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Effect of Some Plant Extracts on Isolated Bacteria from Eyelids of Natural Eye liner Users and Eye Cosmetics Users

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ABSTRACT

This study includes isolation of bacteria from eyelids of ten females use eye cosmetic (A) and ten from those use natural eyeliner (B) in addition to those do not use any things (C: male (3) and female (7)) by swab taken from students of college of Education/ Scientific Dept.-Biology Dept. The bacteria identified depending on cultural, morphological and some biochemical properties and according to their identity they included (*S. aurus* and *S. epidermidis* where the percentage of each of them was 40%, 40% and 20% for group A, B and C respectively and *S. capitis* was 30%, 10% and 60% for group A, B and C), while the Gram negative isolates represented with *E. coli* where the percentage was 70% and 30% for group A and B. Antibacterial activity of nine aqueous plant extracts was tested against twenty selected isolates.. All isolates did not show any susceptibility against extract of ginger and less susceptibility against nutmeg and senna. While all isolates showed moderate sensitivity against the extract of coriander. The antibacterial activity of each of fennel, cubeb and turmeric had a strong effect against the isolates, while black tea and black dry lime extracts showed more strongly effect against growth of isolates. The results showed that *E. coli* isolates were more resistance against the most extracts and in different concentrations comparing with other isolates.

INTRODUCTION

Eye makeup has long been used to enhance personal appearance, to improve self-esteem or attract the attention of others. Among the commonest causes leading to eyelid dermatitis, cosmetics would be playing a major role. Cosmetics used as eyeliners, eye shadow, mascara, eyelash curlers, eye makeup removers…etc could all contribute to eyelid Blepharitis. Researchers showed a 67% to 100% bacterial contamination in the samples, notably Staph, Strep and even *E. coli* bacteria. In addition to misuse it, which is using kohl or eyeliner more than one person or may leave these eyeliners exposed to the air accumulate the dust particles laden contaminate with microbes and fungi and transmitted to the eye while using these eyeliners (Al-Aany *et al*., 2009). Medicinal plants are an important therapeutic aid for various ailments. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Dahanukar *et al*., 2000, Nair and Chanda, 2006).

In many developing countries traditional medicine is one of the primary health care systems. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Satish *et al*., 2008). Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments (Joy *et al*., 1998). The antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that in order to find active compounds, a systematic \study of medicinal plants is very important (Akin *et al*., 2010).

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The genus Piper of family Piperaceae, with over 1,000 species, is distributed in both hemispheres. *Piper cubeba* Linn., commonly known as cubeb. The fruits of this plant are used as a spice and have medicinal value, being often used for the treatment of many diseases (Aneja *et al*., 2010).

Fennel (*Foeniculum vulgare Miller)*, a plant belonging to the family Apiaceae, has a long history of herbal uses. Traditionally, fennel seeds are used as anti-inflammatory, analgesic, carminative, diuretic and antispasmodic agents. Recently there has been considerable interest in the antioxidant potential and antimicrobial activities of fennel seed extracts (Anwar *et al*., 2009). It is also used as a constituent of cosmetic and pharmaceutical products (Shahat *et al*., 2011). Tea (*Camellia sinensis* L.) has been shown to have a wide range of beneficial physiological and pharmacological effects. In one of the earliest reports, an army surgeon recommended the use of tea in soldiers' water bottles as a prophylactic against typhoid (Hamilton-Miller, 1995). Ginger (*Zingiber officinale Rose*) has a long history of medicinal use dating back 2500 years in China and India for conditions such as headaches, nausea, rheumatism and colds. Ginger is used as a food seasoner, and flavouring material in the food, cosmetics and pharmaceutical industries. Ginger had antibacterial activity against respiratory tract Pathogens (Tahereh and Mahsa, 2010).

Coriander (*Corianderum sativum*) seeds have a health-supporting reputation that is high on the list of the healing spices. It has traditionally been referred to as an anti-diabetic anti-inflammatory and recently been studied for its cholesterol-lowering effects. In addition, it is also used as carminative, diuretic, tonic, stimulant, stomachic, refrigerent, aphrodisiac and analgesic (Chaudhry and Tariq, 2006).

Citrus aurantifolia (Lime fruit) is very much employed in herbal medicine. It is an essential ingredient in the preparation of most herbal concoction; it is also used to suppress stomachache the juice has been found to be an excellent cough relieving mixture (Aibinu *et al*., 2007).Turmeric (*Curcuma longa* Linn.), a plant of the family Zingiberaceae, grown mainly in Thailand, has been used in Thai herbal medicine for the treatment of various skin diseases.

There are several reports indicating a variety of pharmacological uses of turmeric, including: as an antioxidant, anti-protozoal activity, anti-microbial activity (Lawhavinit *et al*., 2010). *Myristica fragrans* Houtt (nutmeg) is one of the plants commonly found in Asian medicinal ingredientsM. fragrans extract has been shown to contain antibacterial activity against different genera of bacteria and antiviral activity against rotavirus (Chirathaworn *et al*., 2007). Senna (*Cassia angustifolia*) is one of plants mainly cultivated in Arab Saudi Arabia, Egypt, Sudan, Yemen and India, and it has a strong activity against bacteria and fungi (Mahalingam *et al*., 2011). The aim of current study is to use some plant extracts to reduce the bacteria reached to eye or around area through applying the cosmetic by contaminated hands or by contaminating the area around eyes with dust.

MATERIAL AND METHODS

Bacteria under study

Isolation the normal flora microorganisms from eyelid of 30 persons (includes 10 eye cosmetic users (group A), 10 natural eyeliner (natural alkohl) users (group B), and 10 control (group C: don't use anything of male (3) and female (7)) by swab. Then cultured by streaking method on (nutrient agar, blood agar and MacConkey agar), and were incubated for 24, 48 hours at 37℃, different colonies were obtained; these were identified depending on cultura (for Staphylococci in addition to mentioned cultures Manitol Salt Agar was used; for *E. coli* EMB Agar was used as differential media), morphological (Gram stain) and some biochemical tests (catalase, oxidase, IMViC).

PLANT EXTRACTION

Collection and preparation of plant samples

The plants (bitter fennel, black tea, ginger, turmeric, nutmeg, coriander, cubeb, dry black lime and senna) were obtained from market in Erbil city, then were washed with tap water, then with distill water, then left for air drying until become completely drying, after drying the plants converted into powder form and stored in polyethylene sacks in refrigerator at 4^0C for further process.

Extracts preparation

150 ml of sterilized distilled water was added to 15 g of ground dried plant, heated below the boiling point and stirred for 2 ½ - 3 h. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1) and then stored in the refrigerator at 5 ◦C for using (Al-Neemy and AL-Jebury, 2006, Babpour *et al*., 2009).

Preparation of inoculums

Two to three colonies from pure growth of each tested organism were transferred to (5) ml of nutrient broth. Broths were incubated overnight at 37 ºC. The suspension was diluted with sterile distilled water to obtain approximately $1*10^6$ CFU/ ml (Alhaj *et al*., 2008).

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique (Kivanc and Kunduhoglu, 1997). The Nutrient agar (NA) plates were seeded with (0.1) ml of the inoculums of each tested organism. The inoculums were spread evenly over plate with loop. A standard cork borer of (8) mm diameter was used to cut uniform wells on the surface of the NA and (100) µl of each concentration of plant extracts was introduced in the well, the plates were incubated for 24 hours at 37 ºC, and the zones of inhibition was measured to the nearest millimeter (mm).

Agar dilution method (determination of MIC)

The agar dilution method followed that approved by the NCCLS with slight modification. Briefly a series dilution of each

extract ranging from (0.025%, 0.05%, 1% (v: v) to 10% (v: v)) was prepared in nutrient agar. After solidification of media the plates were inoculated with bacterial suspension. Inoculated plates were incubated at 37 ºC for 24 h. Minimum inhibitory concentrations (MICs) were determined after 24 h., as lowest concentration of extract inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded (Hammer *et al*., 1999). All experiments were applied in triplicates.

RESULTS AND DISCUSSION

The bacteria isolated from eye lids in this study were (*S. aurus* and *S. epidermidis* where the percentage of each of them was 40%, 40% and 20% for group A, B and C respectively and *S.capitis* was 30%, 10% and 60% for group A, B and C respectively), while the Gram negative isolates represented with *E. coli* where the percentage was 70% and 30% for group A and B, in addition to obtain the fungi that identified presumptively (preliminary) as *Candida* (only depending on cultural characteristics on nutrient agar) as in Table (1). Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. Approximately 80% of the word inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. The potential of higher plants as a source of new drugs is still largely unexplored; hence last decade witnessed an increase in the investigation on plants as sources of new biomolecules for human disease management. And the use of plant extract to treat infectious diseases has been extensively applied by people (Ibrahim *et al*., 2011). The antibacterial activity of nine aqueous plant extracts was tested against selected bacterial isolates by presence or absence of inhibition zone and measuring the diameter of inhibition zone around the well. Table (3) reveals that all isolates did not show any susceptibility against aqueous extract of ginger at the serial concentrations used in current study and very less susceptibility against nutmeg and senna at 100%. While Table (3) reveals moderate activity of coriander against the twenty tested isolates. On the other hand the antibacterial activity of each of fennel, cubeb and turmeric was strong against the isolates, while black tea and black dry lime extracts showed more strongly effect against growth of isolates. These results suggesting that antibacterial activity of plant extracts against isolates was decreasing when used in lower concentrations, and showed that E. coli isolates were more resistance against the most extracts and in different concentrations comparing with other isolates. Table (4) shows the antibacterial activity of plant extracts represented as MIC against isolates. The results of non activity of ginger and low activity of nutmeg agree with that of other authors (Nanasombat and Lohasupthawee, 2005) they found that ginger and nutmeg did not have antibacterial effect against bacteria tested. While the strong activity of extract of bitter fennel against all isolates may return to presence of phenols, and these are considered as antienzymatic activities and able to settle down on some proteins and enzymes and change their enzymatic stability and inactivation of the membrane enzymes would involve a modification of the cellular permeability, followed by a lyses of the bacterial cell (Zaidi-Yahiaoui *et al*., 2008). The antibacterial activity of Black Tea, Lime and Senna against these isolates may regard to their chemical composition, where they contain flavonoid (Catechin) and alkaloids and flavonoids respectively; and it was mentioned by some researches the antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and complex with bacterial cell walls (Abo *et al*., 1999, Cowan, 1999, Dhanavade *et al*., 2011). The antibacterial activity shown by the fruit extract of *P. cubeba* against all isolates in this study may be due to the presence of piperine and cubebi n in the berries (Aneja *et al*., 2010). The results of aqueous extract of turmeric revealed that this activity may return to the antibacterial activity of curcumin and turmeric oil which found in turmeric composition (Cowan, 1999). In present study Gram positive bacteria (Staphylococci) were found to be more susceptible than Gram negative bacteria (*E. coli*). This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lacks the natural sieve effect against large molecules due to the small pores in their cell envelop (Seyyednejad *et al*., 2008). Literature and our research works revealed great potential of plant for therapeutic purposes in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new antimicrobial compounds once extracted and used in new therapeutic treatments, they should have their toxicity in vivo (Ibrahim *et al*., 2011).

Table. 1: Identification of isolates.

Microorganism	characteristics	No. of infection/ percentage		
		A	B	C
S. epidermidis	G^+, cocci. Colonies are white, round, regular, concave colonies on Nutrient agar and Blood agar.	4 (40%)	2 (20%)	4 (40%)
S. aureus	G^+, cocci. Colonies are golden yellow, round, regular, concave colonies on Nutrient agar, Blood agar and Manitol salt agar.	4 (40%)	2 (20%)	4 (40%)
S. capitis	G^+, cocci. Colonies are white, round, regular, concave colonies on Nutrient agar	3 (30%)	6 (60%)	1 (10%)
E. coli	G^-, bacilli. Colonies are smooth, round, regular, concave colonies on Nutrient agar and MacConkey agar, and hemolytic on Blood agar. Metalic green sheen on EMB agar.	7 (70%)	-	3 (30%)
Candida	It identified presumptively (preliminary) according to its growing on nutrient agar where its colonies appeared as small, white and circular.	3 (30%)	-	2 (20%)

Table. 2: Selected Bacterial isolates.

Serial number	Bacterial isolate	Source of isolation
1	*Staphylococcus epidermidis*	Eye cosmetic users
2	*Escherichia coli*	Eye cosmetic users
3	*Staphylococcus aureus*	Eye cosmetic users
4	*Staphylococcus epidermidis*	Eye cosmetic users
5	*Staphylococcus epidermidis*	Control
6	*Escherichia coli*	Eye cosmetic users
7	*Staphylococcus aureus*	Eye cosmetic users
8	*Staphylococcus epidermidis*	Natural eye liner users
9	*Staphylococcus aureus*	Control
10	*Escherichia coli*	Eye cosmetic users
11	*Staphylococcus epidermidis*	Natural eye liner users
12	*Staphylococcus capitis*	Eye cosmetic users
13	*Staphylococcus capitis*	Control
14	*Escherichia coli*	Natural eye liner users
15	*Staphylococcus capitis*	Control
16	*Staphylococcus capitis*	Control
17	*Escherichia coli*	Natural eye liner users
18	*Staphylococcus capitis*	Natural eye liner users
19	*Staphylococcus aureus*	Natural eye liner users
20	*Staphylococcus aureus*	Natural eye liner users

Table. 3: Antibacterial activity of plant extracts against selected bacteria.

Con. Of extract (v/v)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Zone of inhibition (mm)																			
C:100%	13	13	15	13	16	12	13	13	21	-	20	8.5	12	-	-	-	-	15	-	-
C:75%	10	11	10	9	13	9	9	9	16	-	17	-	10	-	-	-	-	12	-	-
C:50%	-	-	-	-	9	-	8.5	-	13	-	11	-	9	-	-	-	-	8.5	-	-
C:25%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T:100%	21	-	19	17	23	16	19	22	29	17	23	20	18	15	19	19	25	16	19	20
T:75%	15	-	13	15	18	13	15	16	15	14	20	15	1`2	10	14	15	17	14	16	13
T:50%	10	-	10	11	13	11	12	13	10	11	16	13	10	-	10	10	12	11	12	9
T:25%	-	-	-	9	9	9	9	10	9	9	9	10	-	-	8.5	10	9	9	10	-
F:100%	25	20	15	21	17	15	11	13	17	9	19	23	19	15	9	17	19	30	17	
F:75%	22	16	11	11	18	15	14	-	11	16	-	14	20	16	13	-	12	16	22	15
F:50%	20	15	11	10	11	12	11	-	-	10	-	11	12	14	9.5	-	9	11	19	12
F:25%	17	11	-	-	-	12	9	-	-	-	-	9	10	10	8.5	-	-	9	15	-
Cu:100%	19	12	33	21	27	21	19	15	-	-	20	13	25	20	21	27	-	27	20	14
Cu:75%	17	-	27	16	23	17	16	11	-	-	15	-	22	14	18	25	-	22	16	-
Cu:50%	11	-	21	12	19	12	10	-	-	-	13	-	18	11	13	20	-	15	11	-
Cu:25%	-	-	17	9	16	9	-	-	-	-	10	-	14	9	9	14	-	11	-	-
L:100%	33	28	22	27	18	17	29	29	29	17	16	17	16	20	18	16	19	13	22	21
L:75%	29	24	17	25	13	13	27	24	26	14	13	14	14	16	15	14	10	10	17	17
L:50%	22	16	15	19	12	-	19	19	22	11	10	-	11	13	-	11	-	9	11	10
L:25%	17	10	11	15	9	-	17	17	18	-	9	-	9	-	-	9	-	-	-	-
G:100%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G:75%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G:50%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G:25%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N:100%	12	-	10	15	12	-	-	-	-	-	9	17	8.5	-	9	9	-	11	-	-
N:75%	9	-	-	11	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-
N:50%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N:25%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tu:100%	22	20	20	23	15	17	18.5	15	11	11	19	16	17	19	14	17	17	19	20	25
Tu:75%	18	19	17	20	12	11	17	13	9	-	15	13	12	15	10	11	11	12	18	17
Tu:50%	15	14	15	15	9.5	-	14	10	-	-	12	10	-	8.5	-	-	-	-	16	12
Tu:25%	9	9	9	10	9	-	13	9	-	-	9	9	-	-	-	-	-	-	13	11
S:100%	9	-	-	9	-	-	11	-	-	-	13	-	-	-	-	9	-	9	-	-
S:75%	-	-	-	9	-	-	11.5	-	-	-	11	-	-	-	-	-	-	-	-	-
S:50%	-	-	-	-	-	-	9	-	-	-	9	-	-	-	-	-	-	-	-	-
S:25%	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-

C: coriander, T: black tea, F:fennel, Cu: cubeb, L:black dry lime, G:ginger, N: nutmeg, Tu: turmeric, S: senna, -: no inhibition.

Table. 4: MIC of plant extracts (v : v)%.

No. of isolate	Plant extracts								
	C	F	T	Cu	L	G	N	Tu	S
1	ND	<0.025%	1%	<0.025%	<0.025%	ND	ND	3%	10%
2	ND	<0.025%	0.05%	<0.025%	<0.025%	ND	ND	3%	8%
3	ND	<0.025%	1%	<0.025%	<0.025%	ND	ND	3%	8%
4	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
5	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
6	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
7	ND	<0.025%	0.05%	<0.025%	<0.025%	ND	ND	3%	10%
8	ND	<0.025%	0.05%	<0.025%	<0.025%	ND	ND	3%	8%
9	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	6%	9%
10	ND	<0.025%	<0.025%	<0.025%	2%	ND	ND	6%	9%
11	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	10%
12	ND	<0.025%	0.05%	<0.025%	<0.025%	ND	ND	3%	10%
13	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	10%
14	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
15	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
16	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
17	ND	<0.025%	<0.025%	<0.025%	0.05%	ND	ND	3%	>10%
18	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	8%
19	ND	<0.025%	<0.025%	<0.025%	<0.025%	ND	ND	3%	10%
20	ND	<0.025%	4%	4%	2%	ND	ND	3%	.10%

C: coriander, T: black tea, F: fennel, Cu: cubeb, L:black dry lime, G:ginger, N: nutmeg, Tu: turmeric, S: senna. ND: not determined .

CONCLUSION

It concluded that the aqueous extracts of bitter fennel, black tea, cubeb, black dry lime and turmeric are good antibacterial agents, and it recommended to add these extracts to the cosmetic materials to inhibit the bacteria comes with contaminated hands through applying these cosmetics, in addition to their benefit to beautifulness of the skin.

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