COMPARATIVE ANTI-OXIDANT STUDY OF FRUITS AND LEAVES OF *AVERRHOA BILIMBI L.* AND DEVELOPMENT OF AN ANTI-OXIDANT CREAM

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ABSTRACT

A common problem that occurs due to on-going exposure to UV rays is photo aging. Minimal ultraviolet radiation is considered beneficial to human health, however, prevalence of various diseases is associated with solar UV radiation and has been alarmingly increasing Such damages include skin cancers, sunburns, cataracts and accelerated skin aging. The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. *Averrhoa bilimbi L*. has strong antioxidant activity, and can scavenge a variety of free radical. The main objective of the present study was to formulate and evaluate the herbal cream containing *Averrhoa bilimbi L*. including stability studies. Aqueous and alcoholic extracts were used for comparison of activity and it showed that alcoholic extract has more activity compared to aqueous one. Stability studies were performed for a period of two months and there was no significant variation in the properties of prepared herbal antioxidant cream.

Keywords: *Averrhoa bilimbi L*. Antioxidant activity, DPPH, Stability.

INTRODUCTION

Antioxidants are man-made or natural substances that are used to prevent or delay certain types of cell damage and are found in many fruits, vegetables and also available as dietary suppliments. Human beings, possesses antioxidant defenses that protect against oxidative damages, frequent damage removal and repair enzymes to eliminate or repair damaged molecules. However, several studies have been reported that these natural antioxidant mechanisms can be inefficient and some synthetic antioxidant, including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in processed foods, which have some adverse effects. Therefore, recent researches focused to discover natural originated antioxidant has been increased nowadays.

Many fruits and vegetables contains antioxidants such as ascorbic acid, hydroxycarboxylic acids, flavonoids and caratenoids and besides showing antibacterial, anticarcinogenic, antiinflammatory,antioxidant properties,they are also exhibit antiviral, antiallergic, estrogenic and immune-stimulating effects. Antioxidants are capable of reducing, delaying or preventing auto-oxidation where the antioxidants bind together with free radicals to decrease their estructive power, hence, reduce the harmful effect of oxidants.

Averrhoa bilimbi L. belonging to the family Oxalidiaceae has been widely used in traditional medicine as a cure for cold, itches, boils, cough, diabetes, whooping cough, hypertension, syphilis and rheumatism. The phytochemical constituents of Averrhoa bilimbi consists of phenolics, amino acids, citric acids, cyanidin-3-0-h-D-glucoside, potassium ion, sugars and vitamin A . It also has flavonoids, saponins and triterpenoids with flavones such as luteolin and apigenin and physicochemical characteristics such as total soluble solids, oxalic acids and vitamin C. There is a number of research works published for the assessment of total phenolic content determination and antioxidant activity of Averrhoa bilimbi fruits using its crude extracts; however there is no research work for the application of the extracts in cosmetic creams. The present study aimed to develop an antioxidant cream containing *Averrhoa bilimbi* exracts.

MATERIALS AND METHODS

Chemicals

2,2 -Diphenyl-1-picryl hydrazyl (DPPH) was obtained from S.D. Fine Chem, Ltd (Mumbai, India). All other chemicals used were of analytical grade and purchased from Merck Ltd.India.

Collection and Identification

Leaves and fruits of *Averrhoa bilimbi L*. were collected from different localities of Thrissur, Kerala and washed thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying for 5-7 days and then made to coarse powder and stored in an airtight container.

Preparation of extract

The alcoholic extract was prepared by Soxhlet extraction using methanol. 500 gm leaves and fruits of *Averrhoa bilimbi* have to be successively extracted individually in a soxhlet extractor at elevated temperature using 200 ml of distilled methanol (40-60°C). All extracts have to be filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvent from the extract to get dry extracts. The dry crude extracts have to be weighed and stored in air-tight container with necessary markings for identification and have to be kept in refrigerator for future investigation.

The aqueous extract was prepared in water. The fresh fruits and leaves have to be washed under running tap water, for about one minute before the fruits and leaves are to be sliced and dried using cabinet oven. 20g of the ground sample have to be extracted with 600 ml of boiling water for 10 minutes before being blended and filtered with filter paper, Whatman No.4. The water have to be removed by using Rotary Evaporator at 70°C and the extracts are to be stored in amber bottles at 4ºC prior to further analysis.

Phytochemical Screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These chemicals were identified by characteristic color changes using standard procedures described in elsewhere.

DPPH Free Radical Scavenging Activity

A stock solution of DPPH was prepared by dissolving 2mg in 500ml of methanol. 100 mg of crude herb extract was dissolved and make up the volume to 100 ml. From this 20 ml was taken and make up to 100 ml. Different test concentrations was prepared by taking 1,2,3,4, and 5 ml of above solution and make up the volume to 10 ml respectively. To each 2ml of test sample, 4ml of DPPH was added. Control and blank is prepared in an identical manner using ascorbic acid and methanol respectively. The mixtures were shaken vigorously using vortex and left to stand for 20 minutes at room temperature in a dark room and absorbance was taken at 517 nm in a Spectrophotometer. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The scavenging effects on the DPPH radical are to be calculated using the following equation:

Percentage inhibition = [(control-test)/control] x100

Preparation of Antioxidant cream

The formulation was given in table.1. The oily phase and aqueous phase components are to be heated separately up to 70 °C and are to be mixed using homogenizer by addition of methyl paraben, extract and perfume. Care have to be taken for constant and even mixing, the remaining deionised water is added with continuous stirring until the mixture cools and formed as cream. Base cream is prepared in the same method as formulation without extract.

Table:1 Composition of antioxidant cream

Active Ingredient	**Concentration(% w/w)**
Averrhoa bilimbi extract	2.5%
Oily Phase	
Stearic acid	7.00%
Cetyl alcohol	2.00%
Mineral oil	20.00%
Aqueous Phase	
Glycerin	10.00%
Methyl paraben	0.05%
Triethanolamine(TEA)	2.00%
Deionised water	q.s 100%

Evaluation of Antioxidant Cream

The standard procedure has to be followed to evaluate all the parameters.

Physical Properties

The cream have to be observed for color , odour and appearance. To determine the pH, pH meter have to be calibrated using standard buffer solution. About 0.5 g of the cream have to be weighed and dissolved in 50 ml of distilled water and its pH have to be measured.

Determination of Emulsion Type -Dye test

The emulsion type has to be determined by using dye test. The cream is mixed with scarlet red dye and placed a drop on a microscopic slide have to be covered with a cover slip and examined it under a microscope. If the dispersed globules appears colourless the ground is red, the cream is oil in water type. The reverse condition occurs in water in oil type cream.

Homogenecity

The formulations are to be tested for the homogenicity by visual appearance and by touch.

Loss on Drying

1 g of cream has to be taken in china dish and kept in an oven at 105 °C for 2 hours.

Rheological Studies

Take a fixed quantity 10 g of cream in a 10 ml beaker. Keep it impact for 1 hr. The beaker was inclined to one side see whether consistency has changed or not. The beaker was again tilted and checked for pourability of the cream.

Stability Studies

Stability studies were carried out as per ICH guidelines. The cream filled bottle has to be kept in humidity chamber maintained 30 + 2 °C with 65 + 5 % RH for two months. At the end of the studies, samples are to be analysed for the physical properties.

Test for Microbial Growth

The creams are to be inoculated on the plates of Muller Hinton agar media by streak plate method. The plates were placed in the incubator at 37 °C for 24 hours. After the incubation period, plates were checked for the microbial growth by comparing it with the control.

RESULTS AND DISCUSSIONS

DPPH Radical Scavenging Activity

The antioxidant activity of methanolic extracts of fruits and leaves from *Averrhoa bilimbi* were assessed using DPPH radical scavenging activity. The results were shown in the Table 2. The highest radical scavenging activity was recorded in the leaf methanolic extract. Leaf extract had the lowest concentration to exhibit 50 % of the percentage inhibition when compared to that of fruit extract. However, the extracts were found to be less active compared to the standard ascorbic acid. From the above result,it shows that the maximum antioxidant activity was observed in methanolic leaf extract.

Table.2 Antioxidant activity by DPPH assay

Sl.no	Concentrations	Percentage inhibition of			
		Leaves in methanol	Leaves in water	Fruits in methanol	Fruits in water
1	20	41	52	41	21
2	40	58	59	46	26
3	60	36	65	45	31.6
4	80	86	60	73	59
5	100	99	79	90	80

Evaluation of Cream

The methanolic leaf extracts of *A.bilimbi* were chosen to formulate the cream because of its higher antioxidant activities when compared to fruit. The dye test confirms that the formulated creams were o/w type of emulsion cream. The pH of the formulated creams was found to be 4.6 to 6.2.

The formulated antioxidant cream were evaluated for several physicochemical tests and the resuls given in table 3. It showed pleasant odour and light brownish color. The formulated cream was not greasy after application to the skin and easily removable by washing with tap water. The cream shows homogenous distribution of extract which was confirmed by visual examination. There was no change in colour of formulated cream upon keeping for long time. The loss on drying of the formulated cream was found to be within the limit to standard procedure.

All the physicochemical parameters were well maintained during the period of accelerated stability studies at temperatures 80 C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40 °C ± 1 °C in incubator for 8 weeks for formulation. The formulation showed good stability in colour and consistency until the end of accelerated study period.

Table: 3 Evaluation of antioxidant cream

Sl.no	Parameter	Formulation
1	Homogeneity	Homogenous
2	Appearance	Light brown semisold cream
3	Odour	Good

4	Loss on drying	0.11%
5	Preadability	Good
6	Removal	Easily removed
7	Stability	Stable for two months
8	Microbial limit test	<100 colonies

Conclusion

The antioxidant property of leaf and fruit extract of *Averrhoa bilimbi* was determined in methanol and water and it was found that the alchoholic extract shows better antioxidant property than aqueous extract. A cream was formulated with alcoholic extract *Averrhoa bilimbi* and the evaluation test reveals that the formulated cream from methanolic leaf extract showed that it is safe to be used in the skin to protect from extrinsic oxidation sources. The trend of using herbal skin cream is becoming in demand since it is proven that topical application of anti-oxidant cream will be effective against UV radiation and protect the skin from major consequence of UV damage. In conclusion, the topical application of the formulated cream from *A.bilimbi* extract will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values.

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