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EFFECT OF SOME ESSENTIAL OILS ON MICROBIOLOGICAL QUALITY OF COSMETICS PRODUCTS

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ABSTRACT

Medicinal plant essential oils (lemon grass, cumin, chamomile, green tea, peppermint, thyme and wheat germ) were evaluated to their efficiency as nature preservatives on microbiological quality of cosmetic products. Antibacterial activity of the tested medicinal plant oils against pathogenic bacteria namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* spp. isolated from the tested cosmetic samples were studied. All the tested medicinal plant oils, except green tea and wheat germ oils, had broad spectrum of antibacterial activity against all tested pathogenic bacteria . The maximum antibacterial activity was observed with lemon grass oil compared to oils, which gave 30 mm inhibition zone followed by cumin, peppermint, chamomile and thyme oils. The effect of medicinal plant oil additions (0.25, 0.5 and 1.0 mg/ml) to cosmetic samples against microbial contamination of cosmetic samples was studied. Separately additions of all the tested medicinal plants oils (0.25%) to the tested cosmetic samples caused a dramatic decreases in total microbial counts. Pathogenic bacteria not detected after the incubation periods at 37±2 °C. In addition, the rate of microbial count decreasing was influenced by the type and concentration of tested medicinal plant oil. Lemon grass, cumin and peppermint oils were the most efficient medicinal plant oils against microbial contamination. Results showed that using of tested medicinal plants oils (Lemon grass cumin, peppermint, chamomile and thyme) as natural preservatives markedly contributed in decreasing of microbial contamination of cosmetic products, instead of chemosynthetic preservatives which have harmful side effects on human body.

INTRODUCTION

Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. Essential oils such as aniseed, calamus, camphor, cedarwood, cinnamon, citronella, clove, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver and wintergreen have been traditionally used by people for various purposes in different parts of the world . Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity cinnamon oil also possesses antidiabetic property (Ouattara *et al.*, 1997).

Many cosmetics companies are now exploring the use of natural preservatives such as essential oils and vitamins in place of potentially harmful parabens. Not only some natural preservatives are effective antibacterial substances, but also they are in line with the all-natural image that many cosmetic companies these days are trying to promote. Certain vitamins and essential oils, including vitamin E, tea tree oil and grape seed extract, can be used to reduce bacteria. Essential oils and vitamins may be paired with other natural substances to make for an effective preservative. One drawback of these natural preservatives when compared to parabens is that they must be used in high concentrations to have a long-term anti-bacterial effect, which could result in skin irritation (Varvaresou *et al*., 2009).

The oil has been tested as preservative in two topical products, a cosmetic cream and a shampoo. The results of the challenge test clearly demonstrated that the *Calamintha officinallis* essential oil at 2.0% (w/w) concentration reduced the microbial inoculum, satisfying the criteria A and B of the European Pharmacopoeia regarding the oil-water cream and shampoo, respectively (Nostro *et al*., 2004).

However, the investigation of natural substances with preservative properties is relevant due to the possibility of decreasing or substituting the concentration of synthetic preservatives applied in pharmaceutical and cosmetic products, providing a way for the development of safer formulas for consumers use (Ostrosky *et al.*, 2011).So ,the aim of this work is to screen medicinal plant essential oils for antimicrobial activity against pathogens isolated from the selected cosmetic samples. Also,

evaluation of the selected medicinal plant essential oils as natural preservatives.

MATERIALS AND METHODS

Cosmetic samples

One hundred and forty local manufactured cosmetic samples (shampoo, solutions, hair gels, hair oils and skin creams) were collected from Cosmetic Lab, National Organization for Drugs Control and Research (NODCAR) and stored at 4°C for determination of total microbial counts and pathogens as well as prepared treatments.

Medicinal plant oils

Medicinal plants oils (lemon grass, cumin, chamomile, green tea, peppermint, thyme and wheat germ) were purchased from Cosmetic lab, National Organization of Drugs Control and Research (NODCAR).

Media used

All media of this study were obtained from Difco® Company except blood agar media (Atlas, 2004).

Nutrient agar M18 was used for studying the antibacterial activity of medicinal plant essential oils against different genera and spices of pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella spp*.) the previous medium was sterilized at 121°C and 15 psi pressure for 15 minute.

The following media were used for total viable bacterial counts, yeast and molds of the tested cosmetic samples, weights of media components were given in gram per liter distilled water. Soybean-casein digest broth medium was used for cosmetic samples preparation for total microbial counts. Sabouraud's dextrose agar medium was used for total yeast and mold counts.

Preparation of pathogenic bacteria inocula

Four or five colonies of each isolated pathogenic bacteria were separately picked up by sterile loop and transferred into 20ml tube containing 10 ml of sterile nutrient broth medium. The inoculated tubes were incubated at 37 ±2^{o}C for 24 h. After incubation, 100 µL of each isolated pathogenic culture were separately inoculated 100ml of sterile nutrient agar medium previously milted and cooled to (45-55 ^{o}C). About 15ml of inoculated medium were poured into Petri plates (90 mm in diameter) and incubated at room temperature until solidified. Then,

wells were made with sterile cork borer (6 mm) on sold inoculated medium and plate were preserved in refrigerator until using.

Antibacterial activity of the tested medicinal plant essential oils

Screening of antibacterial activity of medicinal plant essential oils (lemon grass, cumin, chamomile, green tea, peppermint, thyme and wheat germ) against the isolated pathogenic bacteria isolated from the tested cosmetic samples were performed by agar diffusion method. The stock solution of each essential oil was prepared by dissolving 1 g of the tested oil in 100 ml of dimethyl sulphoxide (1%). Further dilutions were made from the stock solution as 1.0, 0.5 and 0.25%. The solvent was served as control.

One ml of each diluted tested oils was used to fill the well and plates were incubated at 37±2^{o}C for 24 h. After incubation, inhibition zone diameters were measured to the nearest millimeter (mm).

Challenge test of selected medicinal plant oils against microbial contamination of selected cosmetic samples

The selected cosmetic samples in this study were contaminated with higher total microbial counts and pathogens than should be encountered in naturally contaminated products, thus, lower concentration of tested oils might suffice for naturally contaminated products. Therefore, the selected cosmetic samples had not be needed to apply the artificial inoculation with pathogenic bacteria to study the challenge test

Twelve replicates of each selected cosmetic samples were prepared as followed: 10 g of each selected cosmetic samples were aseptically transferred into 4 sterile tubes (20 ml). One ml of each tested oil dilution (0.25, 0.5 and 1%, w/v) was separately transferred into 3 tubes (ml/tube) while the fourth tube was amended with one ml of dimethyl sulphoxid solution (1%) as control, homogenized the tube's contents by vortex for 3 min and incubated for 7 days at 37±2^{o}C. After each incubation period, total microbial counts and detection of pathogens were monitored on each three replicates of cosmetic samples contained the same tested oil. Total microbial counts were calculated by the average of each three replicates.

Detection of pathogenic bacteria

From pathogenic bacteria that should not be allowed to be found in cosmetic products are *Staphylococcus aureus, Salmonella spp.* and *Pseudomonas aeruginosa. Escherichia coli* is an opportunistic pathogen and it was considered as bioindicator of samples contamination with human wastes as well as contamination with pathogenic bacteria.

Detection of *Escherichia coli* and *Salmonella*

Ten gram or ml of each cosmetic sample (according to nature of cosmetic)were aseptically suspended with 100 ml of lactose sterile broth medium in conical flask (250ml), shake vigorously for 15 min on rotary shaker (200 rpm) at room temperature and incubated for 24 h at 37 ±2^{o}C. After incubation, loopfuls of inoculated flask were streaked on sterile plates of MacConkey, Levine eosin-methylene blue and triple sugar- iron tubes agar media for detection of *Escherichia coli*. Also, loopfuls of inoculated flasks were streaked on sterile plates of bismuth sulphate, brilliant green, xylose–lysine desoxycolate and triple sugar-iron tubes agar media for *Salmonella* spp. detection.

All the cosmetic samples positive for *Escherichia coli* and *Salmonella pp.* contamination were confirmed using Gram staining (Trevino, 2002).

Detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Ten grams or ml of each cosmetic sample were aseptically suspended with 100 ml of sterile soybean-casein digest broth medium in conical flask (250ml), shaked vigorously for 15 min on rotary shaker (200 rpm) at room temperature and incubated for 24 h at 37 ±2^{o}C. After incubation, loopfuls of inoculated flasks were streaked on sterile plates of Vogel- Johnson, mannitol-salt and Barid-Parker agar media and incubated for 24 h at 37 ±2^{o}C for detection of *Staphylococcus* sp. The conformation test for detection of *Staphylococcus aureus* in cosmetic samples were done on blood agar medium and coagulant test. Also, loopfuls of inoculated flasks were streaked on sterile plates of cetrimide agar medium, *Pseudomonas* agar media for examination of fluorescein and pyocyanin and incubated for 24 h at 37 ±2^{o}C for detection of *Pseudomonas aeruginosa*.

RESULTS AND DISCUSSIONS

Antimicrobial activities of medicinal plant oils against some pathogenic bacteria.

Data in Table (1) revealed that all the tested medicinal plant oils, except green tea and wheat germ oils, had antibacterial activity against the tested pathogenic bacteria isolated from the cosmetic samples. The maximum antibacterial activity was observed with lemon grass oil compared to other tested medicinal plant oils, which gave 30 mm inhibition zone followed by cumin, peppermint, chamomile, thyme and wheat germ oil, which gave 28, 25, 20, 17 and 13mm, respectively. The maximum antibacterial activity of lemon grass oil was observed against *E. coli*-9 which gave 30 mm inhibition zone followed by *Ps. aeruginosa*-130 which resulted in 23 mm inhibition zone While, the maximum antibacterial activity of cumin oil was observed against *E. coli* -9 which gave 28 mm inhibition zone followed by *Staph. aureus*-129 which resulted in 27 mm inhibition zone. The maximum antibacterial activity of peppermint oil was observed against *E. coli* -9 which gave 25 mm inhibition zone followed by *Staph. aureus*-3 which resulted in 22 mm inhibition zone.

Table (1): Antimicrobial activities of some medicinal plant oils against some pathogenic bacteria isolated from cosmetic samples

Pathogenic bacteria isolated	The antimicrobial activities (mm) of medicinal plant oils (0.25 mg/ml)						
	Cumin	Chamomile	Green tea	Lemon grass	Pepper mint	Thyme	Wheat germ
Staph. aureus-3	13	17	-	20	22	12	-
E. coli -9	28	16	-	30	25	13	-
Staph. aureus -13	16	18	-	18	20	15	13
Staph. aureus -129	27	15	-	17	21	14	-
Ps. aeruginosa -130	21	14	-	23	15	17	-
E. coli -131	18	20	-	20	16	14	-
Ps. aeruginosa -134	17	18	-	17	14	16	11
E. coli -68	16	16	-	14	15	12	10
Staph. aureus -28	22	15	-	15	17	13	12

The current results are in good agreement with many investigators which supported the antimicrobial properties of the tested medicinal plants oils against pathogenic bacteria (Cervenka *et al.*, 2006; Magro *et al.*, 2006; Prabuseenivasan *et al.*, 2006; Owlia *et al* ., 2007 ;Kirbaslar *et al.*, 2009).

Effect of medicinal plant oils against microbial contamination of cosmetic samples

Effect of thyme oil on microbial contamination of cosmetic samples

Data in Fig (2 and 3) show that the addition of thyme oil (0.25, 0.5 or 1.0 mg/ml) as a natural preservative in tested cosmetic samples caused a decreasing in total microbial count of various tested cosmetic samples, as well as the pathogenic bacteria were not detected at the end of incubation period (14 days) at 37±2 ^{o}C. In addition, slight differences were observed among various thyme oil concentrations on total microbial count of the tested cosmetic samples.Total bacterial counts of shampoo-3, shampoo-9, shampoo-13, shampoo-129, shampoo-130, shampoo-134, shampoo-131, cos. gel-68 and cos.sol-28, which previously supplemented with 0.25 mg /ml thyme oil and incubated for 7 days decreased by 99.9, 99.6, 100.0, 100.0, 99.5, 99.9, 98.2, 99.8 and 95.1% respectively compared to control.

While, total count of the same tested cosmetic samples under the same previous conditions decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 98.8, 99.9 and 95.2% respectively, with addition of 0.5 mg/ml of thyme oil. Furthermore, total count of the same tested cosmetic samples under the same previous conditions decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 98.8, 99.7 and 98.3% respectively, with addition of 1.0 mg/ml thyme oil. Moreover, studying the effect of 1.0 mg/ml of thyme oil under the same previous conditions showed a decreasing effect on total bacterial counts by 53.5, 53.1, 59.6, 57.9, 12.5, 39.4, 55.6, 51.0 and 30.0%, respectively.

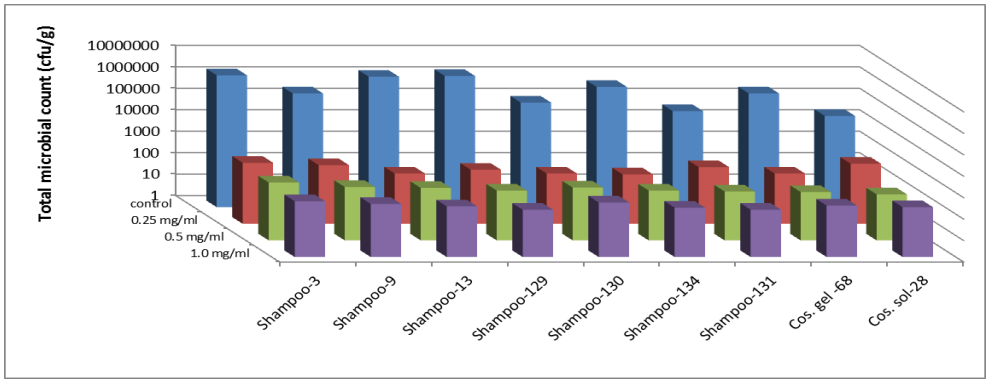

Fig. (1): Effect of thyme oil on microbial contamination of cosmetic samples.

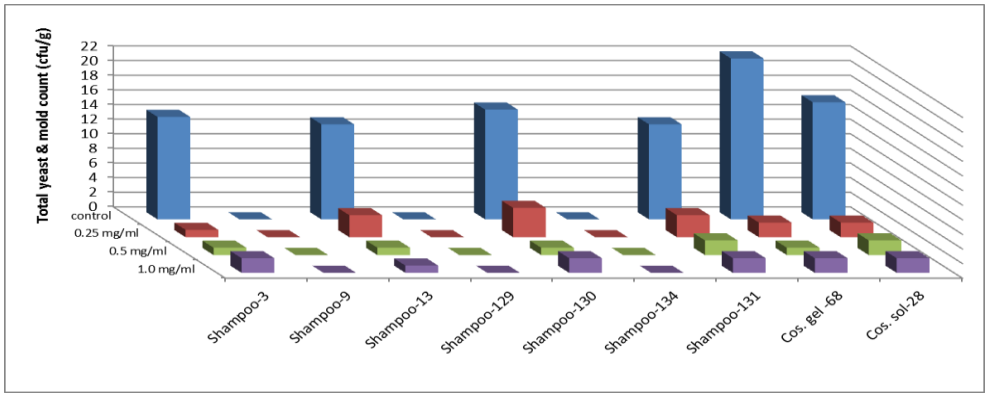

Fig. (2): Effect of thyme oil on yeast and mold contamination of cosmetic samples.

Results illustrated by Figs. (1 and 2) indicated that there were slight differences among the various thyme oil concentrations (0.25, 0.5 and 1.0 mg/ml) on decreasing total microbial counts of the tested cosmetic samples after 7 days of incubation. On the light of all abovementioned results, it could be concluded that the thyme oil is an efficient mean for decreasing total microbial counts in the tested cosmetic samples because it decreased the population of total microbial counts below the official limit of United State Pharmacopeia (1000 cfu/ g).

Current results are in good agreement with those mentioned by many investigators, who found that the addition of thyme oil as natural preservative in some food and cosmetic products exhibited beneficial effects on decreasing total bacterial and yeast and mold counts, as well as the pathogenic bacteria were not detected in the tested samples (Manou *et al.*, 1998; Shetty and Labbe, 1998 ;Farrag *et al.*, 2000). Moreover,

presence of 0.3 mg/ml in tested cream formulation gave anticandida activity compared to control (without thyme oil addition) and that could be further exploited for the treatment of skin infections caused by *C. albicans* (Manou *et al.*, 1998).Also he found that the thyme oil had moderate effects against *Aspergillus niger* and *Candida albicans* in some cosmetic cream samples.

Effect of chamomile oil on microbial contamination of cosmetic samples

Data illustrated in Fig (3 and 4) reveal that the addition of 0.25 mg /ml chamomile oil concentration to shampoo-3, shampoo-9, shampoo-13, shampoo-129, shampoo-130, shampoo-134, shampoo-131, cos.gel-68 and cos.sol-28 decreased total bacterial counts by 99.9, 99.6, 99.9, 99.9, 99.3, 99.8, 98.8,99.7 and 97.4% respectively compared to control. While count decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 98.5, 99.8 and 98.3% respectively with addition of 0.5 mg/ml of oil. Total counts decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 99.2, 99.9 and 98.9 respectively, with addition of 1.0 mg/ml chamomile oil after 7 days of incubation.

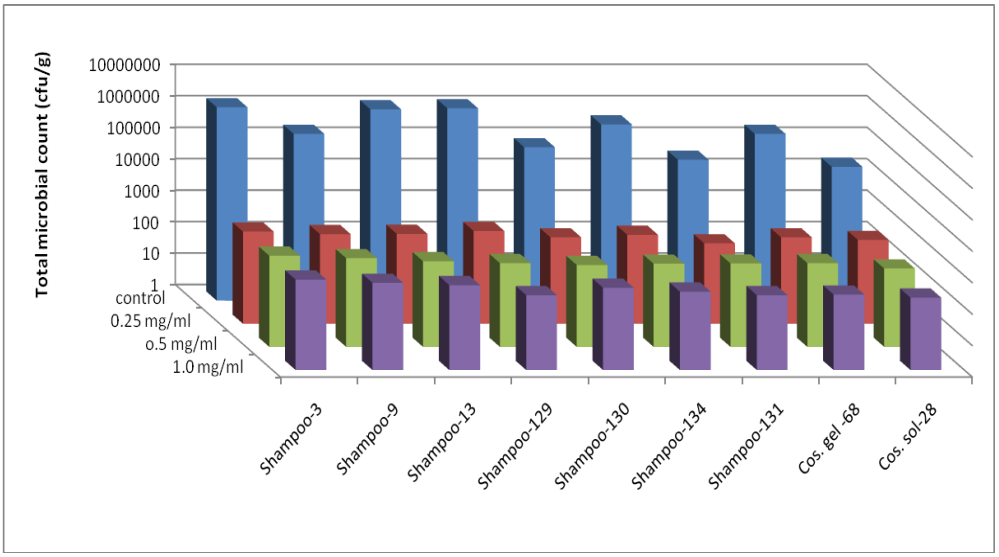

Fig. (3): Effect of chamomile oil on microbial contamination of cosmetic samples.

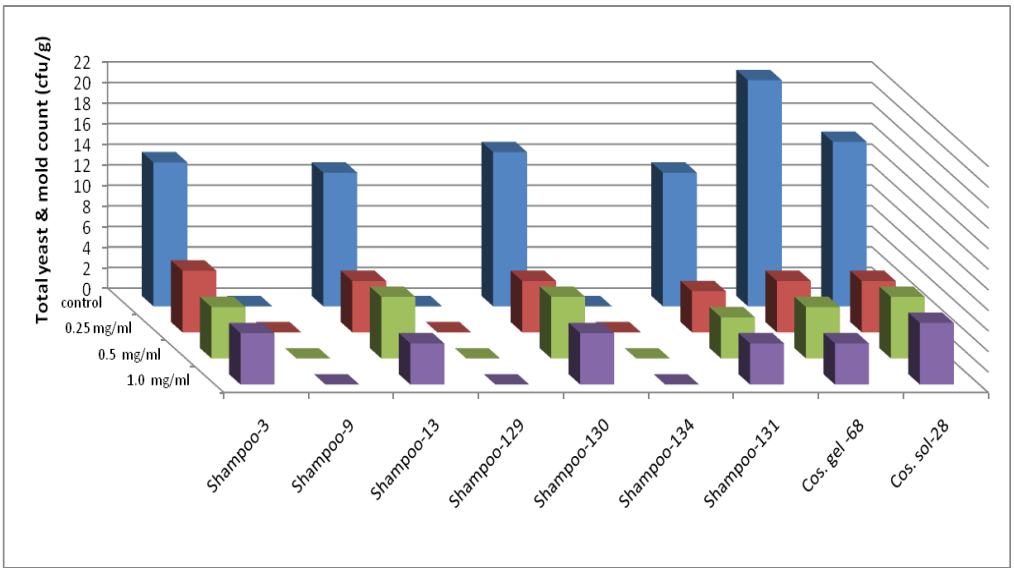

Fig. (4): Effect of chamomile oil on yeast and mold contamination of cosmetic samples.

Total yeast and mold counts of shampoo-3, shampoo-13, shampoo-130, shampoo-131, cos. gel-68 and cos.sol-28 decreased from 14, 13, 15, 13, 22 and 16 to 6, 5, 5,4, 5 and 5 cfu/g, respectively at the same previous conditions.

Data obviously reveal that the addition of chamomile oil at different concentrations (0.25, 0.5 and 1.0 mg/ml) reduced total bacterial counts of the tested cosmetic samples. In addition, total microbial counts of the tested cosmetic samples gradually decreased with increasing chamomile oil concentration (0.25, 0.5 and 1.0 mg/ml).

The abovementioned results strongly suggest that strong antimicrobial activity of chamomile oil against the tested pathogenic bacteria and its inhibitory power in total bacterial count reduction in the tested cosmetic samples pave the way for possible using it as a natural cosmetic preservative.

Current results are in agreement with those found by Bail *et al.* (2009) who studied the antimicrobial activity of an essential chamomile oil against various strains of Gram positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae* and *Salmonella* spp.) and yeast (*Candida albicans*) and they reported that chamomile oil showed high antimicrobial activity against all strains of

the tested microbes. In addition, many investigators mentioned that the chamomile essential oil had the strongest activity on Gram positive, Gram negative bacteria and some pathogenic fungi such as *Trichophyton mentagrophytes, T. rubrum* and *Candida albicans* (Cinco *et al.*, 1983; Wagner *et al.*, 1985; Kedzia, 1991; Turi *et al.*, 1997; Lu *et al.*, 1998; Annuk *et al.*, 1999; Cervenka *et al.*, 2006; Magro *et al.*, 2006 and Owlia *et al.*, 2007). Moreover, Isaac and Kristen (1980) reported that, chamomile essential oil showed a bacteriostatic effect on Gram positive bacteria and some fungi and low activity on Gram negative bacteria.

Effect of peppermint oil on microbial contamination of cosmetic samples

Data illustrated in Fig (5 and 6) revealed that the addition of 0.25 mg /ml peppermint oil concentrations to shampoo-3, shampoo-9, shampoo-13, shampoo-129, shampoo-130,shampoo-134,shampoo-131, cos.gel-68 and cos.sol-28 decreased total bacterial counts by 99.9, 99.6, 99.9, 100.0, 99.4, 99.9, 98.8, 99.7 and 97.1% respectively. While, total bacterial counts decreased by 99.9, 99.7, 100.0, 100.0, 99.6, 99.9, 98.3, 99.9 and 97.2% respectively with addition of 0.5 mg/ml of chamomile oil. Moreover, total bacterial counts decreased by 99.9, 99.7, 100.0, 100.0, 99.7, 99.9, 99.0, 99.8 and 97.6% respectively, with addition of 1.0 mg/ml peppermint oil after 7 days of incubation at 37±2°C.

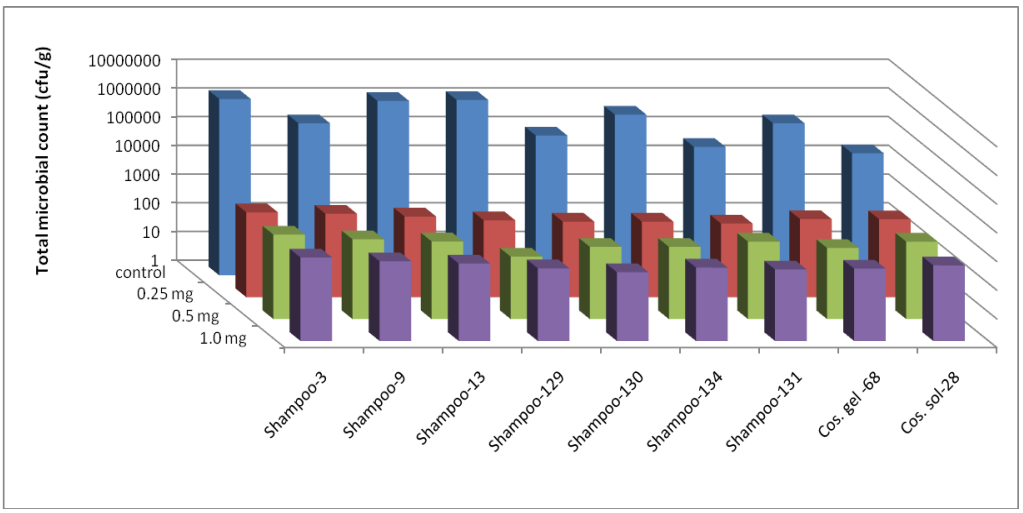

Fig. (5): Effect of peppermint oil on microbial contamination of cosmetic samples.

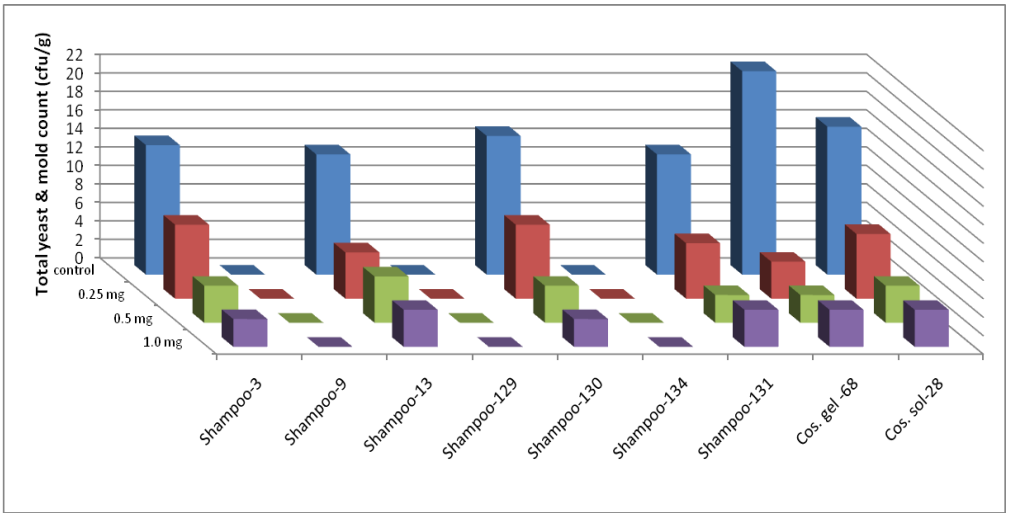

Fig. (6): Effect of peppermint oil on yeast and mold contamination of cosmetic samples.

Total fungal counts (yeast and mould) in shampoo-3, shampoo-13, shampoo-130, shampoo-131, cos.gel-68 and cos.sol-28 decreased from 14, 13, 15, 13, 22 and 8 to 8, 5, 8,6, 4 and 7 cfu/g, respectively, at the same previous conditions. Thus, slight differences were detected among various peppermint oil concentrations on total microbial counts of various tested cos. samples after 7 days of incubation at 37±2 ^{o}C.The obtained data clearly show that the using of different concentrations (0.25, 0.5 and 1.0 mg/ml) of peppermint oil as a natural preservative in tested cosmetic samples resulted in dramatic decreases in total microbial counts of the various tested cosmetic samples, besides pathogenic bacteria were not detected at the end of incubation periods at 37±2 ^{o}C. Slight differences were detected between 0.5 and 1.0 peppermint oil in their activity against microbial contamination of tested cosmetic samples.

The results of the present work confirm the potential role of peppermint oil as a natural preservative in cosmetic products and that related to its bactericidal activity against pathogenic bacteria and its inhibitory power in reducing total microbial counts lower than the permissible limits (not more than 1000 cfu/g, Tirumalai (2007$_{a\&b}$). These results are in agreement with those reported by Mimica-Dukic *et al.* (1993) and Imai *et al.* (2001) mentioned that peppermint essential oil exhibited very strong antibacterial and antifungal activity, in particularly against *Esherichia coli, Shigella sonei, Micrococcus flavus*

ATTC 10,240, *Trichophyton tonsurans* and *Candida albicans*. In addition, peppermint oil possesses antibacterial activity *in vitro*. Different commercial preparations exhibit various activities. The essential oil and its constituents displayed activity against *Escherichia coli*, *Helicobacter pylori*, methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*, *Pseudomonas* spp., *Enterobacter aerogenes*, and *Salmonella enteritidis* (Lis-Balchin and Deans, 1997; Osawa *et al*., 1999; Ezzat, 2001 ;Iscan *et al*., 2002).

Effect of cumin oil on microbial contamination of cosmetic samples

Data showed in Fig (7 and 8) revealed that total bacterial counts of shampoo-3, shampoo-9, shampoo-13, shampoo-129, shampoo-130, shampoo-134, shampoo-131, cos. gel-68 and cos.sol-28 decreased by 99.9, 99.7, 100.0, 100.0, 99.4, 99.9, 98.8, 99.7, 97.8%respectively with the addition of 0.25 mg /ml cumin oil and incubation for 7 days at 37±2 ^{o}C, compared to control. While, the tested cosmetic samples decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 98.5, 99.7and 97.2% respectively, with the addition of 0.5 mg/ml of cumin oil and incubation at the same previous

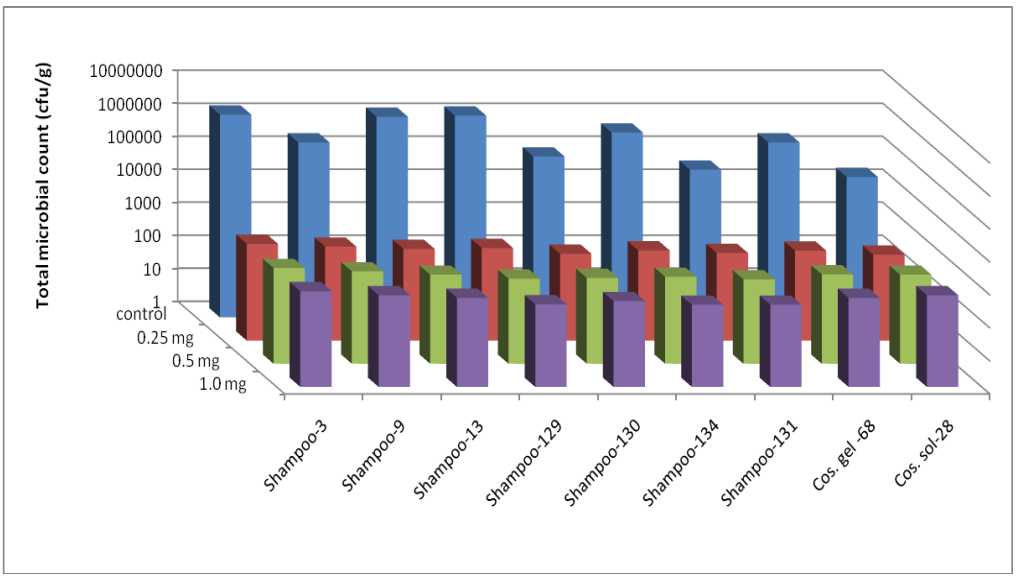

Fig. (7): Effect of cumin oil on microbial contamination of cosmetic samples.

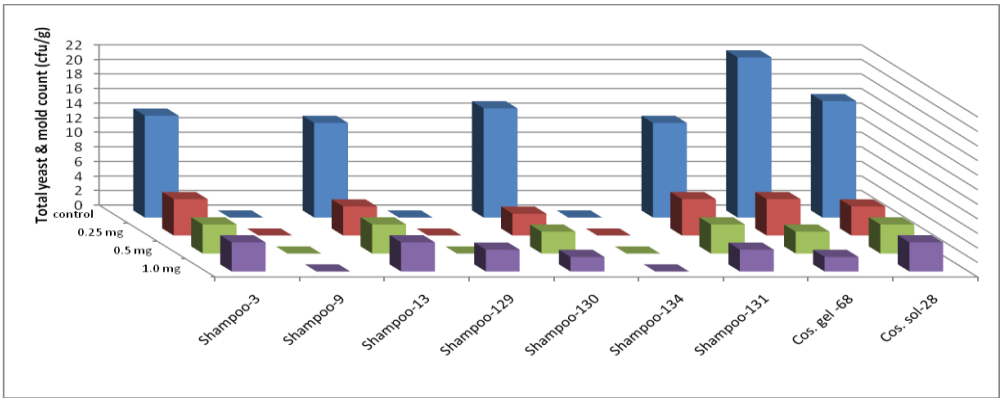

Fig. (8): Effect of cumin oil on yeast and mold contamination of cosmetic samples.

conditions .Total bacterial counts decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 99.0, 99.8 and 96.7% respectively, with addition of 1.0 mg/ml cumin oil and incubation at the same conditions.

Total yeast and mold counts of shampoo-3, shampoo-13, shampoo-130, shampoo-131, cos. gel-68 and cos.sol-28 decreased from 14, 13, 15, 13, 22 and 8 to 5, 4, 3, 5, 5 and 4 cfu/g, respectively at the same previous conditions Slight differences were detected among the various cumin oil concentrations on total microbial count of the various tested cosmetic samples after 7 days of incubation at 37±2 ^{o}C.

Data obviously indicate that using of different concentrations (0.25, 0.5 & 1.0 mg/ml) of cumin oil as a natural preservative in tested cosmetic samples gave dramatic decreases in total microbial counts of the various tested cosmetic samples, pathogenic bacteria were not detected at the end of incubation periods at 37±2 ^{o}C. In addition, slight differences were detected among the various tested cumin oil concentrations (0.25, 0.5 1.0 mg/ml) against microbial contamination at the same incubation period. Total microbial counts of the various tested cosmetic samples gradually decreased with increasing incubation period using the same concentration of cumin oil. The abovementioned data are in agreement with those found by Akgul (1989), who reported that 0.05 and 1% concentrations of black cumin essential oil had antibacterial and antifungal effects. Also, Farrag *et al.* (2000) found that the fixed cumin oil had an inhibitory effect against Gram positive bacteria such as *Staphylococcus aureus* and *B. cereus* and Gram negative bacteria. In addition, De *et al.* (1999) and Khan *et al.* (2003) pointed that the extract

from *Nigella sativa* seeds had antifungal activity against *Aspergillus parasiticus, Candida albicans* and *Saccharomyces cerevisiae*.The inhibitory effects of black cumin were previously determined against bacteria, yeasts and moulds by some researchers. Akgul (1989) reported that 0.05 and 1% concentrations of black cumin essential oil had antibacterial and antifungal effects. Hanafy and Hatem (1991) announced that the diethyl ether extract of *Nigella sativa* seeds (25 – 400 mg extract / disc) caused concentration dependent inhibition of *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*, (but not *Salmonella typhimurium*). Farrag *et al.* (2000) found that the fixed oil of black cumin had an inhibitory effect against Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* and Gram negative ones. Ozcan (1998), De-Billerbeck *et al.* (2001) and Khan *et al.* (2003) reported that the extract from *Nigella sativa* seeds had antifungal activity against *Aspergillus parasiticus, Candida albicans* and *Saccharomyces cerevisiae*. Arici *et al.* (2005) studied the antimicrobial activity of a series of 5 different cumin oils isolated from different types of Turkish black cumin plants against 24 pathogenic, spoilage and lactic acid bacteria. Results revealed that all tested oils showed antibacterial activity against all the bacteria used in the assay. Also, oils at 2.0% concentration were more effective than of the other concentrations. In additiom, the most sensitive bacterium against all of the tested oil concentrations was *Aeromonas hydrophila*, while the most resistant was *Yersinia enterocolitica*. In contrast, lactic acid bacteria had more resistance than pathogenic and spoilage bacteria against black cumin oils.

Effect of lemon grass oil on microbial contamination of cosmetic samples

Total bacterial counts of shampoo-3, shampoo-9, shampoo-13, shampoo-129, shampoo-130, shampoo-134, shampoo-131, cos. gel-68 and cos.sol-28 decreased by 99.9, 99.6, 99.9, 99.9, 99.4, 99.9, 98.2, 99.8 and 95.2% respectively with addition of 0.25 mg /ml lemon grass oil, compared to control. In addition, total bacterial counts of the same tested cosmetic samples decreased by 99.9, 99.8, 100.0, 100.0, 99.4, 99.9, 98.5, 99.8 and 97.2% respectively, with addition of 0.5 mg/ml of lemon grass oil (Fig 9).

While total bacterial counts of the same tested cosmetic samples decreased by 99.9, 99.7, 100.0, 100.0, 99.5, 99.9, 98.9, 99.8, and 97.9% respectively, with addition of 1.0 mg/ml lemon grass oil under the tested condition. Total yeast and mold counts of the same tested cosmetic samples decreased from 14, 13, 15, 13, 22 and 8 to 4, 3, 4, 5, 4 and 4 cfu/g, respectively, at the same previous conditions. (Fig. 10).

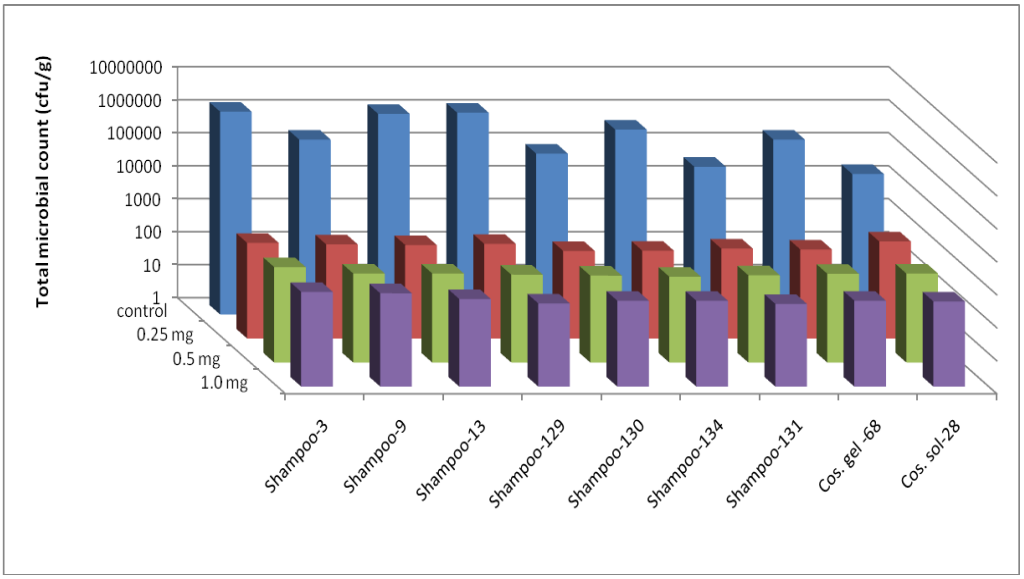

Fig. (9): Effect of lemon grass oil on microbial contamination of cosmetic samples.

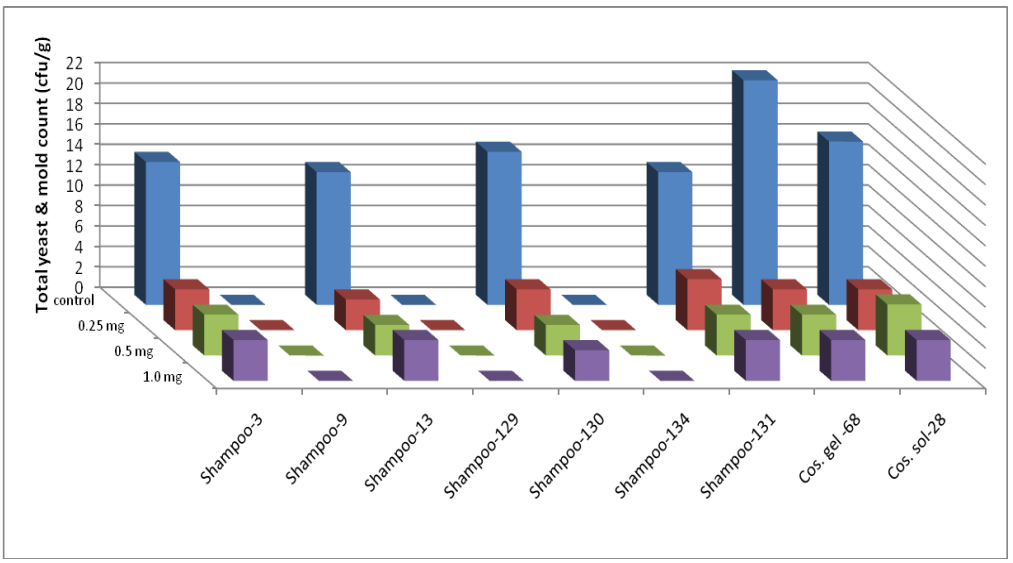

Fig. (10): Effect of lemon grass oil on yeast and mold contamination of cosmetic samples.

Addition of 1.0 mg /ml lemon grass oil decreased total bacterial counts by 70.0, 45.0, 22.3, 25.0, 30.8, 33.3, 31.8, 27.3 and 15.5% respectively.

Data obviously indicated that the using of different concentrations (0.25, 0.5 and 1.0 mg/ml) of lemon grass oil as a natural preservative in tested cosmetic samples gave dramatic decreased in total microbial counts of the various tested cosmetic samples. Slight differences were detected between 0.5 and 1.0 lemon grass oil in their activities against microbial contamination after the incubation at 37±2^{o}C.

Current results are in agreement with those found by Kirbaslar *et al.* (2009), who found that the lemon grass oil had antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067and *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* La 2971); Gram negative (*Escherichia coli* ATCC 11230, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427) and the pathogenic yeast (*Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432). On the contrary, Prabuseenivasan *et al. (*2006) reported that moderate effects were seen in lemon grass oil against *P. aeruginosa, B. subtilis, P. vulgaris, K. pneumonia and S. aureus.*

The obtained data in Table (2) indicateed that all pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella spp*.) not detected at the end of incubation periods at 37±2 ^{o}C when using of different concentrations (0.25, 0.5 & 1.0 mg/ml) of all tested oils as a natural preservative in the tested cosmetic samples.

Table (2): Detection of pathogenic bacteria in the selected cosmetic samples.

Cosmetic Samples	Pathogenic bacteria*														
	Lemon grass			Cumin			Peppermint			Chamomile			Thyme		
	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
Shampoo-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-129	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-130	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-131	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Shampoo-134	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cos. gel -68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cos-sol-28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

***Pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella spp*.**

CONCLUSION

From all abovementioned results, it could be concluded that using of tested medicinal plants oils (Lemon grass, cumin, peppermint, chamomile and thyme) as natural preservatives successfully contributed in decreasing microbial contamination of cosmetic products, instead of, chemosynthetic preservatives that have harmful side effects on human body.

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الملخص العربي

تأثير بعض الزيوت الطيارة علي الجودة الميكروبيولوجية لمستحضرات التجميل

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في هذا البحث تم دراسة تأثير بعض الزيوت الطيارة مثل (lemon grass, cumin, chamomile, green tea, peppermint, thyme and wheat germ) كمواد حافظة طبيعية بهدف تقييم الجودة الميكروبيولوجية لبعض مستحضرات التجميل ، حيث تم دراسة النشاط التثبيطي لهذه المستخلصات ضد بعض البكتريا المرضية المعزولة من هذه المستحضرات. و لقد أوضحت النتائج ان الزيوت العطرية المستخدمة لها تأثير تضادي عالي للبكتريا الممرضة ما عدا مستخلص الشاي الاخضر و زيت جنين القمح اللذان اظهرا نشاط تضادي منخفض. و اوضحت النتائج ايضا ان زيت حشيشة الليمون كان من اكثر الزيوت العطرية تأثيرا بالمقارنة بالزيوت الاخري ثم تلاه تنازليا زيت الكمون ، النعناع الفلفلي ، البابونج ، الزعتر علي الترتيب.

و عند إضافة هذه الزيوت بتركيز ٠.٢٥ مجم/ مليليتر حدث تثبيط حاد لأعداد الميكروبات الكلية و البكتريا الممرضة في هذه المستحضرات ، كذلك أوضحت النتائج ان معدل انخفاض الأعداد الميكروبية قد تأثر كثيرا بنوع الزيت العطري حيث أظهرت الزيوت العطرية الخاصة بحشيشة الليمون و النعناع الفلفلي و الكمون انخفاض معنوي في أعداد البكتريا المرضية.

وفي ضوء هذه النتائج يمكن التوصية باستخدام الزيوت العطرية الطيارة مثل (lemon grass, cumin, chamomile, green tea, peppermint, thyme and wheat germ) كمواد حافظة طبيعية كبديل للمواد الحافظة الكيميائية ، حيث انها اكثر امانا و ليس لها تأثيرات جانبية علي جسم الأنسان مقارنة بالمواد الحافظة الكيميائية.