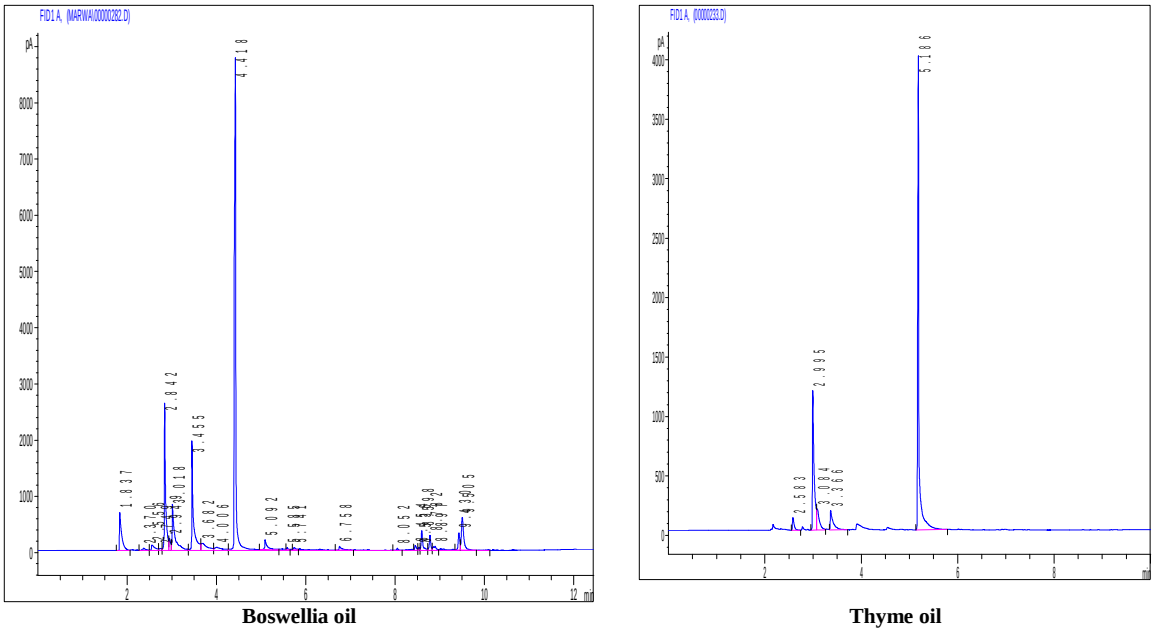
Table (4) Minimum inhibitory concentration (MIC) for Boswellia and Thyme oils

	MIC (µg/ml)	
Tested organisms	**Boswellia oil**	**Thyme oil**
Candida maltos	25	< 3
P. aeruginosa	250	< 3
Proteus vulgaris	> 800	< 3
Salmonella typhi	> 800	< 3
S. Aureus 4330	25	< 3
S. Aureus 3242	3	< 3
Enterocomlus faecalis	< 3	< 3
Sarcina lutea	< 3	< 3
Bacillus subtilis	< 3	< 3
E.coli 25928	> 800	< 3
E.coli 5087	> 800	< 3
E.coli bl21	50	< 3



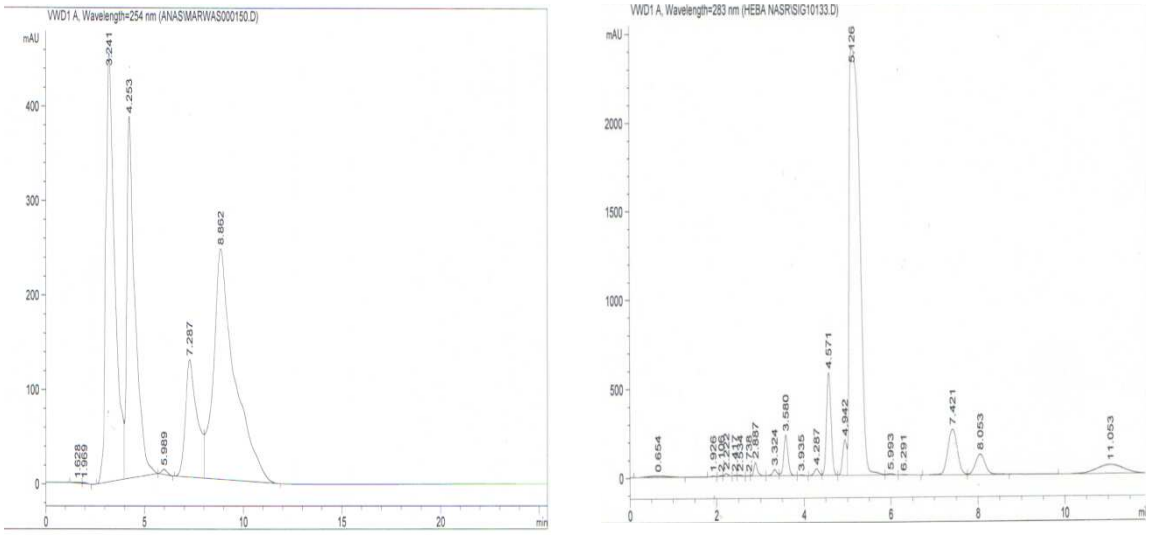
Boswellia oil Thyme oil

Fig. (1) GC/Mass charts for boswellia and thyme oil



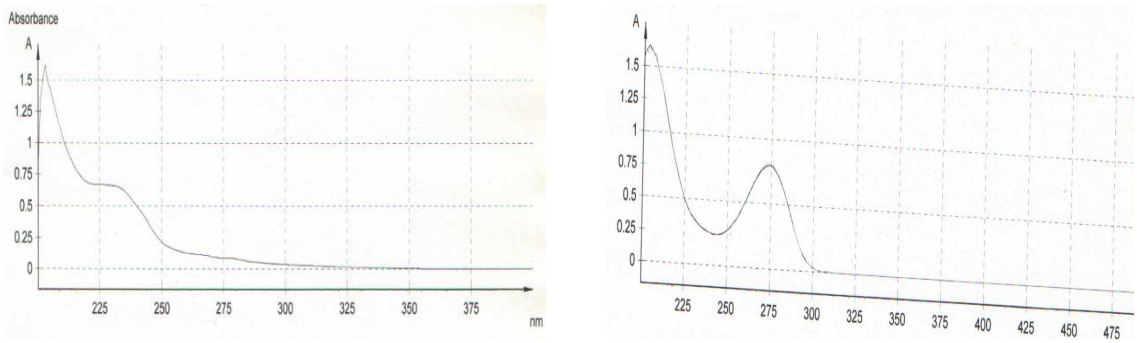
Boswellia oil **Thyme oil**

Fig. (2) GC charts for boswellia and thyme oil



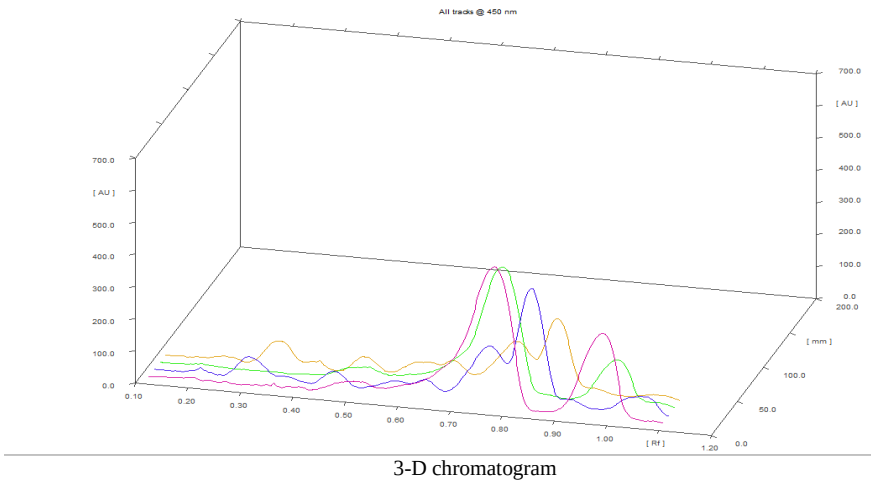
Boswellia oil **Thyme oil**

Fig. (3) HPLC charts for boswellia and thyme oil

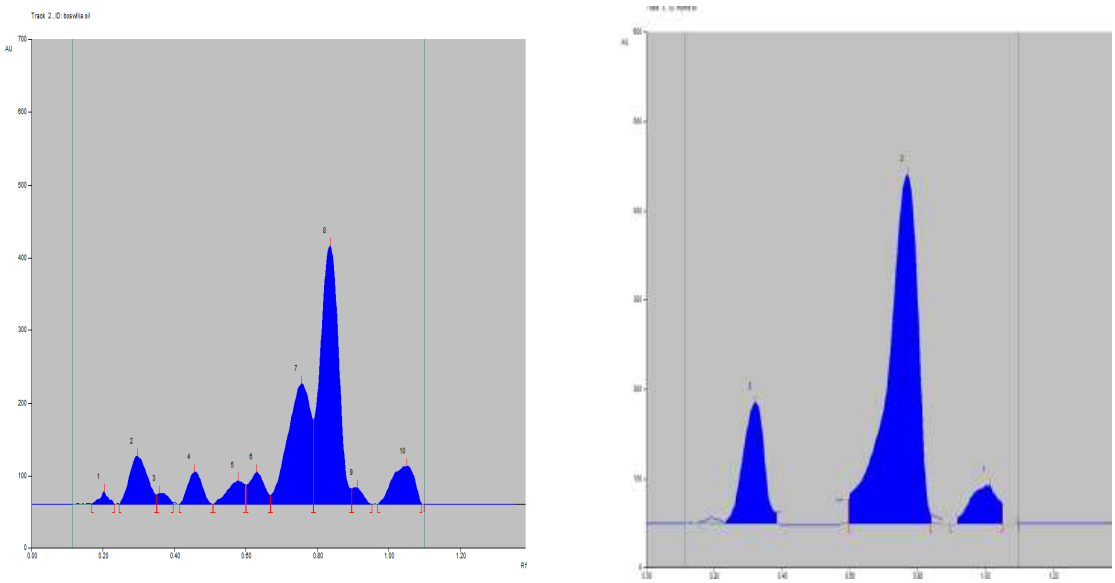


Boswellia oil **Thyme oil**

Fig. (4) U.V spectrum for boswellia and thyme oil



3-D chromatogram



Boswellia oil **Thyme oil**

Fig. (5) HPTLC charts for boswellia and thyme oil and 3-D chromatogram

Pharmacological study

Acute toxicity study

No mortality occurred after 24 hrs. The red and white blood cells counts and platelets were significantly lowered in the group treated with 0.04 cc/kg Boswellia oil when compared to negative control, as shown in Table 5 and Fig.6-9.

Boswellia oil when given orally (0.04 cc/kg) to rats caused significant elevation in serum GPT, GOT, Urea and Creatinine levels when compared to negative control rats. As shown in Table 6 and Fig. 10-11.

Table (5) Results of acute toxicity test of *Boswellia* oil 0.04cc/kg on complete blood count (CBC) in albino rats

CBC Group	RBCs	HB%	Platelet count	WBCs
Negative control	7.05±0.019	13.05±0.4	948.5±15.28	8.32±0.8
0.04 cc/kg Boswellia oil	6.31±0.22*	12.1±0.29	514.5±27.5*	5.87±0.28*

Statistical analysis was carried out using one tailed t-test. Values represent mean ±S.E.
**Significant different from negative control ,n=5, p<0.05.*
RBCs: red blood cells, HB: hemoglobin, WBCs: white blood cells

Table (6) Results of acute toxicity tests of Boswellia oil (0.04 cc/kg) on liver function tests (GPT &GOT) and kidney function tests (Urea & Creatinine) in rats

Group Parameter	Negative control	Boswellia oil (0.04 cc/kg)
GPT U/L	45.3±1. 938	50.881±0.93*
GOT U/L	38.08±1.78	48.68±2.42*
Urea mg/dl	25.35±0.5	39.05±1.6*
Creatinine mg/dl	1.56±0.04	1.89±0.06*

Statistical analysis was carried out using one tailed t-test. Values represent mean ±S.E.
**Significant different from negative control , n=5, p<0.05*

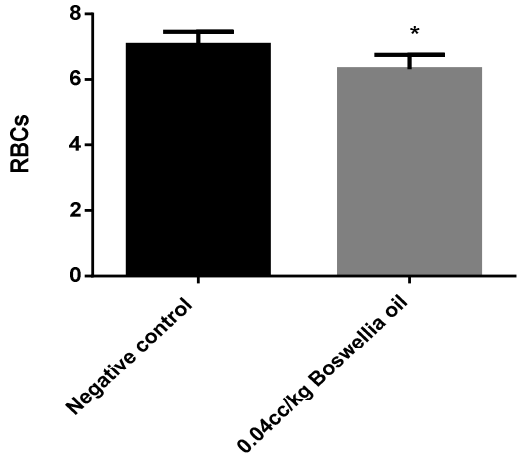


Fig. (6) Study of Acute toxicity effect of boswellia oil on Red Blood cells count in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
**Significant different from negative control,n=5, p<0.05*

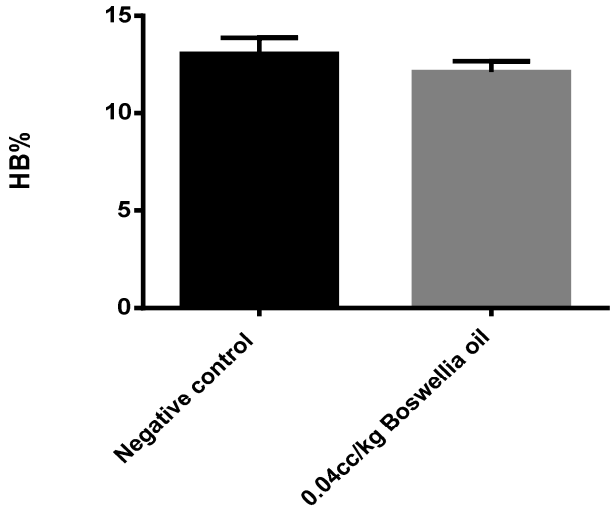


Fig. (7) Study of Acute toxicity effect of boswellia oil on HB% in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
n=5, p<0.05

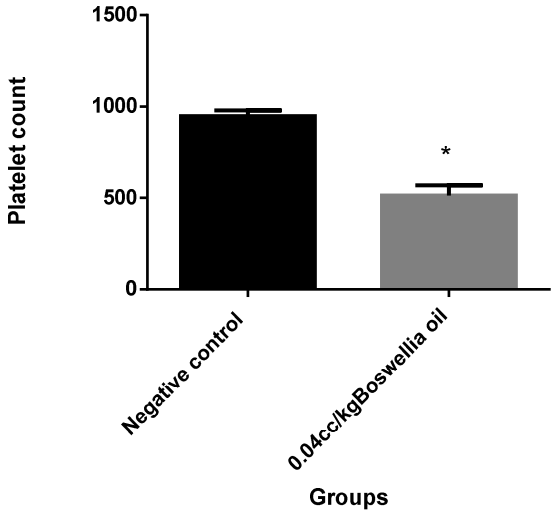


Fig. (8) Study of Acute toxicity effect of boswellia oil on Platelets count in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
**Significant different from negative control, n=5, p<0.05*

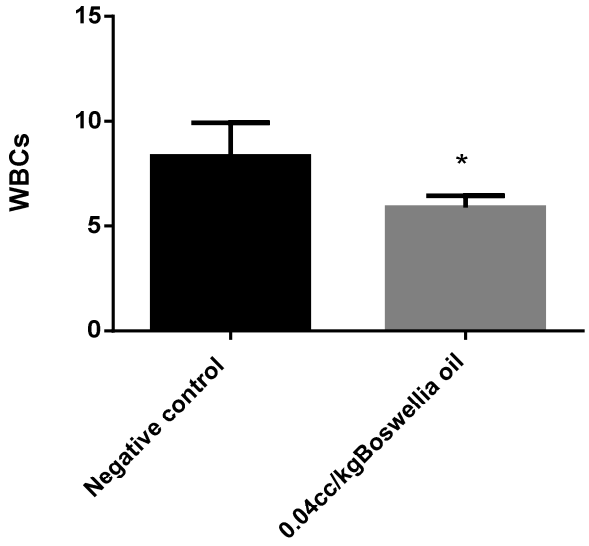


Fig. (9) Study of Acute toxicity effect of boswellia oil on White Blood cells count in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
**Significant different from negative control ,n=5, p<0.05*

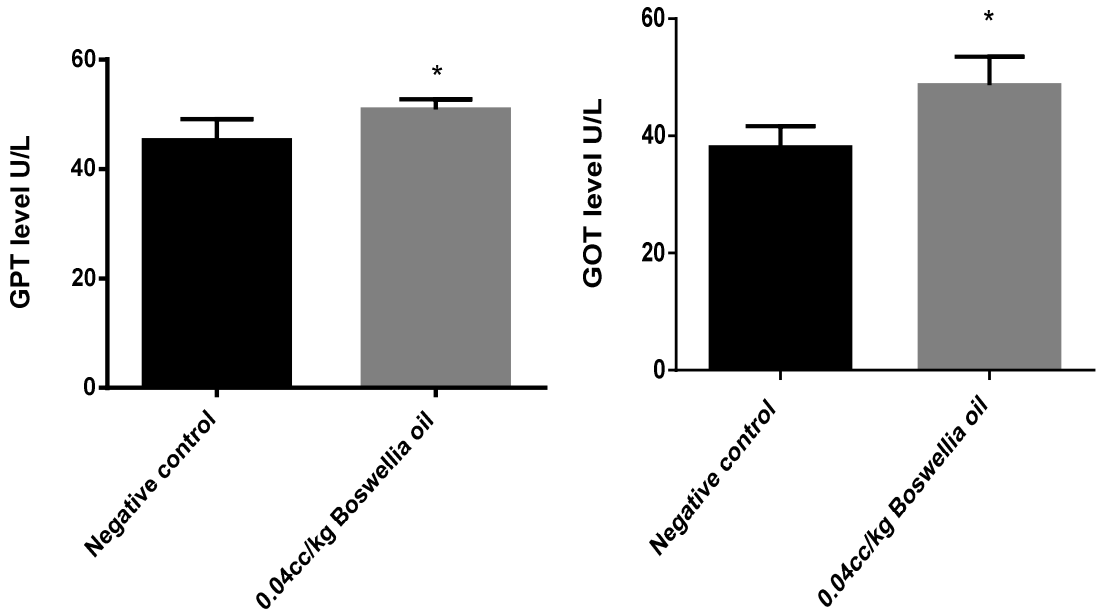


Fig. (10) Study of Acute toxicity effect of boswellia oil on GPT & GOT levels in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
**Significant different from negative control ,n=5, p<0.05*

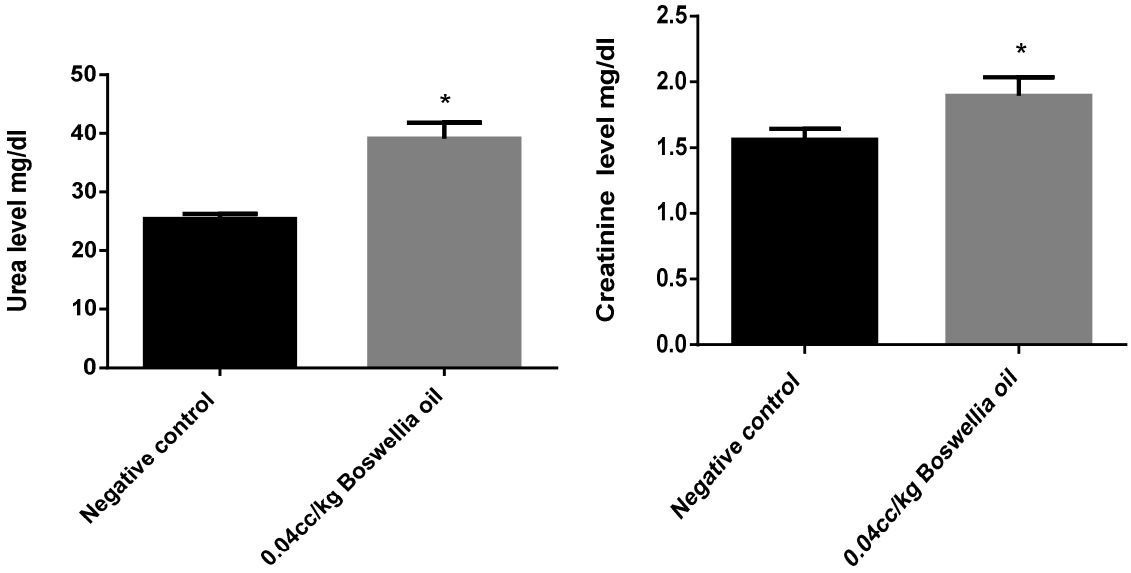


Fig. (11) Study of Acute toxicity effect of Boswellia oil on Urea & Creatinine levels in rats
Statistical analysis was done using one tailed t-test. Values represent mean ± S.E.
**Significant different from negative control ,n=5, p<0.05*

Histopathological findings

Results showed that there is no histopathological alteration in liver or kidney for negative control rats as recorded in (Fig.12a and 12b) and the group administered Boswellia oil at 0.04cc/kg in 0.36 cc/kg Olive oil as presented in (Fig.13a and 13b). However any biochemical change in hepatic or renal function tests takes place before histopathological changes were detected.

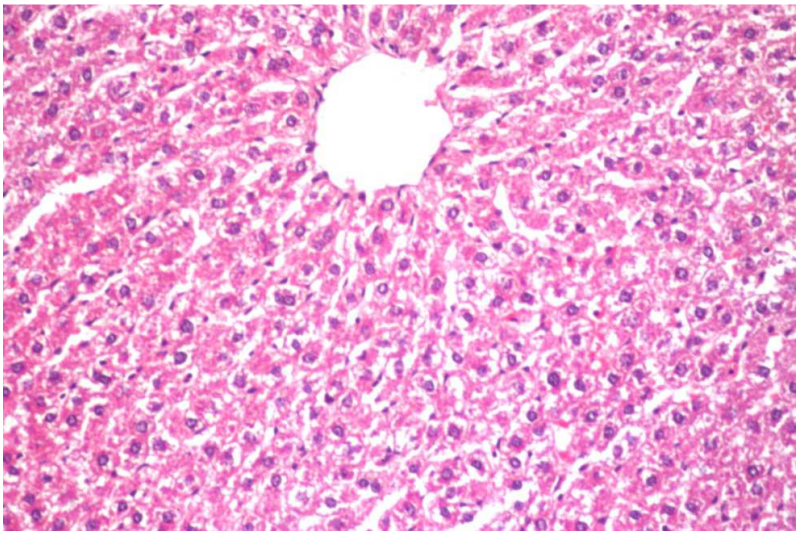


Fig. (12a) photography of liver section of normal group of Wistar albino rats reveled that no histopathological alteration in liver (H&E x 40)

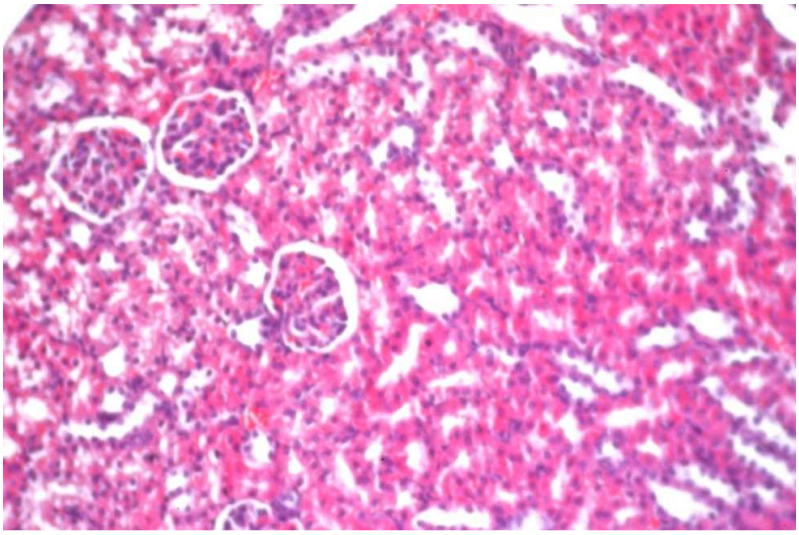


Fig. (12b) photography of kidney section of normal group of Wistar albino rats reveled that no histopathological alteration in kidney (H&E x 40)

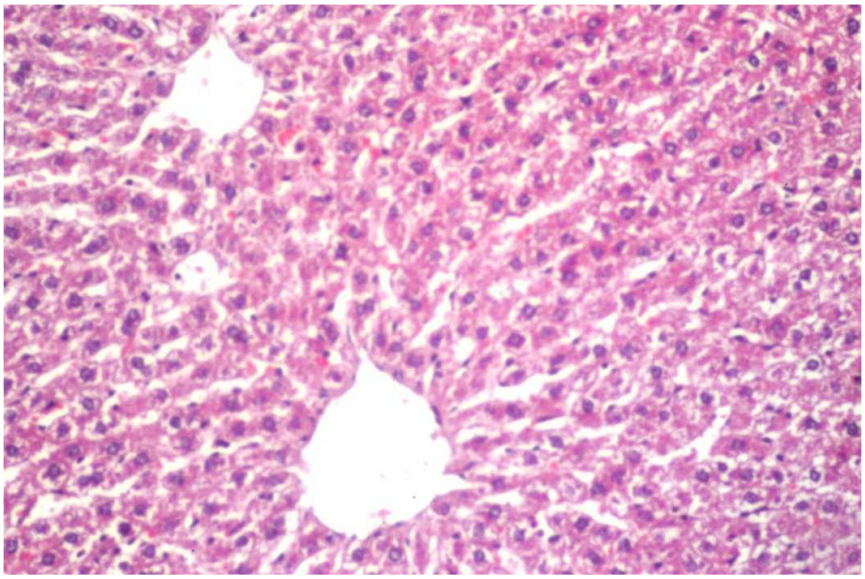


Fig. (13a) photography of liver section of group administered boswellia oil 0.04cc/kg in olive oil 0.36 cc/kg reveled that no histopathological alteration in liver (H&E x 40)

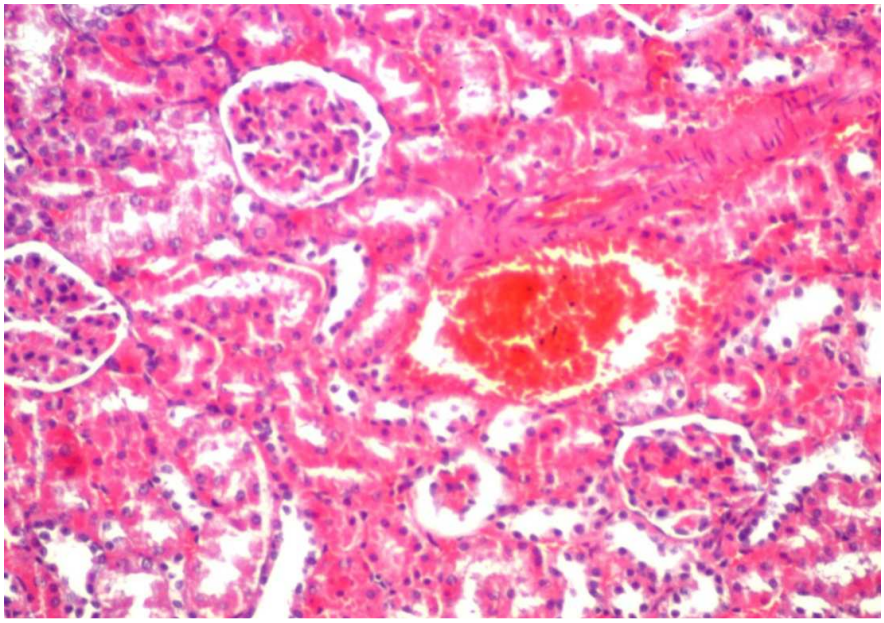


Fig. (13b) photography of kidney section of group administered boswellia oil 0.04cc/kg in olive oil 0.36 cc/kg reveled that no histopathological alteration in kidney (H&E x 40)

Antitussive effect:

Depending on acute toxicity study which revealed that no mortalities were detected among rats receiving 0.04 cc/kg of Boswellia oil equivalent to 5 gm/kg of Boswellia powder, the selected doses of boswellia oil for the efficacy study of its antitussive effect were (0.004 & 0.008 cc/kg).

By using the dose of Thyme oil (0.5cc/kg) according to [25], it was found to produce marked hypnotic effect so we used a fraction of it (0.2 and 0.1 cc/kg) in the present study. Citric acid 10% was used for induction of cough. The negative control rats which weren't exposed to citric acid didn't cough at all. While the positive control rats which weren't given Boswellia or Thyme oils prior to exposure to citric acid exhibited significant increase in cough frequency when compared to all treated groups. Rats treated with both doses of Boswellia and Thyme oils 0.1 cc/kg showed significant decrease in cough frequency when compared to the non treated groups. Thyme oil 0.2cc/kg showed the highest potency as it was 89.3% followed by Thyme oil 0.1 cc/kg 69.81% then Boswellia oil 0.008 and 0.004 cc/kg potencies were 59 and 48% respectively referring to the reference drug DH+Terbutaline as shown in Table 7 and Fig 14.

Table (7) Results of antitussive study of Boswellia and Thyme oils

Groups	Cough count/5minutes	% of reduction of cough	Potency %
Negative control	0	100	
Positive control	101±2.16		
DH+Terbutaline	38.4±1.03*	61.98	
Boswellia oil (0.004 cc/kg)	80±1.7*#	20.79	48
Boswellia oil (0.008 cc/kg)	65±1.7*#	35.64	59
Thyme oil (0.1cc/kg)	55±1.7*#	45.54	69.81
Thyme oil (0.2cc/kg)	43±0.94*	57.42	89.3

Statistical analysis was carried out using one way analysis of variance (ANOVA), followed by Tukey Kramer multiple comparison test. Values represent means±S.E.

**Significant different from positive control group*

#Significant different from Diphenhydramine hydrochloride (DH) +Terbutaline treated group

n=8, $p<0.05$

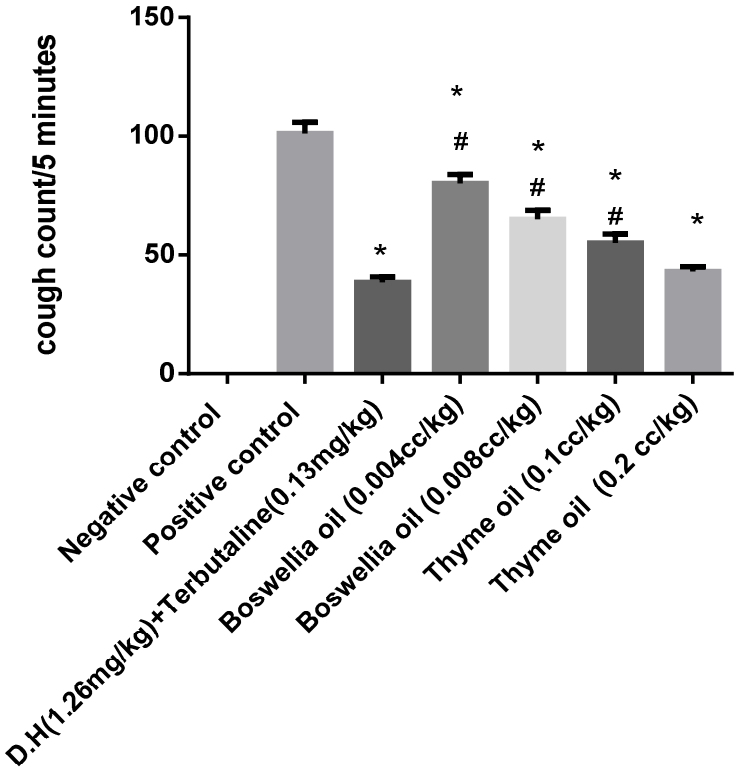


Fig. (14) Study of the Anti-tussive effects of Boswellia oil (0.004 & 0.008cc/kg) and Thyme oil (0.1 & 0.2 cc/kg)
Statistical analysis was done using one way analysis of variance ANOVA, followed by Tukey Kramer multiple comparison test. Values represent mean ±S.E.
**Significant different from positive control group*
#Significant different from Diphenhydramine hydrochloride (DH) +Terbutaline treated group.
n=8, p<0.05.

Although cough doesn't necessarily mean the presence of bronchial asthma, yet it is an important symptom of asthma, which requires relief in combination with antiasthmatic medication, *Boswellia serrata* was used to relief of asthmatic symptoms. Therefore the results of the present study are in accordance with a previous double blind placebo control clinical study with 300 mg *Boswellia serrata* given in a thrice daily dose for 6 weeks, where the anti- asthmatic potential of alcohol extract of *Boswellia serrata* where 70% of the patients with prolonged history of asthma showed improvement in physical symptom and signs of dyspnoea, ronchi, number of attacks, increase in forced expiratory volume (FEV) subset l, forced vital capacity (FVC) and peak expiratory flow rate (PEFR) were established by [13], as well as decrease in eosinophilic count and erythrocyte sedimentation rate (ESR), increase in stimulation of mitogen activated protein kinase and mobilization of intracellular Ca_2 as reported by[41].

Also *Boswellia serrata* extract showed anti-anaphylactic and mast cell stabilizing or inhibiting mast cell degranulation activity in passive paw anaphylaxis and induced mast cell degranulation in the study done by [42]. Moreover it was stated that boswellic acids in *Boswellia serata* inhibited the leukotriene synthesis via 5-lipoxygenase, but did not affect the 12-lipoxygenase and the cyclooxygenase activities [43]. Additionally, boswellic acids did not impair the peroxidation of arachidonic acid by iron and ascorbate. The data suggest that boswellic acids are specific, non-redox inhibitors of leukotriene synthesis either interacting directly with 5- lipoxygenase or blocking its translocation. These may most probably contribute to the antitussive effects of Boswellia.

Thymus vulgaris L. (Thyme) (Lamiaceae) acts as an expectorant and spasmolytic agent for the bronchi [44]. It was reported that the phenolic compounds thymol and carvacrol were better for avoiding the formation of hydroperoxydienes, that is, the first step of the degradation process of a lipid matrix, whose final products are hydroperoxydienes; than preventing the formation of malondialdehyde, one of the secondary lipid peroxidation products, whose quantification provides a measure of the extent of lipid degradation [45].

The common constituents of boswellia and thyme essential oils are pinene, lianool, limonene and cymene which suggest their common antitussive and antimicrobial activities ,but since a previous study revealed that the

antioxidant activity of Linalool was less than that of thymol and carvacrol [46], this may explain the better effect of Thyme than Boswellia oils.

CONCLUSION

It could be concluded that Thyme oil has higher antitussive and antimicrobial activity than Boswellia oil. *Boswellia* oil has antitussive and antimicrobial activity and can be used in cough herbal mixture but with caution.

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