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CFS OF *LACTOBACILLUS:* A NATURAL AGENT AGAINST BACTERIAL CONTAMINATION OF COSMETICS TOOLS

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ABSTRACT
A study was conducted to investigate the potential of cell free supernatant (CSF) of *Lactobacillus* to prevent microbial growth in cosmetic tools. A total of 34 samples including mascara and face sponge were used. Results revealed that microbial contamination was detected in 31 samples. Bacterial and fungal contaminations were defined in these products with 29 and 2 samples respectively. In 29 samples, a total bacteria numbers were distributed by *staphylococcus, proteus, E.coli, Shigella, Citrobacter, Klebseilla, Pseudomonas aeruginosa*. The highest number was recorded by *staphylococcus*. 16 sample of *staphylococcus aurus* were differentiated from 13 samples of *staphylococcus epidermides*. CFS of *Lactobacillus was* able to inhibit all bacterial isolates with high inhibition zones, so these filtrates can be used as added agent to cosmetics tools to increase their resistance and to prevent the contamination.

KEYWORDS: Cosmetic tools, CFS of *Lactobacillus*, contamination.

INTRODUCTION

The cosmetics industry achieved stupendous growth within a short span of time & is now a multi-billion dollar industry. It would continue to grow as long as people are ready to spend a fortune to look their best. Cosmetics are defined by the U.S. food & Drug Administration (FDA) as an "articles intended for beautifying, cleansing, promoting, and attractiveness or altering appearance[1]. Makeup can do wonders for women, but it can be dangerous to their health, if not handled property [2]. Cosmetic contamination leads to several types of infections that range in severity from mild to serious, the ability of organisms to grow & reproduce in cosmetic products has been known for many years & makeup can easily by contaminated by the repetitive use to the skin using an applicator, or finger & also by poor handling procedures during manufacturing that can cause defect in the preservative capacities [3]. Small sponges made of cellulose or nylon are commonly used in daily hygiene as exfoliative beauty aids & in removing makeup, after use , these sponges are generally cleansed under running water & reutilized, these natural & synthetic sponges are reported mostly caused by gram negative & positive bacterial[4]. Contamination of microorganism in cosmetics may cause spoilage of the product and when pathogenic, they represent a serious health risk for consumers [5], contamination of cosmetic by micro- organism such as clostridium tetani, staphylococcus aureus, moulds and yeasts *etc*. may cause serious disease of the eye , skin and mucous membrane which are difficult to cure in sereval cases[3]. Lactic acid bacteria (LAB) are a group of gram – positive bacteria including many of genera[19] .Within the group of LAB *Lactobacillus* species due to their potential beneficiary properties as probiotics , the activity of this bacteria is known to inhibit a large number of pathogenic bacteria, the use of Lactobacillus products to control certain infections has started gaining acceptance, the alarming rise of inappropriate antibiotic use and antimicrobial resistance, along with renewed interest in ecological natural methods to prevent infections, make CFS (products) a very interesting field for research[20]. The present study focuses on isolation & detection of human skin pathogens, on selective media for the assessment of microbes contaminating the cosmetics tools. In the same time , there is a lot of research about antimicrobial activity and antibiotic resistance of LAB but no research was dictated towards occurrence the inhibitory activity of CFS of *Lactobacillus* against bacterial contamination of cosmetic tools and used it as industrial natural agent with these tools to reduce their contamination by resistance the microorganisms

MATERIALS & METHODS

Cosmetic samples

In the study[2] mascara[11] blushers [21] sponges, that were used before were investigated in case of microbial contamination , none of the samples had a reported expiry data . These samples were taken from January to march 2012.

- Media used:blood agar, eosin methelyene blue (EMB), brain heart infusion, mannitol salt, mac Conkey agar was used for isolation of bacteria and fungi .All of the media mentioned above were prepared under aseptic conditions according to the manufactures specifications.
- Methodology :- (34) samples put in (2ml) normal saline , swabs were taken (1ml) from each of them and cultured on brain heart infusion, incubated in 37C^{o} , for 24 hr. vortex was done to make the dilution. Last dilution cultured on nutrient agar for 24 hrs in 37 C^{o}. Selective media were chosen like macConkey agar, EMB, mannitol salt agar to separate and

purification the bacterial isolates, then cultured each of them alone on nutrient agar to identification [6].

- Identification of bacterial isolation: All bacterial isolates were identified based on their gram reaction & biochemical reactions as described [7].
- Identification of fungal isolation: - All fungal isolates were identified based on their macroscopic and microscopic appearance with reference to manuals of [8].

• *Lactobacillus* isolate: From Al- Mustansiriay university / science collage / biology department / high studies laboratories, this isolate was obtained and identified again according to [21].

• Cell free supernatant (CFS) preparation : CFSs were obtained by centrifugation (4000x g, 10 min) of Lactobacillus cultures grown in 20 ml MRS broth at 37 c for 24 h. the supernatant was filtered through a 0.22 mm filter to remove cells [22].

•Determination of inhibitory activity of CFS against microorganisms:

Agar well diffusion assay was made to determined the antimicrobial activity of CFS against test bacteria, depending on this method, streaking of Muller Hinton agar medium by 0.1 ml of bacterial inoculums , tow wells were made by using cock poorer on each plates and filled with CFS and control (media just), these plates incubated at 37 c for 18-24 h. The results were read by measuring the inhibition zone with mm.

RESULTS & DISCUSSION

In the present study,[31] from [34] samples makeup sponges and mascara were observed for microbial contamination.

Of bacterial (29 sample) and fungi (2 sample just), staphylococcus was found in these sample alone and with two species staph. Aureus[13] samples and staph. Epidermidis[16] samples. Table (1,2) show that face sponge brusher , eye lashes had 91.1 % microbes contamination 93.5% and 6.4% contamination for the microbes types (bacteria and fungi) respectively was observed , the percentage of bacterial contamination among all samples in area in the makeup samples were klebseilla, *E. coli* , proteus, citrobacter, shigella, pseudomonas, and staphylococcus found in over experience, as previously reported by other studies, true pathogenic bacterium found was, all the others were considered opportunistic pathogenic bacteria. [4] Matching the result of the table (3) it was evident that the most frequently found bacteria were the same in kind of sponges, then brushers, this observation seemed to demonstrate that the environment conditioned the bacterial growth more than the body area , even if the possibility of hand carry-over of bacteria should be taken in consideration , environmental factors like humidity and high temperature favour bacterial growth ; in normal use ,people keep their sponges in the shower box and it is possible that the interval between showers is not sufficient to allow them to dry completely [1].

TABLE 1: Percentage of microbes in total samples

Presence / sample	Microbes	No result
Faces ponge, brushers	31	3
Mascara.	91.1 %	8.8 %

* Total count of sample (31).

TABLE 2: Percentage of microbes types in microbes samples

Microbe type / Sample	Bacteria	Fungi
Face sponge	29	2
Brushers & mascara	93.5%	6.4%

* Total count of sample (31)

TABLE 3:- percentage of G –ve bacterial isolates in total sample

Presence / Sample	Bacteria					
	Citrto	*Proteus*	*Klebsiella*	*Shigella*	*E.coli*	*Pseudomonas aeru ginosa*
Sponge	1	3	7	2	2	1
	4.3%	13%	30.4%	8.6%	8.6%	4.3%
Brusher	3	1	0	1	1	1
	13%	4.3%	0%	4.3%	4.3%	4.3%
Mascara	0	0	0	0	0	0
	0%	0%	0%	0%	0%	0%

Table (3) & figure (1) also reveal the differential tests between staphylococcus isolates by blood and mannitol salt agar and coagulase test using that all staphylococcus aureus isolated were grown on blood and mannitol salt agar appeared their heamolysis to blood and positive test to coagulase opposite of staphylococcus epidermides isolated that were not heamolysis to blood and negative to coagulase test.

In Table (5) & figure (2) Almost 55.1% of the total staphylococcus isolates revealed staph. Epidermides, whereas 44.8% contamination was caused by staph. aureus .Other there was another reason, may be explain by cosmeticians have to treat a large number of costumers in

a limited time, the repeated use of same brush or face sponge to apply facial makeup causes spread of microbial contamination as these pathogens are reported to adhere to the (poly ethylene oxide) - PEO face sponge coatings very well.

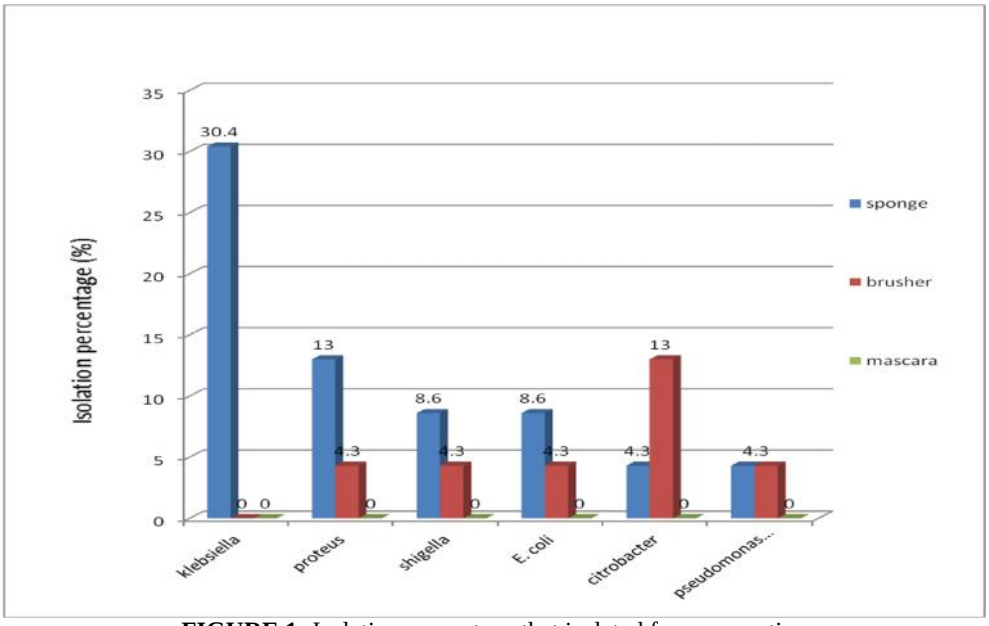

FIGURE 1- Isolation percentage that isolated from cosmetics

This cause may explain contamination the cosmetics samples with microbes[9]. Also in the present study staphylococcus contamination was seen in large number for two type of cosmetic tools that are frequently used , many authors have reported the isolation of pathogens from different parts of the face, in one such study[10]. *Staphylococcus aureus* overcome on staph. Epidermides in contamination that obtained from eyelashes, it was also reported that cosmetics tools items in possession of persons harbor Staphylococcus *aureus* in these samples. Similarly on face sponge samples, *Staphylococcus aureus* presence. Inadequate preservation or outdated products can lead to microbial deterioration and also favors growth and proliferation of skin pathogens after use[11]. Many authors have reported the presence of coaulase-positve Staphylococcus in cosmetics tools and explained that may be from unpreserved cosmetic products after use , lending importance to adequate preservation or from external factors like hands, dust, skin [12]. Behravan *et al.* [13] have reported the incidence of contamination by gram- positive bacteria , and staphylococcus aureus was higher for used cosmetics tools eyelashes and face sponge .in the other side contamination of microorganism in cosmetics may cause spoilage of the products and when pathogenic , they represent a serious health risk for consumers [14,15] in addition to that these contaminated tools will contaminate the original cosmetics when touch it in repeat use, zhang has investigated the creams that were tested before and after use contaminated cosmetic tools became not prepare for using because presences pathogens in this cosmetic product[16]. Many other author have also reported contamination of cosmetics tools with skin pathogens, cosmetic applicators can be an instrument of accidental trauma that introduces potentially hazardous microorganisms [14,15].

TABLE 4 :- Differential test between staphylococcus isolates

No.of sample	Result	Coagulase test	Culture	
			Blood agar	Mannitol salt agar
1	*Staph.aureus*	+	B+	+
2	*Staph.aureus*	+	B+	+
3	*Staph.aureus*	+	B+	+
4	Staph. Epidermidis	___	_____	___
5	Staph. Epidermidis	-	-	+
6	staph. Epidermidis	-	-	+
7	*Staph.aureus*	+	B+	+
8	*Staph.aureus*	+	B+	+
9	*staph. Epidermidis*	-	_____	____
10	*Staph.aureus*	+	B+	+
11	*staph. Epidermidis*	-	-	+
12	*staph. Epidermidis*	-	-	+

13	*Staph.aureus*	+	B+	+
14	*Staph.aureus*	+	B+	+
15	*Staph.aureus*	+	B+	+
16	*Staph.aureus*	+	B+	+
17	*Staph. Epidermidis*	-	-	
18	*Staph. Epidermidis*	_	-	+
19	*Staph.aureus*	+	B+	+
20	*Staph. Epidermidis*	_	-	+
21	*Staph. Epidermidis*	_	-	+
22	*Staph. Epidermidis*	_	-	+
23	*Staph. Epidermidis*	_	_	+
24	*Staph. Epidermidis*	_	_	+
25	*Staph.aureus*	+	B+	+
26	*Staph. Epidermidis*	_	_	+
27	*Staph. Epidermidis*	_	_	+
28	*Staph.aureus*	+	B+	+
29	*Staph. Epidermidis*	_	_	+
30	*Staph. Epidermidis*	_	_____	____
31	*Fungi*	____	_____	____
32	*fungi*	____	_____	____
33	*Staph. Epidermidis*	-	_	+
34	*Staph. Epidermidis*	-	_	+

TABLE 5: Percentage of G+ bacterial isolates in total samples

Bacteria type / sample	*Staphylococcus*		Other isolate
Face sponge , brushers and mascara	29		0
	100%		0%
	Staphylococcus Aureus	Staphylococcus Epidermidis	
	13	16	
	44.8%	55.1%	

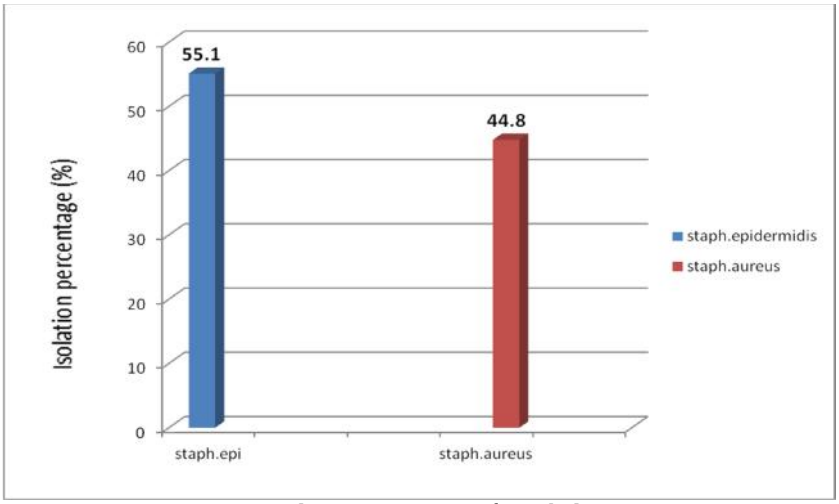

FIGURE 2: Isolation percentage of Staphylococcus sp.

The inhibitory activity of CFS against microorganisms

During the experiment, all isolated bacteria from contaminated cosmetics were sensitive to CFS activity, these results means a good activity for LAB Lactobacillus, as the results indicate in table (5), the diameter of inhibition zones were varied, it ranged between (11- 22) mm , in spite of occurrence low and high degree of inhibition , all test bacteria was respond to the product of Lactobacillus. The mechanisms underlying *Lactobacillus inhibition* of test bacteria may be due to presence many fraction containing proteins with a molecular mass below 5.000 Da and the finding of in vitro pointed to the peptideic nature of the *Lactobacillus* linked to bacteria inhibition[20]. However, there are also reports of compounds of proteinaceous nature with antagonistic activity against all bacteria (these proteinaceous inhibitors

target the cell membrane and depolarize it, and also inhibit synthesis of the cell wall, there are one of those peptides were characterized as Bacteriocin called plantracin [21].
Diacetyl, hydrogen peroxide {H_2O_2 can have a strong oxidizing effect on membrane lipids and cellular proteins } ,organic acids such as lactic acid ,acetic and propionic acids ,the most documented kind of metabolites .The antagonistic actions of acids are believed to be :1.interferance with the maintenance of cell membrane potential , 2. Inhibition of active transport, 3. Reduction of intracellular pH and 4.inhibition of various metabolites functions .They have a broad mode of action and inhibit both Gram-negative and positive bacteria as well as yeasts and molds by provide an acidic environment an unfavorable for the growth of many pathogenic bacteria [23, 25]. Besides the production of inhibitory compounds *Lactobacillus have* ability to compete with the pathogens for nutrients during the growth, the combined influence of large numbers of competing *Lactobacillus* and the resulting decrease in pH produce also an unfavorable environment for many pathogens such as studied bacteria [24, 26, 27]. All of the reasons above give clear reasons for the high activity that obtained in our results. All these factors enable *Lactobacillus* to use as probiotic that used in many lines like (food preservative , alternative drugs , flavored foods and others [28,29,30] so we suggest to use these product industrial as alternative agent from chemical agent that used in cosmetics because it is safe and have high activity against pathogens.

TABLE 5: the inhibitory activity of CFS against microorganisms

Isolates	Inhibition zons (mm)
Klebseilla	12
Proteus	11
Pseudomonas	17
Staphylococcus	22
Salmonella	19
E.coli	18

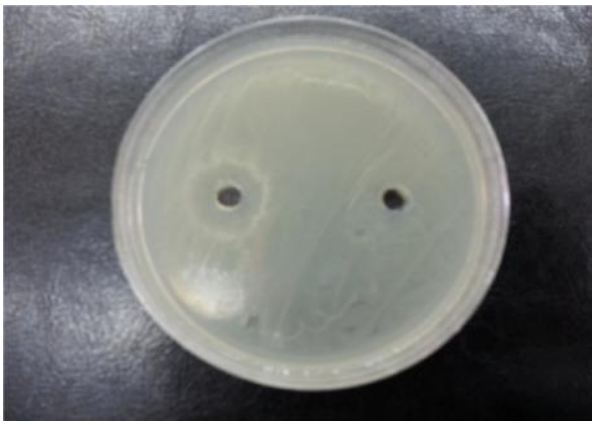

FIGURE 3- anti- *E. coli* activity of Lactobacillus CFS

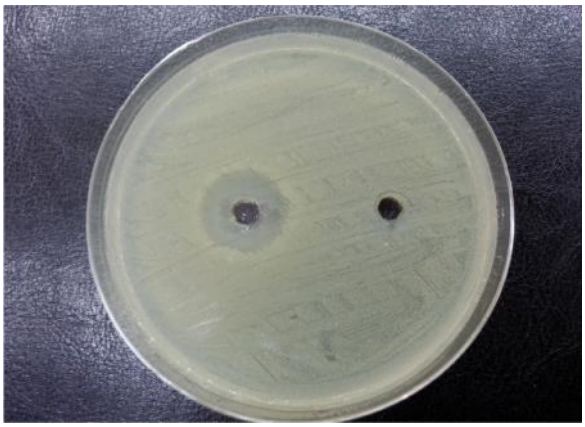

FIGURE 4- anti – Salmonella activity of Lactobacillus CFS

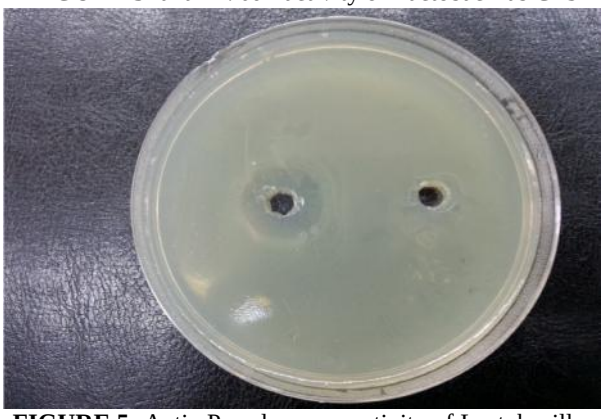

FIGURE 5- Anti –Pseudomonas activity of Lactobacillus CFS

FIGURE 6- anti- Proteus activity of Lactobacillus CFS

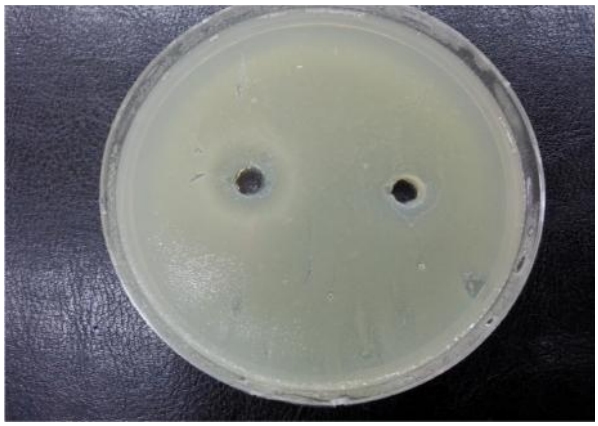

FIGURE 7- anti- Klebseilla activity of Lactobacillus CFS

FIGURE 8- anti –Staphylococcus activity of Lactobacillus CFS

CONCLUSIONS & RECOMMENDATIONS

From the present study it can be concluded that repetitive use of cosmetics tools harbors large number of pathogens that cause serious skin infections. By adapting the proper preventive precautions such as sterilization and proper washing of these tools the microbial contamination from one person to other can be controlled. Use the CFS of Lactobacillus as industrial agent with cosmetic tools (as preservative) to protect them from contamination by inhibitory the microorganisms.

REFERENCES

[1]. Naz, S., Iqtedar, M. ul Ain, Q. and Aftab, K. (2012) Incidence of Human Skin Pathogens from Cosmetic Tools used in Beauty Saloons from Different Areas of Lahore, Pakistan. J. Sci. Res. 4 (2), 523-527.

[2]. Gattuso, D.J. (2011)The True Story of Cosmetics. Exposing the Risks of the Smear Campaign. Issue Analysis 2011 No. 6.

[3]. Dashen, M., Chollom, P., Okechalu, J. and Ma'aji, J.(2011) Microbiological quality assessment of some brands of cosmetics powders sold within Joes Metropolis, Plateau State. J. Microbiol. Biotech. Res., 1 (2): 101-106.

[4]. Corazza, M., Carla, E., Rossi, M. and other (2013) Face and body sponges: beauty aids or potential microbiological reservoir? Eur J. Dermatol., 13: 571–573.

[5]. Onurdağ, F., Ozgen, S. and Abbasoğlu, D. (2010) Microbiological Investigation of Used Cosmetic Samples. *Hacettepe University Journal of the Faculty of Pharmacy*, 30(1): 1-16.

[6]. Forbes, B.A. , Sahm, D.F. & Weissfeld, A.S.(2007) "Bailey and Scott's Diagnostic Microbiology.12th ed.", Mosby Elsevier, China.

[7]. Atlas, R.M., Parks, L.C., and Brown, A.E. (1995) Laboratory Manual of Experimental Microbiology (1st ed.) Mosby. USA.

[8]. Baron, E.J., Petersond, L.R. and Finegold, S.M. (1994) Baily and Scott's diagnostic microbiology . (9th ed.), Mosby company, USA.

[9]. Roosjen, A. Busscher, H.J., Norde,W. and Vander, H. C. (2006) Microbiology. 152, 2673 (2006) .http: //dx.doi.org/10.1099/mic.0.29005-0PMid: 6946262

[10]. Onurdag, F., Ozgen, S. and Abbasoğlu, D. (2010) The True Story. *Hacettepe University Journal of the Faculty of Pharmacy*, 30,1.

[11]. Abdelaziz, A. Ashour, S. E. and EL-Tayeb, O. M. (2008). J. Clinl. Pharm. Therape. 14, 21 (2008) http://dx.doi.org/10.1111/j.1365-2710.1989. tb00217 .x PMid:2921299.

[12]. Ashour, M., Abdelaziz, A. & El-Tayeb, O. M. (2008) Microbial contamination of cosmetics and personal care items in Egypt-eye shadoes, mascaras and face cream. J. Clinl. Pharm. Therape. 14, 207.

[13]. Behravan, J., Bazzaz, F.B. and Malaekeh, P. 44, 482 (2005). http: //dx. doi.org /10.1111/ j.1365-4632.2005.01963.x PMid:15941436 .

[14]. Campana, R., Scesa, C., Patrone, V. and Baffone, E. (2006) Microbiological Study of Cosmetic Products during their use by Consumers: Health Risk and Efficacy of Preservative Systems, Letters in Applied Microbiology, 43, 301-306.

[15]. Ravita, T.D., Tanner, R.S., Ahearn, D. Crockett, P.W.(2009). Post-consumer Use Efficacies of preservatives in Personal Care and Topical Drug Products: Relationships to Preservative Category, J Ind Microbiol Biotechnol, 36, 35-38.

[16]. Zhang, W., Lu, B., Guo, R. and Chen, X. (2010) Study on hygienic microbe pollution for imported cosmetics during 2003-2006, Chinese Journal of Health Laboratory Technology, 3: 79.

[17]. Dendle, C., Mulvey, S., Pyrlis, F., Johnson, D. R. (2007).Clinl. Infect. Dis. 45, 29.http://dx.doi.org/ 10.1086/519425 PMid:17599301.

[18]. Anelich, L.E. and Korsten, L. (2007) Int. J. Cosmet. Sci. 18, 25 .http://dx.doi.org/10.1111/j.1467-2494. 1996.tb00133.x.

[19]. Rattanachaikunsopon, P. and Phumkhachorn, P. (2010) Lactic acid bacteria: their antimicrobial compounds and their uses in food production . Annals of Biological Research, 1(4) : 218-228.

[20]. Ahmed, A. A. (2013) IN vitro screening of Lactobacillus species from homemade yoghurt for antagonistic effects against common bacterial pathogens ., Jordan Journal of Biological Sciences ., 6(3) : 211-213.

[21]. Forbes, B. A. and Daniel, F. S. Alice, S. W. Baily and Scotts diagnostic microbiology.12th ed ., mosby Elsevier company . USA, 2007.

[22]. Mirnejad, R., Vahdati, A.R., Rashidiani, J., Erfani, M., Piranfar, V. (2013) The antimicrobial effect of lactobacillus casei culture supernatant against multiple drug resistant clinical isolates of Shigella sonnei and Shigella flexneri in vitro. Iranian Red Cresent Medical Journal. 15(2):122-126.

[23]. Al-Allaf, M. A. H. (2009) antimicrobial activity of lactic acid bacteria isolated from minced beef meat against som pathogenic bacteria . Iraqi Journal of Veterinary Sciences, 23(1) : 115-117.

[24]. Bilkova, A., Sepova, H.K., Bukovsky, M., Bezakova, L. (2011) Antibacterial potential of Lactobacilli isolated from a lamb . Veterinarni Medicina, 56 (7): 319 -324.

[25]. Tahmourespour, A. & Kermanshahi, R. (2010) The effect of a probiotic strain Lactobacillu acidophilus on the plaque formation of oral Streptococci, Bosnian Journal of Basic Medical Science, 11(1): 37-40 .

[26]. Ravaei, A. Poor, Z. H., Salehi, T.Z., Tamai, I.A., Ghane, M., and Pour, J. D. (2013) Evaluation of antimicrobial activity of three Lactobacillus spp. against antibiotic resistance *Salmonilla typhimurium*, Advanced studies in biology, 5(2): 61-70.

[27]. Giang, H. H; Viet, T. Q. ; Ogle, B. ; Lindberg, J. E. (2011) Effects of supplementation of probiotics on the performance , nutrient digestibility and fecal microflora in growing –finshing pigs., Asian-Aust. J. Anim. Sci., 24(5) : 655-661.

[28]. Darsanaki, R. K. Rokhi, M.L., Aliabadi, M.A., and Issazadeh, K. (2012) Antimicrobial activities of Lactobacillus strains isolated from fresh vegetables, Middle – East Journal of Scientific Research ., 11(9) : 1216-1219.

[29]. 29. Dhanasekaran, D. (2009) Probiotic effect of Lactobacillus isolates against bacterial pathogens in fresh water fish, Journal of Coastal Development., 13 (2):103-112.

[30]. Al-Ani, N., Hadi, S. A., Nazar, R. (2013) Antimicrobial activities of with aniasomnifera extract. Advance in Agriculture and Biology:1:1-4.