Comparative Study Of Synthetic And Herbal Cosmetic Products For Their Toxicity Assessment By Microbial Bioassays

Maithili Agarwal, Anuradha Singh, Nupur Mathur, Sakshi Sharma

Abstract: The main components in personal care and beauty products can be natural or artificial, but their effects on consumer's health is mainly determined by the chemical compounds present in them. The chemical components of these cosmetics build up toxins in our body over a long period of use which results in adverse effects and long term severe systemic illness. This reason has driven us to design a satisfactory toxicological study for evaluating potential toxicity caused by these products. The purpose of the present study is to screen cosmetic products formulated with synthetic chemicals and products formulated with herbal extracts for their toxic effects using short-term microbial bioassays and compare them with each other. The study has been done by using one prokaryotic (*Pseudomonas fluorescens* growth inhibition assay) and one eukaryotic (*Saccharomyces cerevisiae* respiration inhibition assay) bioassay. Synthetic cosmetics products have shown comparatively higher toxicity than the herbal ones in both the bioassays. Through this study, an effort has been made to enlighten consumers and scientific community about the grave consequences on health by excessive use of cosmetics and personal care products. The study also enlightens us towards the selection of the products. The samples found to be toxic based on the baseline data obtained by these assays can further be taken for specific and more advanced assessment procedures.
Index Terms: Cosmetics, Eukaryote, Herbal products, Microbial bioassays, Prokaryote, Synthetic chemicals, Toxicity.

1. **INTRODUCTION**

We all get exposure of toxic chemicals through our everyday routine for instance through the air, water, food as well as through cosmetics and personal care products. All of this ends up with our bodies loaded with toxic industrial chemicals. In present scenario, the use of cosmetics has become such a necessity that one can hardly avoid their use. The increase in social activities and gatherings has multiplied their demand to many folds. Over the past several years, the cosmetic industry has shown steady growth in many developing countries [1]. Now people have many choices to select from according to age, skin colour, profession and with many brand options. This sudden rise in popularity of cosmetics has created wide room for research and social surveys.

Cosmetics are formulated by a complex combination of chemical ingredients [2]. There is a wide list of synthetic compounds present in them, such as sodium lauryl sulfate, phthalates, nitrosamines, parabens and formaldehyde releasers, heavy metals, hydroquinone, nanoparticles, benzophenone, mineral oil, colour pigments, alcohol, ammonium lauryl sulfate and many others [3].

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Some common ingredients can be a part of cosmetics as well as cleaning products and other household essentials for instance, phthalates can be a part of a personal care product and at the same time, it can be present in a plastic ware. This might end up with exposure that is more frequent for such harmful agents.

Synthetic chemicals are mainly present in the form of preservatives and fragrance agents. It is true that not all of the synthetic chemicals present in the personal care products cause adverse health effects but these might have some dangerous ingredients that are being classified as carcinogen i.e. it might cause cancer in consumer's body [4].Some of the ingredients can be neurotoxins, reproductive toxins that have been proven to affect brain development and reproduction [5]. Some ingredients can be cytotoxic that cause impairment to the cell existence which might result in immediate cell death by losing cell membrane integrity (necrosis) or the cell might undergo apoptosis [6]. With all benefits, cosmetics and personal care products come as a parcel of never ending list of allergies. It can be skin allergy (permanent discoloration of skin), breathing allergy (damage to nose and nasal passage) and hair allergy (redness in scalp, hair fall, excessive dandruff, thinning of hair). Some agents can be labelled as hypo-allergic, even though they might have potential carcinogenic property [7]. Many studies have been conducted on patient's cosmetic products to study the undesirable effects caused by them [8], [9].

Carcinogens in personal care products are more likely to produce cancer risk in comparison with food contaminated with industrial carcinogen or pesticides. As compounds enter in the body through mouth are absorbed by intestine and then pass into venous blood, from there they make their way to liver, where various enzymes detoxify them before reaching to rest of the body. However, compounds that are absorbed by the skin can bypass liver process and enter into the blood stream that can further reach to body organs [10]. Therefore, gathering extensive data on toxic chemicals used to manufacture these cosmetic products should be the priority of concern for the researchers dealing with toxicology studies.

An appropriate solution to this current problem has emerged as the use of herbal cosmetic products. This is a fast growing area and will increase many folds in coming years because of much awareness of consumers towards the product's selection. Herbal cosmetics are generally referred as natural cosmetics in which herbs are being used in crude or extract form to make them free from side effects. In addition to being free from ill effects they also provide nutrients and other useful minerals to the body [11]. They contain natural antioxidants like vitamin C [12]. Many other benefits can be seen with the use of herbal products such as; they are suitable for all skin types, more affordable than synthetic ones and wide selection to choose from [13]. The purpose of the present study was to screen cosmetic products formulated with synthetic chemicals for their cytotoxic effects, create a baseline data using short-term microbial bioassays and compare them with data obtained from herbal cosmetic products.

2. MATERIALS AND METHODS

2.1 Sampling

Samples were collected in two categories:

Category 1) Cosmetic products formulated with synthetic chemicals

a) Face pack
b) Gulab jal (Rose water)

Category 2) Herbal cosmetic products

a) Face pack
b) Gulab jal (Rose water)

The samples were collected twice from grocery stores of Jaipur city (Rajasthan, India). Sampling 1 was done in month of September 2016, while sampling 2 was done in month of March 2017. All the samples were stored according to the instructions on the packaging. The list of ingredients present in these samples can be seen in Table 1. A 25 mg/ml stock of all the samples was prepared and taken as 100% dose and further dilutions *viz*. 50%, 20%, 10%, 5% and 2% were prepared in order to obtain a concentration- response profile of the samples for both the assays. All of these sample concentrations were prepared in sterile distilled water.

2.2 Tester Strains- The bacterial tester strain *Pseudomonas fluorescens* MTCC 103 which is equivalent to ATCC 13525 was procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India and stored at -20 ºC. The eukaryotic test organism (*Saccharomyces cerevisiae*) used in second bioassay was a commercial brand of dried Baker's yeast and purchased from a grocery store of Jaipur, India.

Table 1 List of ingredients present in synthetic and herbal cosmetics taken for the study

Sample	Ingredients
Face pack (Synthetic)	R.O. water, fullers Earth, ceto stearyl alcohol, glycerine, titanium dioxoide, bentonite clay, kaolin, bees wax, *Aloe vera* extract, macrogal cito stearate ether 1000, cetyl alcohol, light liquid paraffin, Niacinamide (Vitamin B3), Turmeric extract, Sesame oil, Sandal wood powder, Jatamansi Extract, Licorice extract, perfume, Swertia Chirata extract, xanthum gum, sodium benzoate, methanol, methyl paraben, *Withania somnifera* extract, potassium sorbate, Tea tree oil, propyl paraben, EDTA di sodium
Face pack (Herbal)	Fullers earth, mixed clay, Aloevera, aqua, pack base, Methyl paraben, propyl paraben, sugandhit dravya
Gulab jal (Synthetic)	Aqua, rooh gulab extract, Rose oil, methyl paraben
Gulab jal (Herbal)	Each 10 ml of rose water contains *Rosa centifolia* aqueous distillate of petals

The above list is based on the details provided by the manufacturer on the packaging of the product

2.3 Bioassays

Bioassays used in this study were for cytotoxicity evaluation. Cytotoxic bioassays hold an important place in the field of toxicological assessment. These bioassays are *in vitro* tests to screen potential toxicants that are capable to affect viability, cell membrane integrity and metabolic activity of cells.

2.3.1 *Pseudomonas fluorescens* growth inhibition assay

This simple and rapid test for cytotoxicity was executed according to the method described by Dutka and Kwan in 1981 [14]. It facilitates the effect of potential toxicant on the growth of a pure culture of *Pseudomonas fluorescens* by inhibiting the growth rate of this bacterium. A prime test inoculum was prepared by adding 15 ml of an overnight logarithmic phase culture of *P. fluorescens* to 1000 ml of fresh sterile nutrient broth medium in aseptic condition. After that, 25 ml of this test inoculum was dispensed equally into the conical flask with the capacity of 125 ml. To this 25 ml of the sample or negative control (sterile distilled water) was added. The mixture was then incubated in an orbital shaker at 30 ºC for 10 h. The growth inhibition of *P. fluorescens* was evaluated by taking the absorbance at 650 nm using a spectrophotometer. The results are presented as percent growth inhibition of the tester strain.

The equation used to calculate the % growth inhibition:

$\%I = OD_c - Od_t/OD_c \times 100$

Where %I is the percent growth inhibition, OD_t is the optical density of the culture incubated with the test sample and OD_c is optical density of the culture incubated with the negative control.

2.3.2 *Saccharomyces cerevisiae* respiration inhibition assay

The assay holds much importance to assess potential toxicant due to involvement of eukaryotic system by using yeast as test organism. Thus, it has more advantage as the results can be better correlated with higher organisms and humans. The test is commonly known as baker's yeast assay due to the use of commercially available baker's yeast as a tester strain. The assay is used to assess cytotoxicity caused by heavy metals and other organic xenobiotics present in the test agent.
For this assay 1% (v/v) suspension of baker's yeast in 0.85% NaCl was taken and then this suspension was stirred on a magnetic stirrer to prevent flocculation of yeast cells. To 800µl of this suspension 200µl of test sample was added and incubated on a shaker for 30 min at 30 ºC. To this 0.1 ml of 0.2% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazoliumchloride (INT) solution and 0.1 ml of 10% yeast extract solution were added and again incubated to start the reaction on a shaker in dark for 1 h. To discontinue the reaction 0.1 ml of 37% formaldehyde solution was added. The INT formazan crystals are formed inside the respiring cells in this reaction after reduction as it works as an artificial electron acceptor in electron transport chain during cell respiration. The respiring cells can be seen in green colour with red crystals inside them. After staining10µl of this suspension with 0.025% Malachite green for one min on a slide, 500 cells were counted by bright field microscope at 100X to examine number of respiring and non-respiring cells. Respiring and non respiring cells can be differentiated by using Malachite green as the dye and the results are demonstrated in terms of percent respiration inhibition [15].

All the sets of the experiment were performed in duplicates with both the samplings for both the assays. All reagents used for the assays were of analytical grade, provided by HiMedia Laboratories Limited and Sigma-Aldrich (Mumbai, India) and stocked at 4 ºC. Fresh master plates of tester strains were prepared every month throughout the experiment.

In cytotoxicity bioassays, the growth patterns of bacterial cells can be observed spectrophotometrically or by using chemical endpoint methods [16]. To evaluate results obtained from these tests, the first necessary step is to evaluate median lethal concentration value of each test sample. The results can be interpreted in a significant manner by using statistical methods and dose response curve. The common parameters considered were EC_{50} and EC_{20}, an estimated concentration of test sample at which 50% and 20% of test organisms have shown an effect after a given exposure time respectively. These results are analyzed statistically using a software XLSTAT 2018; Addinosoft. The results are depicted graphically plotting percent inhibition (%I) against the corresponding concentration (%) using MS excel for both the assays.

3. RESULTS AND DISCUSSION

To our best knowledge, there is no such comparative study on synthetic cosmetic products with herbal cosmetic products using microbial bioassays for their toxicity evaluation. Through this study, an attempt has been made to evaluate the effects of constant exposure of synthetic chemicals present in cosmetic products. Hence, the study holds much importance in terms of consumer awareness. Satisfactory designing of toxicological study is important criterion to assess a potential toxicant. Short term microbial bioassays are perfect choice for preliminary screening of these test agents as they are easy to standardize, take a lesser amount of time, easy to reproduce and are cost effective. Furthermore, in this study eukaryotic structure has been used as a biological test agent having similar genetic material (i.e. DNA) to higher species. Therefore, results of a cytotoxicity evaluation based on these cells can be better correlated with human cells. The samples found to be toxic based on the baseline data obtained by these assays can be further taken for specific and more advanced assessment.

P. flourescens growth inhibition assay

As the dose response profile showed in Fig. 1, the average % growth inhibition increased with the increase in concentration for both the categories of the samples and for both of the samplings. The values of EC_{20} and EC_{50} for all the four samples are depicted in Table 2. These values were computed using logistic regression of inhibition in growth of test organism by the log of the sample concentration (%). Data interpretation of results obtained in this assay depicted that herbal cosmetic products were showing very less toxicity when compared to the products formulated with synthetic chemicals. Both the samples of the cosmetic products formulated with synthetic chemicals have shown considerable levels of toxicity in this assay.
EC_{20} and EC_{50} values of synthetic gulab jal were 8.40% and 34.07% respectively for sampling 1 and 10.90% and 42.94 % respectively for sampling 2, which means that low concentration of this sample was able to cause 20% and 50% cell death of tester strain. On the other hand herbal gulab jal showed higher values of EC_{20} and EC_{50} as compared to the synthetic gulab jal. EC_{50} values for this product were 75.20% and 79.89 % during two respective samplings. Face pack synthetic also showed significant toxicity with low values of EC_{20} and EC_{50}. EC_{20} values for this product were 17.19% and 13.84% for the two respective samplings. However, EC_{50} values were 69.48% and 53.46 % during sampling 1 and 2, respectively. Herbal face pack has given highest values of EC_{20} and EC_{50}, which were 33.98% and 90.69% respectively for sampling 1, and 29.09% and 84.74% respectively for sampling 2. These values depicted that herbal face pack has shown very low cytotoxicity among all four tested samples.

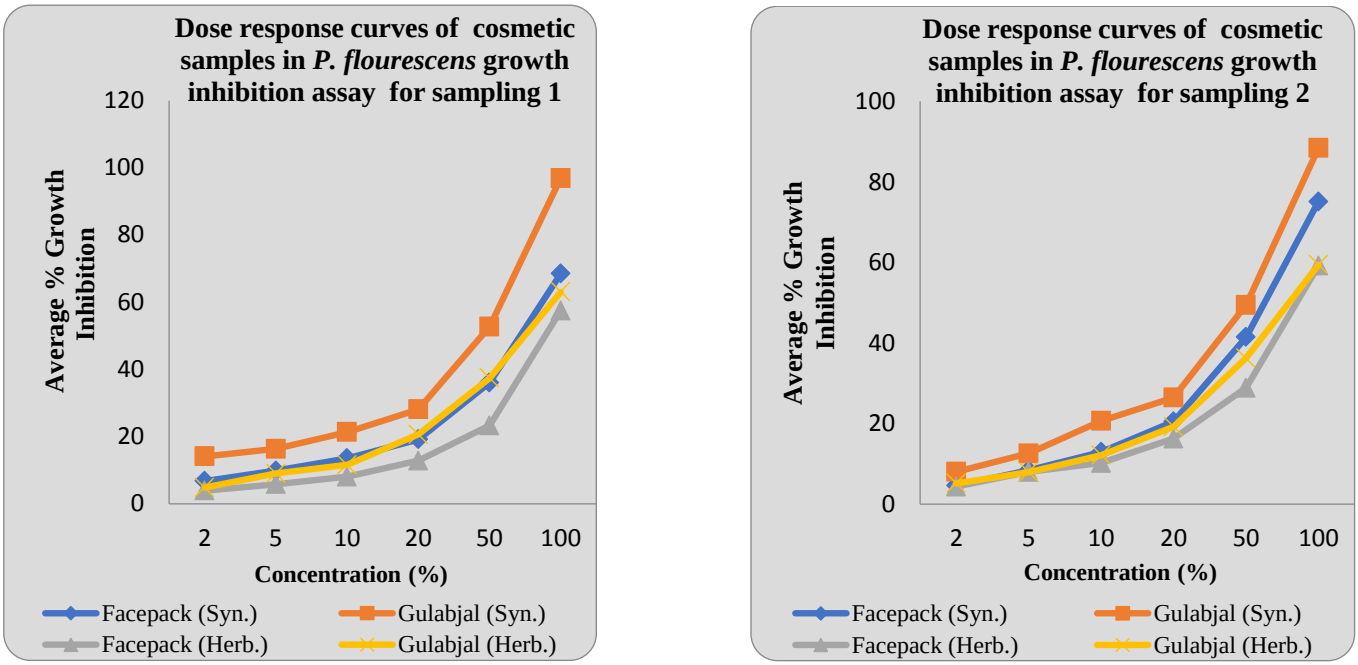

Fig. 1 Dose response curves of herbal and synthetic cosmetic samples using *Pseudomonas flourescens* growth inhibition assay for sampling 1 and 2.

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Table 2 EC_{20} and EC_{50} values of Synthetic and Herbal cosmetics for sampling 1 and 2 using *Pseudomonas flourescens* growth inhibition assay

Samples	EC_{20} (%)		EC_{50} (%)	
	Sampling 1	Sampling 2	Sampling1	Sampling 2
Face pack (Syn.)	17.19	13.84	69.48	53.46
Gulab jal (Syn.)	8.40	10.90	34.07	42.94
Face pack (Herb.)	33.98	29.09	90.69	84.74
Gulab jal (Herb.)	23.65	25.04	75.20	79.89

**Syn. = Synthetic* **Herb. = Herbal*

Saccharomyces cerevisiae respiration inhibition assay

Herbal products were found to be less cytotoxic compared with the synthetic products in this bioassay as well. Both the herbal samples have shown higher EC_{20} and EC_{50} values in comparison with synthetic products. EC_{20} values of herbal face pack and herbal gulab jal were 34.62% and 41.14% respectively for sampling 1 while for sampling 2 these values were 34.72% and 37.39% respectively. However, EC_{50} values could not be calculated as less than 50% respiration inhibition was found to be at 100% sample concentration (Table 3).

Table 3 EC_{20} and EC_{50} values of Synthetic and Herbal cosmetics for sampling 1 and 2 using *Saccharomyces cerevisiae* respiration inhibition assay

Samples	EC_{20} (%)		EC_{50} (%)	
	Sampling1	Sampling 2	Sampling1	Sampling 2
Face pack (Syn.)	0.737	4.45	14.425	37.210
Gulab jal (Syn.)	6.713	11.515	-	-
Face pack (Herb.)	34.62	34.72	-	-
Gulab jal (Herb.)	41.14	37.39	-	-

**Syn. = Synthetic* **Herb. = Herbal*
'-' indicates very high EC_{50} values more than 100%

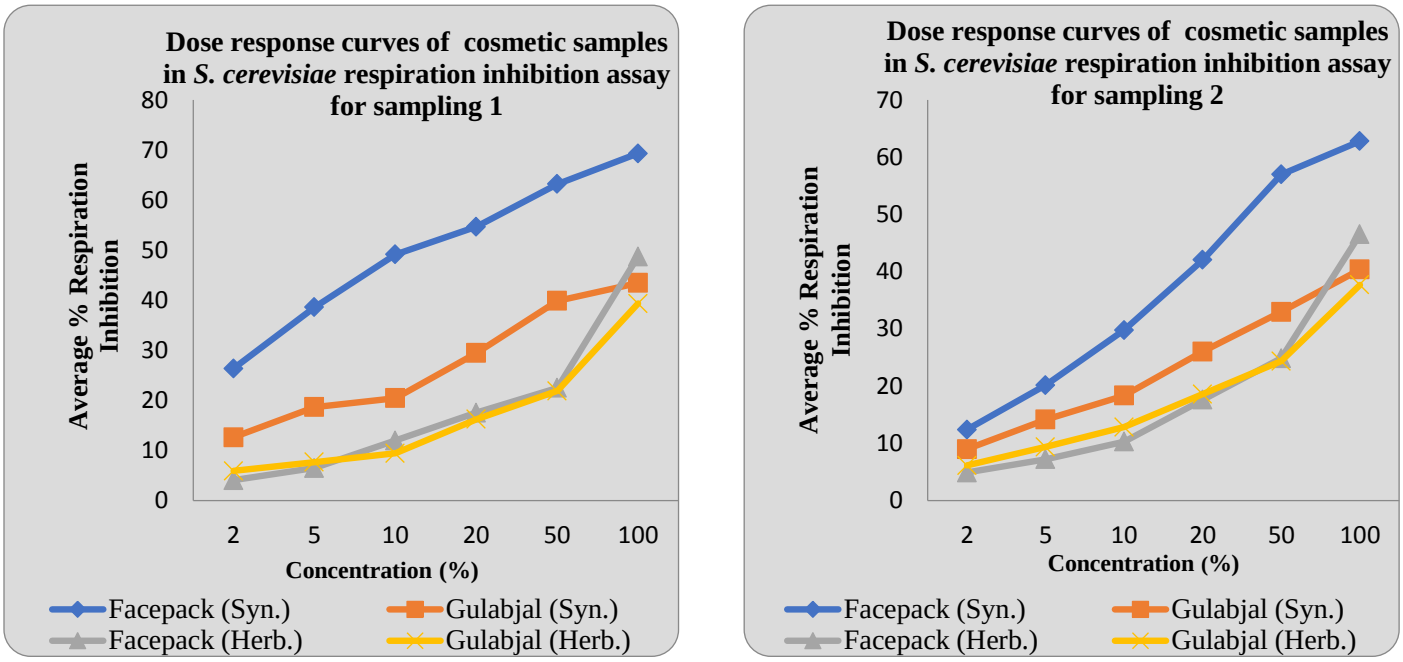

Fig. 2 Dose response curves of herbal and synthetic cosmetic samples using *Saccharomyces cerevisiae* respiration inhibition assay for sampling 1 and sampling 2

Face pack formulated with synthetic chemicals was found to be highest cytotoxic among all four tested samples with lowest EC_{20} and EC_{50} values. EC_{50} values during sampling 1 and 2 were 14.425% and 37.210 %, respectively. Gulab jal formulated with synthetic chemicals was also found to be significantly cytotoxic with EC_{20} values 6.713% and 11.515 % for sampling 1 and 2, respectively. However, for this sample as well EC_{50} could not be calculated as 50% respiration inhibition was found to be at higher than 100% sample concentration indicating that the sample was less toxic compared to the synthetic face pack. Fig. 2 showed dose response profile of all the tested samples for both the samplings for this bioassay.

The data obtained from both the bioassays imply that the herbal cosmetic products were found to be safer than the modern cosmetic products as synthetic ones have shown significant cytotoxicity in both the bioassays. Moreover, both the synthetic products were showing same level of toxicity during both the samplings which indicates that the toxicity is not an outcome of unintended contamination of a single batch of the product. Each herbal product was giving higher values of EC_{20} and EC_{50} as compared to both the synthetic products in both of the bioassays. It should be noted that the cause of cytotoxicity in face pack formulated with synthetic chemicals could be due to the presence of titanium dioxide (TiO_2) in its chemical composition (Table 1). Titanium dioxide has been well known to cause cytotoxic and other toxicogenic effects in many studies [17], [18], [19].

Further, it should be noted that products with chemical constituents taken for this study have more shelf life (*viz*. 24 to 36 months from the date of manufacturing) but the shelf life of herbal products was 12 to 18 months from the date of manufacturing. This illustrates that the herbal products are formulated with less preservatives than the synthetic ones. Hence, with all these observations it can be stated that the prime cause of toxicity to the bacterial cells was chemical ingredients present in the tested synthetic products. A mixture of chemicals may interact with each other and might become toxic [20]. Moreover, tested herbal products having very less chemicals and more natural extracts as listed on their packaging which could run the fact that they were found to be comparatively less toxic.

As compared with synthetic cosmetic products, herbal products have low toxicity potential as well as they are mild and biodegradable [21]. There are many alternatives available in herbal zone that can serve similar purpose for cosmetic use for instance herbal oils *viz*. olive oil and peppermint oil have shown highest SPF (Sun Protecting Factor) values [22].

We have manipulated the natural world around us so that new substances can serve our needs. This dependency may lead to too many troubles. To avoid the toxic effects on health, consumers should be educated about the impact of complex chemical names listed on the product; this will help them to choose the least harmful products. This small change in their lifestyle to decrease the load of toxins will help in their greater good and wellbeing. This field of research needs more attention and concern of the researchers and other scientific and non-scientific communities for consumer awareness and to promote preference for cosmetics which lack toxic ingredients and also to encourage traditional herbal cures through different health care programs. More studies should be published frequently on safety assessment of cosmetic products because a product that is going to be applied on considerable part of the body on daily basis should be safe at first place.

4. CONCLUSIONS

In present scenario, human culture is deeply infused with the importance of cosmetics and personal care products. The contemporary cosmetic products formulated with harmful synthetic agents are being used on the human body extensively and consistently for long time in human lifespan and this result in the possible long-term effects. Thus, requirement of a satisfactory design of toxicological studies has become the topic of concern. From this study, it is concluded that synthetic chemicals used in the cosmetic products can cause significant toxicity. To meet the demand of users, cosmetic formulations should be produced in an organic and hypo-allergic form. Further cell line based studies and other *in vivo* studies need to be accomplished on cosmetic products formulated with synthetic chemicals and development of proper regulation and standardization of herbal extracts that have cosmetic value. The results should be published more often in both scientific and non-scientific publications for the consumer awareness. By avoiding toxic chemicals and opting for natural, organic cosmetic products that have fewer preservatives, we can make better choices in every day routine.

Acknowledgements
The authors would like to thank Dr. P. J. John, Department Head, Department of Zoology for providing complete support during the study and for the financial support from the UGC-BSR meritorious fellowship file no. F.25-1/2013-14(BSR)/5-96/2007 (BSR).

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