Research Article

Microbial Analysis of Cosmetics and Design of an Effective Preservative

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ABSTRACT

Cosmetics play an important role in human beings. The usages of cosmetics are increasing day by day, although it is contaminated by various microorganisms like *Staphylococcus aureus*, *E.coli*, Psudomonas, clostridium, and Aspergillus, Candida etc. The preservation of cosmetics was done by adding chemical preservatives but it is not very efficient against the microorganisms. In this study the microorganism was identified from the used cosmetic samples (Fair and lovely and Lactocalamine) which is before the expiry date. Natural Essential oil was used as a preservative against the microorganism and it shows the high efficiency against the microorganism than the antibiotic.

Keywords: Cosmetics, Essential oil, Antibiotic resistance.

INTRODUCTION

The field of cosmetics and microbiology had not come into contact much before the 1930's and cosmetic microbiology became more important in 1940's (Curry *et al*., 2006) The first contamination of cosmetics was reported in 1946 by several cases of neonatal death from talcum powder containing *Clostridium tetani*[2] Since 1960's, opportunist organisms, such as *Klebsiella pneumoniae*, *Pseudomonas sp*., *Serratia sp. and Enterobacter sp*., have been isolated from cosmetic products to a certain extent [3] Creams are external preparations, are both liable to microbial contaminations either in the course of their preparation, transportation. This spoilage may lead to alteration in organoleptic properties of creams which may manifest in terms of changes in colour, odour and/or taste as well as biodegradation of active constituent of such creams. However, spoilage may result in loss in term of cost on the part of manufacturer and infection on the part of consumers of such spoilt products in a situation whereby a nutritionally rich pharmaceutical or cosmetic product is severely contaminated, rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of infection to consumers of the product

A spoiled product may be described as one that has been rendered unfit for use. As pharmaceuticals and cosmetics are consumed by or applied to the user, manifestations of spoilage are essentially subjective, spoiled can be caused by bacteria, yeast or fungi which are all extremely versatile in their metabolic activities Reports of the microbial quality evaluation of cosmetic products have been from temperate countries and often in response to outbreaks of infectious diseases preparation, by the personnel, storage environment, during transportation and or use by the consumers which may lead to their spoilage. Contaminating microorganisms in cosmetic eye preparations may cause spoilage of the product and when pathogenic, they represent serious health risk for consumers worldwide [4]

The incidence of skin diseases is likely to be frequent in the developing countries due to the unhygienic environment, dense population favoring contagious diseases, lack of awareness on cleanliness, improper sanitation practices, and finally the massive use of contaminated processing water

A wide range of preservatives has been developed to combat the contamination from the repeated use by the consumer. Nowadays, maintaining a balance between protection against microbial contamination and limiting the health risks of preservatives has constituted the art of preservation.

In this project, two different used cosmetic samples (Fair and Lovely and Lactocalamine) were taken and their antimicrobial load was analysed and the preservatives capacity against contamination was assed and the activity of essential oil as a preservative was studied.

MATERIALS AND METHODS

Sample Collection

Cosmetic samples like Fair and Lovely and Lactocalamine was collected from Hindusthan ladies hostel. Tested samples were having manufacture date and expiration date (Fair and lovely- july 2016-2018, Lactocalamine -April 2015-2020) were recorded. The surface of sample container was disinfected with aqueous mixture of 70% ethanol and 1% HCl before opening and removing contents. These samples were collected in sterile glass container the samples were analysed after arrival.

Assessment of Contamination

In order to assess the degree of contamination, 1g of material was dispersed in 4 ml sterile Ringer solution containing 0.25% Tween 80. . From this 1ml of sample can taken and it mixed with 9ml of sterile water from this 10^{-1} to 10^{-6} series of dilution were done and plated out on Nutrient agar, Mannitol salt agar, Certimide agar and Macconkey agar, to isolate and determine the bacterial load of the sample. Sabouraud dextrose agar, potato dextrose agar was used for the isolation and enumeration of yeasts and moulds using the surface viable method (spread plate method).

Identification of Contaminants

Identification of isolates in mannitol salt agar plate,

Yellow colour colonies were observed in mannitol salt agar plates.

Identification of isolates in Eosine methylene blue agar,

Dark metallic sheen colonies and colour less colonies were observed in eosine methylene blue agar.

Identification of isolates in Macconkey agar,

Lactose fermenting colonies were observed.

Identification of isolates in Nutrient agar,

Different types of colonies were observed.

Identification of isolates in Certimide agar,

There are no colonies were observed in the certimide agar.

Microscopy Identification

Gram negative rod, and gram positive cocci microoorganisms were observed from Mannitol salt agar, Eosine methylene blue agar, Nutrient agar and Maconkey agar.

Phenotypic Identification

As per bergey' s manual of systemic bacteriology the isolates were identified based on biochemical test as *Escherichia coli* and staphylococcus aureus.

The Microbial Challenge Test

Microbial challenge test was applied through the method reported by Campana *et al.,* Samples were placed into sterile containers they were dispersed with Tween-80 samples were inoculated with the standard strain of *S. aureus,*. Tryptone soy broth was added onto the samples that contain bacteria and Sabouraud dextrose broth was added onto the samples that contain fungi. Samples were shaken and maintained at room temperature. After a contact time of 0, 3, 7, 14, 21 and 28 days, 1 ml aliquots were removed and placed onto 9 ml of neutralizing medium distilled water it was placed in to nutrient pour plate. Cell viability was determined in this plate after 24 h incubation at 37 ºC.

Antibiogram of Isolates

Antibiogram of the isolates were done by Kirby bauer disc diffusion method using antibiotics like Norfloxacin(NF-10mcg), Amoxicillin(AX-30mcg), Ofloxacin(OF-5mcg), Nitrofurantoin(N-300mcg).

A sterile swab was dipped into the bacteria suspension, this swabbed on to the entire surface of the Mueller-Hinton agar. Sterile forceps were then used to place the multiple antibiotic discs in a circular pattern on the media. The process was carried out for all the presumptively identified isolates, and the plates incubated at 37°C for 24 hours. After incubation the zone of inhibition for each antibiotic was measured from the center of the disc to the point where clearing stopped

RESULTS AND DISCUSSION

Preliminary Identification

Bacterial isolates

The Bacterial isolates confirmed by, Gram staining, motility, plating on to selective media, IMViC, and catalase test. Growth was observed at 24 hours at 37ºc. The biochemical characteristics of all the isolate are recorded.

The viable count in 10^3, 10^4, 10^5 of 2 samples were done and the results are shown in Table No: 1 and Table No: 2 Fatma kaynak too have recently published comprehensive reviews on the microbial investigation of used cosmetic sample in which the same results have been reported.

Fungal isolates

Fungal isolates were confirmed by Lactophenol Cotton Blue staining and colony morphology are tabulated in Table No: 3 Kamal kanta Das studies on bacteria, fungal contamination and the risk of microbial contamination of cosmetic products in which some of the isolates have been reported.

Confirmation of the Isolates

Isolate-1

Primary isolation was done using selective medium Eosine Methylene Blue Agar medium. Suspected colonies from Eosine Methylene Blue plates were subcultured and were used for identification and characterization. Identification of *Escherichia coli* cultures were made on the basis of morphology by microscopic examination (Gram negative rod) and cultural characteristics, biochemical reaction based on Bergey's manual of systemic bacteriology [5]

Isolate-2

Primary isolation of *S.aureus* from cosmetic samples was done by plating on to the selective medium Maninitol Salt Agar plate and after incubation at 37ºc for 24 hours the isolates which fermented mannitol were observed as yellow colour colonies and those colonies were sub cultured and Gram staining was done in that the Gram positive cocci clusters of bacteria were isolated and the

catalase test and coagulase was performed to the isolate. Then the isolates were sub cultured to used in further identification tests in reference to the the work of Fatma kaynak onurdag by standard microbiological methods.

Microbial Challenge Test

Microbial challenge test was done by the method reported by Campaina the degree of contamination decreased day by day. Efficacy of preservative in cosmetic formulation is evaluated in a challenge test according to the European Pharmacopoeia guidelines (The viable count was observed on 7thday and 15th day, and 21st day and 30th day. The comparisons of each plate are tabulated. In that first sample (Fair and lovely) microbial growth was decreased eventually and in the second sample (Lactocalamine) microbial growth does not decrease eventually. Peter Hugbo in which he studied the ability or power of that product to consistently maintain low and acceptable levels of microbial contaminants when such product is challenged with fresh microbial load and he concluded that the preservatives employed in cosmetic products did not possibly possess adequate preservative capacity which are able to bring about acceptable low levels of microbial contamination.

Antibiogram of Isolates

Antibiogram of the isolates were done by Kirby bauer disc diffusion method using four different antibiotics Norfloxacin (NF-10mcg), Amoxicillin (AX-30mcg), Ofloxacin (OF-5mcg), Nitrofurantoin (N-300mcg). The antibiogram pattern of the isolates present study is depicted in Table:No:4 and 5 and it correlates with antibiogram pattern observed among the antibiogram of bacterial isolates associated with creams in which higher resistance and sensitivity of isolates were noted.

Efficacy of Essential Oil as a Preservative

The efficacy of preservative capacity of Natural Essential oil (Lemon grass oil) against the isolates were done in which the Lemon grass oil has highly Antimicrobial activity against *E.coli* than *staphylococcus aureus*. In this Mariola studied about the antimicrobial property of the essential oil and the antimicrobial activity against the microorganisms. The results are summerized in Table No 6.

Table 1: Viable Count- Sample 1 (Fair and Lovely)

S.NO	MEDIA	10^3	10^4	10^5
1	SDA	+	+	+
2	PDA	+	+	+
3	MSA	+	+	_
4	EMB	+	+	+
5	CERTIMIDE	_	_	_
6	NUTRIENT	+	+	+
7	MACCONKEY	+	+	+

+ Presence of Colony, Absence of Colony, SDA - Sabourad Dextrose Agar, PDA – Potato Dextrose Agar, MSA – Mannitol Salt Agar, EMB – Eosine Methylene Blue

Table 2: Viable Count- Sample 2 (Lactocalamine)

S.NO	MEDIA	10^3	10^4	10^5
1	SDA	+	+	+
2	PDA	+	+	+
3	MSA	+	+	_
4	EMB	+	_	_
5	CERTIMIDE	_	_	_
6	NUTRIENT	258	178	98
7	MACCONKEY	+	+	+

+ Presence of Colony, Absence of Colony, SDA - Sabourad Dextrose Agar, PDA – Potato Dextrose Agar, MSA – Mannitol Salt Agar, EMB – Eosine Methylene Blue

Table 3: Fungal Colony Morphology in Cosmetic Sample

S.NO	MEDIA	COLONY MORPHOLOGY	
1	SABOURAD DEXTROSE AGAR	White mycelium black spores	Velvety dark green colony
2	POTATO DEXTROSE AGAR	velvety to orange cream with green spores	Oliva ceous green with sterile white

Table 4: Antibiogram for *E.Coli*

S.NO	ANTIBIOTICS	ZONE SIZE	SENSITIVE/ INTERMEDIATE
1	Norfloxacin	0.5mm	Intermediate
2	Amoxacillin	1mm	Sensitive
3	Ofloxacin	1.5mm	Sensitive
4	Nitrofurantoin	0.8mm	Sensitive

Table 4: Antibiogram for *Staphylococcus aureus*

S.NO	ANTIBIOTICS	ZONE SIZE	SENSITIVE/ INTERMEDIATE
1	Norfloxacin	0.5mm	Sensitive
2	Amoxacillin	No zone	Resistance
3	Ofloxacin	Not clear zone	Resistance
4	Nitrofurantion	No zone	Resistance

Table 5: Antibiogram of Lemon Grass Oil against *E-coli*

S.No	Concentration	Zone Size	Sensitive/ Resistance
1	Oxyetracycline(O)	0.3mm	Sensitive
2	0.25	No zone	Resistance
3	0.50	0.4mm	Sensitive
4	0.75	0.8mm	Sensitive

Table 6: Antibiogram of Lemon Grass Oil against *Staphylococcus aureus*

S.NO	Concentration	Zone size	Sensitive/ resistance
1	Oxytetracycline (O)	0.5mm	Sensitive
2	0.5cm	No zone	Resistance
3	0.25cm	No zone	Resistance
4	0.75cm	1cm	Sensitive

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