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BIOCHEMICAL AND MICROBIOLOGICAL STUDIES ON SOME NATURAL AND SYNTHETIC PRESERVATIVES OF SOME COSMETIC PRODUCTS

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ABSTRACT

Some natural preservatives (thyme and clove essential oils, their basic constituents thymol, carvacrol and eugenol) as well as synthetic preservatives (glydant, methyl paraben and their mixture in a ratio of 2:1,v/w) were added to gram-negative bacteria (*Escherichia coli, Pseudomonas fluoroscens and Serrattia marcescens*) and gram-positive bacteria (*Staphylococcus aureus, Micrococcus* spp. and *Bacillus cereus*) to evaluate their anti-bacterial activities as model systems.The aforementioned materials were also added to a sterilized shampoo to demonstrate their activity against the growth of *Bacillus cereus* as natural systems. The physico-chemical constants and the volatile constituents of thyme and clove essential oils were studied. Two methods were used for the measurements of bacterial activity, *i.e.*, total bacterial counts and disc diffusion. The results showed that the highest contamination with bacteria was seen for shampoo (with no added preservative) followed by toothpaste and cold cream prepared under laboratory conditions. In general, the effectiveness of different preservative materials towards the inhibition of various bacterial growth showed that glydant and thyme had the strongest anti-bacterial effect among the other preservatives. Also, the results of this set of experiments recommend the addition of thyme as a natural preservative as opposed to synthetic preservatives because of their potential side

effects on human health. The mode of preservative action against the bacterial growth is discussed.

***Key words**: anti-bacterial activity, cosmetic products, gram-positive and negative bacteria, natural and synthetic preservatives.*

1. INTRODUCTION

During the last two decades, cosmetic products have been developing rapidly. The chemical industry introduced unlimited new basic and active substances and every year a further development appears. The quantity and quality of biological materials used for cosmetic formulation are also steadily improving (Baran and Maibach, 1998). However, the Food and Drug Administration (FDA) classifies cosmetic ingredients according to their degree of toxicity and prohibits the use of certain ingredients proven to be unsafe for use on humans. In some perfumed preparations the scent is soon accompanied by the rancid odor. The rancid cosmetic preparations that contain rancid ingredients act as skin irritants (Harry, 1982). The presence of heavy metal traces such as copper and iron, exposure to light, free fatty acids.... etc. effectively promotes rancidity (Allen *et al.* 1979 and Farag and Bassiuny,2002). Also, certain micro-organisms induce ketonic rancidity which cause skin diseases as well as unpleasant body odors (Van Cleef and Arpels, 1982). There are several means to prevent rancidity including: avoidance of contact of cosmetic products with air or heavy metals, packing in coloured containers and the addition of preservatives (Harry, 1982).

The search for and the development of preservatives are therefore highly desirable. These compounds are added to cosmetic products to protect against residual contamination introduced from new materials and during manufacture and to protect the cosmetic products against microbial contamination during use (Duke, 1978). However, any preservative ought to be non-toxic, not irritant or induce sensitizing effect at the concentrations used on skin and mucous membrane. In the case of oral administration, the preservatives must not induce deleterious effect on the gastrointestinal system (Harry, 1982). In addition, these compounds have to tolerate heat at prolonged storage period and free from gross incompatibility with other ingredients in the cosmetic formula and with the packing materials(El-Wakeil *et al.*,1986).

There are several chemicals which act as antimicrobial agents For instance, BHA and BHT are added to Brut lotion and cream body lotion (Faberge, 1987 and Van Cleef and Arpels, 1982). These synthetic chemicals convert some of the ingested material into toxic or carcinogenic substances by the increase of microbial enzymes (Johnson and Cort, 1985 and Wurtzen *et al.*, 1986). Consequently, alternative preservatives are needed which possess antimicrobial activity and in the mean time cause no health problems to the handler and consumer. In recent years, the use of certain plant products as preservatives has shown encouraging results (Farag *et al.*, 1989a, 1989b, and 1990). The main objective of the present work was to demonstrate whether the natural preservatives could provide a more potent effect than the synthetic ones. In other words, the essential oils of thyme and clove plants and their basic compounds (thymol, carvacrol and eugenol) were added individually to some cosmetic products (shampoo, cream and toothpaste) instead of the synthetic preservatives glydant (1,3-bis-hydroxy methyl-5, 5- dimethyl hydantoin) and methyl paraben (methyl-p- hydroxy benzoate) and a mixture of both in a ratio of 2: 1 (v/w) to compare their effectiveness on the growth of some bacteria as the main cause of cosmetic spoilage.

2. MATERIALS AND METHODS

2.1.Sources of essential oils

Thyme (*Thymus vulgarius*, labiatae) and clove (*Eugenia caryophllus*, Myrtaceae) essential oils were obtained from Kato-Aromatic company, Egypt. These oils were obtained by steam distillation of leaves, flowers and buds of thyme and clove plants, respectively. Carvacrol (isomer of thymol), thymol and eugenol were obtained from Conroth-GmbH company, Germany.

2.2.Sources of synthetic preservatives

Glydant (1,3-bis- hydroxy methyl-5,5-dimethyl hydantoin) and methyl paraben (methyl-p- hydroxy benzoate) were purchased from the International Specialty Products (ISP) Company (Surrey Research Park, Guildford, Surrey, England (GU 5 YF). Glydant and methyl paraben mixture in a ratio of 2:1 (v/w) are designated in the text as a synthetic mixture.

2.3.Preparation of some cosmetic products

The ingredients of balsam shampoo (brut 33), toothpaste and cold cream are presented in Table (1). The preparations of the aforementioned cosmetic products were followed as reported by Faberge (1987), Harry (1982) and Ernest (1989), respectively.

Table (1): Ingredients of balsam shampoo, cold cream and toothpaste.

Ingredient	Concentration (g%)
Balsam Shampoo	
Soduim lauryl sulfate	25.00
Lauramide diethanolamine	8.00
Propylene glycol	1.70
Citric acid	0.25
Methyl paraben	0.20
Polyethylene glycol-10- sorbitan laurate	5.70
Menthol	0.01
1,3- Bis-(hydroxy methyl)- 5,5- dimethyl hydantoin	0.30
Perfume (Brut oil N 7-59)	0.79
Deionized water	58.05
Toothpaste	
Methyl paraben	0.10
Hydroxy propyl methyl cellulose	1.00
Glycerine	15.00
Calcium carbonate	25.00
Saccharin	0.10
Amino flouride	0.10
Sodium lauryl sulphate	1.50
Spearment	0.80
Menthol	0.20
Deionized water	56.20
Cold Cream	
White mineral oil	50.00
Bees wax	16.67
Borax	0.83
Methyl paraben	0.20
Perfume	0.30
Deionized water	32.00

2.4.Some physico-chemical properties of thyme and clove essential oils

Some physical (refractive index and specific gravity) and chemical (acid value) constants were determined using the standard American Oil Chemists Society methods (A.O.C.S. 1990).

2.5.Chemical composition of thyme and clove essential oils

The essential oils of thyme and clove were analyzed by a GCV Pye Unicam gas chromatograph (GC) equipped with dual flame ionization detector and dual channel recorder. The chromatograph was fitted with a coiled glass column (1.5 m X 4mm) packed with diatomite C(100-120 mesh) and coated with 10% PEGA. The column oven temperature was programmed at 4^0 C/min from 60° C to 180^0 C for 30 min. Detector and injector temperatures were 220^0 C and 300^0 C, respectively.

An aliquot from the essential oil (ca. 0.1 µl) was injected without any dilution with gases flow rates of 30 ml/min., 33 ml/min and 330 ml/min. for N_2, H_2 and air, respectively. Peak identification was performed by comparing the relative retention time of each peak with those of the reference compounds. Also, the essential oils were mixed with their major compounds and injected again into GC in order to verify the peak identity. Quantitative results were obtained by using Philips PU 4810 computing integrator. All samples were analyzed in triplicates and the mean values are presented in Table (2).

2.6.Microbiological examination

2.6.1.Bacteria and antibacterial activities

Three microbes representing gram- negative bacteria (*Escherichia coli, Pseudomonas fluorescens and Serrattia marcescens*), and three gram- positive bacteria (*Staphylococcus aureus, Micrococcus spp* and *Bacillus cereus*) were used in the present study. All strains were obtained from the Microbiology Department, Faculty of Agriculture, Cairo University. These micro-organisms were checked for purity and identity. All strains were grown in a nutrient agar medium (tryptone, 5 gm; yeast extract, 2.5 gm; glucose, 1.0 gm and agar, 15 gm/L) and adjusted to pH 7.

The disc diffusion method was used to measure the antimicrobial activity (Wurtzen *et al.* 1986) of thyme, carvacrol, clove, glydant, methyl paraben and the synthetic mixture. Plates were provided with filter paper discs (7mmΦ) previously autoclaved, dried and immersed in

the neat oil and the tested materials diluted to half its concentration using ethanol (1:2, v/v). Inoculated plates were incubated at 30^0 C for 24-48 h and the inhibition zones of the microbial growth produced by the tested materials were measured in mm.

Table (2): Volatile components of thyme and clove essential oils.

Component	Clove		Thyme	
	RRT *	%	RRT *	%
α-pinene			0.08	1.10
β-pinene			1.10	0.30
Limonene			0.20	0.30
γ-terpinene			0.13	0.10
Phellendrene			0.17	1.50
P-cymene			0.27	36.00
Caryophyllene	1.08	10.20		
Eugenol	1.00	80.60		
Thymol			1.00	42.20
Thujone			0.41	0.70
Borneol			0.52	0.70
Linalayl acetate			0.19	1.00
Terpinyl acetate			0.16	0.10
Eugenol acetate	1.26	3.40		16.00
Unidentified compounds		5.80		0.00

*RRT refers to the relative retention time of the basic compound for each essential oil and is given a value of 1.0.

2.6.2.Viability of *Bacillus cereus* in shampoo

Appropriate amounts of the thyme and clove essential oils, carvacrol and the synthetic mixture were added to a sterilized shampoo (20 ml) to obtain final concentrations of 0.06, 0.12 and 0.24 ppm. *Bacillus cereus* was inoculated at a level of about 10^5 cells / ml and stored at $30^0C \pm 1^0C$. Samples of the aforementioned materials were microbiologically examined after 1day, 2 days and 7 day intervals.

3.RESULTS AND DISCUSSION

Thyme and clove essential oils, their main components (thymol, carvacrol and eugenol, respectively) and some synthetic preservatives (glydant, methyl paraben and the mixture of them) were added individually to various cosmetic products (shampoo, cream and toothpaste) in order to evaluate their anti-bacterial effect.

3.1.Some physico-chemical properties of thyme and clove essential oils

The most important physical and chemical characteristics of thyme and clove essential oils were determined. Clove oil was characterized by laevo rotation (-0.30) while thyme oil possessed dextro rotation (+5.32). The specific gravity and refractive index values of clove oil (1.1268 and 1.5449) were higher than that of thyme oil (0.9549 and 1.4942). The acid values were 1.50 for thyme oil and 1.69 for the clove oil. All the aforementioned results are in agreement with the data reported by Farag *et al.* (1986) and Katsiotis and Iconomou (1986).

3.2.Chemical composition of essential oils

The essential oil compositions of thyme and clove were identified by gas-liquid chromatography against standard compounds and the results are shown in Table (2). The identified materials represented 93.9% and 100% of the compositions of clove and thyme oils, respectively. The lack of certain standard volatile compounds did not allow the complete identification of the essential oil composition.

The concentrations of various essential oil components were classified into 3 categories, *i.e.*, major (>10%), minor (>1-<10%) and trace (>1%). Thyme oil contained thymol (42.20%) and p-cymene(36.00%) as the major compounds. α-pinene and phellendrene were present as minor compounds while β - pinene, limonene, γ - terpinene, thujone, borneol, linalyl acetate and terpinyl acetate occurred as trace compounds. Clove oil contained two basic components, *i.e.*, eugenol (80.60%) and caryophyllene (10.20%) and one minor constituent (eugenol acetate, 3.4%). The results of the composition of the essential oils under investigation are in accordance with other reports (Farag *et al.*, 1986 and Katsiotis and Iconomou, 1986). Also, Daouk *et al.* (1995) found that the major compounds of the essential oil of zaater plant (*Origanum syriacum* L.) were carvacrol and thymol. The composition of these oils were found to vary with geographical origin, climatic conditions, stage of plant maturity (Franz *et al.*, 1984 and Putievsky and Ravid, 1984).

3.3.Microbiological examinations

3.3.1.Total bacterial counts of some cosmetic products

The total bacteria present in cream, toothpaste and shampoo prepared under laboratory conditions without adding any preservatives

and stored at room temperature ($30^0C \pm 1^0C$) are given in Table (3). No bacterial growth was detected after 2 and 4 weeks of storage for shampoo and toothpaste, respectively. At the beginning of this set of experiments, the highest contamination with bacteria was seen for shampoo (8.0×10^5) followed by toothpaste (2.6×10^5) then cream (1.1×10^4). It seems that the ingredients of shampoo had no obvious effect on the inhibition of bacterial growth at the beginning and after 1-week storage period. On the other hand, the ingredients of cream possessed antibacterial activity. This set of experiments demonstrated that a preservative agent has to be added to the ingredients of cosmetic products to prevent bacterial growth.

Table (3): Total bacterial counts of cream, toothpaste and shampoo without adding preservatives and stored at room temperature ($30^0C \pm 1^0C$).

Incubation period (week)	Cream	Toothpaste	Shampoo
Zero	1.1×10^4	2.6×10^5	8.0×10^5
1	4×10^3	1×10^4	4.9×10^4
2	3.5×10^3	1.2×10^3	ND
4	1.2×10^3	ND	ND

ND refers to not detected bacterial growth.

3.3.2. Influence of some natural and synthetic materials on the growth of various bacteria (model systems)

Preliminary screening was performed on the antibacterial activities of thyme, carvacrol, clove, glydant, methyl paraben and their mixture against three gram- negative bacteria (*Escherichia coli*, *Pseudomonas fluorescens* and *Serrattia macrcescens*) and three gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus* spp. and *Bacillus cereus*) and the results are shown in Table (4).

The natural preservatives (thyme, carvacrol and clove) always possessed inhibition zones greater than of the diluted ones on *E. coli* growth. Thyme and carvacrol had nearly the same effect. One would expect to achieve this finding since carvacrol is the isomer of thymol the basic constituent of thyme oil. In general, thyme and carvacrol

exhibited potent effect on *E. coli* growth than the synthetic preservatives either alone or in a mixture. It is worth to mention that the synthetic mixture possessed an additive effect on *E. coli* growth and not a synergistic behavior. The effectiveness of both undiluted and diluted natural and synthetic compounds towards the inhibition of *E. coli* growth followed the sequence: thyme > carvacrol > glydant > synthetic mixture >methyl paraben > clove.

The effect of natural and synthetic compounds on the *P. fluorescens* growth raise the following points. The natural preservative compounds did not show any obvious effect towards the prevention of *P. fluorescens* from growth. On the contrary, the synthetic ones had a profound inhibitory effect against *P. fluorescens* growth. In addition, glydant possessed about 3-fold inhibitory effect as that produced by methyl paraben. The synthetic mixture exhibited a strong inhibitory effect on *P. fluorescens* growth and the effect of this mixture was additive. In connection with *Serrattia marcescens*, glydant possessed the most powerful effect and induced an inhibitory effect about 2.3 times that produced by methyl paraben. In general, the synthetic preservatives induced more inhibitory effect than that of the natural preservatives. For instance, glydant produced an inhibitory effect on *Serrattia marcescens* growth being about 1.2 times as great as that of thyme or carvacrol.

It is worth mentioning that the synthetic compounds produced more inhibitory effect on *Micrococcus* spp. growth than that of natural compounds. For instance, glydant produced an inhibitory effect of about 1.16, 1.20 and 2 times as great as that of thyme, carvacrol and clove, respectively. On the other hand, the results for diluted natural compounds demonstrated that thyme exhibited the most potent activity. The effectiveness of different materials under study towards the inhibition of *Micrococcus* spp. growth can be arranged in the following sequence: glydant >thyme> carvacrol>synthetic mixture> clove > methyl paraben. The efficiency order of the natural and synthetic materials against *Staphylococcus aureus* growth was as follows: glydant > synthetic mixture > thyme > carvacrol >clove >methyl paraben.

Regarding the inhibition of *Bacillus cereus* growth, the order of arrangement is as follows: carvacrol > thyme > glydant > synthetic mixture > clove > methyl paraben. Generally speaking, the most powerful compounds against the microbial growth were glydant and thyme whereas the least potent materials were methyl paraben and

Table (4) :*Bacillus cereus* counts in shampoo samples containing different concentrations (mg / ml) of thyme carvacrol , clove and synthetic mixture.

Incubation period (Day)	Control	Thyme			Carvacrol			Clove			Synthetic mixture*		
		60	120	240	60	120	240	60	120	240	60	120	240
Zero	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$
1	$3.0x10^5$	$8.8x10^4$	$4.3x10^4$	$4.3x10^4$	$2x10^4$	$9.7x10^4$	$9.0x10^4$	$8.5x10^4$	$1.2x10^4$	$8.0x10^4$	$3.0x10^3$	ND**	ND**
2	$2.0x10^5$	$6.0x10^4$	$5.0x10^4$	$5.0x10^4$	$9x10^4$	$9.0x10^4$	$8.0x10^4$	$7.0x10^4$	$6.0x10^4$	$6.0x10^4$	ND**	ND**	ND**
7	$1.5x10^5$	$8.0x10^4$	$2.5x10^4$	$2.5x10^4$	$3x10^3$	$8.0x10^4$	$6.0x10^4$	$5.0x10^4$	$2.0x10^4$	$1.0x10^4$	ND**	ND**	ND**

*Synthetic mixture comprised of glydant and methyl paraben at 2:1 ratio (v/w).

**ND refers to not detected *B. cereus* growth.

Table (5): Inhibition zones (mm) of microbial growth in a media containing some natural and synthetic preservatives.

Micro-organism	Thyme		Carvacrol		Clove		Glydant		Methyl paraben		Synthetic mixture	
	a	b	a	b	a	b	a	b	a	b	a	b
				Gram-negative bacteria								
Eschrichia coli	41	34	40	32	19	17	37	26	22	21	31	27
Pseudomona fluorescens	T	0	T	0	T	0	46	32	15	14	35	30
Serrattia marcescens	30	22	29	26	17	12	35	10	15	14	38	37
				Gram-positive bacteria								
Micrococcus spp	50	42	45	36	22	17	60	52	22	20	42	39
Staphyllcoccus aureus	30	20	30	27	18	13	47	33	10	9	40	33
Bacillus cereus	51	45	55	41	27	23	41	30	14	14	33	28

a and b represent the neat and diluted substances to half its original concentration in ethanol.

Synthetic mixture indicates a mixture of glydant and methyl paraben in 2: 1 ratio (v/w).

T indicates trace inhibitory effect.

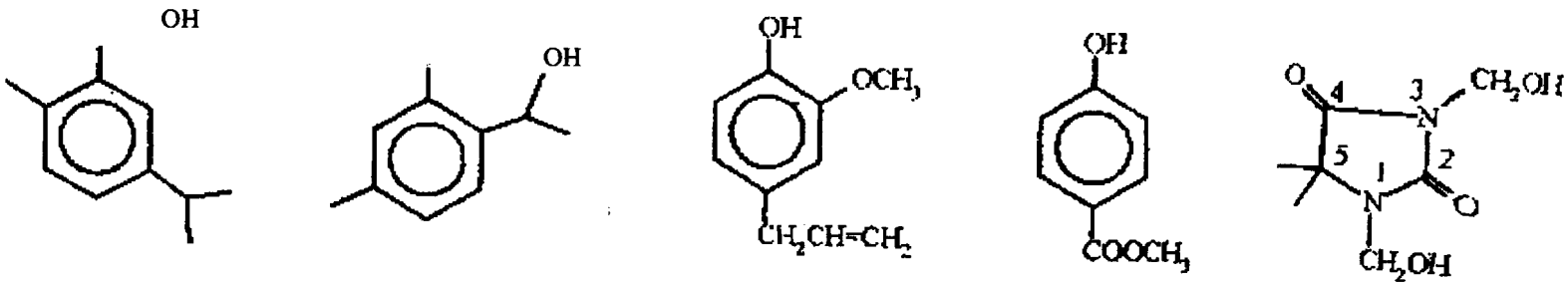
clove. Generally, the data (Table 4) demonstrate that gram-positive were more sensitive than the gram-negative bacteria.

According to the disc diffusion method, the sequence of tolerance of the gram-negative bacteria is in the descending order:glydant > synthetic mixture > thyme > carvacrol > methyl paraben > clove. The tolerance for gram-positive bacteria is in the decreasing order :glydant > thyme > carvacrol> synthetic mixture > clove > methyl paraben. In general, thyme, carvacrol and glydant had strong potent antibacterial effect. The results of this set of experiments recommend the addition of thyme or carvacrol as opposed to synthetic materials (glydant and methyl paraben) because of possible harmful side effects on humans.

3.3.3. Influence of some natural and synthetic substances on *Bacillus cereus* growth (natural systems)

In this set of experiments thyme, carvacrol, clove (natural preservatives) and the synthetic mixture were added at 60, 120 and 240 mg/ml to a nutrient agar medium containing *B. cereus*. The later bacterium was selected in this set of experiments since it is widely present in most cosmetic products, (Harry, 1982). The results for the bacterial inhibition zones produced by different preservatives are shown in Table (5).

For simplicity, the bacterial counts after 7 days were taken as an indicator for the efficiency of different natural and synthetic materials. In general, the data show differing degrees of inhibition by using various preservatives at 60 mg/ml against *B. cereus* growth. For instance, the counts of this bacterium after 7 days for control shampoo (without any preservatives) and shampoo containing thyme, carvacrol, clove and synthetic mixture were 1.5×10^5 and 8.0×10^4, 3×10^3, 5.0×10^4 and not detected, respectively. These results show that the synthetic mixture completely suppressed the *B. cereus* growth after 7 days of incubation. The degree of effectiveness of the used preservative materials against *B. cereus* growth can be arranged in the following order: synthetic mixture > clove > thyme > carvacrol > control. The present data show that as the level of any preservative increased the bacterial counts decreased.



Carvacrol | Thymol | Eugenol | Methyl-p-hydroxy benzoate (Methyl paraben) | 1,3 bis-hydroxy methyl-5,5-dimethyl hydantoin (Glydant)

Fig. (1): Chemical structures of some natural and synthetic preservatives.

3.4.Structure activity relationship

It appears that there is a relationship between the chemical structures of the tested natural and synthetic preservatives and the anti-microbial activity. Generally, the extent of the inhibitory effects of these compounds could be attributed to the presence of a cyclic nucleus containing hydroxy groups. The wide spread use of phenol and chlorophenols and related compounds as disinfectants is well established. It is well known that the OH group is quite reactive and easily forms hydrogen bonds with the active sites of target enzymes. In this respect, Chandravadans and Nidiry (1994) studied the antifungal activity of geranium oil and its major constituents (linalool, geraniol and citronellol) against *Colletotrichum glesporuides*. Geraniol and citronellol were more active (100% alcohols inhibition) than geranium oil (60% inhibition) at a concentration of 250 ppm while linalool (isomer of geraniol) showed considerably lower activity causing only 8% inhibtion. An examination of the structures of constituents revealed that geraniol and cirtronllol are primary alcohols while linalool is a tertiary alcohol. This suggests that the primary alcoholic group may be necessary for the antifungal activity.

The highest inhibitory effect of glydant (1,3- bis-hydroxy methyl-5,5- dimethyl hydantoin) might be due to the presence of two hydroxyl groups, whereas the other compounds contain only one hydroxy group. The high inhibitory action of thyme and its isomer (carvacrol) might be due to the presence of phenolic OH group. It seems that the second substituent on the aromatic nucleus affect the antimicrobial activity. For instance, the presence of electron donating group at ortho position (alkyl) increases the possibility of OH to form hydrogen bond with the active sites of the enzymes and consequently increases the antibacterial activity. On the other hand, the presence of a substituent at the para position (electron withdrawing group, carboxyl in methyl paraben) decreases the ability of OH to form hydrogen bond with the active sites of enzymes and hence decreases the microbial inhibitory effect (Fig.1).

Farag *et al.* (2001) evaluated the safety of some synthetic preservatives {germaben Π (diazolidinyl urea, 30%,methyl paraben 11%, propyl paraben 3%, and propylene glycol 56%) and euxyl k 100 (1,2-dibromo-2,4-dicyano butane) } by determining the activities of some enzymes in rat liver and kidney. Also, they examined the clastogenic effects of the aforementioned compounds on somatic cells. They found that these synthetic preservatives affected the activities of

some liver enzymes. In addition, euxyl k 100 induced the highest chromosomal aberrations followed by germaben II and this effect was much higher than that of some natural preservatives (thyme white oil, thyme red oil and tea tree oil). The results of the present work and the data of Farag *et al.* (2001) highlight the importance of substituting the synthetic preservatives by natural ones for human health safety.

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تأثير إضافة بعض المواد الحافظة الطبيعية والمخلقة لبعض مستحضرات التجميل

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ملخـــص

تم في هذا البحث دراسة تأثير إضافة بعض المواد الحافظة الطبيعية (الزيت العطري للزعتر والقرنفل ومكوناته الأساسية من الثيمول و الكارفاكرول و الإيجينول علي التوالي) و بعض المواد الحافظة المخلقة (جليدانت و ميثيل بارابين ومخلوط منهما بنسبة ١:٢ وزن/حجم) إلى أنظمة محتوية على مستحضرات التجميل والمضاف أليها أنواع من البكتيريا الموجبة و السالبة لجرام لتقييم نشاطها كمضادات بكتيرية. كما أضيفت هذه المواد إلى عينة شامبو معقمة لدراسة تأثيرها على نمو بكتيريا باسيلس سيرياس . تم دراسة بعض الثوابت الطبيعية والكيماوية والمكونات المتطايرة للزيت العطري لكل من الزعتر والقرنفل و قياس النشاط البكتيري للأنظمة تحت الدراسة بطريقتي العد البكتيري و تشرب القرص.

أوضحت الدراسة أن أعلى تلوث بكتيري ظهر في نظام الشامبو(بدون آي إضافات) يليه نظام معجون الأسنان ثم نظام الكريم وهذه الأنظمة تم تحضيرها تحت الظروف المعملية . وقد أظهرت النتائج أيضاً أن الجليدانت والزيت العطري للزعتر كان لهما أقوى تأثير كمضادات لنمو البكتيريا . ولذلك توصي الدراسة بإضافة زيت الزعتر كمادة حافظة طبيعية لمستحضرات التجميل لعدم وجود أي آثار جانبية له على صحة الإنسان.

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