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"Formulation & Evaluation of Anti-oxidant Cream by Using Tea Tree oil And Fenugreek seeds Extract"

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Abstract: The skin is the largest and outermost organ of the human body. Skin aging is a biological process induced by both intrinsic and extrinsic factors. The process of skin aging involves numerous structural and functional changes including the skin wrinkles, thinning and roughening of skin as it ages. Skin care products could be solid, semi - solid, or liquid. The semi – solid preparations includes creams, ointments, and pastes. Cream is an emulsion of oil in water, prepared for skin applications. Antioxidants are a chemically heterogeneous group of compounds which lowers or prevents the harmful effects of free radicals on human tissues. Fenugreek (Trigonella-Foenum Graecum) acts as powerful antioxidant mainly due to presence of flavonoids and polyphenols. Tea tree oil, an essential oil derived from the Australian plant Mecaleuca alternifolia contains more than one hundred different compounds in it. Tea tree oil shows antioxidant properties due to the presence of phenols in the essential oil of tea tree. In this following research we are going to formulate and evaluate an herbal antioxidant cream while using tree tea oil and fenugreek seeds extracts.

KEYWORDS: Skin, Skin – aging, Creams, Antioxidants, Fenugreek (Trigonella-Foenum Graecum), Tree tea oil, etc.

1. INTRODUCTION:

The skin is the largest and outermost organ of the human body. As such, the skin represents the major protective barrier between the internal and external environment and protects the body from environmental damages. Additionally, the skin is important for the regulation of body temperature and water loss and participates in certain immune response [1]. Skin has an extensive surface area of 1.5- 2 m^2. The structure of skin and its cellular composition work in harmony to prevent infections and to deal with physical and chemical challenges from the outside world [2].

Skin aging is a biological process induced by both intrinsic and extrinsic factors. Intrinsic aging is caused by changes in the elasticity of skin over time (Genetic, Chronological), while extrinsic aging is attributed to UV radiation, pollution, and smoking. Exposure to UV radiation (photo aging) is a predominant factor [3]. The process of ageing involves numerous structural and functional changes including the skin. Due to its aging-related loss of functional capacity, the skin becomes more susceptible to develop adverse skin conditions and dermatological diseases (e.g.; Xerosis cutis and itch, fungal infections, skin cancer and dermatitis) [4].

Skin cells are constantly exposed to the harmful effects of the free radicals that are generated by both endogenous and exogenous factors. Although skin has natural defense mechanism against free radicals, it is susceptible to their effects when they are produced in excess amount. Free radicals may damage the skin by destroying lipid components of sebum and ceramides of the intercellular cement of the stratum corneum or by oxidation of polyunsaturated fatty acids of cell membrane phospholipids. Free radical reaction leads to skin lesions, which are characterized by a disruption of defense and restoration mechanisms in skin. Free radicals adversely affect the condition and functioning of the skin, and oxidative stress is one of the major mechanisms of skin aging. Wrinkles, thinning and roughening of the skin are some of the symptoms that affect the skin as it ages. Reactive oxygen species induced oxidative stress plays a major role in skin aging by modulating the elestase enzyme level in the skin [5].

The pharmaceutical formulations used for skin care, termed as the cosmetic, could be herbal in nature. The herbal cosmetics might contain the isolated bioactive compounds or the crude phytoextracts [6]. There are three types of bioactive compounds present in various phytoextracts; the compounds includes polyphenols, flavonoides, and carotenoids. These compounds exert both antioxidants and the UV protection effects [7].

Skin care products could be solid, semi-solid, or liquid. The semi- solid preparations includes creams, ointments, and pastes. A cream is an emulsion of oil and water, prepared for skin applications [8]. The creams are topically used to protect and treat the skin problems including hyper pigmentation and wrinkles. The preparations of herbal

creams may involve the modified methodology using isolated photochemical or the extracts along with appropriate An antioxidant is a substance whose presence in low concentration relative to the substrate susceptible to oxidation significantly lowers or prevents the harmful effects of free radicals on human tissues. Antioxidants are a chemically heterogeneous group of compounds that can be classified according to their structure, solubility (in water or fat), and the kinetics of the reaction in which they are involved [10].

Fenugreek (Trigonella-Foenum Graecum) is known as one of the traditional and most promising medicinal herb belongs to the leguminous family. Fenugreeks have medicinal purposes as anti-diabetic, anti-cancer, anti-microbial, anti-oxidative, and anti-inflammatory activities [11] [12]. Fenugreek is a plant whose seeds and leaves are used in traditional medicine. Fenugreek acts as powerful antioxidant mainly due to the presence of flavonoids and polyphenols. Rutin is a compound of fenugreek that has been investigated for its anti-aging and antioxidant activities, as well as inhibition of collagen degradation [13]. Fenugreek has been reported to posses anti-inflammatory activity mainly due to the presence of the flavonoides as it acts as antioxidant and potential inhibitors of cyclo-oxygenase, lipoxygenase, and nitric oxide synthase [14] [15].

compositions of the mandatory constituents essentially employed for creams with desirable features [9-15].

Essential oils are natural products derived from raw plant materials, including flowers (clove, turmeric, lavender, orange), leaves (rosemary, marjoram, oregano, basil, mint, parsley, tea tree), roots (turmeric, gingers), seeds (coriander, cumin, fennel, almond), fruits (peppers, star anise, tamarind), wood (sandalwood, rosewood), bark (cinnamon), and resin (myrrh) for their various biological properties and medicinal uses. Essential oils extracted from aromatic plants are a potential alternative to antibiotics [16].

Tree tea oil, an essential oil derived from the Australian plant Melaleuca alternifolia, contains more than one hundred different compounds mainly monoterpenes and their derivatives. The main components of Tree tea oil includes terpinene-4-ol, r-terpinene, α-terpinene, 1, 8-cineole, and α-terpineol. Tea tree oil extracts exhibits anti-inflammatory, antioxidant, and anti-bacterial properties. Tea tree oil is also known by other common names such as ‘Cajupat oil’ or ‘Australian tree tea oil’. Tree tea oil exhibited better antioxidant properties than butylated hydroxyl-toluene, which is commonly used as synthetic antioxidant. The antioxidant properties of tree tea oil are due to the presence of phenols in the essential oil of tea tree [17]

2. MATERIALS AND EQUIPMENTS:

2.1. MATERIALS:

2.1.1. Fenugreek

- Botanical name : Trigonella-Foenum Graecum
- Synonym : Bird’s foot, Greek hay, Methi
- Family : Fabaceae
- Chemical constituents : Diosgenin, Saponins, Flavonoides, Galactomanan
- Uses : Antioxidant and anti-microbial agent

FIGURE 1: Fenugreek seeds

2.1.2. Aloe Vera gel:

- Botanical name : Aloe barbadensis
- Synonym : Aloe, Musabbar, Ghrit kumari
- Family : Asphodelaceae (Liliaceae)
- Chemical constituents: Vitamin (A, C, E), Saponins, lignin, oleic acid, stearic acid, b-sitosterol, polysaccharides, minerals.
- Uses : Moisturizing, Anti-microbial, soothing & cooling effects

FIGURE 2: Aloe Vera

2.1.3. Tree Tea Oil:

- Botanical name : Melaleuca alternifolia
- Synonyms : Australian tea tree oil, Cajupat oil
- Family : Myrtaceae
- Chemical constituents: 1, 8-Cineole, terpinen-4-ol, essential oils, α-terpineol, γ-terpinene.
- Uses : Antioxidant, Anti-inflammatory, Anti-acne

FIGURE 3: Tree tea oil

2.1.4. Almond oil:

- Botanical name : Prunus dulcis
- Synonyms : Almond hull extract, Almond oil bitter
- Family : Rosaceae
- Chemical constituents : Oleic acid, Linoleic acid, Palmitic acid, Stearic acid and a little of palmitoleic acid
- Uses : Moisturizer, lessens sun damage, Fragrance

FIGURE 4: Almond oil

2.1.5. Vitamin – E

- Botanical name : Tocopherol
- Synonyms : Alpha – Tocopherol
- Chemical constituents : α-, β-, γ- and δ-tocopherols
- Uses : Emollient

FIGURE 5: Vitamin-E capsules

2.1.6. Bees wax

- Synonyms : Cera alba, candle wax, crude wax
- Family : Apidae
- Chemical constituents : Esters of fatty acids and fatty alcohols, paraffinic hydrocarbons, and free fatty acids
- Uses : As stiffening agent, A natural emulsifier, and as base

FIGURE 6: Bees wax

TABLE 1: ROLE OF INGREDIENTS

S.NO	INGREDIENTS	ROLE OF INGREDIENTS
1	Fenugreek seeds	Antioxidant, antimicrobial, anti-inflammatory
2	Aloe Vera gel	Moisturizer, anti-acne, anti-microbial
3	Tea tree oil	Antioxidant, anti-aging, anti-inflammatory
4	Almond oil	Fragrance
5	Vitamin- E capsule	Emollient
6	Bees wax	Emulsifying agent, stabilizer, and base

2.2. EXTRACTION PROCESS:

2.2.1. Fenugreek seeds extraction:

In a clean beaker add 8-10 spoonfuls of fenugreek seeds. We can also crush these seeds a little in a blender and then use them. Then add 40-50 ml of water along with fenugreek seeds at 75° C for 20-30 minutes. After that filter the remaining filtrate by using muslin cloth to remove the particles and impurities in it. Then the filter extract of fenugreek is used for preparation.

FIGURE 7: Fenugreek seeds extract

2.2.3. Aloe Vera gel extraction:

Mature, healthy and fresh aloe vera leaves were collected washed with distilled water. Then after proper drying of leaves in hot air oven, the outer part of the leaf was dissected longitudinally using a sterile knife. Then the aloe vera gel that is the colorless parenchymatous tissue was removed using the sterile knife. Then it is filtered using muslin cloth to remove the fibers and impurities. Then the filtrate or the filter product which is a clear aloe vera gel was used in preparations.

FIGURE 8: Aloe vera gel extract

2.2. EQUIPMENTS:

TABLE 2: LIST OF EQUIPMENTS

Sr.NO.	LIST OF EQUIPMENTS
1	Slab and spatula
2	Digital pH meter
3	Viscometer
4	Spreadability slides

3. METHOD OF PREPRATIONS:

3.1. FUSION METHOD:

Fusion is the act or procedure of liquefying or melting by the application of heat. By fusion method, all or some of the components of an API are combined by melted together and cooled with constant stirring until congealed. In small scale, the fusion process conducted in a porcelain dish or glass beaker where as in large scale, it is carried out in large steam-jacketed kettles.

3.2. GEOMETRIC SLAB TECHNIQUE:

It is a technique which is to be used when a small amount of a potent substance is to be mixed with a large amount of diluents; the geometric slab method is used to ensure the distribution of the potent drugs. By this method, the potent drug is placed with the approximately equal volume of diluents in a slab with the help of spatula and is mixed thoroughly by trituration. In this technique the compounding slab provide a hard surface for better blending of the mixture.

FIGURE 7: Slab and spatula

4. FORMULA:

TABLE 3: FORMULATION OF THE CREAM

Sr.NO	INGREDIENTS	QUANTITY GIVEN(100gm)	QUANTITY TAKEN(30gm)	USES
1	Fenugreek seeds extract	42 ml	10 ml	Antioxidant, antimicrobial
2	Aloe Vera extract	19 ml	7.5 ml	Anti-bacterial, moisturizer
3	Tree tea oil	7.0 ml	1.0 ml	Antioxidant, anti-inflammatory
4	Almond oil	8.0 ml	1.0 ml	Fragrance, moisturizer
5	Vitamin-E	3.0 ml	1.5 ml	Nutrients, Emollient
6	Bees wax	20 gm	9.0 gm	Base
7	Distilled water	Q.S	Q.S	Q.S

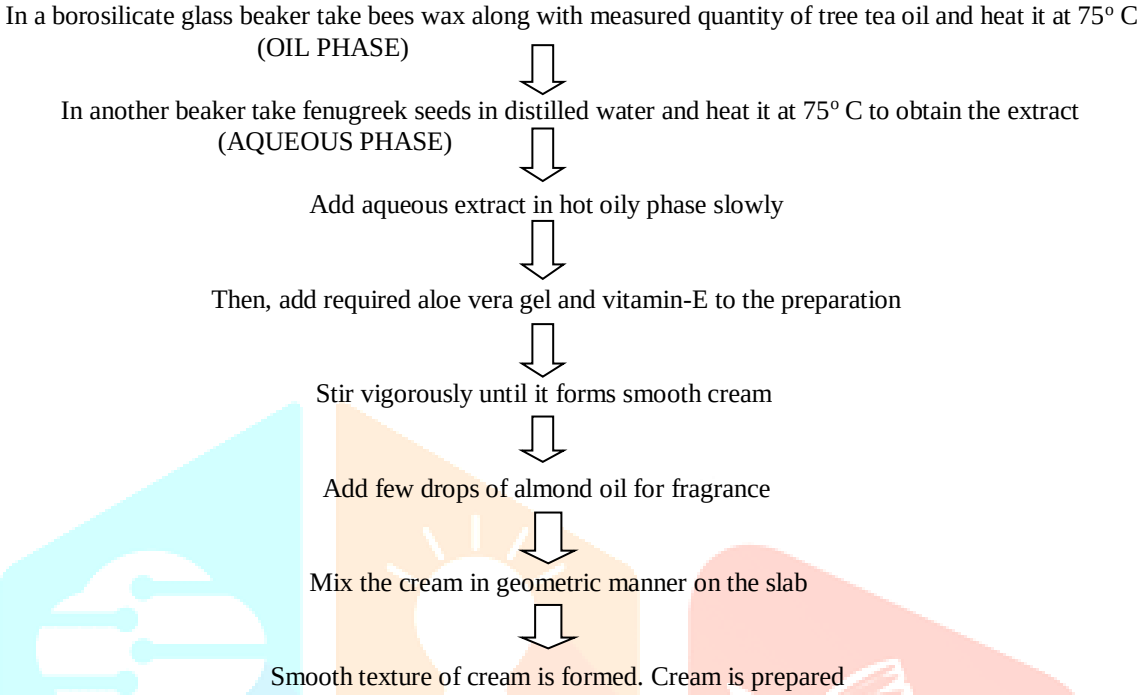
5. FORMULATION:

Heat bees wax along with tea tree oil in a borosilicate glass beaker at 75° C and maintain that heating temperature[oil phase]. In another beaker take fenugreek seeds in distilled water, then heat this beaker to 75° C for 20-30 minutes to obtained the fenugreek seeds extracts [aqueous phase]. Then add this aqueous phase to the oily phase slowly.

Then add measured amount of aloe vera gel and vitamin-E and stir vigorously until it forms a smooth cream. Then add few drops of almond oil as fragrance. Put this cream on slab and add few drops of distilled water if necessary and mix the cream in a geometric manner on a slab to give a smooth texture to the cream and to mix all the ingredients properly. This method is called as slab technique method of preparation of creams.

FIGURE 8: CREAM FORMULATION

FLOW CHART FOR FORMULATION OF CREAM



6. EVALUATION:

6.1. PHYSICAL EVALUATION: [18]

In this evaluation test, the cream was observed visually for its colour, odour, texture and state.

6.2. SPREADABILITY: [18]

The spreadability of cream was determined by the parallel plate method. Two glass slides of 20/20 cm were selected. About 1 gm of the cream formulation was placed over one of the slides. The other slide was place upon the top of the cream such that the cream was sandwiched between the slide and 125 gm weight was placed upon the upper side so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the smear formed was measured.

6.3. DIFFUSION TEST: [18]

Diffusibility gives the amount of cream diffused with the body surface. A small amount of cream is applied on the skin and it is observed to easily diffuse in skin surface.

6.4. STABILITY TEST: [18]

The stability test of the cream is carried out to check for any visible disturbances and phase separation from time to time over a period of time.

6.5. IRRITANCY TEST: [19]

An area (1 cm^2) on the dorsal test hand surface was marked. The cream was applied to the specified area and the time was noted. Irritancy, erythema, oedema were checked for regular intervals upon 24 hours.

6.6. WASHABILITY TEST: [18]

A small amount of cream was applied on the hand and it is then washed with tap water.

6.7. HOMOGENITY: [18]

The cream formulation was tested for homogeneity by visible inspection after the cream was set in a container.
In this homogeneity test they were tested for their appearance and presence of any aggregates.

6.8. VISCOSITY: [18]

Viscosity of the cream was measured after a regular interval of time. Changes in the viscosity were determined by using the viscometer. Viscosity is measured at room temperature and at elevated temperature, 45° C.

6.9. pH: [18]

The pH of the cream was determined by using the pH paper.

6.10. GREASINESS: [18]

Here the cream was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like. According to the result, the cream formulation is non-greasy.

6.11. TYPE OF EMULSION DYE TEST: [19]

The scarlet red dye is mixed with the cream. A drop of the cream was placed on a microscopic slide, and then it was covered with a covered slip and examined under the microscope. If the dispersed globules appear red and the ground is colourless, the cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appear colourless in the red ground.

6.12. MICROBIAL LIMIT TEST: [19]

Microbial analysis was carried out for the formulation as a procedure of Indian Pharmacopoeia 2010 and WHO Guidelines. It includes that bacterial count, total fungal count, and presence of Escherichia coli (E.coli), Staphylococcus aureus (S.aureus), Klebsiella pneumonia (K.pneumonia) and Bacillus cereus (B.cereus).

TABLE 4: PHYSICAL EVALUATION OF CREAM

S.NO	PARAMETER	OBSERVATION
1	Colour	Faint green
2	Odour	Pleasant
3	Texture	Smooth texture
4	State	Semi-solid

TABLE 5: EVALUATIONS OF CREAM

S.NO.	TESTS	OBSERVATIONS
1	Spreadability test	Easily spread
2	Diffusibility test	Easily diffused
3	Stability testing	NO phase separation
4	Irritation effect	NIL
5	Edema	NIL
6	Erythema	NIL
7	Washability test	Easily washable
8	Homogeneity	NO aggregates formed (Good homogeneity)
9	pH	6.8 – 7.1
10	Dye test	O/W type cream

TABLE 6: VISCOSITY OF CREAM

S.NO.	VISCOSITY TEST	OBSERVATION	
		Room temperature	AT 45^{O} C
1	0	1.201	1.208
2	5 Hrs	1.237	1.240
3	10 Hrs	1.283	1.288
4	20 Hrs	1.286	1.292

TABLE 7: MICROBIAL ASSAY

S.NO	TEST	Total bacterial count	Total fungal count	E.coli	S.aureus	B.cereus	K.pneumonia
1	Cream sample	3 X 10^2	1 X 10^2	Absent	Absent	Absent	Absent

6. RESULT:

6.1. Antioxidant activity of Fenugreek seed extract:

The potential of antioxidant activity in fenugreek seed extract was determined using DPPH scavenging essay. The DPPH radical-scavenging test is a commonly employed assay in antioxidant studies. The antioxidant effect of plant extracts on DPPH radical scavenging may be due to their hydrogen-donating abilities,
which reduce the stable violet DPPH radical to yellow DPPH-H. The potential of free radical scavenging can be indicated by the degree of discolouration of the sample or extract. In the present study, the degree of antioxidant activity of extract is given based on the 50% inhibition concentration (IC_{50}). The fenugreek seed extract indicated an IC_{50} equal to 172.6+-3.1µg/ml. This value shows a good antioxidant activity due to the existence of palmitic acid and phytol present in it. They are well known for their antioxidant and some other biological activities. Moreover, the extract of fenugreek seed has also shown some compounds with anti-inflammatory activities. [12]

6.2. Antioxidant activity of Tea tree oil:

The antioxidant activity of Tea tree oil is investigated, using two complimentary in-vitro assays: The 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging assay and the hexanal/hexanoic acid assay In addition, some tea tree oil components showed strong antioxidant activity, when separated from the crude tea tree oil by silica gel open column chromatography and C18-high pressure liquid chromatography and identified by gas chromatography-mass spectrometry. The antioxidant potential of tea tree oil was evaluated by DPPH, thiobarbituric acid reactive species (TBARS) assay and hydroxyl radical scavenging activity method. Tea tree oil was able to reduce DPPH with an EC_{50} (concentration for 50% of maximal effect) of 48.35µg/ml, inhibit the lipid hydroxyl radicals with an EC_{50} of 43.71µg/ml, which indicated that the tea tree oil could serve as a very powerful natural based tool in oxidative stress reduction in skin. [20]

The cream was prepared by the described methods, and their evaluation was carried out for physical (Table- 4), spreadability, diffusibility, stability, irritancy, washability, homogeneity, pH, and dye test (Table-5). In table-6 it shows the viscosity of the cream kept at room temperature and at elevated temperature 45° C. For assessment of the thermal stability of the cream, half portion of the cream is kept at room temperature and remaining half kept at 45° C.

The cream does not show any sudden change in viscosity at room temperature and at an elevated temperature which indicates the stability of the developed cream at higher as well as lower temperature. Regular inspection of the cream is done over a period of time for thermal stability that is stable, without any changes of liquification and phase separation.

- An ideal cream with good consistency, smooth and shining texture, good spreadability, non–greasy in nature, easily washability and good stability is formulated.

6. CONCLUSION :

It is concluded that the cream prepared from tea tree oil and fenugreek seeds extract showed statically significant better antioxidant or anti-aging efficacy as compared with the control. Thus the cream prepared from tea tree oil and fenugreek seeds extract proved for having strong antioxidant activities which further proved for retarding the symptoms of aging. The result obtained in this research is only directional in view and further investigation can be made on this basis to get additional data and information about the fenugreek seeds extract, and combined effects of various herbal extracts can be studied on skin renewal.

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