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Formulation and Evaluation of Herbal Antiaging Cream Extract Containing Annona Squamosa Leaf Extract

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ABSTRACT:
Skin is the largest organ of the integumentary system. The overall well-being& perception of health in humans, very much depends on skin health &beauty.Ageing occurs due to the intrinsic factors like genetics, cellular metabolism, hormone & metabolic process or extrinsic factors like sun exposure, smoking, diet and pollution. In this era of modern science, people choose natural herbs rather than plastic surgery or laser therapy for not only looking younger but also to reduce complications. Herbs help in biological functioning of the skin & supplies nutrients required for healthy skin. Herbs contain several phytochemicals like carotenoids, terpenoids, polyphenols which possesses anti-aging activity. A few herb which shows anti-aging.

KEYWORDS: Anti-ageing, Skin, Herbs, Phytochemicals

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I. INTRODUCTION:

The skin is the largest organ of the body with a total area of about 20 square feet. The skin protects us from microbes and the elements, helps regulate body temperature, and permits the sensations of touch, heat, and cold[1].

Skin has three layers:

- The epidermis, the outermost layer of skin, provides a waterproof barrier and creates our skin tone.
- The dermis, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands.
- The deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue.Theskin"scolour is created by special cells called melanocytes, which produce the pigment melanin. Melanocytes are located in the epidermis. The epidermis renews itself constantly, with new epidermal cells arising from the lower cell layer of the epidermis. As the epidermal cells mature, they gradually rise to the surface of the skin where they ultimately die and are shed.Beneath the epidermis is the dermis, which contains blood and lymph vessels , nerves ,sweatglands and oil glands. Hair follicles are extensions of the epidermis that reach into the dermis. The dermis is made up of networks of elastic fibres (elastin) for suppleness and dense fibres (collagen) for strength. Finally, a layer of fatty tissue lies below the dermis to gives the skin its structure.[11]Ageing or aging is the process of becoming older.

Some of the signs of healthy ageing skin include:

- Thinning – the basal cell layer of the epidermis slows its rate of cell production and thins the epidermis. The dermis may become thinner. Together, these changes mean skin is more likely to crepe and wrinkle.
- Sagging – older skin produces less elastin and collagen, which means it is more likely to sag and droop. Older skin is particularly vulnerable to the effects of gravity. For example, jowls along the jaw and bags under the eyes are simply skin that has yielded to gravity. [10]
- Wrinkles -reduced elastin and collagen and the thinning of skin, mean those.high traffic" areas of the face (like the eyes and mouth) are especially prone to lines and wrinkles.
- Age spots – the remaining pigment cells (melanocytes) tend to increase in number and cluster in certain areas, forming what's known as age or liver spots. Areas that have been exposed to the sun, such as the backs of the hands, are particularly prone to age spots.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

 Dryness– older skin has fewer sweat glands and oil glands. This can make the skin more prone to conditions related to dryness, such as roughness and itching.
 Broken blood – blood vessels in older, thinner skin are more likely to break and bruise. They may also become permanently widened. This is commonly known as broken vessels. [10]

1.1 Molecular and cellular hallmarks of ageing:
In a detailed review, Lopez-Otin and colleagues (2013), who discuss ageing through the lens of the damage theory, propose nine metabolic "hallmarks" of ageing in various organisms butespecially mammals:[9]
 Genomic instability (mutations accumulated in nuclear DNA, and in the nuclear lamina)
 telomere attrition (the authors note that artificial telomerase confers non-cancerous immortality to otherwise mortal cells)
 epigenetic alterations (including DNA methylation patterns, post-translational modification of histones, and chromatin remodelling)
 loss of proteostasis (protein folding and proteolysis)
 deregulated nutrient sensing (relating to the growth harmone factor 1 signalling pathway, which is the most conserved ageing-controlling pathway in evolution and among its targets are the FOXO3/Sirtuin transcription factors and the mTOR complexes, probably responsive to caloric restriction)
 mitochondrial dysfunction (the authors point out however that a causal link between ageing and increased mitochondrial production of reactive oxygen species is no longer supported by recent research).[2]
 cellular senescence (accumulation of no longer dividing cells in certain tissues, a process induced especially by p16INK4a/Rb and p19ARF/p53 to stop cancerous cells from proliferating)
 stem cell exhaustion (in the authors' view caused by damage factors such as those listed above)
 altered intercellular communication (encompassing especially inflammation but possibly also other intercellular interactions
 inflammageing, a chronic inflammatory phenotype in the elderly in the absence of viral infection, due to over-activation and a decrease in the precision of the innate immune system.
 Dysbiosis of gut micro biome (e.g., loss of microbial diversity, expansion of enteopathogens, and altered vitamin B12 biosynthesis) is correlated with biological age rather than chronological age.[12]

1.2 Effect of aging on body:
 Aging changes in skinr
Aging makes changes in your skin, these are common conditions, it increases skin change when people grow older. Skin is the biggest organ in the body. Skin is the visible part of the body and skin is the most visible sign of aging.

 Role of Skin in the body:
The skin has a lot of nerve receptors that help to feel touch, pain, and pressure, Skin helps to control fluid and electrolyte balance. Skin control your body temperature. The skin protects from viruses, bacteria etc from the environment[13]

Skin Layers
 The outer part (epidermis)
 The middle part(dermis)
 The subcutaneous layer (The inner layer under the dermis)

The outer layer of skin contains skin cells,,pigment and proteins. The middle layer contains sweat glands, hair follicles, blood vessels, and fat. The inner layer of skin contains skin cells, blood vessels, nerves, hair follicles, and oil glands. The dermis provides nutrients to the epidermis.Each layer also contains connective tissue with collagen fibers to give support and elastin fibers to provide flexibility and strength.

II. LITERATURE REVIEW:

Annona squamosa is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar-apples or sweetsops.

Kingdom: Plantae
Phylum: Tracheophytes
Division: Angiosperms
Class: Magnoliids
Order: Magnoliales
Family: Annonaceae
Genus: Annona
Species: Annona squamosa

2.1] SYNONYMS:
ENGLISH-CUSTARD APPLE
HINDI - SEETAPHAL
SANSKRIT - SEETAPHALAM
TAMIL - SITAPPLAM

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

TELGU - SITAAPANDU
BENGALI- ATA

2.2] MACROSCOPICAL STUDIES OF LEAF:
Size-10-15 cm long ,3-5cm width
Shape-Altrnate,Bilateral,,Petiolate,Ovate to Lanceolate
Taste - Bitter
Base- Asymmetric
Margin- Simple
Colour- Dark Green
Surface Glossy Smooth
Odour- Aromatic

2.3] MACROSCOPICAL STUDIES OF FRUIT:
Shape- Round Shape
Size - 5-10cm
Taste - Sweet
Colour- Greenish yellow
Smell- Sweetly Aromatic
Stalk - Thickened

The fruit of Annona squamosa (sugar-apple) has sweet whitish pulp, and is popular in tropical markets.

2.4] STEMS AND LEAVES

Branches with light brown bark and visible leaf scars; inner bark light yellow and slightly bitter; twigs become brown with light brown dots (lenticels – small, oval, rounded spots upon the stem or branch of a plant, from which the underlying tissues may protrude or roots may issue).Thin, simple, alternate leaves occur singly, 5 centimetres (2.0 in) to 17 centimetres (6.7 in) long and 2 centimetres (0.79 in) to 6 centimetres (2.4 in) wide; rounded at the base and pointed at the tip (oblong-lanceolate). Pale green on both surfaces and mostly hairless with slight hairson the underside when young. The sides sometimes are slightly unequal and the leaf edges are without teeth, inconspicuously hairy when young. Leaf stalks are 0.4 centimetres (0.16 in) to 2.2 centimetres (0.87 in) long, green, and sparsely pubescent.

2.5] FLOWERS:

Solitary or in short lateral clusters of 2–4 about 2.5 centimetres (0.98 in) long, greenishyellow flowers on a hairy, slender 2 centimetres (0.79 in) long stalk. Three green outer petals, purplish at the base, oblong, 1.6 centimetres (0.63 in) to 2.5 centimetres (0.98 in) long, and 0.6 centimetres (0.24 in) to 0.75 centimetres (0.30 in) wide, three inner petals reduced to minute scales or absent. Very numerous stamens; crowded, white, less than 1.6 centimetres (0.63 in) long; ovary light green. Styles white, crowded on the raised axis. Each pistil forms a separate tubercle (small rounded wartlike protuberance), mostly 1.3 centimetres (0.51 in) to 1.9 centimetres (0.75 in) long and 0.6 centimetres (0.24 in) to 1.3 centimetres (0.51 in) wide which matures into the aggregate fruit.Flowering occurs in spring-early summer and flowers are pollinated by nitidulid beetles. Its pollen is shed as permanent tetrads.

2.6] FRUITS:

Aggregate and soft fruits form from the numerous and loosely united pistils of a flower which become enlarged and mature into fruits which are distinct from fruits of other species of genus (and more like a giant raspberry instead).The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous on a thickened stalk; 5 centimetres (2.0 in) to 10 centimetres (3.9 in) in diameter with many round protuberances and covered with a powdery bloom. Fruits are formed of loosely cohering or almost free carpels (the ripened pistels).The pulp is white tinged yellow, edible and sweetly aromatic. Each carpel containing an oblong, shiny and smooth, dark brown to black, 1.3 centimetres (0.51 in) to 1.6 centimetres (0.63 in) long seed

International Journal of Pharmaceutical Research and Applications
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Fig 1:-Images of Annona sqamosa leaves and flowers

2.7] DISTRIBUTION:

Annona squamosa is native to the tropical Americas and West Indies, but the exact origin is unknown. It is now the most widely cultivated of all the species of Annona, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand, Taiwan, and China as far north as Suzhou it was introduced to southern Asia before 1590. It is naturalized as far north as southern Florida in the United States and as southas Bahia in Brazil, Bangladesh, and is an invasive species in some areas.

2.8] ETHNOPHARMACOLOGY:

All portions of A. squamosa tree, which is similar to other species within the same genus, are widely used as ethnical medicine against various ailments and human diseases, especially for cancer and parasitism (Gajalakshm et al., 2011). In Ayurveda, srikayas, the fruits of A. squamosa, are reported to be good tonic. It was stated that

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

srikayas have the capacity toenrich blood and to increase muscle strength. It can also be used as apophlegmatisant and can help cool, relieve burning perception and tendency to biliousness. In addition, srikayas are sedative to the heart and alleviate vomiting (Vijayalakshmi and Nithiya, 2015). The seeds are deemed to be abortient and good at eliminating lice in hair according to Yunani medicine. Seed yields oil and resin, which act as a decontaminant, and mixed with gram-flour, are good for hair wash (Gajalakshmi et al., 2011). In the south of China, seed extraction was used as a folkloric remedy for "malignant sores" (cancer) (Wu, 2004). Seeds are powerful irritant of conjunctiva and thus can trigger ulcers in the eye. Several research studies in our laboratory cured cornea injury with seeds. Leaves are made into poultice to heal boils and ulcers, and leaf infusion is proved efficacious in treating prolapse in children. A cataplasm, made from bruised leaves with salt, is applied for extraction of guinea-worms (Gowdhami et al., 2014). In Cuban medicine, leaves are taken to lower uric acid levels. Leaves, bark, and unripe fruit were used for diarrhea and dysentery (Kirtikar and Basu, 1918). Folkloric record presented the use of A. squamosa as an insecticidal, an anticancer agent, antidiabetic, anti-oxidant, antilipidimic and anti-inflammatory agent, which have been confirmed by recent investigations

Fig 2:- (a) Annona squamosa plant

(b) Annona squamosa fruit

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

Fig:-3 (a) Tree (b) Fruit and (c) Fruits with seeds of Annona squmosa

2.9] PHYTOCHEMISTRY:

The custard apple is very sweet contains up to 28% sugar in which sucrose 2.53% dextrose 5.05% and laevulose 0.04% with aromatic flavours. It contains significant quantities of Vitamin C, iron, calcium, thiamine, amino acid, potassium, carotene, riboflavin, niacin and ascorbic acid, magnesium and dietary fibres. Despite its high sugar content, the glycemic index of custard apple is low and the glycemic load moderate. Specific chemicals extracted include aliphatic ketones like palmitone. Organic acids like hexanoic and octanoic acid and purines 20 .GC-MS analysis of leaf oil yielded 59 compounds. Main components were βcaryophyllene (31.4%) (natural bicyclic sesquiterpene) δ-cadinene (6.7%), α-murlen (5.5%), α-cadinol (4.3%) and isoquinoline alkaloids. Two acetogenins, annoreticuin and isoannoreticuin isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. The leaves and stems also gave alkaloids dopamine, salsolinol and coclaurine. Others are anonanine, aporphine, coryeline, isocorydine, and glaucine . The alkaloid isolated from the plant was samoquasine, aporphine, benzylisoquinoline, protoberberine and tetrahydro isoquinoline. Other constituents of plant are oxophoebine, reticuline, atidine, histisine, hetidine, hetisine, heterophyllisine, heterophylline, heterlophylline, isoatisine, dihydroatisine, hetisinoneand benzoyl heteratisine. In root and stem, bark oxoaporphines compound like liriodenine, oxoanalobine were identified by NMR spectra. Different chemical constituents like borneol, camphene, camphor, car3-ene, carvone, β-caryphyllene, eugenol, farnesol, geraniol, 16- hetriacontanone, hexacontanol, higemamine, isocorydine, limonine from stems root extracts of plant . Stem bark yielded one acetogenin, solamin, two triterpenoids, stigmasterol, sitosterol and different bullatacin, bullatacinone. About 30 acetogenins were isolated from the seeds squamocins B to N, coumarinoligans, annotemoyin-1, annotemoyin-2, squamocin and cholesteryl, glucopyranoside. These compounds showed remarkable antimicrobial and cytotoxic activities.[13]

III. 3)MATERIALS AND METHODS:

1) COLLECTION OF LEAVES:

The fresh leaves of Annona squamosa were collected from Thube farm Donwade, of donwade region of bhagur. Than after collection leaves were washed by the tap water to remove the soil and dirt.They have been dried under shade for 4-5 days . dried leaves were subjectd to pulverization with the help of mechanical grinder. The obtained dried powder were store in a air tight container.

2) EXTRACTION PROCESS

The extraction of the dried powder of leaves of Annona squamosa was carried out with thehelp of simple distillation process. The process continues for 4 hours, the obtained extractwas filtered by muslin cloth and after by filter paper . the obtained filterate was poured in a china dish and heated on water bath for evaporation of solvent. The dried brown colour extract was then scratches from the china dish stored in air tight container.[18] [12]

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

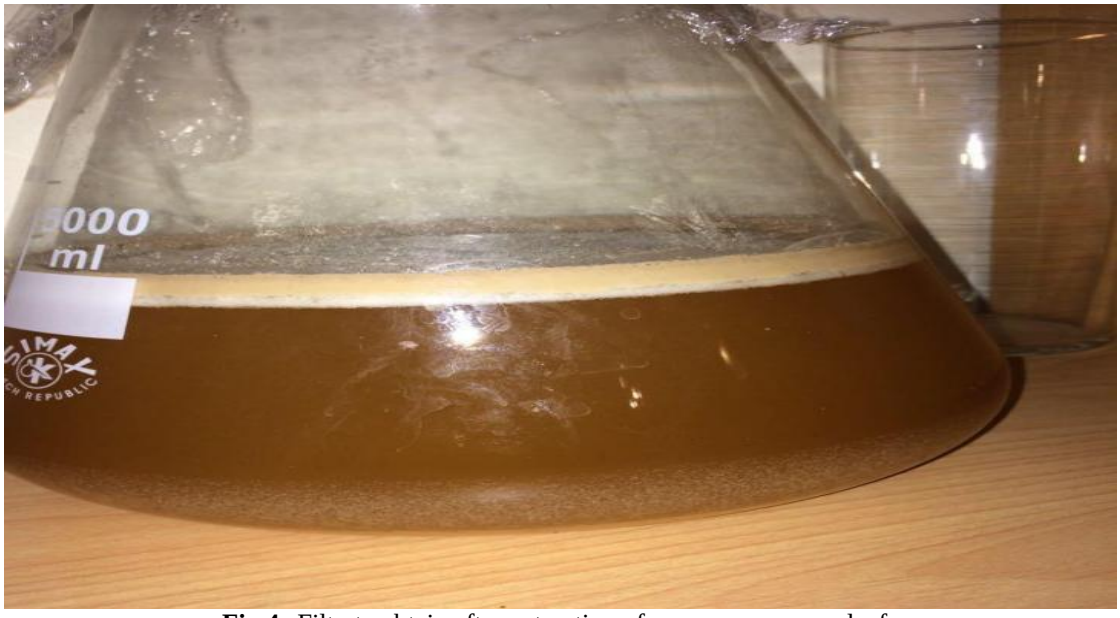

Fig 4:-Filtrate obtain after extraction of annona squamosa leaf

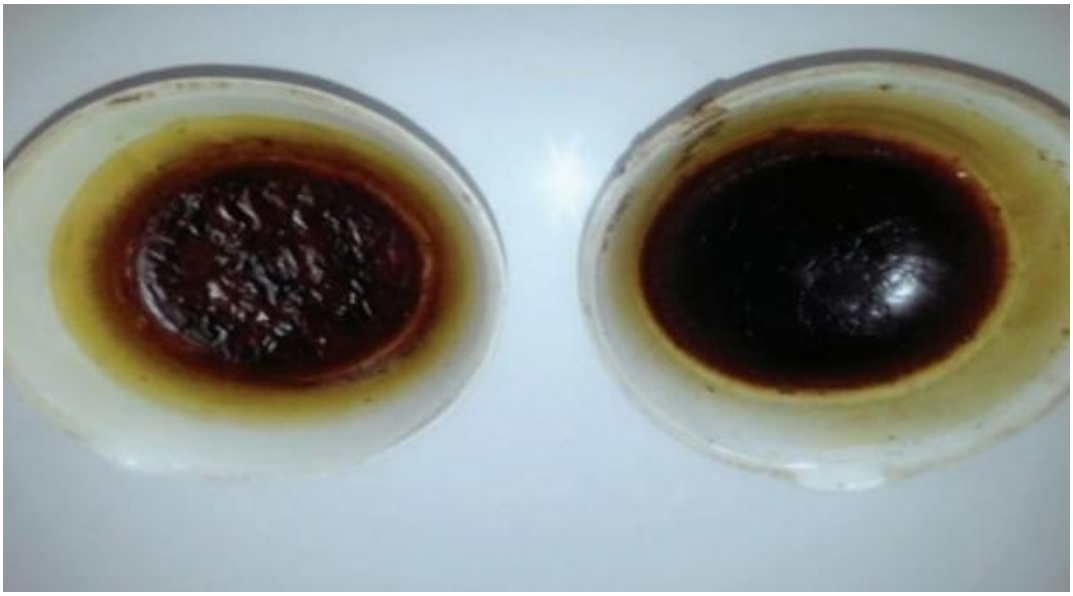

Fig 5:- Dried extract of Annona Squamosa

3.2 : Ingredients Used For Formulation:

1) Annona squamosal (g): The herbal extract of Annona sqamosa which contain antioxidant activity which scavenges the free radicals and prevent ageing process. It isthe main active ingredient of the formulation.

2) Stearic acid (g): The role of steric acid is prevent the water loss and helps to decrease the sign of aging.

3) Cetyl alcohol (g): The role of cetyl alcohol in formulation is that it prevents the separation cream into two phases.

4) Olive oil (ml) :-The main olive of olive oil is it reduce the aging and wrinkle due to its antioxidant content.

5) Polyethylene glycols (ml) : The role of polyethylene glycol it facitilates the penetration of cream in skin. It act as penetration enhancer.

6) Glycerine (ml) : its role is to moisturize the skin and prevent it from getting dry.

7) Triethanolamine (ml) : It act as a stabilizer in formulation.

8) Tween 80 (ml) : It acts as a emulsifier.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

9) Sodium benzoate (gm) : it is a preservative in this formulation.
10) Ethylene diamine tetra acetic acid (gm) : It is a chelating agent. It helps to maintain stability of formulation.
11)Rose water (ml) : It reduce wrinkles because it have antiaging action. It also act as a cleanser.
12) Water : Act as vehicle and keep skin hydrated and refreshed.

TABLE -1: Composition of herbal anti aging cream containing Annona squamosa extract.

INGRIDIENT	F1	F2
Annona sqamosa	3 Gm	4 Gm
Steric acid (g)	6.2 Gm	5 Gm
Cetyl alcohol (g)	0.75 Gm	0.75 Gm
olive oil (ml)	1 ML	1 Ml
Polyethylene glycol (ml)	1.5 ML	1.5 ML
Glycerine (ml)	2 ML	2 ML
Triethanolamine (ml)	0.5 ML	0.5 ML
Tween 80 (ml)	2.5 ML	2..5ML
Sodium benzoate (g)	0.25 Gm	0.25 Gm
ethylene di amine tetra acetic acid (g)	0.020 GM	0.020 GM
Rose water (ml)	3 ML	ML
Water (ml	Q.S	Q.S

PROCEDURE FOR FORMULATION OF CREAM:

An anti-aging cream contains an aqueous phase and oil phase.Oil phase like (stearic acid an emulsifier and other oil miscible components like olive oil, and cetyl alcohol) was mixed (Part A) and heated up to 75°C.
Components of aqueous phase (Part B) mixed together and warmed to about the same temperature of an oil phase.
After heating, the aqueous phase and oil phase were mixed by continuous stirring in a magnetic stir.
The therapeutically active ingredient and preservative sodium benzoate were added after cooling to 40°C.
Then Perfume was added at last. [14][16]

IV. RESULTS AND DISCUSSION:

Phytochemical screening of leaf extract of A. squamosa (custard apple) Phytochemical screening of the freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, and it was performed using the following reagents and chemicals

1) Test for flavonoids

A few drops of dilute sodium hydroxide were added to 1 ml of the extract. An intense yellow color was produced in plant extract, which becomes colorless in an addition of a few drops of dilute acid indicates the presence of flavonoids.

2)Test for terpenoids

Chloroform (2 ml) and concentrated H2 SO4 were added with plant extract to form a layer. The presence of terpenoids is determined when a reddish-brown coloration at the interface was formed.

3)Test for tannins and phenols

The test extract was taken in water, warmed and filtered. 5 mL of the filtrate were allowed to react with 1 mL of 5% ferric chloride solution. Dark green or deep blue color shows thepresence of tannins and phenols.

4) Test for the steroid

From 0.5 ml of acetic anhydride to 0.5 ml of chloroform, the extract was treated and then concentrated solution of sulfuric acid was added slowly, and the green-bluish color was produced which shows the presence of steroids.

5)Test for alkaloids

About 1 ml of extract adds to 2 ml of Wagner's reagent (iodine in potassium iodide) a reddish brown precipitate confirms the presence of alkaloids

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

TABLE - 2: Various chemical constituents present in Annona squamosa

PHYTOCHEMICAL CNSTITUENT	PROCEDURE	OBERVATION
FLAVONOIDS	1 ml extract + NaOH+Dil HCL	Intense yellow than colourles after addition of dil HCl
STEROIDS	0.5ml acetic anhydride +0.5ml chloroform + extract +conc H2SO4	Green bluish colour
ALKALOIDS	Extract + Wagner's reagent	Reddish brown ppt
TANNINS AND PHENOLS	Extract + water warm fiter to fiterate + 1 ml 5% FeCl3 solution	Dark blue

(a)

(b)

Fig :-6(a) Test for steroids, (b) Test for tannins and phenols

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

(a)

(b)

Fig 7: (a) Test for flavonoids, (b) Test for alkaloids:

Phytoconstituents	Pet. ether (40°-60°)	Benzene	Chloroform	Propanone	Ethanol	Water
Alkaloids	-	-	-	-	-	-
Flavonoids	-	-	+	-	+	-
Phenolic	-	-	-	+	+	+
Steroids	-	-	+	-	+	+
Tannins	-	-	-	+	+	+

'+' presence, '-' absence

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

These are observations of the Phytoconstituents in herbal antiaging cream like Tannins steroids, terpenoids flavonoids are present shown in following table 2

Evaluation parameters of anti-aging herbal cream

Organoleptic properties:

The organoleptic test of a cream preparation was performed visually including colour, odour, and texture.

Stability of a formulation

Stability of a formulation was evaluated by placing formulation at different storage conditions, that is, 8, and 40°C for 1 months.

Washability

To determine the washability of a formulation a small amount of cream applied to the skin and wash under tap water with minimal force to remove the cream

Homogeneity

The prepared cream formulation was evaluated by visual appearance for its homogeneity. After feel The quantity of cream formulation left after the application and slipperiness and emolliency was checked

pH of the cream

To determine pH of anti-aging cream formulation a pH meter is used. Put 50.0 ml waterin abeaker and dissolved the weighed amount of cream (5 g) in it and its pH was Measured the pH study of a cream formulation was determined in triplicate, and the average of three reading is recorded.

Irritancy test

The cream was applied on the skin, and time was noted. Irritancy, erythema, was checked after application of cream formulation on a specified area of skin and reported at regular intervals up to 2-4 hrs.[18][15][17]

TABLE - 3: Physical properties of herbal anti aging cream formulation

S.R NO	PROPERTIES	F1
1.	COLOR	BROWN
2.	ODOUR	CHATACTERSTICS
3.	TEXTURE	SMOOTH
4.	STATE	SEMISOLID
5.	GRITTINEES	NO GRITTINESS

Formulation and evaluation of herbal antiageing cream containing annona squamosa leaf extract was prepared and evaluated.

V. CONCLUSION:

Due to constant exposure of human skin to the UV radiations present in sunlight, several pathobiological alterations in cells occur such as irregular pigmentation, increased wrinkling,loss of elasticity, dryness, and roughness. For the protection of this signs of aging herbal cosmetic are used as a therapy. Various active constituents such as flavonoids and phenolic acids appear efficient against UV radiation-induced damage.The result showed that the formulated antiaging creams and it's ingredients were consistent in quality and can be easily used .From the above result,it is concluded that the formulation is safe and usable for the skin

VI. FUTURE SCOPE:

Nowday's peoples are not affording the surgery or lesor treatment and the synthetic creams will affects our skin so, mostly peoples are prefering the herbal creams and herbal formulations.In recent era people focuses on herbal products. Herbal antiageing cream contain herbal plant extract in it. Being natural, there is least Noccurrence of harmful effect on the skin or other body parts. Herbal creams contain N-phytochemical constituents which plays an important role to protect the skin.Thepreparation of herbal creams involves the modified methodology using isolated phytochemical or the extract along with appropriate composition of the mandatory constituents essentially employed for creams with desirable features . Due to the presence of numerous bioactive ingredients in phyto extract loaded cream areconsidered more efficacious with lesser side effects.In under age ageing is occur due to some reasons and the synthetic creams has more side effects compare to herbal.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 468-479 www.ijprajournal.com ISSN: 2249-7781

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