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A Review on Ethnopharmacology and Methodology of cream: Averrhoa bilimbi

¹Rinki Mehta, ^{2*}Panshul Sharma, ³Jyoti Gupta

^{1.} Rinki Mehta, Student of bachelors of pharmacy, School of Pharmacy, IEC University, Baddi. ^{2.*}Assistant Professor, School Of Pharmacy, IEC University, Baddi.

^{3.} Associate Professor, Head of Department, IEC School of Pharmacy. IEC University, Baddi.

ABSTRACT

Averrhoa bilimbi is used as an antibacterial, astringent, anti -inflammatory, postpartum pills. Averrhoa bilimbi has medicinal properties to the effective treat a number of human diseases. Averrhoa bilimbi has different parts of the plant are used in the different conditions. This fruits are used as a remedy for cough. The syrup from this fruit is used not only to treat the inflammation and fever but also to stop rectal bleeding and relieve internal haemorrhoids The infusion of the flower is believed to be effective for the coughs and mouth ulcers. The fruits are combined with pepper are eaten to cause sweating when people are feeling under the weather. Rubbing the pickled bilimbi paste all the over your body will speed up your recovery from fever. Acidic fruits are employed to clean the blade of a dagger, and they serve as mordants in the preparation of an orange dye for silk fabrics. Because of its oxalic and content, fruit juice is useful for the bleaching stains from hands and rust from white cloth, and also tarnishes from brass. They are applied on bites of the poisonous creatures. Malasiysians take the leaves fresh or fermented as a treatment of venereal disease. A leaf decoction is taken to relief rectal inflammation. A leaf infusion is a remedy for coughs and is taken after child birth as a tonic.

Keywords: Averrhoa bilimbi, haemorrhoids, antibacterial, inflammation.

1. INTRODUCTION

Averrhoa bilimbi Linn is essentially developed for the medicinal purposes in numerous tropical and subtropical countries of the world. Averrhoa bilimbi is mainly used as a medicine in the treatment of hypertension, anti-diabetic, antioxidant, and as an anticancer agent. It is an

attractive, long-lived Averrhoa bilimbi tree, reaching 5-10 m in height. It has a short trunk and a number of upright branches. The fruit is the administered as the treatment for cough and beriberi. Syrup has prepared from the fruit is taken as a cure and inflammation and stop rectal bleeding.[Belimbing Wuluh] The leaves are applied as a paste or swellings of the mumps or poultice on itches, and on skin eruption. A flower infusion is said to be effective against cough and thrush. Averrhoa bilimbi leaves and fruits are containing phytochemicals, such as alkaloids, flavonoids, tannins, saponins and phenols. Averrhoa bilimbi is used as the traditional remedy for the many symptoms .It is also used for the treatment of fever, cough, acne, rectal inflammation, diabetes, itching, ulcers, high blood pressure, and as a cooling drink.[Dragendorf and Wagner reagent] Averrhoa bilimbi is used as an antibacterial, astringent, anti inflammatory, postpartum pills. Averrhoa bilimbi has medicinal properties to the effective treat a number of human diseases. Averrhoa bilimbi has different parts of the plant are used in the different conditions. This fruits are used as a remedy for cough. The syrup from this fruit is used not only to treat the inflammation and fever but also to stop rectal bleeding and relieve internal haemorrhoids.[Belimbing Wuluh] Malaysians use fresh or fermented leaves to treat sexually transmitted diseases. The infusion is a cough suppressant and is taken as a tonic after childbirth. A decoction of the leaves helps relieve inflammation of the rectum. The infusion of the flower is believed to be effective for the coughs and mouth ulcers. [Follin Ciocalteu] The fruits are combined with pepper are eaten to cause sweating when people are feeling under the weather. Rubbing the pickled bilimbi paste all the over your body will speed up your recovery from fever. Acidic fruits are employed to clean the blade of a dagger, and they serve as mordants in the preparation of an orange dye for silk fabrics. Because of its oxalic and content, fruit juice c. It is cultivated throughout in Indonesia, Malaysia, and as exotic in Argentina, Brazil, Cuba, Australia, Colombia, Bangladesh and India. The other common names of A. Bilimbi are bilimbi, cucumber tree, tree sorrel, pickle tree, bilimbim, biri-biri, and bilimbing Assam. In the Philippines, the leaves are applied as a paste or poultice on itches, swelling of mumps and rheumatism, and on skin eruptions. They are applied on bites of the poisonous creatures. Malasiysians take the leaves fresh or fermented as a treatment of venereal disease. A leaf decoction is taken to relief rectal inflammation. A leaf infusion is a remedy for coughs and is taken after child birth as a tonic. A flower infusion is said to be effective against coughs and thrush. Fruit combined with pepper are eaten to the cause sweating when people are the feeling under the weather. A paste of pickled bilimbiis is smeared all over the body to the hasten recovery after a fever. The fruit is administered as a treatment for the biliousness, beri-beri, and

coughs. Syrup prepared from the fruit is taken as the cure for the fever and the inflammation and to stop rectal bleeding and the alleviate internal hemorrhoids. The organic soluble materials of the bark extracts of *Averrhoa bilimbi* were evaluated for photochemical screenings, antimicrobial activity, and anti-thrombolytic activities.



Fig: Flower of Averrhoa bilimbi

1.1 Scientific Classification

- Kingdom: Plantae Plants
- Subkingdom: Tracheobionta Vascular plants
- Superdivision: <u>Spermatophyta</u> Sseed plants
- Division: Magnoliophyta Flowering plants
- Subclass: Rosidae
- Class: Magnoliopsida Dicotyledons.
- Order: Geraniales
- Family: Oxalidaceae Wood-Sorrel family
- Genus: Averrhoa Adans Averrhoa
- Species: A. bilimbi L. bilimbi.

Bilimbi probably originated from Maluku and grew up throughout Indonesia. It has been cultivated and raised wild throughout the Philippines. Distributed in the Ceylon and Burma. It is very common in Thailand, Malaya and Singapore. Often in gardens was Indian Plains. It was introduced in Queensland around 1896 and could be easily integrated and distributed into production networks ductive.

1.2 ETHNOMEDICINAL USES

Averrhoa bilimbiis in traditional medicine for treatment of various diseases. The infusion and decoction of the leaves are used as antibacterial, antiscorbutic, astringent, postpartum protectant in the treatment of fever, inflammation of the rectum and diabetes. Paste of leaves are used to treat itching, boils, rashes, poisonous bites, rheumatism, coughs, colds, mumps, and syphilis. Grated fruit with a little salt is added applied to the face to treat acne. The fruit juice is used to treat scurvy, biliary colic, whooping cough, high blood pressure, obesity, and diabetes.

2 PHYTOCHEMICAL CONSTITUENTS:

2.1 FRUIT:

Analysis of the Malaysian A. bilimbi fruit revealed 53 different ingredients as a volatile. The 12 identified compounds were esters, of which butyl nicotine and hexyl nicotine were present in large quantities.

Fruit extracts contain saponins, flavonoids, and triterpenoids. It also contains Amino acids, Citric acids, Cyanidin-3-O-h-D-glucose Phenolics, Potassium ion, Sugars, Vitamins.

2.2 LEAVES:

Phytochemicals screening of leaf extracts showed the presence of alkaloids, saponins, tannins, cardiac glycosides, glycosides, phenols, triterpenes, flavonoids, and carbohydrates.

Gunawanet al. investigated the isolation of seven constituent from the leaves extract of A. bilimbi. These include squalene, 3, 4-Dihydroxyhexanedioic acid, malonic acid.

2.3 NUTRITION VALUE:

Rich in vitamin C, In addition to vitamins and minerals, fruits contain fiber, ash, protein, moisture and minerals.

Riboflavi Vitamin B2	0.026mg
Ribbinavi vitanini D2	0.02011g
Vitamin B1	0.010mg
V Italiiii D1	0.010111g
Niacin	0.302mg
Tudelli	0.3021115
Ascorbic Acid	15 6mg
	13:0115
Carotene	0.035mg
Carotone	0.0351115
Vitamin A	0.036mg
	0.050115

Table 1. Vitamin per 100mg: Vitamin composition of bilimbi fruit

Table : 2 Minerals per 100 g: Mineral composition of bilimbi fruit

Phosphorus	11.1mg
Calcium	3.4mg
Iron	1mg

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3. PHARMACOLOGY

The traditional claims, as well as roles for the efficacy of *A. bilimbi* in the treatment of various infectious and noninfectious diseases, have been confirmed by several relevant scientific studies. Numerous pharmacological investigations including *in vitro* and *in vivo* (animal) studies have been carried out on the leaves and fruits of *A. bilimbi*. A wide range of pharmacological activities such as antidiabetic, antihypertensive, antithrombotic, hypolipidemic, hepatoprotective, cytotoxic, antimicrobial, wound healing, anthelminthic, and antioxidant have been reported by different researchers so far.

3.1 ROLE OF AVERRHOA BILIMBI

Antioxidants are compounds that interact with and neutralize free radicals, thus preventing them from causing cellular damage. The therapeutic potential of antioxidants in diseases associated with oxidative stress e.g. cancer, diabetes mellitus, and neurodegenerative disorders has gained much attention in recent years. Abas *et al.* studied the antioxidant properties and the effect on nitric oxide production in lipopolysaccharide -activated macrophages of A. *bilimbi* and 11 other Malaysian traditional vegetables. The result obtained revealed that A. precious *et al.* studied the photo protective effect of leaves ethanol extract of *A. bilimbi* against the ultraviolet light induced oxidative damage in albino mice. The study revealed that topical application of extract decreased the effect of UV light-induced photo-aging in mice skin by decreasing malondialdehyde level by up to 50% compared to an irradiated control group. The extract treated animals also showed minimal signs of histological changes and dermatitis compared with the untreated group. This finding suggests that the leaves may possess some anti-aging agent.

Benny et al has investigated the hypoglycemic activity of the semi-purified fractions of an ethanolic leaf extract of Averrhoa bilimbi in high fat diet streptozotocin induced diabetic rats. The long term of aqueous fraction at a dose significantly lower blood glucose and triglyceride concentrations when compared to the vehicle. The hepatic glycogen content was significantly higher in AF treated rats when compared to diabetic control. They found better amelioration of the hyperglycemia and hyperlipidemia in HFD fed-STZ diabetic rats by the aqueous fraction of leaf extract. Hence they have proposed AF as a potential source for the isolation of active principles for oral anti-diabetic therapy. AF-treated rats when compared to diabetic control. They found better amelioration of hyperglycemia and hyperlipidemia in HFD fed-STZ diabetic rats when compared to diabetic control.

rats by the aqueous fraction of leaf extract. Hence they have proposed AF as a potential source for the isolation of active principles for oral anti-diabetic therapy. Another study by Pushparajet al., investigated the possible mechanisms of the hypoglycaemic action of hexane, ethyl acetate, butanol, and aqueous fractions of the ethanol extract of A. bilimbi leaves in male SpragueDawley rats with STZ-diabetes. The results showed that administration of an oral aqueous fraction to rats with STZ-induced diabetes significantly increased insulin secretion and glucose tolerance, while simultaneously decreasing the activity of glucose-6- phosphatase in the liver. The resulting increase in serum insulinis believed that the possible mechanism of action plant.

3.3 Antihypertensive activity:

High blood pressure is considered a major risk factor for various cardiovascular diseases such as arteries, heart failure, stroke, coronary artery disease, and kidney failure. In recent years, much attention has been paid to the use of herbal preparations as an alternative to the treatment and prevention of cardiovascular complications. Traditionally, A. bilimbi berries and leaves have been effectively used as a symptom of blood pressure. Bipatet al. used an isolated in vitro organ model to scientifically investigate the ability of aqueous extracts of A. bilimbi and other plant leaves to lower blood pressure. The aqueous extracts of the leaves were found to significantly reduce norepinephrine-stimulated a trial contractility without affecting the heart rate of guinea pigs. The leaf extract has also shown significant antihypertensive effects in invivo experiments in cats, indicating that the leaf extract may be a potential hypertensive drug.

3.4 Antimicrobial activity:

The development of widespread antibiotic resistance in recent years has created a new demand for new antimicrobial agents for the treatment of infectious diseases. A. Bilimbi ethanol extract has significant antimicrobial activity against 6 pathogens against 2 gram-positive bacteria , 2 gram-negative bacteria and 2 fungi Whole borrowed non-fruit juices and blended dull without juice at 1: 2and 1:4 w/v concentrations, It shows significant activity against Listeria monocytogenes Scott A and Salmonella typhimurium in an in vitro antimicrobial test. The extracts of fruitwasalso found to reduce the microbial load of L. monocytogenes Scott A and S. typhimurium on raw shrimp after washing and storage. This shows the possibility that A. bilimbi will be recognized as a natural method for decontaminating shrimp immediately prior to consumption. Another study showed that A. bilimbi fruit and root extract showed positive activity against Mycobacterium tuberculosis with a MIC of 1600 μ g / ml.

3.5 Anticancer activity:

Cytotoxicity tests are used to find whether a molecule or extract is toxic to cells. Cytotoxicity tests are routinely used in screening for anti-tumor drugs. When analyzing shrimp mortality, it was found that the ethanol extract of A. bilimbi leaves had moderate cytotoxic activity. In another study, methanol fruit extract and its fractions CCl4 and petroleum ether showed significant cytotoxic potential, compared to vincristine sulfate . In another similar study, LC50 values of chloroform and water-soluble fraction were found 5,691 and 6,123 μ g / ml, respectively

3.6 Antifertility:

Studies in mice have shown that bilimbi is a potential source of fertility drugs. It has been shown that the butanol fraction of the ethanol extract reduces fertility. This activity can be caused by one or both of the steroidal glucoside and potassium oxalate components.

3.7 Wound healing:

Several medicinal plants have been shown to have important healing properties. In this context, the use of Averrhoa bilimbi for the treatment of oral injuries has also been scientifically studied. Igaa conducted ainvestigation the effect of A. bilimbi leaf extract on wound healing.

3.8 Antihypertensive activity

High blood pressure is considered a major risk factor for various cardiovascular diseases such as arteries, heart failure, stroke, coronary artery disease, and kidney failure. In recent years, much attention has been paid to the use of herbal preparations as an alternative to the treatment and prevention of cardiovascular complications. Traditionally, A. bilimbi berries and leaves have been effectively used as a symptom of blood pressure. Bipatet al. used an isolated in vitro organ model to scientifically investigate the ability of aqueous extracts of A. bilimbi and other plant leaves to lower blood pressure. The aqueous extracts of the leaves were found to significantly reduce norepinephrine-stimulated atrial contractility without affecting the heart rate of guinea pigs. The leaf extract has also shown significant antihypertensive effects in invivo experiments in cats, indicating that the leaf extract may be a potential hypertensive drug.

3.9 Antihyperlipidemic activity

Ambiliet: al., has investigated antihyperlipidemic activity of the fruit Averrhoa bilimbi in rats using a model of Triton induced hypercholesterolemia. The fruit and its aqueous extracts exhibit remarkable anti hypercholesterolemic activity. The active fraction exhibiting activity at a low dose of 0.8 mg/kg was purified from the aqueous extract. The active ingredient is separated from the active fraction and exhibits optimal activity at a dose of 0.3 mg/kg. The fruit's efficacy was tested on a chronic high-fat diet fed to hyperlipidaemic rats. Fruit and aqueous extract have been shown to be effective in reducing body fat in high-fat rats. They concluded that this fruit can be used as a food ingredient to prevent and treat hyperlipidaemia. Pusparajet al., also effective. Ethanol extract of bilimbi fruit significantly increased the antiatherogenic index and the ratio of HDL cholesterol to total cholesterol. It also significantly reduces the rate of lipid peroxidation in the kidney. Their study demonstrated hypotriglyceridaemia, lipid peroxidation, and anti-atherogenic activity in STZ diabetic rats.

3.10 Thrombolytic activity:

Anticoagulant herbs are used as antithrombotic. Anticoagulant herbs are effectively used for angina, hepatitis, coronary artery disease, dysmenorrhea, rheumatoid arthritis, traumatic injuries, tumors, depression, kidney failure, stroke prevention, and post-stroke syndrome. The anticoagulant effect of A. bilimbi has been demonstrated by Daud et al. in normal and alloxan-induced diabetic rats. In their experiment, they found that oral administration of ethanol extract from leaves and fruits 14 days produced a significant anticoagulant effect, as observed with an increase in prothrombin time.

4. Methodology of Averrhoa Bilimbi

4.1 Plant materials and reagents

The fresh leaves of Averrhoa bilimbi L. was collected on Augest-May 2019 in Dawuan District, Subang city, Indonesia. The samples were than the voucher specimen was deposited at Laboratory of Food and Agro Chemistry, Research Center for Appropriate Technology.Gallic acid, aluminium chloride, ethanol, sodium hydroxide, sodium bicarbonate, phenol were obtained from Sigma-Aldrich Pte Ltd., Singapore. All reagents are used as an analytical grade.

4.2 Preparation plant powder and extracts

The fresh leaves of the Averrhoa bilimbi were washed by the tap water, air dried. The powder was then the soaked in the distilled water or ethanol by maceration methods. The dried crude extracts were stored in the air tight container and kept in a refrigerator for the further analysis.

4.3 Determination of physicochemicals properties of Averrhoa bilimbi leaves dried extracts

Physiochemicals properties viz, pH, yields, total solids, total acid titration, and the colour were analyzed following the methods reported previously.

4.5 Determination of mineral contents of Averrhoa bilimbi leaves powder

The digestion and minerals of the Averrhoa bilimbi L, leaves powders were measured by the atomic absorption spectroscopy following the method reported previously.

4.6 Determination of total phenolic contents

The total phenolic contents of Averrhoa bilimbi leaves extracts were determined by using a Folin-Ciocalteu assay with mirror modification. The measurement of the sample to conducted at the blank using a spectrophotometer.

4.7 Determination of total flavonoids contents

The total flavonoids contents of Averrhoa bilimbi leaf extracts were determined by the aluminium chloride assay as the reported previously. The measurement against of the blank carried out by the spectrophotometer.

5. Antioxidant assay

The antioxidant activity of Averrhoa bilimbi leaf extracts was measured by the using reported previously with the slight modification. The measurement against of the blank was carried out by a spectrophotometer.

5.1 Antibacterial activity

Antibacterial activities of Averrhoa bilimbi leaf extracts were determined by the using disc diffusion method. The sample was dropped to the sterile paper disc and the incubated for 24 hours. Commerical antibiotics, amoxilin and cefadroxill were used as positive control a dose of both antibiotics. Identification of chemical functional group by Fourier transforms infrared spectrophotometer Dried powder, ethanol and water extracts of Averrhoa bilimbi leaves were used for FTIR analysis. The samples were loaded directly in the FTIR spectroscope with a scan range from 450 to 4000cm and each sample was scanned in triplicate.

5.2 Chemicals

2,2-Diphenyl-1-picryl hydrazyl was obtained from S.D fine Chem, Ltd. All other chemicals used were of the analytical grade and purchased from the Merck Ltd. India.

5.3 Collection and identification

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

5.4 Preparation of extract

The alcoholic extract was prepared by Soxhlet extraction using methanol. 500gm leaves and the fruits of Averrhoa bilimbi have to be successively extracted individually in the soxhlet extracter at elevated temperature using 200 ml of the distilled methanol. All the 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 the air tight container with necessary markings for the identification and have to kept in the refrigerator for future investigation.

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

5.5 Phytochemical Screening

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

5.6 DPPH Free Radical Scavenging Activity

A stock solution of the DPPH was prepared by the dissolving 2mg in 500ml of methanol. 100mg of the crude herb extract was dissolved and make up the volume to 100ml. from this 20ml was taken and make up to 100ml. Different test concentractions was prepared by taking 1,2,3,4 and 5ml of above solution and make up the volume to the 10ml 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. This mixture was shaken vigorously using vortex and left to the stand for the 20 minutes at the room temperature in a dark room and absorbance was taken at the 517 nm in a spectrophotometer. A lower absorbance value of the reaction mixture indicates higher free radical scavenging activity. The scavenging effects on the DPPH radical are the calculated using the following equation:

Percentage inhibition =
$$\frac{Control - test}{Control} \times 100$$

5.7 Preparation of antioxidant cream

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

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

Table:3 Composition of a	ntioxidant cream
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6. Evaluation of Antioxidant cream

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

6.1 Physical Properties

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

6.2 Determination of Emulsion Type – Dye test

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

6.3 Homogenecity

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

6.4 Loss on Drying

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

6.5 Rheological Studies

Take a fixed quantity 10g of cream in a 10ml beaker. Keep it impact for 1hr. The beaker was inclined to one side see weather consistence has changed or not. The beaker was again titled and checked for pours ability of the cream.

6.6 Tests for Microbial Growth

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

6.2 Evaluation of Cream

The methanolic leaf extracts of A.bilimbi were chosen to formulate the cream because of its higher antioxidant activities compared to the fruit. The dye test confirms that formulated creams were oil in water type of the emulsion cream. Formulated cream of the pH was found to be 4.6 to 6.2.

Formulation of antioxidant cream was evaluated for several physiochemical tests and results. This shows the light brownish color and pleasant odour. Cream formulated was not greasy after application to the skin and easily removable by the washing with tap water. This cream shows the homogenous distribution of the extract which was confirmed by the visual examination. This formulated cream was not change in colour upon keeping for long time. This formulated cream is loss on drying was found to be limited the standard procedure.

All physicochemical parameter was well maintained during the period of the accelerated stability. The formulation showed good stability in the colour and consistency until the end of accelerated study period.

Parameter	Formulation	
Homogeneity	Homogenous	
Appearance	Light brown semisolid	
Odour	Good	
Loss on drying	0.11	
Preadability	Good	
Removal	Easily removed	
Stability	Stable for two months	
Microbial limit test	<100 colonies	

Table: 4 Evaluation of antioxidant crea	m
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7. Conclusion

Antioxidant property of the leaf and fruit extract of the Averrhoa bilimbi was determined in the methanol and water and it was found that the alcoholic extract showed better antioxidant properties then the aqueous extract. This cream was formulated with alcoholic extract Averrhoa bilimbi and the evaluation test reveals that the formulated cream and from methanolic leaf extract shows that it is safe to be used in the skin to protect from extrinsic oxidation sources. Herbal skin cream trend is using becoming the demand so it is proven that the tropical application of antioxidant cream will be effective against UV radiation and protect the skin from measure consequence of UV damage. In conclusion, the tropical application of this formulated cream from Averrhoa bilimbi extract help in the reducing oxidative damage and the antioxidant effect to our skin due to its high antioxidant values.

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