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PHYSICAL EVALUATION AND ANTI-MICROBIAL ACTIVITY ON POLY HERBAL VANISHING CREAM

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ABSTRACT

Herbal cosmetics are the preparations used to enhance the human appearance. The aim of the present research was to formulate the herbal Cream for the purpose of Moistening, Nourishing, lightening & Treatment of various diseases of the skin. Different crude drugs; Aloe barbadensis (Aloe Vera leaves), Ocimum Sanctum (Tulsi-leaves), *Azadirachta indica* (Neem-leaves), Curcuma longa (Turmeric-rhizomes), Cedro Oil (Lemon Peel), Myristica fragrans (Nutmeg seeds), Olium rosae (Rose Oil), Orange Oil, Prunus dulcis (Almond oil) were taken. Accelerated stability testing of two final sample has been conducted in the environmental chamber with temperature 25 ± 10C and humidity 60 ± 10% RH. All the products were found to be stable with no sign of phase separation and no change in the color. The patch test for sensitivity testing has also been done and no evidence of skin irritation and allergic signs. This work mainly focuses on the assessment of the microbial quality of Formulated cosmetic preparations. To the surprise, both formulations were found to comply with the microbial limit tests as per the international specifications. Thus herbal cosmetics formulation is safe to use was proved and it can be used as the provision of a barrier to protect skin.

Keywords: Vanishing Cream, Herbal Extracts, Phytochemical screening, Physical evaluation

INTRODUCTION

The herbal creams containing synthetic preservatives are used in skin care cosmetics have side effects. Herbal cosmetics are used in crude form and powdered form mixed with various ingredients and directly applied on the skin for immediate relief but cannot be preserved and stored for long periods. Herbal extracts can be prepared from plant parts and prepared into different skin care cosmetic creams, lotions and ointments **[1, 2]**. Herbal cosmetics can prevent the skin from different skin conditions, skin allergic reactions and skin diseases. Herbal cosmetics are preferred more than synthetic cosmetic, they have fewer side effects, safe on skin, efficacy and quality and the cost of these products are also economic and affordable to customers **[3-5]**.

Since time immemorial herbal medicine have been used from ancient times in Ayurveda, Siddha and Unani, these herbal cosmetics like aloe vera, amla, henna, neem, tulsi, turmeric, bitter orange peel, soap nut, cypress and sandal wood in the form of fixed oils and volatile oils are used in many cosmetic creams.

Tulasi (*Ocimum tenuiflorum):* Tulsi is a heavy branched having hair all over. It attains the height of about 75 – 90 cm. It has round oval shaped leaves which are up to 5 cm long. The leaves are 2- 4 cm in length. Its seeds are flat. Its flowers are purple creamish in colour. The Tulsi with the green leaves is called the Sri Tulsi with reddish leaves is called the Krishna Tulsi. Its seeds are yellow to reddish in colour. Leaves of Tulsi contain very essential oil.

Medicinal uses: Tulsi has got the great medicinal value. Tulsi is taken as the herbal tea. The oil extracted from the Karpoora Tulsi is mostly used in the herbal toiletry. Its oil is also used against the insects and bacteria. The Rama Tulsi is the effective remedy for the severe acute Respiratory Syndrome. Juice of its leaves gives relief in cold, fever, bronchitis and cough. Tulsi oil is also used as the ea drop. Tulsi helps in curing malaria. It is very effective against indigestion, headache, hysteria, insomnia and cholera.

Neem (*Azadirachta indica*): It is a tall evergreen tree with the small bright green leaves. It is up to 100 feet tall. It blossoms in spring with the small white flowers. It has a straight trunk. Its bark is hard rough and scaly, fissured even in small trees. The colour of the bark is brown grayish. The leaves are alternate and consist of several leaflets with serrated edges. Its flowers are small and white in colour. The olive like edible fruit is oval, round and thin skinned.

Medicinal uses: The indigenous people of Nilgiris consume the dried and powered tubulers of the terrestrial orchids as an energizing tonic. Neem also holds medicinal value. Each part of neem is used in the medicines. It has been used in Ayurvedic medicines for more than 4000 years. Neem oil extracted from its seeds is used in medicines, pest control and cosmetics etc. Its leaves are used in the treat Chickenpox. According to the Hindus, it is believed that the Goddess of the chickenpox, Sithala lives in the Neem tree. Neem tea is usually taken to reduce the headache and fever. Its flowers are used to cure intestinal problems. Neem bark acts as an analgesic and can cure high fever as of malaria. Even the skin diseases can be cured from the Neem leaves.

Cinnamon (*cinnamomum vergum):* Cinnamon trees belong to a large genus of some 250 species, most of which are aromatic. True Cinnamon is native to Sri Lanka, formerly known as Ceylon and the south-eastern coast of India, while the closely related Cassia is native to China. Cinnamon and Cassia are both small tropical evergreen trees that grow up to 20 - 30 feet tall, with aromatic bark and leaves. Young leaves employ a typical trick of tropical trees to make themselves look unappealing to predatory insects by assuming a limp, reddish appearance, as if wilting. Once they mature they perk up and darken to a deep green. The leaves are elongated ovate with a pointed tip, shiny and dark green on the upper surface, lighter below. The inconspicuous whitish flowers grow in panicles, which later develop into bluish berries. The bark is reddish brown and smooth.

Medicinal uses:

In Ayurvedic medicine Cinnamon oil is used in external applications for rheumatism, aching joints and stiffness. It is also used for toothache and sore gums, much like clove oil. Aryuveda makes use of Cinnamon for the same purposes as Disocorides recommends: as a decongestant for the respiratory tract and urinary problems. It is a good addition to teas for coughs and colds and is sometimes used in steam inhalations for respiratory conditions. In India it is used at the first sign of a cold to prevent it from taking hold fully. The essential oil component of Cinnamon has anti-coagulant properties, which helps to thin blood and improves circulation. (Caution is advised for those already on blood thinning medication). It also exhibits anti-microbial and anti-fungal properties. The anti-microbial action helps to preserve food and can be used in place of common food preservatives. It not only helps to prevent food spoilage by common bacteria, but also by yeasts.

Cinnamon is one of the few herbs that can used to treat fungal growth.

Nutmeg (*myristica fragrans*): Nutmeg is known by many names, such *Myristica fragrans*, mace, magic, muscdier, muskatbaum, myristica, noz moscada, nuez moscada, and nux moschata. It is most commonly used as a cooking spice, comes from the fruit of a 50 ft (15 m) tall tropical evergreen tree. This tree grows in Indonesia, New Guinea, and the West Indies. The bark is smooth and grayish brown with green young branches and leaves. The oblong, fleshy fruit, called the nutmeg apple, contains a nut from which nutmeg is made. The dried nut and essential oil are both used as medicine.

Medicinal uses: Nutmeg relaxes the muscles, sedates the body, and helps remove gas from the digestive track. It is most commonly used for stomach problems such as indigestion. It is also used for chronic nervous disorders, kidney disorders, and to prevent nausea and vomiting. In Chinese medicine, nutmeg is used to treat abdominal pain, diarrhea, inflammation, impotence, liver disease, and vomiting. In the Middle East, some cultures are said to use nutmeg in love potions as an aphrodisiac. The essential oil of nutmeg is used for rheumatic pain, toothache.

Figure 1: Images of crude drugs

Preparation of extracts:

The material is collected, thoroughly washed with water, dried under shade to remove moisture. The material is then subjected to maceration using methanol as the solvent system.

About 20gm of each plant material is taken and kept in glass jars separately for maceration. Plant material is macerated for about 3- 7 days with occasional stirring. After maceration the material is filtered and then the solvent is subjected to distillation

for obtaining concentrated crude extract. Then the crude extract is transferred to china dish and kept in desiccators for further concentration. The amount of crude extract obtained from tulasi was 1.6gm. The amount of crude extract obtained from neem was 1.2gm. The amount of crude extract obtained from nutmeg was 0.82gm. The amount of crude extract obtained from cinnamon from cinnamon was 2gm

Phyto-chemical screening of the extracts:

Phyto-chemical analysis: The test samples were subjected to phyto-chemical analysis in order to find out the presence of phytochemical constituents. The phytochemical tests were employed for alkaloids, tannins, saponins, flavonoids, terpenoids etc. **[6, 7]**.

Table 1: Results for Phytochemical Screening of Crude Extracts

S. No.	Secondary metabolites	Result in Tulasi (leaf) extract	Result in Neem (leaf) extract	Result in Cinnamon (bark) extract	Result in Nutmeg (fruit) extract
1	Alkaloids	Negative	Positive	Positive	Negative
2	Tannins	Positive	Positive	Positive	Negative
3	Flavanoids	Positive	Positive	Negative	Negative
4	Saponins	Negative	Negative	Negative	Positive
5	Steroids	Positive	Positive	Negative	Positive
6	Glycosides	Negative	Positive	Positive	Negative
7	Carbohydrates	Positive	Positive	Negative	Positive
8	Starch	Negative	Positive	Positive	Negative
9	Inulin	Negative	Negative	Negative	Negative
10	Amino acid	Negative	Negative	Negative	Negative
11	Terpenoids	Positive	Positive	Negative	Positive
12	Resins	Negative	Negative	Negative	Positive

By performing the chemical test, the crude methanolic extracts are:

Tulasi contain: tannins, flavanoids, steroids, carbohydrates, and terpenoids.

Neem contain: alkaloids, tannins, flavanoids, steroids, glycosides, carbohydrates, Starch

Cinnamon contain: alkaloids, tannins, glycosides, starch.

Nutmeg contain: saponins, steroids, carbohydrates, terpenoids, and resins.

FORMULATION OF VANISHING CREAM

The base of vanishing cream was prepared by weighing 4.5g of Stearic Acid taken into beaker, heated under flame in waterbath at 70^0C. In another beaker 0.2g of Potassium Hydroxide was weighed and dissolved in water. To this borax and glycerine were added and mixed thoroughly. This solution is heated to 70^0C. Then the solution is transferred into melted stearic acid, stirred at same temperature for 10 minutes**.** Then the preservative (methyl paraben) was added. Alcoholic extracts like *Ocimum tenuiflorum, Azadirachta indica, Cinnamomum vergum,*

Myristica fragrans were added in different concentrations along with other oil components like Almond oil or lemon oil. Finally, the different formulations of herbal vanishing cream were evaluated for various physical parameters and biological parametres **[8-13]**.

Table 2: Formula for preparation of vanishing Cream

S. No.	Ingredients	Working formulae
1.	Stearic acid	4.5gm
2.	Pottasium hydroxide	0.2gm
3.	Borax	0.05gm
4.	Glycerine	1.00ml
5.	Perfume	q.s
6.	Purified water	Upto 25ml
7.	Methyl paraben	0.01gm

Table 3: Uses of ingredients

S. No.	Ingredients	Uses
1	Tulasi	Anti microbial agent.
2	Nutmeg	Flavoring agent, carminative, anti spot.
3	Neem	Treatment on eczema, ringworm infection, psoriasis.
4	Cinnamon	Soothness

Table 4: Composition of Cream

S. No.	Ingredient	F1	F2	F3	F4	F5
1	Alcoholic extract of *Ocimum tenuiflorum*	0.1	_	0.1	0.1	0.1
2	Alcoholic extract of *Azadirachta indica*	_	0.1	0.1	o.1	0.1
3	Alcoholic extracts of *cinnamomum vergum*	_	_	0.1	0.1	0.1
4	Alcoholic extracts of *myristica fragrans*	_	_	0.1	0.1	0.1
5	Stearic acid	13.6	13.6	13.6	13.6	13.6
2	Borax	0.4	0.4	0.4	0.4	0.4
3	Potassium hydroxide	0.6	0.6	0.6	0.6	0.6
4	Glycerine	4	4	4	4	4
5	Water	q.s	q.s	q.s	q.s	q.s
6	Methyl paraben	0.01	0.01	0.01	0.01	0.01
7	Almond oil	0.1	0.1	_	_	0.1
8	Lemon oil	_	_	0.1	0.1	_

Note: Evaluation of polyherbal vanishing cream is done by using Himalaya cold cream as standard

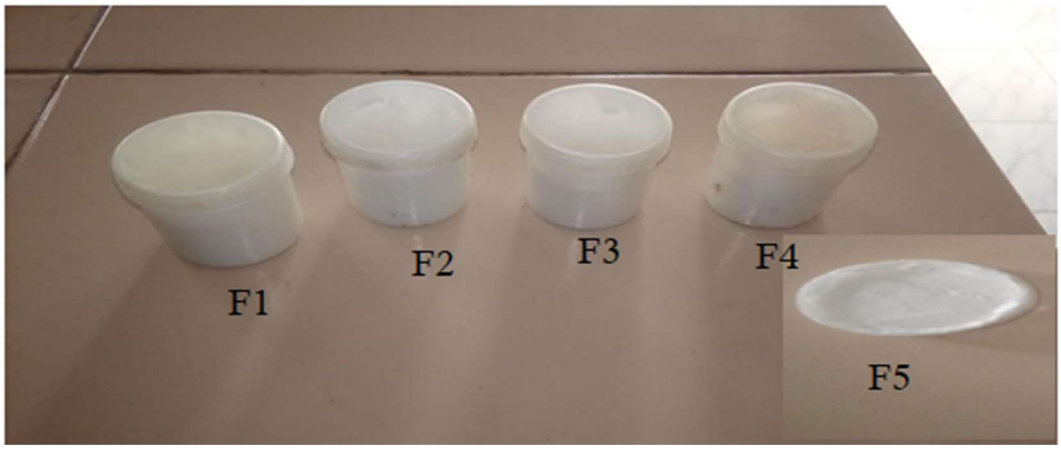


Figure 2: Images of Poly Herbal formulations

Evaluation of Herbal Vanishing Creams

Physical Parameters:

1) **Physical property:** The cream was observed for color, odour, and appearance.
2) **Determination of P^H:** The P^H meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0ml of distilled water and its pH was measured and compared with standard
3) **Homogeneity:** The formulation was tested for the homogeneity by visual appearance and by touch and compared with that of standard.
4) **Appearance:** The appearance of the cream was judged by its color, pearlscence and roughness and graded and compared with that of standard
5) **After feel:** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.
6) **Removal:** The ease of removal of the cream applied was examined by washing the applied part with tap water.
7) **Acid value:** Accurately weigh the 1g of cream and dissolve it in 6ml of mixture of equal volumes of alcohol and ether. Previously neutralize with potassium hydroxide to phenolphthalein solution. Slightly warm the solution of necessary to get a clear solution. Add 2drops of phenolphthalein and titrate with standardized potassium hydroxide and compared with standard.

   Acid value = n*5.61/w

   n = the number of ml of NaOH required.

   w = the weight of substance.

   Acid value titration figure
8) **Irritancy test:** Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time noted irritancy, erythema, and edema, was checked if any for regular intervals up to 24 hrs **[11]**.

BIOLOGICAL EVALUATION OF POLYHERBAL VANISHING CREAM

A) Anti Microbial Activity:

Preparation of test and standard solutions:

The stock solution of ployherbal vanishing cream F3, F4 and F5 were prepared by dissolving in dimethylsulphoxide (DMSO). The stock solution of reference standards (Amoxicillin) was prepared at concentration of 10 mg/ml in

sterile water. Antimicrobial activity was screened by adding 0.05ml stock solution to each cup by micropipette **[12]**.

Culture medium:

Nutrient agar media for bacteria:

Agar……2-2.5%

Meat extract……...0.3%

Peptone……0.5%

Sodium chloride……..0.5%

Distil water……..100ml

P^H adjusted to 7-7.2

The above ingredients are dissolved in distilled water and heated for uniform mixing of agar then adjust the pH 7-7.2 and sterilized at autoclaving at 25lbs for 20 min.

Sterilization:

Sterilization of media, water, etc., was carried out by autoclaving at 15 lbs/ $inch^2$ for 20 minutes. The glassware like syringes, Petri dishes, pipettes, empty test-tubes were sterilized by dry heat in an oven at a temperature of 160ºC for one hour. The sterilized medium was cooled to 40^0C and poured into Petri dishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

RESULT AND DISCUSSION:

PHYSICAL PARAMETERS:

1) **Physical property: (Table 5)**

Table 5: Results for Physical Properties of F1 to F5

S. No.	Color	Odour	Appearance
Standard	White	Characteristic	Semi-solid
F1	Cream	Characteristic	Semi-solid
F2	White	Characteristic	Semi-solid
F3	White	Characteristic	Semi-solid
F4	Cream	Characteristic	Semi-solid
F5	White	Characteristic	Semi-solid

2) **Determination of pH: (Table 6)**

Table 6: Results for pH of Formulations and Standard

Formulations	pH
Standard	6.32
F1	6.20
F2	6.01
F3	6.37
F4	5.73
F5	5.80

3) **Homogeneity and Appearance: (Table 7)**

Table 7: Results for Homogeneity and Appearance of Formulations and Standard

Formulations	Homogeneity	Appearance
Standard	Homogenous and smooth	No color change
F1	Homogenous and Gritty	Color change
F2	Homogenous and Gritty	Color change
F3	Homogenous and smooth	No color change
F4	Homogenous and smooth	No color change
F5	Homogenous and smooth	No color change

4) After Feel, Removal affects and Irritancy: (Table 8)

Table 8: Results for After Feel, Removal affects & Irritancy of Formulations and Standard

Formulations	After feel	Removal	Irritancy
Standard	No residue left	Easily removable	No irritation
F1	No residue left	Easily removable	No irritation
F2	No residue left	Easily removable	No irritation
F3	No residue left	Easily removable	No irritation
F4	No residue left	Easily removable	No irritation
F5	No residue left	Easily removable	No irritation

5) Acid Value: (Table 9)

Table 9: Results for Acid Value of Formulations and Standard:

Formulations	Acid value
Standard	7.1
F1	10.36
F2	10.16
F3	7.6
F4	10.06
F5	9.16

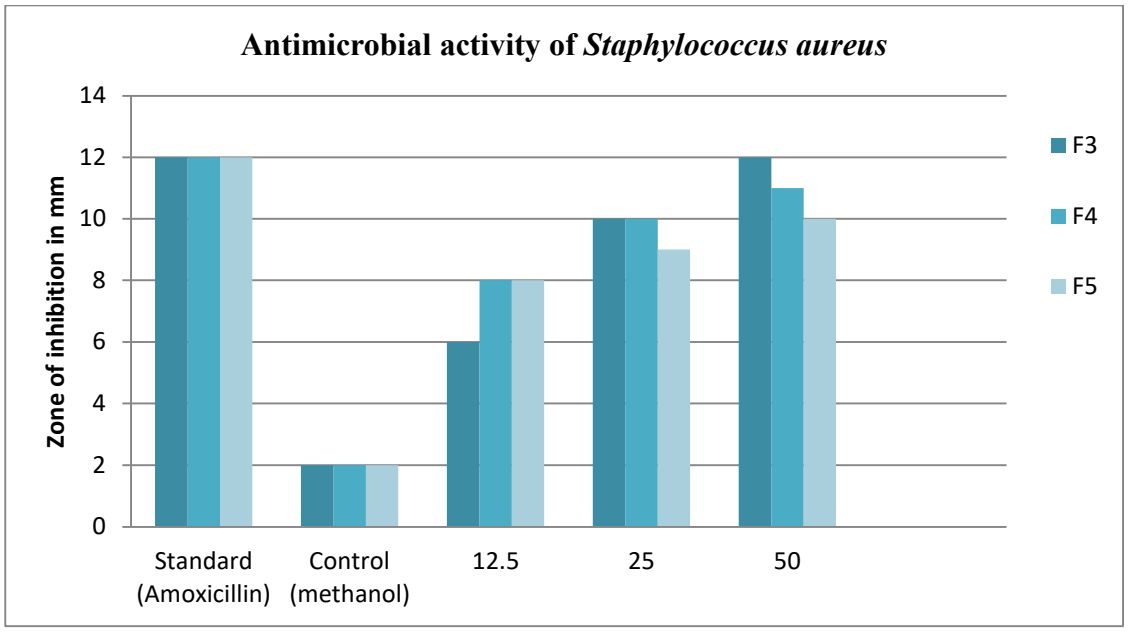
F3, F4 and F5 formulations were preferred for Biological Evaluation as the results of physical parameters were significant for these formulations.

BIOLOGICAL PARAMETERS:

1) Anti-Microbial Activity: (Table 10) (Graph 1)

Table 10: Results for anti microbial activity of *Staphylococcus aureus*

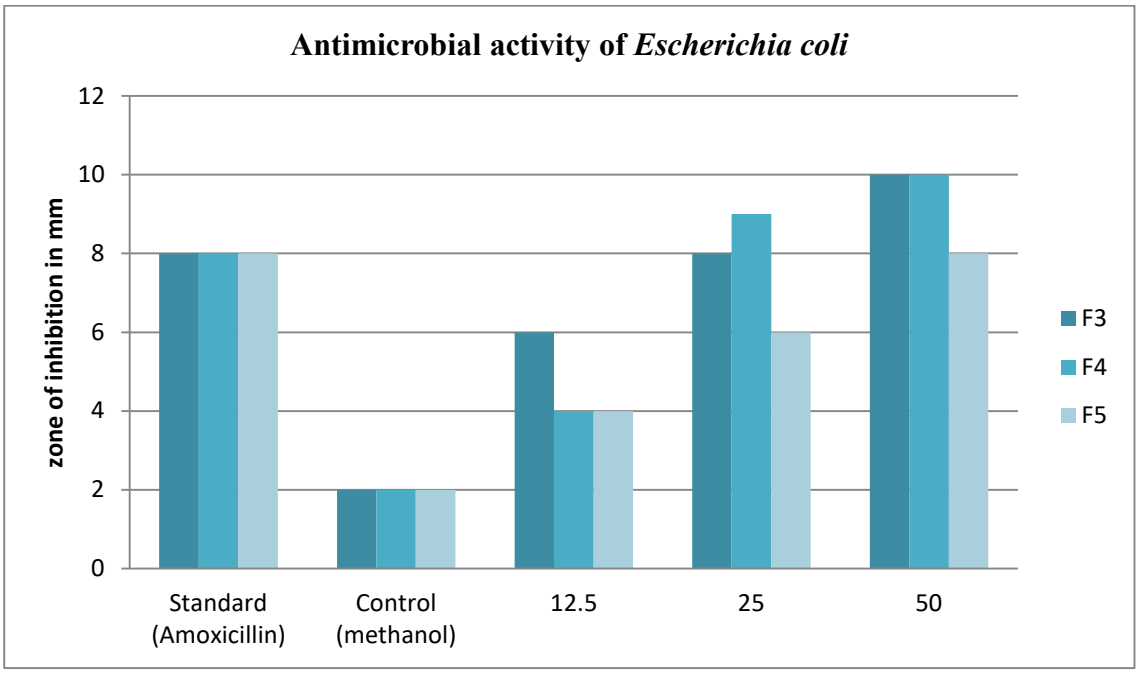
Concentration in mg/ml	Zone of Inhibition of *Staphylococcus aureus* in mm		
	F3	F4	F5
Standard (Amoxicillin)	12	12	12
Control (methanol)	2	2	2
12.5	6	8	8
25	10	10	9
50	12	11	10



Graph 1: Results for anti microbial activity of *Staphylococcus aureus*

Table 11: Results for anti microbial activity of *Escherichia coli*

Concentration in mg/ml	Zone of Inhibition of *Escherichia coli* in mm		
	F3	F4	F5
Standard (Amoxicillin)	8	8	8
Control (methanol)	2	2	2
12.5	6	4	4
25	8	9	6
50	10	10	8



Graph 2

From the above experiment we observed that F3, F4 and F5 formulations of polyherbal Vanishing cream showed antimicrobial activity when prepared at concentrations of 12.5mg/ml, 25mg/ml, 50mg/ml as that of the standard concentration, the concentration of 50mg/ml showed significant activity against *Staphylococcus aureus* and *Escherichia coli* **(Table 11, Graph 2)**.

DISCUSSION:

In the present work, we had decided to extract and formulate herbal Vanishing cream. *Ocimum tenuiflorum, Azadirachta indica, Cinnamomum vergum, Myristica fragrans* are well known for its medicinal values in Indian traditional system of medicine and in herbal preparations. The herbal cream prepared can be easily washed with water that gives better customer compliance. There is growing demand for herbal creams in market and they are invaluable gifts of nature. Therefore we tried to make an herbal cream containing extracts in different concentrations along with almond oil or lemon oil. Our study indicated F3, F4, F5 were found to be more stable and other formulations are not much stable. The

extracts of *Azadiracta indica* and *ocimum tenuiflorum* produces whitening of skin as well as removes marks, acts against pimples. The Almond oil and lemon oil increases the glow on skin and has emollient properties.

CONCLUSION

By performing above experiment, we conclude that we had formulated herbal vanishing cream using alcoholic extracts of different crude drugs. Among the five formulations F3, F4 and F5 showed good results as that of the standard cream. Hence all these properties are beneficial to normal human skin and it is safe and stable too. These studies suggest that composition of creams F3, F4, F5 are more stable and may produce synergistic action.

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