The microbial contamination study of some herbal cosmatics products used in traditional medicine in Iraq.

*Ibrahim S.Abaas[*1]*Haithem R. Mohammed *Ali H. Majeed

*Deparment of pharmacognosy and medicinal plants /Collage of Pharmacy/University of Kerbala.

Keywords : Microbial contamination ,Herbal Cosmetic.

(Received: Oct2013,Accepted: Dec2013)

Abstract:-

Herbal Cosmetic products contain variable amounts of natural material such as nutrients that support microbial growth. Most contaminants in herbal cosmetic products include bacteria sach as *Staphylococcus ,Pseudomenas ,Klebsiella, Achromobacter* and *Alcaligenes*. Contaminated water is likely source of organisms found in herbal cosmetic produets, extractes and crude of natural material.

Herbal cosmetic products sach as shampoo ,hand and body lotion ,facial cleanser,and liquid soaps were analyzed .In this study out of sixty herbal cosmetic products analyzed,27.3% were found to be contaminated.Most of the contamination was from bacteria while no fungal contamination was detected, The highest level of contamination occurred in shampoo. Viable bacterial were not recovered from 100%,92.7, 91.5% and 88.3% of showed herbal bath soaps,herbal facial cleanser ,hand and body herbal lotion and herbal shampoos, respectively .Coliforms were recovered from one sample of herbal shampoos. One isolate of shigella and *paseudomonas earuginosa* was detected from two samples of herbal shampoo.

دراسة التلوث الميكروبي لبعض المنتجات العشبية cosmatics المستخدمة في الطب التقليدي في العراق.

ابراهيم عباس هيثم محمد علي مجيد

مفتاح البحث: التلوث الميكروبي، والأعشاب مستحضرات التجميل

الخلاصة :-

تتعرض مستحضرات التجميل العشبية للتلوث الجرثومي نتيجة المواد الغذائية والطبيعة المضافة اليها والتي تساعد على نمو الملوثات وتشمل هذه الملوثات انواع مختلفة من البكتريا مثل *Klebsilla ,Aachromobcter ,Alcaligenes Staphylococcus ,Pseudomonas*, يعتبر الماء من مصادر التلوث الرئيسية التي تتعرض لها مسحضرات التجميل العشبية مثل الشامبوات ،مستحضرات التجميل العشبية السائلة ،المنظفات والصابون العشبي السائل. شملت هذه الدراسة فحصا مايكروبيا" لستون من المستحضرات العشية التجميلية واظهرت نتائج الدراسة ان حوالي 27.3% من هذه المستحضرات كانت ملوثة وان معظم هذا التلوث كان بكتيرياً وان اعلى معدل للتلوث كان في الشامبوات العشبية ووصل الى حوالي 11% وان بكتريا الـ *coliforms* موجوده في احد انواع الشامبوات وايضا وجدت بكتريا shigella و *puseudomonas* في نوعين اخرين من الشامبوات وهي من البكتريا المرضية (Pathogenic microorgisms)

Introduction:-

The herbal cosmetics are part of every ones daily grooming routine . Most herbal cosmetic products are based on water/oil emulsion or oil /water emulsion and contain variable amount of nutrients on natural extracts that support microbial growth .The herbs as raw materials used in cosmetic products may be grouped into categories (Table 1) .

Table (1) Raw material categories

Water
Acids , alkalis ,salts
Oils ,waxes ,paraffin
Fatty acids ,alcohol,esters
Surfactants ,emulsifier
Talc ,clay
Protein ,starches,botanicals ,gum and resin
Humectants
Colour and pigments
Preservatives ,antioxdants and chelating agents
Fragrances ,essential oils

Source: Adapted from Orth (1989).[1]

Microbial contamination in herbal cosmetics ,toiletries and personal care products is very common and had been a great concern to the industry for many years . Bacteria ,yeast and fungi are extremely diverse in their metabolic activites .The metabolic reaction of the microorganisms can cause health hazards because the metabolic products can be toxic ,mutagenic .Herbal cosmetic products need protection against microbial spoilage first of all in order to protect the consumer against potential dangers arising from pathogens and secondly to guarantee long _ term stability (shelf life)of the formulae. Preservatives play a vital role in product formulations .In many cases, chemicals which are highly active against microbes also have similar effects against mammalian cells .Therefore ,a balance needs to be established with the preservatives of choice between killing oraganisms and not injuring the cells of consumer who uses the herbal product .It is important to keep monitoring the herbal cosmetic product for contamination because an increasing number of products are recalled each year ,and the majority is contaminated with potential pathogenic micro organisms (2).More knowledge of the reasons for contamination is needed.The aim of this study is to investigation the microbial content of unused herbal cosmetic products at the point of sale. The herbal cosmetic products were manufactured in Iraq and were purchased from super market,and saloons .

Microbiological contaminants of herbal cosmetics .

The most frequent contaminants of herbal cosmetic products inclued *Pseudomonas* , *Klebsiella* ,*Achrombacter* and *Alcaligenes* (3). Baird (4) surveyed 147 unused cosmetic products purchased in England .Herecovered viable bacteria from 99 of the 147products (Table2). Gram - negative rods were isolated from 6.1% of the herbal products .

Table 2: Gram - negative rods in herbal cosmetics.

Cntaminant	Product	No.of organisms per ml/g
Pseudomonas aevginosa	Lanolin hand cream	1.2×10^3
P.Maltophitia	Mascara	7.0×10^3
P.pseudoalkaligenes	Cleansing milk	3.1×10^3
P.pseudoalkaligenes	Hair cream	1.9×10^3
p.fluorescens	Hair oil	4.0×10^3
P.putida	Cleasing jelly	2.5×10^3
Moraxella osloensis	Moisture cream	1.3×10^3
Enterobocter cloacas	Dental cream	2.3×10^3
Klebsiella aerogenes	Dental powder	3.4×10^3
K.oxytoca	Dental powder	—
Erwina herbicola	Dental powder	—
Enterobocter clace	Dental powder	—

Source Adapted from Baird (1974)

Anumber of survey (1) reported the incidence of contaminants in unused cosmetic products(5). The clinical and pharmaceutical significance of contamination for cosmetics has been reviewed by (6). Certain herbal products ,notably aqueous prepavation were more susceptible to contamination than others. Overall ,these findings indicated that under the existing manufacturing condition ,some forms of contamination in the final product appeared to be inevitable .Table(3) shows some potentially pathogenic bacteria isolated from cosmetic products and some of these organisms (7)are part of the normal human flora.

Table(3):Potentially pathogenic bacteria isolated from cosmetic preparations

Acinetobacter calcoaceticus	*Escherichia coli*	*Providencia rettgeri*
Citrobacter diversus	*Hafnia alvei*	*Providencia stuartii*
Citrobacter freundi	*Klebsiella oxytoca*	*Pseudomonas ccepacia*
Clostridium spp.	*Klebsiella pneumonia*	*Pseudomonas fluorescens*
Enterobacter aerogenes	*Morganella morganii*	*Serratia liquefaciens*
Enterobacter agglomerans	*Proteus mirabilis*	*Staphylococcus aureus*
Enterobacter cloacae	*Proteus vulgaris*	*Staphylococcus epidermidis*
Enterobacter gergoviae		
Enterobacter sakazakii		

Source :Adapeted from Norman (1984).

Hazards associated with microbial contamination.

-Infection from non –sterile from ducts

The roles of many organisms in cosmetic preparations were studied (8)by Brunch (1972).He concluded that most are objectionable for application to damaged epithelium while others are opportunistic pathogens depending on the species,the site and the health of the recipient.

Contaminants in herbal cosmetic products include bacteria such as *Staphyococcus* ,*Pseudomonas* and other opportunistic bacteria .Contamination of talc with *Closlridium tetani* , infection of neonates with *Pseudomonas aeruginosa* from contaminated cleansing solution and scalp infection from diluted stored shampoo leading to fatality in granulocytopeia patient are some examples .The eye is particulatry vulnearable to infection .The loss of eye sight after the use, during intraocular operation ,of saline solution contaminated with *Pseudomonas aeruginosa* (9) ,and severe eye disorders caused by use of a cortisone ointment contaminated with the same organism are examples of the serious dangers posed by contaminated preparations.

Spoilage

A spoiled product is one that has been rendered unfit for its intended use .Mouldiness, colour change , fronting and packaging that bulge, leak or explode as aresult of gas production are obvious effects of gross contamination and lead to chemical and physical changes in the products. Discovery by the customer of a mould colony is not likely to encourage further purchase of the particular brand .Recent reviews of the spoilage a spects of microbial contamination of medicines and cosmetics have been made by (6). Mixed flora introduced into the product by whatever means are ofren extremely versatile in their metabolic activity and can adapt to a broad rang of environmental conditions . All cases of herbal or natural organic compounds are susceptible to degradation by one organism or another and synthetic material may also be attaked .

Products such as herbal shampoos ,wich contain surfactants are particularly susceptible to contamination by water –borne gram- negative bacteria, wich may cause at the very minimum, avisible loss of lathering a ctivity .Active ingredients may also be rendered infective ,

Visible Effects

Contaminants of herbal cosmatics may be seen as sediment ,turbidity or pellicles in liquid products .On more solid preparations ,coloured colonies may from . The appearance of bright yellow micrococcus colonie on a white cream is an alarming sight .This may result from use of herbal or natural ingredients such as dried egg ,which can carry a large number of organisms ,if improperly treated. Contamination by *Psedomonas spp*. ,wich is metabolically versatile ,cause colour changes . This is due to alteration in product components as a result of direct consequence of metabolism or indirectly because of alteration of parameters such as pH or oxidation –reduction .

The addition of organic material greatly increases the chances of growth and deposits or turbidity due to algae ,mould ,bacteria or yeast in arange of poorly preserved pharmacopoeia solutions .Emulsions can become thin separate ,decolorize or change in pH of the aqueous phase .Herbal products such as shampoos are particularly susceptible to contamination by Gram – negative water –borne bactria (5).Slimy sediments ,pellicles and discoloration may occur .

The degradation of actives constituents

Beveride(10) reported on the inactivation of potent drugs and antimicrobial gents by a wide varity of microorganisms .Penicillin can rapidly be destroyed by B- lactamases. Other antibiotics, preservatives and disinfectants can be metabolized.Atropine in eye drops can be destroyed by *Corynebacterium* and *Pseudomonas spp*.(11) and the transformation of hydrocortisone in the dermatoloical cream to a therapeutically different compound by a contaminant *Cladosporium herbarum*, has been reported by(12).

Methodology

In this study ,various types of herbal cosmetic products were purchased from supermarkets ,saloons. These products were then subjected to microbiological tests to detect the presence of microorganisms at the point of purchase.

Aerobic Plate Count

Sterile materials and equipments were sterilized before use and aseptic techniques were used.The caps of products were wiped with ethanol (70%).Microblogical media were reconstituted and prepared from their dehydrated powder according to manufacturer instructions .By means of a syringe, or sterile spatula , one ml or one gram of the product was disintegrated in tryptic soy broth (9ml) according to B.P 2004 using a flask shaker and suitable serial dilations in tryptic soy broth were prepared .One – ml sample of each dilution was poured in a sterile petridish and then 15ml of sterile tryptic soy agar was poured on the sample ,the plates were gently swirled in round movement to allow a good mixing of the agae with the sample ,then the plates were allowed to solidify on a leveled surface .Triplicate plates for each sample were used and incubated at (35C˚ -37C˚)for two days for bacteria .Sabourand dextrose agar was used instead of tryptic soy agar – for the detection of fungi .The prepared plates were incubated at 25˚C for 5 days .After incubation the number of colonies was counted by estimating the total count of the growing bacteria and fungi then the mean of three plates was calculated .A laboratory control count was perfomed using negative control blank (without product) and with positive control (contaminated product).More than two colonies on the negative control plate invalidated the test. The colonies were counted .Colony count exceeding 1000 were considered too high to count and the product further diluted .Plates with colonies of 30 -300 were selected .The microoganism content per milliliter or gram is the colony count multiplied by the appropriate dilution factor (10 or 100).

Samples

Herbal products used in this study included shampoos , liquid bath soaps,facial cleansers ,hand and body lotions and moisturizers wich were purchasred from supermarkets and saloons .Detection for specific microoganisms such as *Escherichia coli*, *staphylococcus aureus* , *Pseudomonas* and *salmonella* was performed following procedure under isolation and identification tests for specified microorganisms (BP.2004)as shown in table(4).When results showed presence of any of these organisms , appropritate biochemical tests were performed.

microorganisms Table (4): Isolation and Identification tests for specified

Organism	Enrichment	Primary test	Secondary tast	Confirmation
Enterobacteriace	Lactose broth 35 - 37c˚	EEB –Mossel 35 -37c˚ for 24-48 hr	VRBGLA 35 – 37 c˚	GROWTH of Gram negative
E.coli	As above	Mac Conkey broth 43-45c˚ for 18-24 hr	Mac Conkey ager 43- 45c˚ for 18- 24 hr	Indole at 43.5-44.5c˚ biochemical
Salmonella spp.	As above for 5- 24 hr	TBBG broth 42-43c˚for 18-24 hr then subclture on:DCA.XLDA BGA for 35 -37 c˚ for 24 -48 hr	TSI aguar 37c˚ for 18-24 hr	Biochemical serological
P .aeruginosa	Saline peptone 35 - 37 c˚ for 2 -5 hr	Gasen digest broth 35 -37 c˚ for 24 -48 hr	Cetrimid ager 35 -37 c˚ for 18- 24 hr	Oxidase test
Staph. aureus	As for *P.aeruginosa* above	As for *P.aeruginosa* above.	Baird – Parker 35 -37 c˚ for24 -48hr	Coagulose catalase DNase test

EEB-m-mossel. Enterobacteriaceae enrichment broth- Mossel ;VRBG,violet red bile agar with glucose and lactose. TBBG .tetrrathionate bile brilliant green broth ;DCA, deoxycholate citrate agar ;XLDA, xylose lysine deoxy cholat agar BGA, brilliant green agar; TSI,tripl sugar iron agar; DNase ,doxyribonuclease test.

Results and Discussion:

Out of 60 herbal products analyzed for their total aerobic bacterial, coliforms 27.3% were found to be contaminated,(Table 5) .Most herbal products were bacterial contamination and no one were of yeast and mould . Herbal shampoos were more susceptible to contamination than other products presumably because they contain surfactants (Table 5) .Viable bacterial were not recovered from 100%, 92.7%, 91.5% and 88.3% of herbal shower bath soaps ,herbal facial cleanser, hand and herbal body lotion and herbal shampoos ,respectively.Table (6) shows the microbial counts (C.F.U) /gm or ml) and types found in herbal shampoos and herbal body lotions, only 2% of herbal shampoos were heavily contaminated by aerobic spore forming bacteria(bacilli) with move than 10^4 c.fu/gm or ml. while non of the others contamined such as high number of bacteria . With regard to the medium range contamination levels ;5% of herbal shampoos showed bacterial counts ranging from 10^2 to 10^3 C.F.U/gm or ml, compared to 2% of hand and herbal body lotion which were contaminated to same level . Coliforms were recovered from one smple of herbal shampoos ; *staphylococcus* were not recovered from any samples . One isolate of *Pseudomonas aeruginosa* was also detected in sample of herbal shampoo . One isolate of *shigella* was also detected in a sample of herbal shampoo. No fungal contamination was detected , as shown in table (6). The pH of all the tested samples was alkaline Ph (8.5 -9) ,wich is well

known to inhibit fungal contamination . Bacterial contamination in unused herbal cosmetic products is common because of the environment in which the products are manufactured , packed and the ingredients themselves . Herbal cosmetic ingredients are rich in nutrients and these provid organic substrates in the form of sugar , starch , protein , amio acid ,organic acid lipid and etc. for microbial growth. Organisms such as *Pseudomonas putidsses* posse a mixed functions oxidase enzyme that enable them to utilizes substrates that many other organisms are unable to use .The ability of microorganisms to utilize substrates depends on their survival strategies. The nutrients were needed by organisms include nitrogen , sulphur ,phosphorus phosphorus and mineral. These materials ,which are required for enzyme function and cellular osmorgulation are funished as components or raw matererial or in water. Water is amajor media in many cosmetic products and it has been the source of finished product contamination . Malcom and Woodroffe (3) reported that the most frequent contaminants of cosmetic products are *Pseudomonas, Klebsiella* **,***Achrombacter* and *Alcaligenes* They observed that these genera are common residents in water and contaminated water is likely source of the organisms found in contaminated cosmetic products. Some microbes survive by forming endospores , biofilms ,capsules, extracellular enzymes and by exhibiting acid tolerance (14-18).Our result is similar or like the report of okekre(19) ,Gram negative bacilli were seen in these studies , but unlike the report of Hugo (20). Unlike the report of .(19),(20) *salmonella spp*. was isolated in our study. Generally ,microorganisms of interest in raw materials or herbal cosmetic products grow best around neutral pH7.0 and many yeasts and mould are able to tolerat acid pH conditions.Natural herbal cosmetic ingredients supply nutrients for microbial growth .Therefore , herbal cosmetics should be produced in a prefectly clean hygienic environment. Product premises , equipment , instruments , storage tank and containers should accordingly be maintained in a high standard of cleanliness. In order to minimize cotamination , cosmetic manufactures should follow guidelines of good manufacturing practice (GMP). All starting materials should correspond to the agreed standards and be of consistenly good quality [(6)] .Ingredient listing is an important aspect of the labeling of cosmetic products .

Table 5: Shows the microbial counts (C.F.U) /gm or ml ,and types found in herbal shampoos and herbal body lotions .

Sample	Total No.of herbal product	Contaminated herbal products
Herbal Shampoo	15	11.7
Hand and body herbal lotion	15	8.4
Shower bath herbal soaps	15	Zero
Herbal Facie cleaner	15	7.2
Total	60	27.3

Table (6) :Microbial counts(C.F.U. / ml or gm)and types found in herbal shampoos and body herbal lotions.

Herbal Shampoo	Counts	Types diaynoized
2%	$>10^4$ C.F.U /gm	Aerobic spore forming bacteria (bacilli) high level
5%	10^2-10^3C.F.U. /gm	Aerobic spore forming bacteria (bacilli)medium level

1%	10^3- 10^4C.F.U /gm 5.0x 10^2 1.0 x10^3	*Pesudomonas aeruginosa* *Shigella Spp* .
Hand and body lotion	13 x 10^3	*Escherichia*
2%	10^2- 10^3C.F.U /gm	Aerobic spore forming bacteria (bacilli)medium level

Conclstion :

The current study revealed that bacterial contamination is more likely to occur than yeast and mould contamination .Bacterial growth is favored at neutral pH and most herbal or natural products are at this range microorganisms such as *Psudomonas ,Klebsiella , Achromobacter and Alcaligenes* are the most frequently reported contaminantes of herbal cosmetic products. Also , contamination is higher in herbal shampoo than other products .

This may be because they contain organic compounds and they are susceptible to contamination by water –borne gram – negative bacteria . Herbal cosmetic manufactures can prevent contamination by controlling raw materials, validating processes ,instituting effective cleaning and sanitizing procedures, and training personnel .

Even low contamination dose not necessarily mean that the manufactures have followed the newly adopted EU regulation . There might be a possibility that the manufacture used excessive preservations in the product . A further study on preservatives will should be card out to detect the preservative level in herbal cosmetic products marked in Iraq .

Refrences:-

1- Orth , D S. Handbook of Cosmetic Normax F E. The Microbiology .(1989) Marcel Dekker .New York.
2- Lundov ,MD and zacharian, C.Recall of microbiologically contaminated cosmetics is Eu from 2005 to may 2008 .int.J. of cosmetic science ,(2008),30 (6):471 -474.
3- Malcom ,S and Woodroffe ,R. The Susvial of bacteria in Toletries :Inhibition and Inactivation of vegetation microbes (Skinnel ,Found Hugo .Weds).Vol.5 Academic press ,London. (1976),P.305 – 314.
4- Baird ,R M. Microbial contamination of cosmetic products .J. Soc.Cosmet .Chem. (1974), 28: 17 – 20 .
5- Baird ,R M .Drugs and cosmetics .Microbial Biodeterioation (Rose ,A H ed.)Academy Press,London ,(1981),p 387 -429.
6- Russel ,AD ;Hugo , WB ;Aylife ,peter A. Lambert . Principles and practice of *disinfection* preservation and *sterilization* .Academy pres London ,(2004) ,P: 485 -487.
7- Normam ,FE .The cosmetic industry Scientifc regulatory foundation. Cosmetic seience and Tech . series ,(1984). Vol.2 :21 , 301- 320.

8- Brunch ,C W . Possible modifications of USP microbial limits and tests .Drug Cosmet. Ind ., (1972), 110(6):32 -37 ,116 -121 .
9- Ayliffe G A J; Barry D R; Lowbury . E J L ; Roper –Hall ,M J and Walker ,W M . Postoperative infection with *Pseudomonas aeruginsa* in an eye hospital .Lancet ,(1966), 1113 -1117 .
10- Beveridge ,E G, The microbial spoilage of pharmaceutical products .Microbial Aspects of Deterioration of Materials (Lovelock ,D W and /Gilbert ,R J eds .).Academic Press , London ,(1975), p .213 – 235.
11- Kedzia ,W ; Lewon, J and Wismienski ,T . The breakdown of atripine by bacteria .J. Pharma . Phamacoal , (1961),13: 614 – 619 .
12- Cox , P H and Sewel , B S, The metabolism of steroids by Cladosporium herbarum . J. Soc . Cosmet .Chem., (1968),19:461 -467.
13- British phermacopoeaia . H . M. S. O, London Appendix XVI BA 245 ,B , test for microbial contamination ;(2004).
14- Bos ,CE ,van Doorne , Hand derk ,CF , Microbiological stability tableto stred under tropical conditions inter .J. pharm; (1989) 55:175 -83.
15- Baird ,R M . and chrrtan R A. *pseudomonas aerubine* inflactions associated with the use of contaminated medicament . Brmed J.
16- Myas GE and pasutto FM . microbiological contaminated of cosmetics and toiletries .Cem .J . pharm. Sci .(1973)19 -23.
17- Killings Lo,Ringerts O, silverton PE and Ernenfell icrobiological contamination of medical perpartions . Actapharm Sci.(1966);219 -22.
18- Beck vanf Lorenzoni : moisturizing creams and lotion distributed atropical developing country . JSPP Microbiol; (2001),91: 922-928. N *Psedomonas aeruginosa* out break in aneonatal intensium care unit. A possible link to contaminated hand lotion .Amer Jinf control ; (1976) ;2 :349-350. (1995),23 : 396 – 398.
19- Okeke in and Lomikanra A. Bacteriobgied quality of skin.
20- Hugo PG. onyerwd : AO. Microbial contamination and preservation capacity of sone brands of cosmetic creams .Trop. J.of pharmaceutical research; (2003), 2:229 -234.