



“FORMULATION AND EVALUATION OF ANTI -OXIDANT HERBAL FACE SERUM”

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Abstract:

Nyctanthes arbor-tristis, also known as the "Night-Flowering Jasmine," has been used in traditional Ayurvedic medicine for its antioxidant and anti-inflammatory properties. In this study, we developed an antioxidant face serum incorporating *Nyctanthes arbor-tristis* extract as the primary active ingredient. The serum was formulated to protect the skin from oxidative stress, reduce the appearance of fine lines and wrinkles, and improve skin elasticity and firmness. The *Nyctanthes arbor-tristis* extract-based serum significantly scavenged free radicals, reduced inflammation, and improved skin hydration. This novel formulation offers a natural and effective solution for promoting skin health and protection, making it an attractive addition to the growing market of natural and organic skincare products. *Nyctanthes arbor-tristis* Linn, also referred to as Parijat or night jasmine, is one such plant. This plant, which belongs to the Oleaceae family, grows natively in tropical and subtropical climates across the globe. It has several therapeutic and pharmacological qualities, although being mainly valued for its aesthetic qualities. Each portion of this significant traditional plant from India has therapeutic significance and a variety of pharmacological effects, including antibacterial, antifungal, antipyretic, antihistaminic, antimalarial, anti-inflammatory, and antioxidant properties. It is utilized in Ayurveda, Siddha, and Unani medicine. Phytochemicals such as flavanol glycoside, oleanic acid, tannic acid, carotene, friedeline, lupeol, glucose, and benzoic acid compounds are found in *Nyctanthes arbor-tristis*. *Nyctanthes arbor-tristis* may be a less expensive and potentially harmful substitute for pharmaceutical medications. (5)

Key words: *Nyctanthes arbor-tristis*, antioxidant face serum, skin health, protection, natural skincare, Ayurveda, Harsingar.

INTRODUCTION:

Serum is a concentrated product which widely used in Cosmetology. The name comes from itself in professional cosmetology. The cosmetic serum is as concentrated in water or oil as any other cream. Serum, or other concentrated product that contains ten times more organic matter than cream. Therefore, deal with the cosmetic problem quickly and effectively. Rising global cost of living has led to an increase in demand for cosmetic products. The cosmetics industry established in Malaysia is one of the most important economic resources. The value of cosmetics has increased as more and more people want to stay young and attractive. Serum is a skin care product that contains a gel or lightweight lotion or moisturizer and has the ability to penetrate deep to bring the active ingredients to the skin. A good skin serum may give your skin firmness, a smooth texture, make the pores appear smaller and increase moisture levels. Whether it is a moisturizing, anti-aging or anti-wrinkle product or serum for skin, all of these products should contain antioxidants, cell based ingredients and skin-like ingredients. All skin types need these ingredients to stay as healthy as possible. Gel preparations and Liquids are best for oily skin and a combination, serum and light lotion is best for normal dry skin, more emollients and the best moisturizing creams for dry to very dry skin. Skin texture is all about the skin type but the smart ingredient for healthy skin is the same for everyone, no matter what product, texture or preference you have. The skin is the largest and most protective of the

body for 24 hours, but sometimes the skin can become dry for many reasons such as UV rays, dirt, cosmetics left overnight can cause irritation or allergies. Skin serum is a skin care product that you can apply to your skin after cleansing but before moisturizing with the intention of bringing the ingredients directly to the skin. Serum is particularly well suited for this task because it is made up of a small molecule that can penetrate deep into the skin and bring about a very high concentration of active ingredients. This makes them a tool to identify specific skin care concerns, such as colour, signs of aging. (6)

Cosmetic Serum:

Serum is a highly concentrated product based on water or oil as any other cream. Serums, or concentrates, contain approximately ten times more of biologically active substances than creams, therefore quicker and more effectively coping with cosmetic problems. (7)

Skin:

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions including protection against external physicochemical and biological prevention of excess water loss from the body.

Skin has three layers:

Epidermis: The epidermis is outermost layer of the skin, provides a waterproof barrier and creates our skin tone.

Dermis: It contains tough connective tissue, hair follicles and sweat glands and it is present beneath the epidermis.

Hypodermis: It is made up of fat and connective tissue. (8)

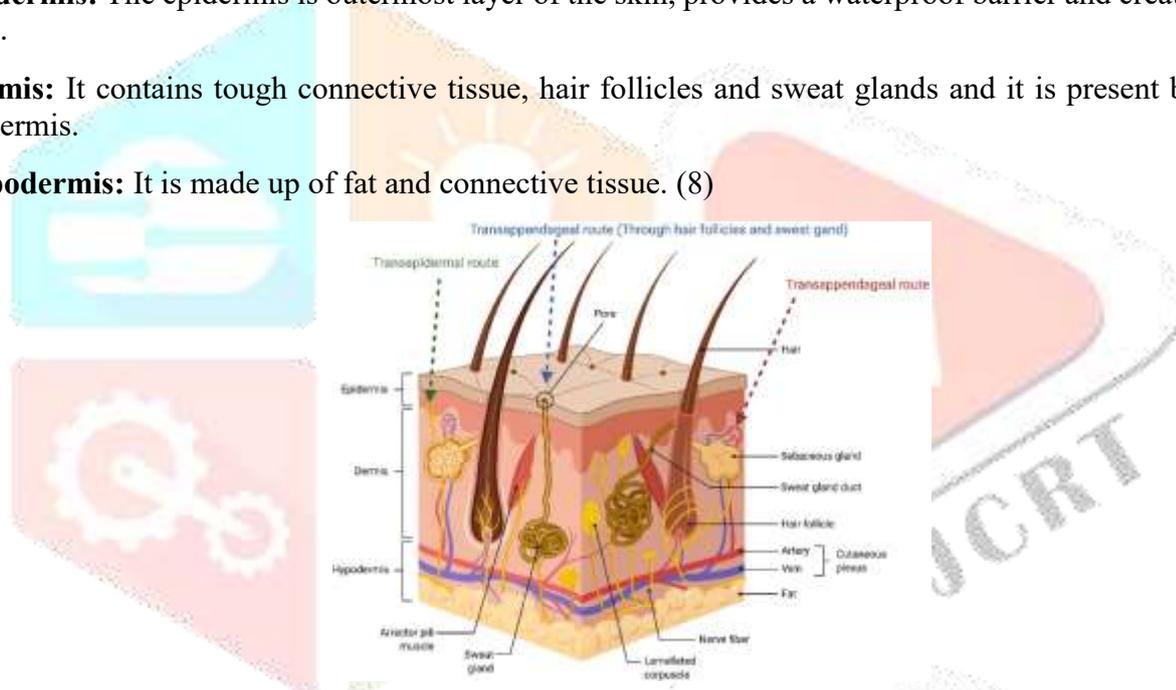


Fig 2. Mechanism of drug penetration through skin.

1. Basics of Serum:

Face serums contain only what we need most. Face serum strips away all of the additives, fillers and fluff that we don't need. As the serum formulation is not necessarily an emulsion, it does not require emulsifiers which could damage the active ingredient. Usually, the serum has few ingredients designed to optimize the availability of the active agent, which may be a vitamin, growth factor, botanical extract, etc. Serums are thinner in consistency as they are made with smaller molecules and no heavy oil or other ingredients. Unlike your face creams and moisturisers, serums could have up to 70 per cent active ingredient concentrations, thus making them perfect for nourishing and layering your skin. Their overall density is allowing for rapid absorption and deeper penetration, also Their thinner viscosity allows for quick dispensing and infusion into the dermis. (9)

Serum Effect:

When concentrates are used the skin immediately gets the necessary amount of active substances in such form which assimilates easier. The active substances in high concentration act in the same way as creams - they moisturise, rejuvenate, lift up etc. The only difference is that in case concentrates are used correctly the noticeable results will be reached quicker. (10)

According to effect produced all serum are strictly divided in following category:

- Lifting up
- Revitalizing
- Moisturizing
- Nourishing
- Anti inflammatory
- smoothening
- Anti stress

Serum act locally upon different body parts: face, neck, decollate, eyelids. Exceptional cases should be taken of the skin around where special preservatives and bases are used, and the doses of active constituent accurately calculated. Serum can be used irrespective of age. When using concentrate you can always get not only quick cosmetics effects. But also, psychological satisfaction after the treatment because they will be seen practically immediately. If the PH level of other serums varies from 5 to 6.5, in eyelids serums it must be neutral – 7 (as PH level of human tears). (7)

2.How to use Serum:

Use of serum also depends on the season and climate where a person permanently lives. In hot climate, concentrates are based on water (for normal and dry skin) or accompanied by antiseptic concentrates (for oily skin as they have a slight drying effect). Use of oily bases is not recommended in this case as it may lead to pore bridging. For this reason, oil bases of any cosmetics whether it is a cream or a concentrate, are recommended for cold or variable climate, in winter. To get the most benefits from a serum, use it as the label recommends. In general, you should put on serum before heavier products. In the morning Apply serum after cleansing, but before moisturizer and sunscreen. In the evening Apply serum after cleansing but before night cream. Always put the serum on first after cleansing, because you want those expensive, active ingredients to penetrate as deeply as they can prior to applying other creams that might create a barrier. (11)

3.How long face serum take to show result:

Face Serums are formulated specially to target specific skin concerns. They contain smaller molecular size ingredients that takes less time to penetrate the skin. Efficacy of a serum & time required to show effect on a particular concern totally depends upon the efficacy of the active ingredient added in it & its base formulation. Various benefits like moisturization, hydration, flakiness, dullness, radiance etc. takes lesser time to appear on application of a serum They can be seen immediately after single application. whereas, intense skin concerns like wrinkles, dark circles, hyperpigmentation, acne etc takes time to show result which may vary from product to product. (12)

4.Face serum benefits:

Generally, face serum is packed with high percentage of Active Ingredients that targets various skin concerns effectively. They have combination of active ingredients that not only target a particular skin concern but solves various other issues related with it. Serums are lighter skin care formulations than moisturizers. The thinner viscosity allows the serum to be absorbed more easily into your skin. This makes a face serum an ideal first step in the layering process. Face serum not only absorbs quickly but they also penetrate into the deep layers of skin to target various areas & give maximum efficacy. They have skin friendly penetrators in the formulation that goes 6-7 layers deeper & replenish the skin with required ingredient. Because they absorb quickly into your skin, a face serum doesn't feel heavy or greasy. The higher concentration of active ingredients may provide more visible results, compared with other types of skin products. (13)

5.Frequency of face serum application:

Anti-pigmentation and antioxidant serums work best when applied in the morning. This helps protect your skin against environmental assaults and free radical production produced by light, including UV and high energy visible light. Anti-aging serums are often best suited for night time application. This allows the ingredients to work with your body's circadian rhythm for skin repair and turnover. Hydrating serums for drier skin can be applied twice daily to help keep your skin dewy and moisturized. (13)

INTRODUCTION OF *NYCTANTHES ARBOR-TRISTIS* LEAVES:



Fig 1 : *Nyctanthes arbor tristis* Plant

Taxonomical Classification: (1)

Kingdom	: Plantae
Subkingdom	: Viridiplantae
Infrakingdom	: Streptophyta
Superdivison	: Embryophyta
Division	: Tracheophyta
Class	: Magnoliopsida
Order	: Lamiales
Genus	: <i>Nyctanthes</i>
Species	: <i>N. arbor tristis</i>
Binomial Name	: <i>Nyctanthes arbor tristis</i>
Family	: Oleaceae

Phytochemical analysis of *N. arbor tristis* :

The physico-chemical properties of *N. arbor tristis* leaves fats had been decided to be 2.10%. These leaves were found to be of dark green colour and produced a viscous, semi-solid substance at room temperature. The acid value of *N. arbor tristis* leaves was found to be 76.27, indicating that this oil is inedible due to a high acid content. The iodine values of *N. arbor tristis* leaves were found to be 134.44, indicating that they are highly unsaturated. Furthermore, these leaves contained 50.01% moisture, 13.98% ash, 15.87% lignin, 9.41% crude fibre, 2.10% fat, 15.02% protein, and 9.48% carbohydrate. (2)

Characteristics Features of *Nyctanthes arbor tristis* :

Nyctanthes arbor-tristis is a shrub or small tree that can grow up to 10 meters (33 feet) in height. It has flaky grey bark and opposite, simple leaves that are 6-12 cm (2.4-4.7 in) long and 2-6.5 cm (0.79-2.56 in) broad, with a smooth edge. The flowers are fragrant, with a white corolla that has five to eight lobes and an orange-red center. They are produced in clusters of two to seven together, with individual flowers opening at dusk and closing at dawn. The fruit is a bilobed, flat brown heart-shaped to round capsule that is 2 cm (0.79 in) in diameter, and each lobe contains a single seed. (3) (4)

Morphology**Leaves:**

Parijat Plant leaves are acute or acuminate, oval in whole leaves are with a few huge Harsinghar leaves are rough and hairy and hard. Leaves and bark are said to be anti-bilious and cough syrup. Leaves are simple, Petiole, and stipulate. So this leaves upper surface is dark green with dotted glands, and the lower surface is pale green and softly pubescent. Leaves contain D-mannitol, β -sitosterole, Flavanol glycosides, Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Tannic acid, Ascorbic acid, Methyl salicylate, resinous substances, Amorphous glycoside, Amorphous resin, Trace of volatile oil, Carotene, Friedeline, Lupeol, Mannitol, Glucose, Fructose, Iridoid glycosides, Benzoic acid. All the important phytoconstituents are being used in Ayurvedic medication and reported for sciatica, arthritis, fevers, and various painful conditions and as a laxative. (14)

PHARMACOLOGICAL APPLICATIONS OF NYCTANTHES ARBOR-TRISTIS:**1. ANTI-ALLERGY ACTIVITY:**

The bark of the plant has additionally been checked for anti-histamine activity. The bark of *Nyctanthes arbor-tristis* was extracted using petroleum ether, chloroform, ethyl acetate, ethanol, and water. Of these extracts, the petroleum ether extract at 50 and 100mg/kg demonstrated the greatest protection against mast cell degranulation by clonidine and resisted contraction (bronchodilation) induced by histamine at 50 and 100 mg/kg more effectively than other extracts, which they suggested might be caused by beta sitosterol. The ability of extracts to inhibit histamine launch appears glaring from the above paintings with the aid of using exclusive groups, with does below proper limits. Though the toxicity studies have not been reported in any of this work, but other reports of toxicity in similar extracts indicate a much safer level. The pretreatment of guinea pigs uncovered to histamine aerosol with a water-soluble part of the alcoholic extract of *N. arbor-tristis* leaves provided sizable safety towards the improvement of asphyxia. Arbortristoside A and Arbortristoside C, two anti-allergic chemicals, have been reported to be present in *N. arbor-tristis*. (15)

2. ANTI-ANXIETY ACTIVITY:

Hydroalcoholic extracts of *N. arbor-tristis* have anxiolytic potential. First, dried plant parts of *N. arbor-tristis* were extracted using a hydro-alcoholic mixture. The extract was then concentrated by distilling off the solvent, evaporated to dryness on a water bath, and stored in an airtight container in a refrigerator until used. (16)

3. ANTI-INFLAMMATORY ACTIVITY:

The extract obtained from the entire plant of *N. arbor-tristis*, as well as the alcoholic extract of its stem and seeds, and the water-soluble part of the alcoholic extract of its leaves, have demonstrated acute and sub-acute anti-inflammatory activity. The acute anti-inflammatory activity was evaluated using different phlogistic agents such as Carrageenan, formalin, histamine, 5-hydroxytryptamine, and hyaluronidase in the hind paw of rats. In sub-acute models, *N. arbor-tristis* was found to significantly reduce granulation tissue formation in the granuloma pouch and cotton pellet test. Additionally, *N. arbor-tristis* was found to inhibit inflammation produced by immunological methods such as Freund's adjuvant arthritis and purified tuberculin reaction. (17)

4. ANTI-VIRAL ACTIVITY:

The ethanolic extract, n-butanol fractions, and two pure compounds, arbortristoside A and arbortristoside C, which are derived from the *Nyctanthes arbor-tristis* plant, have been found to possess strong inhibitory activity against two viruses - the encephalomyocarditis virus (EMCV) and the Semliki Forest Virus (SFV). In laboratory evaluations, the *Nyctanthes arbor-tristis* Linn. Flower extract and its isolated compound have also been tested against the common floral vector, *Culex quinquefasciatus* say (dip). The in-vivo ethanolic extract and the n-butanol fraction, when administered at daily doses of 125 mg/kg weight, have been shown to protect EMCV infected mice against SFV by 40% and 60% respectively. However, it should be noted that only two reports have been found on the anti-viral activity of the ethanolic extract, n-butanol fraction, and Arbortristoside A and C isolated from the seed of the plant against Encephalomyocarditis virus and Semliki forest virus, both in vitro and in vivo. Furthermore, it has been reported that the antiviral activity of arbortristosides B, D, and E was not observed. (18)

5. ANTI-BACTERIAL ACTIVITY:

Infectious diseases are responsible for the majority of premature deaths globally. Pathogens are increasingly developing resistance to antimicrobial agents, and multiple drug resistance is becoming more common. Organisms such as *Staphylococcus aureus*, *Staphylococcus epidermis*, *Salmonella typhi*, and *Salmonella paratyphi A* are among those that have developed resistance. However, a recent study found that the methanolic extract of leaves from *N. arbor-tristis* has significant antibacterial activity against these organisms, with the minimum inhibitory concentration (MIC) ranging between 1-8 mg/ml. The researchers compared the zone of inhibition and MIC of the extracts with those of standard drugs such as ciprofloxacin and fluconazole. They found that the chloroform extract had both antibacterial and antifungal activities, while the petroleum ether and ethanol extracts only had antibacterial activity. (19)

6. ANTI-FILARIAL ACTIVITY:

The flowers' chloroform extract and a compound from *N. arbor-tristis* plant possess larvicidal activity against *Culex quinquefasciatus*, a common filarial vector. (20)

7. ANTI-LEISHMANIAL ACTIVITY:

The anti-leishmanial activity of *N. arbor-tristis* can be attributed to the presence of iridoid glucosides, namely arbortristosides A, B, and C and 6-b-hydroxyloganin. These compounds have demonstrated antileishmanial activity both in vitro and in vivo against amastigotes in macrophage cultures and hamsters test systems, respectively. (21)

8. ANTI-AGGRESSIVE ACTIVITY:

It was discovered that the plant's fresh leaf juice had anti-malarial properties. It has been demonstrated that the plant's 50% ethanolic extract of the seeds, leaves, roots, flowers, and stem possesses anti-amoebic and antiallergic qualities. The plant's leaf extract exhibited ulcerogenic, analgesic, antipyretic, and anti-inflammatory properties. It has been stated that the plant's leaves, seeds, and flowers have immunostimulant properties. It has been demonstrated that the water soluble portion of the ethanolic extract exhibits purgative, antihistaminic, calming, and tumor necrosis-depleting properties. Antitumor activity was discovered for the arbortristoside A that was separated from the seeds. (16)

9. ANTIOXIDANT ACTIVITY:

In vitro evaluation of the different *N. arbor-tristis* leaf extracts' capacity for unbound radical scavenging was conducted using the diphenyl-picryl-hydrazyl (DPPH) test technique. Plant extracts combined with the stable free radical DPPH to produce 1, 1-diphenyl-1,2-picryl hydrazine, which had a wavelength of 517 nm. The following is a decrease in the DPPH radical scavenging effect of plant extracts and standard (ascorbic acid and BHT): (Ascorbic acid > Butanol > Ethyl acetate > BHT > Pet ether). Ascorbic acid was discovered to be the most abundant substance at a concentration of 10 mg, followed by butanol, ethyl acetate, BHT, and pet ether, which were found to be the least abundant at a concentration of 100 mg, 95.22%, 84.63%, and 82.04%, respectively. According to the results of this investigation, several *N. arbor-tristis* leaf extracts have concentration-dependent free radical scavenging activities. According to the results of this investigation, several *N. arbor-tristis* leaf extracts have concentration-dependent free radical scavenging activities. (22)

10. ANTI-ANEMIC ACTIVITY:

Hematological research was conducted on ethanolic extracts of the plant's flowers, barks, seeds, and leaves, and it was observed that the concentration of red blood cells and hemoglobin in rats increased in a dose-dependent manner. Additionally, the extracts prevent the hemogram profile in anemic rats from declining. (23)

11. WOUND HEALING ACTIVITY:

For 16 days, Wistar albino rats were given a 2% w/w *N. arbor-tristis* methanolic extract to investigate the efficacy of *N. arbor-tristis* in healing wounds. It was discovered that both excision and incision wounds need roughly 16 days to fully epithelize before they healed. It was determined that *N. arbor-tristis* extract at a dose of 300 mg/kg would be a good way to treat both kinds of wounds. (22)

12. IMMUNO-STIMULANT ACTIVITY:

Humoral and cell-mediated responses demonstrate the powerful immunomodulatory properties of aqueous leaf extract of *N. arbor-tristis*. Additionally, flower has demonstrated immuno-stimulant action, which turns on the immune system through cells. The ethanolic extracts of *N. arbor-tristis* from the seeds and roots shown immunomodulator effect against systemic candidiasis in mice. Arbotristosides A and C, two iridoid glucosides, were extracted from the plant's seeds. (15)

13. ANTI-ULCEROGENIC AND ULCER-HEALING PROPERTY:

One of the main gastrointestinal illnesses, peptic ulcer, is caused by an imbalance between defensive (primarily mucus-bicarbonate secretion and prostaglandin) and offensive (mostly acid, pepsin, *H. pylori*, and bile salts) components. The two main therapeutic strategies for gastric ulcer disorders are decreasing the production of stomach acid and protecting the gastric mucosa. It has been found that arbotristoside-A and 7-O-trans-cinnamoyl-6 β -hydroxyloganin from *N. arbor-tristis* exhibit anti-ulcerogenic and ulcer-healing properties. These two promote the healing of stomach ulcers and inhibit the development of irritant-induced ulcers. (1)

14. SEDATIVE ACTIVITY:

A hot infusion of the flowers was used to investigate the sedative effects on rats. Male rats in this experiment exhibited a dose-dependent conscious sedative activity, but female rats showed no effects at all. Blood glucose levels were unaffected by these dosages, even at the maximum dosage, nor were muscular strength or coordination *Nyctanthes arbor-tristis*. However, there was a discernible drop in the amount of glucose absorbed by the small intestine. The sedative effect was partially attributed to the antioxidant and membrane-stabilizing activities of the extract. (14)

15. ANTI-CHOLINESTERASE ACTIVITY:

In mice, *N. arbor-tristis* aqueous extract increased acetylcholine esterase activity, counteracting malathion's suppression of the enzyme. The serum exhibited more effects than the brain. The isolated rabbit ileum's weak anti-muscarinic effect against acetylcholine-induced contractions has previously been documented. (23)

AIM: Formulation and Evaluation of Herbal face Serum using *Nyctanthes arbor-tristis*

OBJECTIVE:

1. To harness the antioxidant properties of *Nyctanthes arbor-tristis* extract to neutralize free radicals and protect the skin from oxidative stress and environmental damage.
2. To reduce the appearance of fine lines, wrinkles, and age spots, promoting a smoother and more youthful complexion.
3. To improve skin elasticity and firmness, giving the skin a more radiant and supple appearance.
4. To soothe and calm the skin, reducing inflammation and redness.
5. To enhance skin hydration and moisturization, leaving the skin feeling soft, supple, and nourished.
6. To protect the skin from pollution, UV radiation, and other environmental stressors.

7. To promote even skin tone and brighten the complexion.
8. To reduce the appearance of pores, giving the skin a more refined and even texture.
9. To provide a natural and organic alternative to synthetic skincare products.
10. To develop a product that is gentle, non-irritating, and suitable for all skin types, including sensitive skin.

EXCIPIENT PROFILE:

1. Aloe vera Gel:



Fig 3. Aloe vera gel

Using aloe vera on the face can help moisturize skin. Regularly applying a small amount of aloe vera to the face can help treat various skin conditions, including acne, eczema, and sunburn.

- Kingdom: Plantae
- Order: Asparagales
- Family: Asphodelaceae
- Subfamily: Asphodeloideae
- Genus: Aloe
- Species: Aloe vera
- Botanical name: Aloe barbadensis miller

Vernacular name

- English: India aloe.
- Hindi: Ghrīt kumara, Gwar Patha.

Since prehistoric times, aloe Vera has been used to treat wounds and infections. Aloe Vera is now recognised as a crucial ingredient in makeup thanks to advancements in beauty. It has nearly 20 amino acids, enough amounts of nutrients like calcium, magnesium, and sodium, enzymes, vitamins, and Its polysaccharides, nitrogen, and other ingredients make it a wonder plant for attractiveness. Here is a short explanation of some of the most significant uses of aloe Vera for cosmetic purposes. (24) (25)

2. Sandalwood Oil:

Sandalwood oil is an essential oil used for centuries in various cultures for its therapeutic properties. Traditionally, it is extracted from the heartwood and roots of the East Indian sandalwood tree, one of the world's most valuable trees. Sandalwood oil has been used in traditional medicine for its anti-inflammatory, antiviral, and anti-ageing properties. It is also used in cosmetics and perfumery due to its unique fragrance. The oil contains more than 90% sesquiterpene alcohols, mainly α -santalol and β -santalol, responsible for its therapeutic properties. (26)



Fig 4. Sandalwood Oil.

Worldwide, there are more than a dozen species of sandalwood, most of which have served as sources of essential oil. However, the International Organization for Standardization (ISO) has issued standards for only two species: *Santalum album* and *Santalum spicatum* (West Australian sandalwood). Of the two species, *S. album* produces oil with much higher concentrations of alpha- and beta-santalol. SAO was previously produced from wild-grown trees in India, but over-harvesting and poaching has led to *Santalum album* trees being pushed to the brink of extinction in their native habitats. Since 1998, the trees have been listed as Vulnerable by the International Union for the Conservation of Nature, and the harvesting and export of wild-grown Indian trees is highly restricted. (27)

3. Olive Oil:



Fig 5. Olive Oil

Olive oil is a popular natural ingredient for skin care that has been used for centuries. It contains antioxidants, vitamins, and fatty acids that nourish and moisturize the skin. Olive oil is especially beneficial for dry, sensitive, or aging skin.

Olive Oil For Skincare

It helps to soothe irritation, reduce inflammation, and improve skin texture. The antioxidants in olive oil can also help protect the skin from environmental damage and premature aging. Olive oil can be used as a moisturizer, makeup remover, and hair treatment. Regular use can leave the skin feeling soft, supple, and rejuvenated. Olive oil is a natural ingredient that nourishes and moisturizes the skin, soothes irritation, reduces inflammation, and improves skin texture. It also helps protect the skin from environmental damage and premature aging. Virgin olive oil can be used as a moisturizer, makeup remover, and hair treatment. (28)

4. Glycerine:

Glycerine is naturally occurring in all animals and plant matter in combined form as glycerides in fats and oils or in intracellular spaces as lipids.³ While the chemicals are identical, there is naturally occurring glycerine derived from plants and animals and synthetic glycerine obtained from non-triglyceride sources.



Fig 6. Glycerine.

Glycerine is extremely beneficial to the skin since it serves as a humectant, allowing it to retain moisture, relieve dryness, increase hydration, and regenerate the skin from within. It's also a rich emollient that may soothe skin and help with rough or dry spots caused by psoriasis or eczema. Aside from that, glycerine's significant antibacterial properties protect the skin from hazardous infections. Furthermore, it can mend, restore, and accelerate wound healing. It acts as a vehicle between various cosmetic products such as creams, lotions, serums, toners, etc. (29)

5. Tween 80:



Fig 7. Tween 80.

An effective emulsifier and solubilizer, allowing for the blending of water and oil-based ingredients in cosmetic formulations. This makes it valuable in creating stable emulsions, such as creams, lotions, and serums, ensuring proper dispersion of active ingredients.

6. Coconut oil:



Fig 8. Coconut oil

Coconut oil, beloved for its hydrating properties and rich nutrients, is more than just a cooking ingredient—it's a skincare superstar. It contains nourishing fatty acids and linoleic acid which help retain the moisture in the skin and help protect and hydrate your skin. The medium-chain fatty acids in coconut oil have antimicrobial properties that can help protect against harmful microorganisms. This is especially important

for skin health, as many types of skin infections, including acne, cellulitis, folliculitis and athlete's foot, are caused by bacteria or fungi.

6. Distilled water:

Distilled water has a pH of about 4.5-5 which is close to the skin's pH, hence distilled water is effectively used in many skin and hair care products. Water constitutes almost 65-70% in the cosmetic product, hence it is very important that the very best quality of water is used in cosmetic products, so as to avoid any kind of allergic reaction on your skin.

LITERATURE REVIEW:

- ✦ Herbs have been always the main principal form of medicine since traditions and now a days it becoming more popular form of medicine throughout the world. Herbal medicines are not only providing traditional and ethnic medicine but also promising for highly efficient novel bioactive molecules. (30)
- ✦ Cosmetics are also known as make-up products which are commonly used by young aged adults to improve their appearance and to show them pleasantly. The Food and Drug Administration of America states that, "cosmetics are pretended to be applied on the human body for beautifying, cleansing, changing the appearance, attractiveness promotion without influencing or changing the functions of the body's structure". (31)
- ✦ Herbs play a significant role, especially in modern times, when the damaging effects of food processing and over-medication have assumed alarming proportions. They are now being increasingly cosmetics, foods and teas, as well as alternative medicines. The growing interest in herbs is a part of the movement towards change in life-styles. This movement is based on the belief that the plants have a vast potential for their use as a curative medicine.
- ✦ Herbal products in cosmetics or herb in cosmetics can also be referred as botanical origin products in cosmetics. According to the Drugs and Cosmetics Act 1940 cosmetics may be defined as, any substance intended to be rubbed, poured, sprinkled, or otherwise applied to human being for cleansing, beautifying, promoting attractiveness. Cosmeceuticals are the cosmetic products which contain biologically active principles or ingredients of plant origin having effect on user or they are combination product of cosmetics and pharmaceuticals intended to enhance the health and beauty of skin. Herbal cosmetics are defined as the beauty products which possess desirable physiological activity such as healing, smoothing appearance, enhancing and conditioning properties because of herbal ingredient. (32)
- ✦ *Nyctanthes arbor-tristis* (Oleaceae) popularly known as "Parijat" is a plant of great importance in India. It is widely used in Ayurvedic medicines. Each part of this plant has some medicinal value. It possesses extensive medicinal uses, viz., antipyretic, anti-inflammatory, anthelmintic, sedative effect, laxative, and expectorant, in rheumatism. The present review aims to perform a detailed compilation of work done on this plant mainly as a source of the antioxidant and anticancer agent as well as various pharmacological properties from 1987 to till date. All these activities possessed by plants are due to the presence of multiple phytochemicals which can act as a source of active pharmacological agents. (33)
- ✦ An effective emulsifier and solubilizer, allowing for the blending of water and oil-based ingredients in cosmetic formulations. This makes it valuable in creating stable emulsions, such as creams, lotions, and serums, ensuring proper dispersion of active ingredients.
- ✦ Glycerine extensively employed in cosmetic and Herbal industry. Glycerine acts as humectants, help in skin to remain moistened and protect from excessive dryness. Glycerine can help moisturize and protect skin. But, on the face it may dehydrate and cause blistering, especially if undiluted. Glycerine is reported to function in cosmetics as a denaturant, fragrance ingredient, hair conditioning agent, humectant, oral care agent, oral healthcare drug, skin protectant, skin conditioning agent, and viscosity decreasing agent.

PLAN OF WORK:

Literature Review (7 days)

Selection of Herbal Plant/ Drug/Chemicals (12 days)

Authentication of drug (5 days)

Collection of Plant leaves (*Nyctanthes arbor-tristis* leaves) (7 days)

Drying of Leaves (7 days)

Decoction Process (1 day)

Extract of Plant (1 day)

Preliminary Phytochemical tests (2 days)

Formulation of Herbal Face serum (10 days)

Evaluation tests (8days)

Physical evaluation

Determination of pH

Determination of Spreadability

Microbial contamination test



AUTHENTICATION OF PLANT:



भारत सरकार / GOVERNMENT OF INDIA
पर्यावरण, वन एवं जल वायु परिवर्तन विभाग
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
पश्चिमी क्षेत्रीय केंद्र / WESTERN REGIONAL CENTRE
७ कोरेगाँव मार्ग, पुणे / 7- KOREGAON ROAD, PUNE- 411001



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No. BSI/WRC/Iden. Cer./2024/0804240047245

Date : 16.04.2024

CERTIFICATE

This is to certify that **Ms. Simran Prashant Deshmukh, B.Pharmacy Student, from Navsahyadri Institute of Pharamacy, Naigaon (Nasarapur), Pune-412213.** The plant specimen/specimens brought by aforesaid is/are identified and authenticated as :

Specimen No.	Plant Name	Family
SPDNA-1	Nyctanthes arbor-tristis L.	Oleaceae

- * Note :
1. Avoid plastic wrappers for Herbarium sheets, to protect our environment.
 2. Always send specimens in dry form.
 3. Mission LiFE -- "Save Water - Save Life "
 4. Mission LiFE -- "Conserve Plants - Conserve Biodiversity"
 5. Certificate is issued to Ms. Simran Prashant Deshmukh for her academic B. Pharmacy Project purpose.

(Signature)
16.04.2024
(D.L.Shirodkar)
Botanist
BSI,WRC,Pune-1

D. L. SHIRODKAR
BOTANIST
Botanical Survey of India
Western Regional Centre
7, Koregaon Park, Pune- 411001.

EXPERIMENTAL WORK

Material and Methods:

Collection of Plant material;

Fresh leaves of plant were collected from Navsahyadri Institute of Pharmacy, Pune. Sandalwood oil, Olive oil, Glycerine, Tween 80 and Distilled water were collected from college Pharmacognosy lab. Then all the materials collected were identified on the basis of their morphological and microscopical characteristics. The fresh Leaves of *Nyctanthes arbor-tristis* were separated from the plant and allowed to shed dry for up to 7 days.



Fig 9. Fresh leaves of *Nyctanthes arbor-tristis* plant

Preparation of Extracts (Decoction Process)

The fresh leaves of *Nyctanthes arbor-tristis* were washed and allowed for shed drying.



Fig 10. Dried leaves of *Nyctanthes arbor-tristis* plant

The dried leaves of *Nyctanthes arbor-tristis* were then grinded into powder using mixer grinder and kept in well closed container until further use and study.



Fig 11. Coarse powder of *Nyctanthes arbor-tristis*

Now the powdered plant material was added to the distilled water. 20 gm of sample powder was added to the 100ml distilled water and allowed to boil for 90 to 120 mins on 80 °C in a heating mantle. Stir continuously until the water comes to half of its initial volume. Let it cool and then filter the extract using filter paper and funnel. Use the obtained plant extract for further studies and formulation process.

Leaves were kept for drying for 7 days and grinded into fine powder.

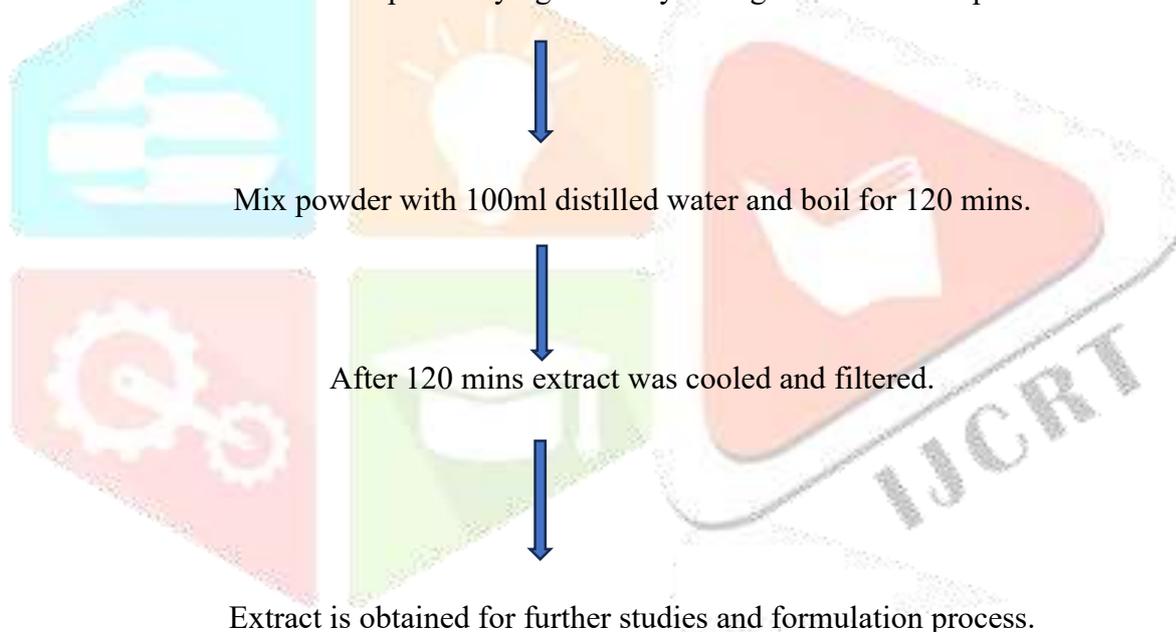


Fig 12. Extraction process of *Nyctanthes arbor-tristis*



Fig 13. Decoction Process



Fig 14. Plant Extract.

PRELIMINARY PHYTOCHEMICAL TESTS:

Obtained extract of *Nyctanthes arbor-tristis* was further used for performing phytochemical tests gives Positive test for Alkaloids, Tannins, Saponin, Cardiac glycoside, Terpenoids, Phenol and Flavonoids and gives Negative test for Carbohydrates test.

Table 1. Preliminary phytochemical tests.

Sr. No.	Test	Observation	Inference
1.	Test for alkaloids- 1.0 ml plant extract + 1.0 ml saturated solution of picric acid.	Yellow colour occurs	Positive/Negative
2.	Test for tannins – 0.5 gm extract +10ml water + boiled in test tube & then filtered +0.1 ml of fecl3 was added.	Brownish green or blue – black colouration	Positive /Negative
3.	Test for saponins – The frothing + 3 drops olive oil + shaken vigorously.	Formation of an emulsion	Positive/Negative
4.	Test for cardiac glycosides – 0.5 ml plant extract +5 ml water + 2ml of glacial acetic acid containing 1 drop of fecl3 +underlaid with 1 ml of conc. sulphuric acid.	Formation of a brown ring at the interface	Positive/Negative
5.	Test for terpenoids – 5ml plant extract +2ml chloroform +3 ml of conc.H2SO4.	A reddish-brown colouration at the interface	Positive /Negative
6.	Test for phenols – 2ml of plant extract + 2 ml of Folin's reagent.	Appearance of violet brown colour	Positive /Negative
7.	Test for flavonoids – 5 ml of diluted ammonia +crude drug extract + addition of conc.H2SO4.	Yellow colouration occurs.	Positive / Negative
8.	Test for carbohydrates-		
	a) Molisch test – 2ml of plant extract + 1 ml of α -naphthol solution + conc. sulphuric acid.	Purple or reddish violet at the junction of two liquids reveals the presence of carbohydrates.	Positive/Negative
	b) Fehling's test – 1 ml of plant extract +add equals solution of Fehling's A and Fehling' B, upon heating.	Formation of brick red precipitate.	Positive / Negative
	c)Benedict's test – 5 ml of benedict's reagent + 1 ml of plant extract + boil for 2 min.& cool.	Formation of red precipitate.	Positive /Negative

FORMULATION PREPARATION

Different batches were prepared according to the Table 2. Mentioned below

1. Solution A: Aqueous phase was prepared by mixing together Aloe vera gel, Glycerine and small amount of Distilled water uniformly in a beaker.
2. Solution B: Oil phase was prepared by mixing together all the oily components consisting of Olive oil, Sandalwood oil, Tween 80 and Coconut oil mixed together for 15 mins to obtain uniform solution.
3. Then Solution B (Oil phase) is added to Solution A (Aqueous phase) dropwise under mechanical stirring to obtain Oil in Water based on biphasic Emulsion.
4. Finally add quantity sufficient of distilled water to make volume up to 15ml formulation.
5. Prepared formulations were filled in a suitable container, labelled accordingly and stored away in a cool & dry place away from direct sunlight exposure.

Table 2. Composition of Face Serum for 15ml

Sr. No.	Name of Ingredients	F1 (ml)	F2 (ml)
1.	Plant Extract	4	5
2.	Aloe-vera gel	2	2
3.	Glycerine	3	3
4.	Tween 80	0.2	0.2
5.	Olive oil	0.6	0.6
6.	Coconut oil	0.4	0.4
7.	Sandalwood oil	0.8	0.8
8.	Distilled water	Q. S	Q. S

EVALUATION PARAMETER

Prepared formulations evaluated for the following tests:

1. Physical Appearance:

Physical appearance was evaluated by observing the Colour, Odour, Texture, Homogeneity of the prepared Herbal Cosmetic Serum.

2. Determination of pH:

Apparatus: pH meter, preferably equipped with glass electrode.

Principle: The Formulation of Serums are meant for topical application. So their pH should be similar to that of the skin. The skin has an acidic range and the pH of the skin serum should be in the range of 5-9. To ensure the required shelf life of skin serum, chemical inertness is essential i.e. it should neither be too acidic nor too alkaline. Based on above point it was through the standard pH of skin should be in the range of 4-5.5

Procedure: Take about 2ml of sample in a beaker and add 16ml of Distilled water in it. Mix it properly until it mixes completely in a water, then the pH of the sample mixture by using pH meter.

3. Determination of Microbial Contamination:

Apparatus: Petri plate, Inoculation Loop.

Principle: The zone of inhibition can be used to measure the susceptibility of the bacteria to the anti-biotic. Larger zones indicate decreased bacterial growth with greater anti-biotic susceptibility. (34)

Procedure: Using the streak plate method, the serum formulation was inoculated into agar medium plates, and a control was set up. After being put in the incubator, plates are incubated for 24 hrs. at 37°C. Plates were removed from incubation period and examined for microbial growth by contrasting them with the control.

4. Determination of Spread-ability Tests:

Apparatus: Wooden block, with a moveable glass slide with one end tied to weighted pan rolled on pulley.

Principle: It is very important for any cosmetic product that after application for the product must be easily spread over the skin. Spread-ability is affected by many factors such as viscosity, temperature, etc. The spreading time must be very less.

Procedure: Excess sample was placed between two glass slides and 100gm weight was placed on the glass slide for 5min to compress the sample to uniform thickness. Weight(250gm) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spread-ability.

$$S=m \cdot l/t$$

m-weight tied on upper slide

l- length of glass slide

t- time in seconds

5. Accelerated Stability Studies:

Cyclic Temperature test: These tests are not carried out at a fixed temperature and humidity. In this test, temperature was changed cyclically everyday e.g. low-high-low-high to stimulate the changes in temperature daily.

Parameter: 1. Room Temperature

2. Freeze Temperature

RESULT AND DISCUSSION

Phytochemical Tests:

Table 3. Results of Phytochemical tests.

Sr. No.	Test	Observation	Inference
1.	Test for alkaloids- 1.0 ml plant extract + 1.0 ml saturated solution of picric acid.	Yellow colour	Positive
2.	Test for tannins – 0.5 gm extract +10ml water + boiled in test tube & then filtered +0.1 ml of fecl3 was added.	Brownish green colouration	Positive
3.	Test for saponins – The frothing + 3 drops olive oil + shaken vigorously.	Formation of an emulsion	Positive
4.	Test for cardiac glycosides – 0.5 ml plant extract +5 ml water + 2ml of glacial acetic acid containing 1 drop of fecl3 +underlaid with 1 ml of conc. sulphuric acid.	Formation of a brown ring at the interface	Positive
5.	Test for terpenoids – 5ml plant extract +2ml chloroform +3 ml of conc.H2SO4.	A reddish-brown colouration at the interface	Positive
6.	Test for phenols –	Violet brown colour	Positive

	2ml of plant extract + 2 ml of Folin's reagent.		
7.	Test for flavonoids – 5 ml of diluted ammonia + crude drug extract + addition of conc.H ₂ SO ₄ .	Yellow colouration	Positive
8.	Test for carbohydrates-	No colouration.	Negative
	a) Molisch test – 2ml of plant extract + 1 ml of α -naphthol solution + conc. sulphuric acid.		
	b) Fehling's test – 1 ml of plant extract +add equals solution of Fehling's A and Fehling' B, upon heating.	No Formation of brick red precipitate.	Negative
	c)Benedict's test – 5 ml of benedict's reagent + 1 ml of plant extract + boil for 2 min.& cool.	No Formation of red precipitate.	Negative



Fig 15. Preliminary Phytochemicals Tests.

Determination of Microbial Contamination:

No bacterial growth observed around the zone of inhibition.



Fig 16. Zone of Inhibition

Physical Evaluation:

The face serum was observed for its colour, odour, texture and homogeneity.

Table 4. Physical evaluation.

Sr. No.	Properties	Appearance
1.	Colour	Muddy yellow
2.	Odour	Pleasant
3.	Texture	Smooth homogenous
4.	Homogeneity	Good

Determination of pH:

Table 4. Determination of pH

Formulation Batches	F1	F2
pH		

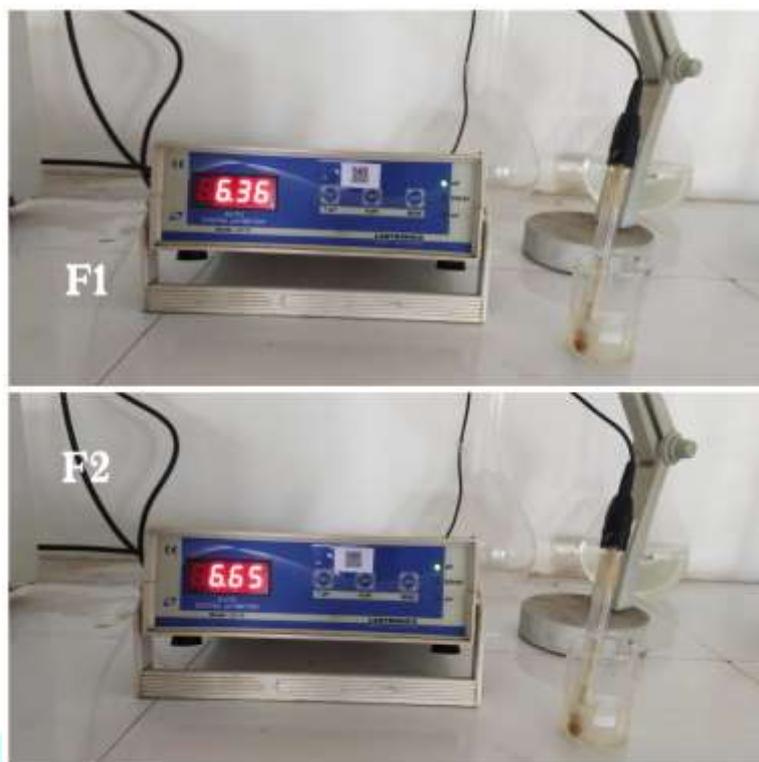


Fig 17. pH of sample.

Determination of spread-ability time:

Table 4. Determination of Spread-ability Time

Formulation Batches	F1	F2
Spread-ability (cm/s)	180.2 ± 0.547	103 ± 0.887

Accelerated Stability Studies:

Cyclical Temperature Tests

Table 5. Cyclical temperature Test

Sr. No.	Parameter	F1	F2
1.	Room Temperature	Stable	Stable
2.	Freeze Temperature	Unstable	Unstable

CONCLUSION

The aim of the study was to formulate different herbals into a serum form moisturizing and glowing activity on skin. Cosmeceuticals are skin-care products that cater both cosmetics and drug. In the serum aloe vera and olive oil are mainly used. The aloe vera gel from the inner central part of the leaf often has a very good action in acne, pimples and other skin problems, burns due to heat, sun exposure and in treatment of radiation dermatitis. Aloe vera is rich in vitamins and minerals that have a good moisturizing capacity and anti-aging effects to maintain healthy- and fresh-looking skin. Olive oil is beneficial for treating sunburn, the antioxidants in the oil used treat damaged caused by the UV rays. It also slow down and prevent premature ageing. It contains fatty acids such as omega 6 & omega 9, which help to prevent dry skin. Stability studies revealed that there was no significant difference in the physical and pH parameter. Thus, the formulation was found to be stable. Microbial examination of serum revealed that the formulation is free from micro-organism and safe for use. The Spread-ability was found to be good. No residues were form and was easy to wash out. The gel stimulates cell growth and as such enhance the restoring of damage skin. So, this serum can be used treat skin related problem.

REFERENCES

1. K.R. Kirtikar, B.D. Basu, B. Singh, M.P. Singh. Indian Medicinal Plants. 1975; 1(2):2392-93.
2. A.K. Singh, Acta botanica Hungarica 1983;29(1-4) :281-92.
3. H.K. Shandhar, M. Kaur, An update on *Nyctanthes arbor-tristis* Linn. Journal of Internationale Pharmaceutica Scientia 2011; 1:77-86.
4. S.A. Nirmal, S.C. Pal, S.C. Mandal. Pharmacognostic evaluation of *Nyctanthes arbor-tristis* bark , Asian and Pacific Journal of Tropical Biomedicine. 2012; 2(2);494-500.
5. B. Gulshan, K.A. Suri, G. Parul, A Comprehensive review on *Nyctanthes arbor-tristis*. Int. J. Drug. Dev. & Res. 2015;7(1):183-193.
6. P.S. Rajdev, S.D. Gaikwad, A.A. Somvanshi, S.S. Gunjal. Formulation and Evaluation of Face serum. International Journal of Advanced Research in Science, Communication and Technology.2022; 2(5)
7. The Ultimate Guide to Face Serum Ingredient Book.
8. A.J. Kolarsick, M.A. Kolarsick, C. Goodwin. Anatomy and Physiology of skin 2000.
9. T. Shukla, N. Upmanyu, M. Agarwal, S. Saraf, A. Alexander. 2018; Biomedical applications of microemulsion through dermal and transdermal rout. Biomedicine and Pharmacotherapy. 2018; 108:1477-1494.
10. S. Sasidharan, J. P. Joseph. Formulation and Evaluation of fairness serum using polyherbal extracts, Int. J. Pharm. 2014; 4(3): 105-12
11. K. Sanders. What's the difference between a serum, creams and lotions ?2022
12. P.A. Axel. 2014. Skin care tips to get Glow: All About Serums. In WebMD, Retrieved 2017; 22.
13. N. Gudwani 2022; Face Serums introduction and benefits of using Face serum.
14. D.M. Kulkarni, R.B. Dhakne, R.R. Patil, 2022. "REVIEW OF NYCTANTHES ARBOR-TRISTIS AS A MEDICINAL PLANT". Ind. J. Res. Methods Pharma. Sci 1 (1):21-26.
15. J.S. Rathee, S.A. Hassarajani, S. Chattopadhyay, Anti-Oxidant activity of *Nyctanthes arbor-tristis* leaf extract, Food Chemistry. 2007; 103 (4):1350-1357
16. A. Abraham, Anti-anxiety evaluation of *Nyctanthes arbor-tristis*,Linn. Indian Journal of Phytoconstituents. 2010; 6:77-79
17. S. Das, D. Sasmal, S.P. Basu, Anti-inflammatory and antinociceptive activity of arbortristoside. A Journal of Ethnopharmacology 2008;116(1): 198-203
18. P. Gupta, S.K. Bajaj, K.Chandra, K.L. Singh, J.S. Tandon, Anti-viral profile of *Nyctanthes arbor-tristis* against encephalitis causing viruses Indian journal of experimental biology. 2005; 43: 1156-1160
19. Y. Mahida, J.S. Mohan, Screening of plants for their potential antibacterial activity against staphylococcus and salmonella sp. Natural product radiance. 2007; 6: 301 -05
20. N.A. Khatune, M.E. Islam, M.A. Rahman, M.A. Mossadik, M.E. Haque In-vivo cytotoxic evaluation of new benzofuran derivative, isolated from *Nyctanthes arbor-tristis* L. on Ehrlich ascite Carcinoma cells in mice. J. Med. Sci. 2003 Mar;3(2): 169-73
21. J. S. Tandon, V. Srivastav, P.Y. Iridiods; a new class of leishmanicidal agents from *Nyctanthes arbor-tristis*, Journal of Natural Products 1991; 54(4): 1102-4
22. B. Gulshan, K.A. Suri, G. Parul, A Comprehensive review on *Nyctanthes arbor-tristis*, Int. J. Drug Dev. Res. 2015 Jan;7(1):183-93
23. R. Jain. M. Mittal, A Review on Pharmacological and Chemical documentation of *Nyctanthes arbor-tristis* Linn. Asian journal of traditional medicine. 2011;6(5):187-02
24. N.K. Dewi, N. Fakhrudin, S. Wahyuono, A Comprehensive review on the Phytoconstituents and biological activities of *Nyctanthes arbor-tristis* L. Journal of Applied pharmaceutical science. 2022 Aug 4; 12 (8): 009-17
25. A. Talal, M. Feda, 2003 plants used in cosmetics. Phyto-therapy research. 17(9): 987-1000
26. R.L. Moy, C. Levenson, Sandalwood oil as Botanical Therapeutic in Dermatology. J. Clin. Aesthet. Dermatol. 2017
27. S. R. Dulal, M. A. Taher, H. Sheikh, Sandalwood oil can be a miraculous tackle on skin aging, appearance and wrinkle skin World journal of pharmaceutical and medical research, 2019; 5(1) 51-55
28. J. Khan, Olive oil for skin care: benefits and recipes. Veda Oils 2023
29. L.C. Beckar, W. F. Bergfeld, B. Heldreth, Safety Assessment of Glycerine as used in cosmetics. International journal of toxicology 2019
30. A. K. Daund, R. S. Jadhav, D.N. Vikhe, Review on *Nyctanthes arbor-tristis* Leaves: Potential Medicinal Herbs. World Journal of Pharmaceutical and Medical Research. 2022, 8(4) 128-132

31. S. M. Kumar, V. Swarnkar, S. Sharma, A. Baldi, Herbal Cosmetics: Used for skin and hair, Researchgate 2012.
32. A.S.Ghuge And R.A.Khandre, Formulation and Evaluation of Mouthwash using Guava leaves for Aphthous ulcer Treatment, World Journal of Biology Pharmacy and Health Science 2024, 17(01),228-241.

