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Full Length Research Paper

Antibacterial activities, chemical constitutes and acute toxicity of Egyptian *Origanum majorana* L., *Peganum harmala* L. and *Salvia officinalis* L. essential oils

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Natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. *Origanum majorana* L., *Peganum harmala* L. and *Salvia officinalis* L. oils had wide range uses as traditional medicinal plants in Egypt. The current study was designed to evaluate the antibacterial activity of *O. majorana*, *P. harmala* and *S. officinalis* essential oils growing in Egypt for first time. The chemical constitutes and toxicity of these oils were also determined to obtain further information on the correlation between the chemical contents and antibacterial activity. The antibacterial effect of the essential oils of *O. majorana*, *P. harmala* and *S. officinalis* oils were studied against some food borne pathogenic bacteria species. The oils of each plant were subjected to gas chromatography-mass spectrometry (GC/MS). The impact of oils administration on the change in rate of weight gain and complete blood picture in hamsters were investigated. *P. harmala* oil had strong antibacterial effect against bacterial species especially at minimum inhibitory concentration (MIC) less than 75.0 µg/ml. From the oil of *P. harmala*, forty one compounds were identified, and the major constituent was 1-hexyl-2-nitrocyclohexane (9.07%). Acute toxicity test was performed on hamsters and showed complete survival after 14 days, and there no toxicity symptoms occurred. This study demonstrated that these essential oils seemed to be destitute of toxic effect which could compromise the medicinal use of these plants in folk medicine.

Keywords: Analysis mass spectrometry, antibacterial activities, acute toxicity, chemical constitutes, gas chromatography, weight gain, *Origanum majorana, Peganum harmala, Salvia officinalis.*

INTRODUCTION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. Traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries, and moreover, the use of herbal remedies has risen in the developed countries in the last decade. In this manner, plants continue to be a rich source of therapeutic agents.

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It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin et al., 1985; Parekh and Chands, 2007b, c)

Gram-positive cocci, particularly *Staphylococcus* species, are predominant among the organisms that are responsible for infective complications, following surgical vascular grafts or the implantation of prosthetic devices (De Lalla, 1999). Treatment of postoperative infections is further complicated by the emergence of antibiotic-resistant pathogens which has contributed significantly to the morbidity and mortality of hospitalized patients. Most *Staphylococcus* infections result in acute diseases. *Staphylococcus aureus* is a facultative anaerobic, gram positive bacterium, which causes food poisoning and usually grows on the nasal membrane and skin. It is also found in the gastrointestinal and urinary tracts of warm-blooded animals (Cheesbrough, 2000). It also causes boils, abscesses, wound infection, pneumonia, toxic shock syndrome, and other diseases (Cheesbrough, 2000).

S. aureus rapidly develops resistance to many antimicrobial agents. *Staphylococcus epidermidis* is the most common cause of nosocomial bacteremia and is the principal organism responsible for infections of implanted prosthetic medical devices such as prosthetic heart valves, artificial joints, and cerebrospinal fluid shunts (Rupp and Archer, 1994). Resistant bacteria representing a challenge in the treatments of various well-known infections necessitated the need to find new substances with antimicrobial properties to be used in the combat against these microorganisms (Martins et al., 2001).

Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases (Clardy and Walsh, 2004). Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno-medicinal use (Verpoorte et al., 2005). Antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher et al., 1987; Selim, 2011). There are several reports regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation to yield active principles (Palombo and Semple, 2001; Parekh and Chands, 2007a).

The use of traditional antibiotics as food additives has led to the emergence of antibiotic resistant strains of bacteria worldwide (Neu, 1992). On the other hand, an upsurge in cases of histomonosis and other infectious diseases is reported in countries where the use of medical prophylactics is very restricted or is banned in some animals (McDougald, 2005). These observations indicate the need and importance for intensive research in the field of alternative prophylactics and therapeutics. Phytotherapy has become an active area of research in this scenario (Cowan, 1999). Antimicrobial effects of medicinal plants and their components is being reported with great frequency, however, their effects against anti-biotic resistant strains of bacteria, particularly of poultry origin, are less well investigated (Barnes and Bradley, 2003). Alkaloids of plant origin are of special interest for conferring antimicrobial activity and their use in modern medicine (Schmeller and Wink, 1998). The search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Davis, 1982).

However, there has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades and in recent times (Rios and Recio, 2005). More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compounds in essential oils with established potent insecticidal (Kambu et al., 1982) and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Rios and Recio, 2005). Santos et al. (1995) remarked that the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubt, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity (Oloke et al., 1988). However, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies that will lead to the development of more potent drugs, with no or minimal toxicity and high sensitivity especially towards the emerging microbial agents (Fabricant and Farnsworth, 2001).

Several studies conducted in the past three decades had focused on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions (Alma et al., 2003). This research project will deal with studying the effect of essentials oils of *Origanum majorana, Peganum harmala* and *Salvia officinalis* wild plants used in folk medicine in Egypt to control food pathogenic microorganisms. Also, investigating the changes rate of weight gain and complete blood picture were performed to animals to be sure that administration of large amounts of plant oils is safe or not.

MATERIALS AND METHODS

Plant material and isolation of essential oil

The aerial parts of wild *O. majorana, P. harmala* and *S. officinalis* were collected from Ismailia villages, Egypt at full flowering stage. The voucher specimen has been deposited in the Botany Department,

Faculty of Science, Suez Canal University, Egypt. Collected plant materials were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a clevenger-type apparatus for 4 h. Hydrodistillation of plants yielded 2.3% (v/w) of essential oil. The yields were based on dry material of plant sample and stored until analyzed.

Antimicrobial susceptibility testing

Antimicrobial activity tests were performed using broth microdilution methods described by National Committee for Clinical Laboratory Standards (NCCLS) (2008). The medium used was Muller-Hinton broth. The essential oils were prepared in dimethyl sulphoxide (DMSO) and the correct volume was put in the first microplate well with Muller-Hinton broth medium for the concentration of each natural compound to be 250µg/ml in that well. The cell suspension was prepared in 0.85% saline, with an optical density equivalent to 0.5 McFarland standard and diluted 1:100 in Muller-Hinton broth to obtain a final concentration of 1×10^4 to 5×10^4 colony-forming units per milliliter (CFU/ml). This suspension was inoculated in each well of a microdilution plate previously prepared with the essentials oils to give concentrations from 250 µg/ml down to 0.4 µg/ml. The plates were incubated with agitation at 37°C for 24 h for bacterial strains. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which the optical density (OD) was reduced to 90% of the OD in the growth control well as measured by spectrophotometer. For the essential oils, the MIC was defined as the lowest concentration able to inhibit any visible microbial growth. Results were analyzed visually and spectrophotometrically. Extracts displaying an MIC less than 75.0 µg/ml were considered to have strong antimicrobial activity, from 75.0 to 150.0 µg/ml, the antimicrobial activity was moderate, from 150.0 to 250.0 µg/ml, the antimicrobial activity was weak, and over 250.0 µg/ml, the extract was considered inactive.

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry analysis (GC-MS) of oils were done at Central Lab in Egyptian Petroleum Research Institute (EPRI), Cairo, Egypt. Analysis of the oils was performed using a Parken Almer (Clarus 500) GC, equipped with a HP 5972 mass selective detector and a HP-5 MS capillary column (30 m × 0.25 mm id, film thickness 0.25 µm). For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, a scan time of 1.5 s and mass range 40 to 300 amu, was used. Helium was the carrier gas at a flow rate of 1.2 ml/min. Injector and transfer line temperatures were set at 250 and 280°C, respectively. Oven program temperature was the same with GC analysis. Diluted samples (1/100 in hexane, v/v) of 1.0 µl were injected manually and in the splitless mode. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature as described by Adams (2001). Further confirmation was done from Kovats Retention Index data generated from a series of n-alkanes retention indices (relative to C9 to C28 on the BP-1) (Hayouni et al., 2008).

Toxic effects of oils

In this experiment, the harmful effects were investigated. Changes in rate of weight gain and complete blood picture were performed on animals to be sure that administration of large amounts of plants oils is safe. Fifty male hamsters (90 to 250 g) were obtained. After they had been acclimatized for one week, they were maintained throughout the study in a specific pathogen-free environment with a temperature of 24 ± 2°C, a humidity of 60 ± 15 and a 12 h light-dark cycle. Hamsters were housed in clean aluminum cages, each of which held 5 hamsters, and they were provided with the pellet diet CE-2 and water *ad libitum*. In this experiment, ten groups of five rats were used each: group 1 (control treated with saline), groups 2, 3, 4 (treated with 80,160 and 320 mg/kg of *S. officinalis* oil, respectively), groups 5, 6, 7 (treated with 80,160 and 320 mg/kg of *O. majorana* oil, respectively) and group 8, 9, 10 (treated with 80,160 and 320 mg/kg of *P. harmala* oil, respectively). Commercial oils were suspended in saline solution (SS) and were administered orally by intragastric route. The experiment continued for 14 days. Body weights were recorded every 3 days (Nagayama et al., 2002).

Statistical analysis

The variations between experiments were estimated by standard deviations, and statistical significance of changes was estimated by student's t-test. Only the probability $P \leq 5\%$ was regarded as indicative of statistical significance.

RESULTS

The patterns of antibacterial effect of oils from *O. majorana* were detected after treatment of the studied bacterial species (Table 1). Moderate effect was observed at all concentration on *Bacillus cereus.* Weak effect was detected at all concentrations of this oil on *Salmonella indica* and *S. aureus.* No effect (-) was recorded at concentrations of 250 µg/ml of this oil after treatment of *Escherichia coli.* The chemical composition of the commercial oil of *O. majorana* was determined by GC/MS method. Seventeen compounds (Table 2 and Figure 1a) were identified, and the following compounds are representing the major constituents: (1) 1,30-dibromotriacontane (41.07%), (2) 11-tricosene (15.35%), (3) 1,38-dibromooctatriacontane (9.78%), (4) 1-pentacontanol (9.05%), (5) O-2-methylpropyl-hydroxylamine (7.02%), (6) 2-piperidinone,N-(4-bromo-N-butyl) (6.39%), (7) 2-methyl-tricosane (3.07%) and (8) 9-cyclohexyl-eicosane (2.17%), respectively.

The effect of *P. harmala* oil on the growth of *B. cereus, S. indica, S. aureus* and *E. coli* are shown in Table 1. The obtained results revealed that concentrations (75 µg/ml) of *P. harmala* oil had the highest antibacterial activity against *B. cereus* and *S. aureus*. The concentration of 250 µg/ml was not effective against *S. indica*. The chemical composition of the commercial oil of *P. harmala* was determined by GC/MS method. Forty one compounds (Table 3 and Figure 1b) were identified and the following compounds are representing the major constituents: (1) 1-hexyl-2-nitrocyclohexane (9.07%), (2) Z-2-octadecan-1-ol (8.13%), (3) 3,5,24-trimethyltetracontane (7.84%), (4) 2-octadecyl-1,3- propane-diol (6.18%), (5) E-2-tetradecen-1-ol (5.89%), (6) 11,14-ecosadienoic acid

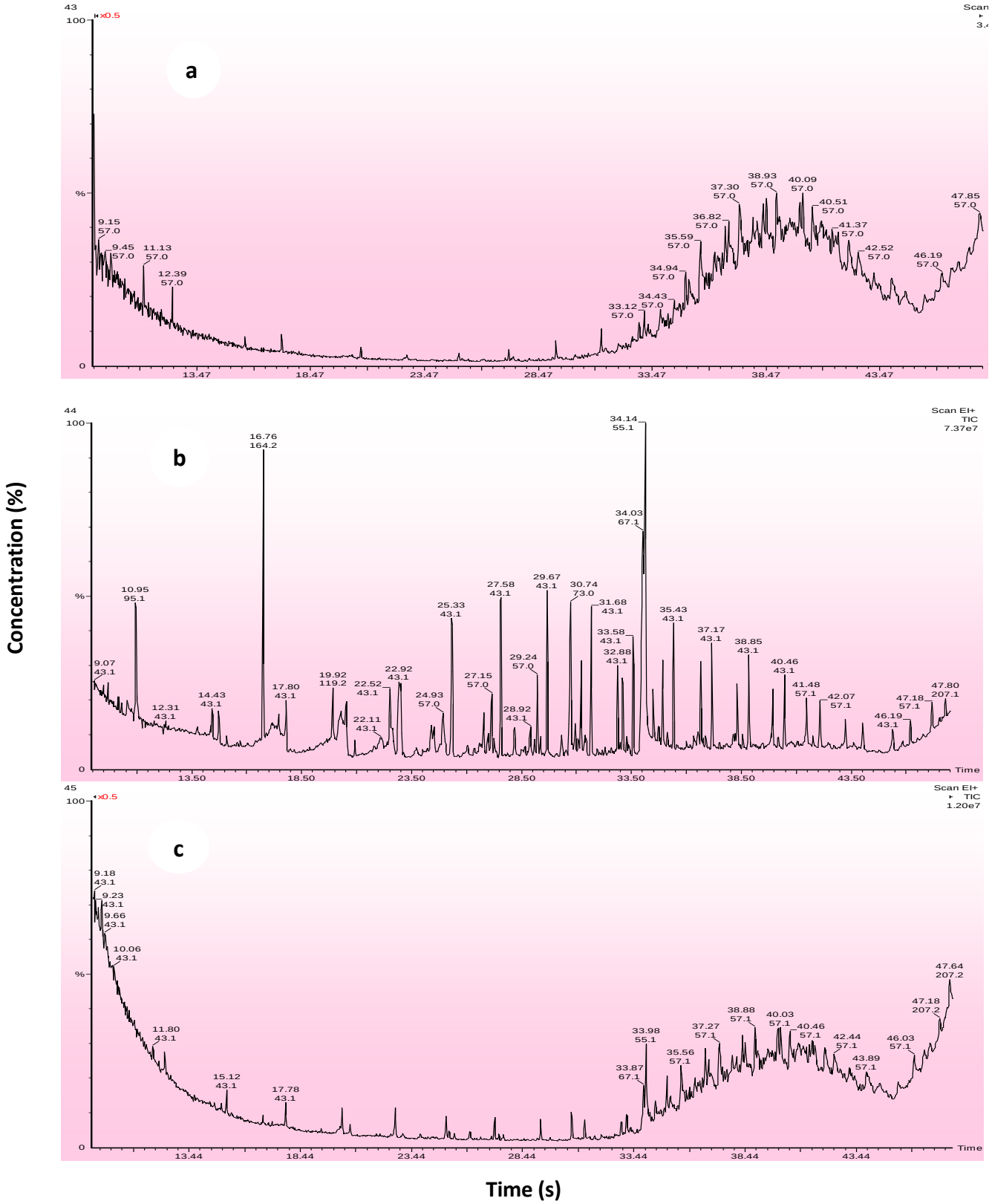

Figure 1. Gas chromatographic profile of the major constituents of (a) *Origanum majorana*, (b) *Peganum harmala* and (c) *Salvia officinalis* oils.

Table 1. Antimicrobial susceptibility of *Origanum majorana* , *Peganum harmala* and *Salvia officinallis* oils against studied bacterial species.

Plant species	Bacterial species			
	Bacillus cereus	***Staphylococcus aureus***	***Salmonella indica***	***Escherichia coli***
Origanum majorana	M	W	W	N
Peganum harmala	S	S	N	M
Salvia officinallis	W	W	W	N

Where MIC less than 75.0 µg/ml was considered to have strong antimicrobial activity (S), from 75.0 to 150.0 µg/ml, the antimicrobial activity was moderate (M), from 150.0 to 250.0 µg/ml, the antimicrobial activity was weak (W), and over 250.0 µg/ml, the extract was considered inactive (N).

Table 2. Chemical composition (%[a]) of the commercial oil of *Origanum marjorana* .

Compound	MW	Formula	RT	wt %
1,30-Dibromotriacontane	578	$C_{30}H_{60}Br_2$	41.85827	41.07
11-Tricosene	322	$C_{23}H_{46}$	38.86413	15.346
1,38-Dibromooctatriacontane	690	$C_{38}H_{76}Br_2$	41.666	9.777
1-Pentacontanol	718	$C_{50}H_{102}O$	36.2113	9.046
O-2-Methylpropyl-hydroxylamine	89	$C_4H_{11}ON$	11.391	7.023
2-Piperidinone, N-[4-bromo-N-butyl]	233	$C_9H_{16}ONBr$	33.71163	6.391
2-Methyl-tricosane,	338	$C_{24}H_{50}$	29.721	3.071
9-Cyclohexyl-eicosane,	364	$C_{26}H_{52}$	37.01467	2.173
1,54-Dibromo-tetrapentacontane,	914	$C_{54}H_{108}Br_2$	38.37167	1.493
2,4,6-Tritert-butyl-4-methyl-2,5-cyclohexadien-1-one	276	$C_{19}H_{32}O$	21.659	1.441
1-Hexyl-2-nitrocyclohexane	213	$C_{12}H_{23}O_2N$	33.323	0.719
2-Azido-2,3,3-trimethylbutane	141	$C_7H_{15}N_3$	15.553	0.699
6-Ethyl-2-methyl-decane	184	$C_{13}H_{28}$	26.7075	0.598
N-(1-phenyl-2-propanyl)-1-decanamine	275	$C_{19}H_{33}N$	27.177	0.597
DL-3,4-dimethyl-3,4-hexanediol	146	$C_8H_{18}O_2$	25.7035	0.308
Taurolidine	284	$C_7H_{16}O_4N_4S_2$	10.999	0.126
2-Aminononadecane	283	$C_{19}H_{41}N$	28.087	0.121
Identified components (%)				100

%[a] peak area of oil components. MW = Molecular weight, RT = Retention time.

methyl ester (5.79%), (7) eugenol (5.22%), (8) 2,6,10,15-tetramethyl-heptadecane, (4.26%), (9) 11-tricosene (4.10%), (10) 2-piperidinone,N-(4-bromo-N-butyl) (3.49%), (11) L-(+)-ascorbic acid 2,6-dihexadecanoate (3.47%), (12) 14-heptadecenal (2.87%), (13) E-9-tetra decenoic acid (2.77%), (14) 1,1-dodecanediol, diacetate (2.64%) and (15) 2-methyl-7-octadecyne (2.45%), respectively.

Two patterns were observed after treatment of *S. officinalis* oil on the studied bacterial species. No effect (N) and weak effect (W). The first one, no effect, was recovered in *E. coli* after treatment with concentration (250 µg/ml) of *S. officinalis* oil (Table 1). The weak effect was also recorded in *B. cereus, S. aureus* and *S. indica* at all concentrations *of S. officinalis* oil. The chemical composition of the commercial oil of *S. officinalis* was determined by GC/MS method. Seventeen compounds (Table 4 and Figure 1c) were identified and the following compounds are representing the major constituents: (1) Docosanoic acid, docosyl ester (16.63%), (2) 2-piperidinone,N-(4-bromo-N-butyl) (15.02%), (3) DL-3,4-dimethyl-3,4-hexanediol (12.82%), (4) 1,30-dibromo-triacontane (10.91%), (5) 11-tricosene (9.03%), (6) 1-pentacontanol (6.50%), (7) 1,38-dibromo-octatriacontane (5.86%), (8) heptane,4-azido (4.15%), (9) 3-ethyl-5-(2-ethylbutyl)-octadecane (4.11%), (10) 3-bromo-decane (3.07%) and (11) 1-hexyl-2-nitrocyclohexane (2.43%), respectively.

Table (5) shows the effect of 80, 160 and 320 mg/kg concentration from different studied oils on hamsters. The increase in body weight was observed in days 3, 6 and 9 when animals were treated with 160 mg/kg *O. majorana*

Table 3. Chemical composition (%[a]) of commercial oil of *Peganum harmala.*

Compound	MW	Formula	RT	wt %
1-Hexyl-2-nitrocyclohexane	213	$C_{12}H_{23}O_2N$	34.087	9.072
Z-2-Octadecen-1-ol	268	$C_{18}H_{36}O$	30.88133	8.125
3,5,24-Trimethyltetracontane	604	$C_{43}H_{88}$	37.48822	7.84
2-Octadecyl-1,3-propane-diol	328	$C_{21}H_{44}O_2$	28.502	6.181
E-2-Tetradecen-1-ol	212	$C_{14}H_{28}O$	28.2885	5.893
11,14-Eicosadienoic acid methyl ester	322	$C_{21}H_{38}O_2$	34.033	5.787
Eugenol	164	$C_{10}H_{12}O_2$	16.758	5.216
2,6,10,15-Tetramethyl-heptadecane,	296	$C_{21}H_{44}$	29.81033	4.261
11-Tricosene	322	$C_{23}H_{46}$	25.97133	4.097
2-Piperidinone, n-[4-bromo-n-butyl]	233	$C_9H_{16}ONBr$	32.403	3.486
l-(+)-Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_8$	30.712	3.465
14-Heptadecenal	252	$C_{17}H_{32}O$	42.193	2.875
E-9-Tetradecenoic acid	226	$C_{14}H_{26}O_2$	34.167	2.765
1,1-Dodecanediol, diacetate	286	$C_{16}H_{30}O_4$	17.186	2.64
2-Methyl-7-octadecyne	264	$C_{19}H_{36}$	10.973	2.448
2,6,10,14-Tetramethyl-heptadecane,	296	$C_{21}H_{44}$	24.927	1.954
E-3-Pentadecen-2-ol	226	$C_{15}H_{30}O$	37.193	1.679
16-Heptadecenal	252	$C_{17}H_{32}O$	38.854	1.576
2-Dodecyl-oxirane,	212	$C_{14}H_{28}O$	17.776	1.446
E-15-Heptadecenal	252	$C_{17}H_{32}O$	32.881	1.323
Dl-3,4-Dimethyl-3,4-hexanediol	146	$C_8H_{18}O_2$	16.3454	1.264
9-Octyl eicosane	394	$C_{28}H_{58}$	44.3045	1.228
1-(1,5-Dimethyl-4-hexenyl)-4-methylbenzene	202	$C_{15}H_{22}$	19.918	0.967
6,10,14-Trimethyl-2-pentadecanone	268	$C_{18}H_{36}O$	28.194	0.82
Docosanoic acid, docosyl ester	648	$C_{44}H_{88}O_2$	36.122	0.738
Taurolidine	284	$C_7H_{16}O_4N_4S_2$	14.401	0.704
Heptafluorobutyric acid,n-tridecyl ester	396	$C_{17}H_{27}O_2F_7$	34.515	0.689
9-Ethyl-9-heptyl octadecane	380	$C_{27}H_{56}$	47.184	0.628
1,1-Dodecanediol, diacetate	286	$C_{16}H_{30}O_4$	14.722	0.616
1-Pentacontanol	718	$C_{50}H_{102}O$	34.89	0.519
4-Methyl cyclopentadecanone	238	$C_{18}H_{36}O$	30.337	0.458
Dl-3,4-dimethyl-3,4-hexanediol	146	$C_8H_{18}O_2$	23.695	0.408
Heptafluorobutyric acid, n-octadecyl ester	466	$C_{22}H_{37}O_2F_7$	30.953	0.364
2,6,10,14-Tetramethyl heptadecane	296	$C_{43}H_{88}$	22.65	0.329
Hexadecanoic acid, (3-bromoprop-2-ynyl) ester	372	$C_{19}H_{33}O_2Br$	33.337	0.321
Oxirane, 2-decyl-3-(5-methylhexyl)-, cis	282	$C_{19}H_{38}O$	36.363	0.27
2-Nonadecanone	282	$C_{19}H_{38}O$	27.284	0.217
Heptadecyl ester heptadecanoic acid	508	$C_{34}H_{68}O_2$	38.131	0.169
Dl-3,4-dimethyl-3,4-hexanediol	146	$C_8H_{18}O_2$	17.7555	0.113
Eicosanoic acid	312	$C_{21}H_{44}O_2$	26.587	0.096
Heptane, 4-azido	141	$C_7H_{15}N_3$	10.598	0.504
Identified components (%)				99.67

%[a] peak area of oil components. MW = Molecular weight, RT = Retention time.

oil. A gradual increase in body weight was recorded after treatment with 80 mg/kg *P. harmala* oil at 3, 6, 9, 12 and 14 days. The weight increase of hamster was recorded at days 3 and 6 after treatment with 160 mg/kg of *P. harmala.* Reduction in body weight was observed in this group which was treated with dose 160 mg/kg in only day 9. At days 12 and 14, the group returns to increase in its body weight more than the control group. The obtained

Table 4. Chemical composition (%[a]) of commercial oil of *Salvia officinallis*.

Compound	MW	Formula	RT	wt %
Docosanoic acid, docosyl ester	648	$C_{44}H_{88}O_2$	43.45	16.625
2-Piperidinone, n-[4-bromo-n-butyl]-	233	$C_9H_{16}ONBr$	34.6328	15.022
Dl-3,4-Dimethyl-3,4-hexanediol	146	$C_8H_{18}O_2$	24.75505	12.821
1,30-Dibromo- triacontane,	578	$C_{30}H_{60}Br_2$	39.9655	10.906
11-Tricosene	322	$C_{23}H_{46}$	38.24571	9.027
1-Pentacontanol	718	$C_{50}H_{102}O$	37.40014	6.497
1,38-Dibromo-octatriacontane,	690	$C_{38}H_{76}Br_2$	41.13075	5.856
Heptane, 4-azido-	141	$C_7H_{15}N_3$	12.20945	4.153
3-Ethyl-5-(2-ethylbutyl)-octadecane,	366	$C_{26}H_{54}$	44.7088	4.11
3-Bromo-decane	220	$C_{10}H_{21}Br$	26.065	3.07
1-Hexyl-2-nitrocyclohexane	213	$C_{12}H_{23}O_2N$	34.12	2.434
2-Methyl-tricosane	338	$C_{24}H_{50}$	31.06	1.929
o-(2-Methylpropyl)-hydroxylamine	89	$C_4H_{11}ON$	9.928	1.802
1-(2-Decyldodecyl)-2,4-dimethyl- cyclopentane	406	$C_{29}H_{58}$	40.555	1.147
2,3,4,5,6,7-hexahydro-3,6-dihexyl-10,11-diphenyl-bis[1,3]oxazino[6,5-f:5',6'-h]quinoxaline	564	$C_{36}H_{44}O_2N_4$	15.3605	1.057
Di-n-undecylamine	325	$C_{22}H_{47}N$	26.078	0.665
3,5,24-Trimethyltetracontane	604	$C_{43}H_{88}$	36.852	0.538
Identified components (%)				99.96

%[a] peak area of components. MW = Molecular weight, RT = Retention time.

Table 5. Body weight (g) of hamsters treated with potent oils (mg/kg) for 14 days and percentage of weight gain.

Day	Weight (g)	Control	*Origanum majorana*			*Peganum harmala*			*Salvia officinalis*		
		0	80	160	320	80	160	320	80	160	320
0	Weight	247.75	221.25	130.31	177.1	148	157.72	210.48	90.52	137	165.66
3	Weight	241.16	185.51	131.2	156.88	163.77	159.9	199.37	92.82	138.12	160.19
	Weight gain (%)	-2.66	-16.15	0.68	-11.42	10.66	1.38	-5.28	2.54	0.82	-3.3
6	Weight	264.54	196.4	132.5	150.12	170.92	160.88	190.2	95.9	140.29	157.84
	Weight gain (%)	6.78	-11.23	1.68	-15.23	15.49	2	-9.64	5.94	2.4	-4.72
9	Weight	253.9	199.8	133.43	143.44	180.96	151.09	183.48	99.93	141.11	152.93
	Weight gain (%)	2.48	-9.69	2.39	-19	22.27	-4.2	-12.83	10.39	3	-7.68
12	Weight	250.8	202.08	127.52	141.83	174.3	158.5	200.36	100.3	130.55	135.57
	Weight gain (%)	1.23	-8.66	-2.14	-19.91	17.77	0.49	-4.81	10.8	-4.7	-18.16
14	Weight	240.42	215.68	129.47	140.86	158.42	167.8	181.13	102.25	144.3	163.75
	Weight gain (%)	-2.96	-2.52	-0.64	-20.46	7.4	6.39	-13.94	12.96	5.33	-1.15

data showed that the administration by high dose of *S. officinalis* (320 mg/kg) reduced body weight of hamsters at days 3, 6, 9, 12 and 14. The decreasing effect of 160 mg/kg of and *S. officinalis* on hamsters was observed only at day 12, while the weight gain was observed in days 3, 6, 9 and 14 (Table 5). In general, the hamsters animal demonstrated both slight increase or decrease in animal body weight after treatment with 80, 160 and 320

Table 6. Hematological parameters of blood of hamsters treated with oils (mg/kg) after 14 day.

Group	Normal value	control	*Origanum majorana*			*Peganum harmala*			*Salvia officinalis*		
		0	80	160	320	80	160	320	80	160	320
RBCs	9-15×10^8 g/l	4.9	4.58	5.53	4.53	5.52	5.47	5.41	6.8	6.84	6.46
HGB	9-15 g/dl	11.6	11.4	11.9	11.2	12.2	11.4	11.6	12.5	14.7	12.8
HCT%	27-45	42.8	37	42.6	36.9	45.8	38.1	39.4	43.6	48.3	43.9
MCHC	31-34 g/dl	27.1	30.9	28	30.2	26.5	29.9	29.5	28.7	30.9	29.2
WBCs	4-12×10^8 g/l	8.38	4.46	4.1	5.3	20.2	4.02	7.64	6.61	12.8	3.06
lymphocyte	2-9×10^8 g/l	3.66	1.35	0.99	1.03	2.44	1.22	3.04	2.07	2.46	0.73
Lymphocyte%	40-75	43.7	30.2	24.3	19.3	12.1	30.2	39.8	31.3	19.2	23.8
Monocyte	ND	0.06	0.04	0.04	0.05	1.2	0.03	0.17	0.23	0.11	0.03
Monocyte%	0-5%	0.8	0.8	0.9	0.9	5.9	0.8	2.2	3.4	0.9	0.9
Granulocyte	0.70-6.00	4.66	3.08	3.07	4.23	16.6	2.77	4.42	4.32	10.2	2.31
Granulocyte%	10.0-50.0	55.6	68.9	74.9	79.8	82	68.9	57.9	65.3	80	75.3

ND; not detected.

mg/kg, which was statistically insignificant with oils of *O. majorana*, *P. harmala* and *S. officinalis* within time of treatment.

The effect of different doses of *O. majorana*, *P. harmala* and *S. officinalis* oils on total counts of red blood cells (RBCs), white blood cells (WBCs) and other hematological parameters after 14 days of treatment on hamsters were recorded in Table 6. It is of interest to note that all treatments of *O. majorana*, *P. harmala* and *S. officinalis* oils had increasing effect on RBCs, haemoglobins (HGB) and hematocrit (HCT%). The maximum values of RBCs (6.84 g/l), HCT% (48.3%) and HGB (14.7%) were recorded after the treatment with 160 mg/kg of *S. officinalis*. All values of mean cell hemoglobin concentration (MCHC) were increased in all treatment by 80, 160, 320 mg/kg of *O. majorana, P. harmala* and *S. officinalis* oils. The concentration of 160 mg/kg of *S. officinalis* had a good effect on MCHC value (30.9).

In general, all doses of *O. majorana* and *S. officinalis* oils reduced the total counts of WBCs of treated hamsters compared to untreated animal except 160 mg/kg of *S. officinalis*. There were remarkable reduction in lymphocytes and lymphocytes percentage as a result of treatment with all doses of oils at days 3, 6, 9, 12 and 14. No change in monocyte percentage was observed in case of treatment with 80 mg/kg of *O. majorana* and 160 mg/kg of *P. harmala*. Slight increase (0.1%) was observed after the treatment with 160 and 320 mg/kg from both *O. majorana* and *S. officinalis*. The highest increase in monocyte values were 5.9, 3.4 and 2.2% after the treatment by 80 mg/kg *P. harmala*, 80 mg/kg *S. officinalis* and 320 mg/kg *P. harmala*, respectively. All values of granulocyte percentage were increased in all treatment. The assumption of weight gain results is supported by hematological analysis (Table 6).

DISCUSSION

Many herbs, essential oils and species have demonstrated some inhibitory effect against spoilage microorganisms in variety of foods (Ellin, 2007). Also, herbs are used as substances enhancing the taste and varieties of regular foods. Some herbs are used as meat additives which have been reported to have bactericidal or bacteriostatic additives (Dyankova et al., 2009). The inhibitory effects of herbs are mostly because of their content of volatile oils (Vazgecer et al., 2004). Many medicinal plants have been known to synthesize active secondary metabolites such as phenolic compounds found in essential oils, with established potent insecticides and antimicrobial activities (Kambu et al., 1982). Many research groups screened various plants extracts as secondary metabolities to detect their biological activities. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus they can be used in the treatment of infectious disease caused by resistant microbes. The essential oils of plants are used as antioxidant, laxatives erosive and in treatment of skin, sleep, cold, cough, urinary system, and nervous system disorders (Abo-Ghalia et al., 2004).

This investigation was directed to study the effect of three essential plant oils on the growth of most potent proteolytic bacterial species *B. cereus, E. coli, S. indica* and *S. aureus.* All oils were tested for their antimicrobial activity using microdilution method. The results revealed that extracts of all plants oils had variations in antibacterial activity against *B. cereus, E. coli, S. indica* and *S. aureus.* It also revealed that *P. harmala* essential oils have a highest antibacterial activity against the studied bacterial species. The plant extracts and the essential oils of plants exhibited various reduction in the growth

according to its chemical composition. This assumption is in accordance with Rota et al. (2004) who reported that the bacterial effect of thymus oils are supposed to be associated with high levels of carvacrol and linalool.

Kalemba and Kunicka (2003) reported that, because of the great number of cell constituents, volatile oils seem to have no specific cellular targets. As typical lipophilis, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layer of poly-saccharides, fatty acids, phospholipids and permeabilized them. The mode of action of antimicrobial agents also depends on the type of microorganisms and their cell wall structure and outer membrane arrangement. Cytotoxicity appears to include such membrane damage. In bacteria, the permeabilization of the membranes is associated with loss of ions, reduction of membrane potential, collapse of the proton pump and depletion of the adenosine triphosphate (ATP) pool (Turina et al., 2006). Moreover the volatile oils can act as pro-oxidant affecting inner cell membranes and organelles such as mitochondria in eukaryotic organisms. Mitochondria like structure and membranes structure in prokaryotic are affected by volatile oil like mitochondria in eukaryotic organisms. Depending on type and concentration, essential oils exhibit cytotoxic effect on living cell but are usually non genotoxic (Bakkali et al., 2008). The antibacterial activity of *O. saccatum* oil was established by using agar diffusion method. Ozcan and Chalchat (2009) showed that *O. saccatum* essential oil exhibits antibacterial activity against *S. aureus and E. coli.*

GC/MS analysis had revealed various compounds in the commercial oils of the three plant species studied (17 *O. majorana*, 41 *P. harmala* and 17 *S. officinalis*). GC/MS analysis of oil components revealed many differences between plant species (*Origanum vulgare and O. majorana*) while those in *O. majorana* were tepiniene-4-ol, α and γ-terpinenes, terpinoline, α and β pinenes, saninene, 1,8 cineol, camphene, β-caryophyllene, α-terpineol and camphene (Novak et al., 2003). The differentiation in the composition between the *O. majorana* is possible; this may be due to biochemical transformation during distillation. It may play a considerable role (Fischer et al., 1987). It might reflect the differences between terpenoid component of the intact plant and composition of distilled *O. majorana* oil (Novak et al., 2003). *Origanum* species showed a strong antibacterial activity against both gram positive and gram negative bacteria (Neslihan et al., 2009). The result showed that antibacterial activity increased depending on the concentration used (Ozcan and Chalchat, 2009). This result is in agreement with obtained result.

P. harmala showed a high antibacterial activity, this result is in agreement with Arshad et al. (2008) who found that *P. harmala* inhibits the growth of all bacteria. Several reports in the literature indicate a great variety of pharmacological activities of *P. harmala* such as anti-microbial, antitumer, antinociceptive and monoamine oxidase (MAO)-inhibiting activities (Shahverdi et al., 2008). The most important component from *P. harmala* seeds are harmine, harmaline, vasicinone and deoxyvsinone (Astulla et al., 2002). These compounds exhibited various bioactivities such as antibacterial activity (Gaviraj et al., 1998) and enzyme inhibition (Sobhani et al., 2002). All the fractions of *P. harmala* showed a good activity against *S. aureus*. The methanolic fraction was the most active against all organisms tested followed by the chloroform fraction (Prashanth and John, 1999). These results are in agreement with the obtained results. Moreover, Shahverdi et al. (2008) reported that the smoke from burning *P. harmala* seeds reduce the viability of tested microorganisms.

S. officinalis is commonly used in traditional medicine to treat various microbial infections. The extracts of *S. officinalis* showed activity against all gram positive and gram-negative bacteria (Kamatou et al., 2007a). The active compounds in *Salvia* spp. extracts are carnsol,7-o-methylepirosmanol, oleanolic acid and its isomer ursolic acid. These active compound displayed moderate to good activity against all the gram positive and gram negative bacterial strains. The essential oils of *Salvia* species however displayed moderate activity against gram positive bacteria. Gono-Byola (2003) demonstrated that methanolic extracts and essential oils of three species of *Salvia* collected at various localities in South Africa inhibited the growth of gram positive bacteria. These results and observation of Kamatou et al. (2007a) are in agreement with the obtained results.

Our results revealed that the change (increasing or decreasing) in body weights of hamster exhibited insignificant variations compared to the control group. Besides, no toxic symptoms or death were observed and they survived being active and healthy, up to 14 days. These results are matching with Abdallah et al. (2009) who reported these observations up to 15 days. These findings could be a good indicator for the non toxicity and safety of *O. majorana*, *P. harmala* and *S. officinalis* at doses 80, 160, 320 mg/kg body weight per day.

Ramirez et al. (2007) did not show any significant variation in both treated and untreated of both sexes of rats after the treatment with *Salvia scutllarioides*, our result confirmed this finding. This action of *S. scutllarioides* was similar with our results. The hamsters demonstrated slight increase and decrease in body weights as shown in Tables 3 and 4. These results are in accordance with Tripathi et al. (2006) who found that the experimental animal demonstrated slight increase in body weight which was statistically insignificant, and he claimed that this effect of oil may be due to its non nutritional value. The body weight of the animals treated with hydroalcoholic extract once a day during 15 days did not show any significant change when compared with the control group, although this had a tendency to decrease

body weight at high concentration of 2000 and 4000 mg/kg (Costa et al., 2011). Costa et al. (2011) also found that the amount of weight loss in animals may be directly related to food ingestion.

Pieme et al. (2006) found that the hydroethanolic extract of *Senna alata* leads to progressive weight gain in rats during 26 days. The administration of aqueous ethanol extract of *S. alata* may indicate an improvement in the nutritional state of animal. The growth response effect could be as a result of increased food and water intake. This explanation is in accordance with our results. The assumption of weight gain results is supported by hematological analysis. The daily oral treatment of *O. majorana*, *P. harmala* and *S. officinalis* oils for 14 days showed no significant variation for RBCs and WBCs. In general, the results showed that the value for the RBCs and WBCs were slightly increased or decreased compared with the untreated animals. These variations were not dose dependent. These results are in line with Pieme et al. (2006) who found that hydroalcoholic extract of *S. alata* has a slight effect on some heamatological parameters. The insignificant increase or decrease in heamatological parameters was recorded by Abdallah et al. (2009). This observation was similar to our results. The increase in HGB content may be due to the effect of oil, Tripathi et al. (2006) reported this finding after treatment of hamsters with oils. Our results showed that heamatological estimation for 14 days revealed an increase of RBCs, HGB and granulocyte%. These results are in accordance with Tripathi et al. (2006) who found that the treatment of oitiver oil (gramineae) revealed the increase in HGB also.

Conclusions

In summary, our study demonstrated that *O. majorana*, *P. harmala* and *S. officinalis* oils seem to be destitute of toxic effect which could compromise the medicinal use of these plants in folk medicine. More detailed study in the future is necessary to clarify exactly the safety of plants extracts and plants oils for human.

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