Formulation and evaluation of moisturizing cream containing *E. hirta* removes the warts

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Abstract

Scientists are seeking for novel molecules with therapeutic potential because of issues with traditional therapies for a variety of dermatological conditions. Among the endeavors have been analyses of natural products like *Euphorbia hirta,* honey, *Hibiscus rosa-sinensis, Rosa damascene, Cassia alata, Aloe* and Shea butter. These are herbaceous plant that has been used extensively by humans for thousands of years as an herbal remedy. Our nation is renowned for its Ayurvedic and medicinal practices. The plant's parts are utilized for a variety of purposes; in this case, warts and skin conditions are treated with the leaves and flower petals. A survey was created and input was gathered prior to using this therapeutic herb. *Euphorbia hirta* was the source of the latex or sap that was harvested and used in the therapy. Once the treatment was finished, a new questionnaire was sent out, and the group's input was gathered. According to the research, warts and fungal diseases were completely cured by *Euphorbia hirta* leaf extracts and other flower, completely painlessly and without any negative side effects. Additionally, leaves and flowers are readily available and plentiful, making them affordable. Rather than using manufactured pharmaceuticals, we can use the numerous therapeutic plants like this one that are all around us, each with a unique chemical composition, and use them safely in our daily lives.

Keywords *Euphorbia hirta,* honey, *Hibiscus rosa-sinensis, Rosa damascene, Cassia alata, Aloe*

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INTRODUCTION

The high frequency of HPV infection and its association with a variety of diseases, from benign skin and mucous membrane conditions to the most common sexually transmitted infection (STI), highlight the significance of HPV in the context of public health and the importance of dermatologists as medical professionals who are trained to identify and treat many of these conditions1. The most common way that they spread is probably by direct contact, though autoinoculation is also a possibility. This illness is comparable to Charmakeela, according to Ayurveda [16,27,28,30]. Hard nail structures called Charmakeela develop as a result of the pathogenesis of this disease, which is caused by the vitiation of Vata and Kapha over the skin. Warts are infectious diseases that spread when keratinocytes become infected with the human papillomavirus (HPV). Viral warts are prevalent, occurring 7–12% of the time. Warts most frequently affect children and young adults[18].

Plant parts have many applications; in this case, warts and skin conditions are treated with the leaves of *Euphorbia hirta*, an Indian species of weed commonly known as the asthma plant and also with some flowering parts like *Hibiscus rosa-sinensis, Rosa damascene, Cassia alata, Aloe* to make the moisturizer cream for smoothy[2]. As opposed to synthetic drugs that cause side effects and are detrimental to the human body, medicinal plant extracts and Ayurvedic medicines do not have any kind of side effects[29]. There are medicinal uses for every part of the plant, including the fruits and seeds. India has acknowledged the therapeutic benefits of over 3000 plants for treating a wide range of illnesses.

Numerous studies on *Euphorbia hirta* have been conducted in the past, and it has been discovered that it contains a large number of chemically active compounds. Myricitrin and quercitrin have been isolated using a methanolic extract. *Euphorbia hirta* leaf extracts in aqueous form demonstrated antioxidant activity, while ethanolic extract demonstrated antifungal activity. *Euphorbia hirta* n-hexane extract exhibited anti-inflammatory properties. In addition to these minute atoxin, *Euphorbia hirta* also contains derivatives of camphol and choline. *Hibiscus rosa-sinensis* traditional medicinal plants, especially their extracts and phytochemicals, are thought to be generally safe, causing little to no adverse effects. They also frequently act on several different target sites at once, which lowers the likelihood that resistance will develop[5].

In Sarawak, Borneo, the natives refer to *Cassia alata* (Linn.) as the candle shrub. This plant holds great value, especially for India's traditional healers. The locals treat ringworm

infections with the plant as a prescription medication[10]. A wide range of bioactive substances, such as triterpenoids, alkaloids, tannin, phenolics, and flavonoids, have been identified as characteristics of *C. alata*[12].

Rose damascena is a perennial bushy shrub that can live up to 50 years. It only reaches a height of 1-2 meters. The flowers are large and vibrant. The leaves are arranged in an odd way, being pinnate with five to seven leaflets[19]. This plant is used to treat erectile dysfunction, cardiovascular disorders, and infections of the respiratory tract[22]. Additionally, it eases constipation and encourages the motility of the digestive system. It has anti-inflammatory, anti-HIV, and antibacterial qualities[6]. The antifungal characteristics of *R. damascena* have been reported[21]. The drug is prescribed for the treatment of jaundice and hepatitis in all its forms [Iqbal, Park]. It is beneficial for osteoarthritis and renal disorders in addition to being used as a deodorant, disinfectant, and cosmetic[7].

Above medicinal plants are well-known ornamental plants used in the fragrance business. In addition to its perfuming effect, its pharmacological effects include antioxidant, astringent, antibacterial, antimicrobial, anti-inflammatory, and analgesic properties. Removal of fungal infections and warts is the primary focus of this research.

MATERIALS AND METHODS

Sample collection

For cream product analysis, three composite samples (leaves, stems) of asthma plant were collected from Rathinam Tech Zone, Coimbatore, Tamilnadu, India. The red flower petals of *H. rosa-sinensis,* rose petal, aloe vera leaves and *Cassia alata* were collected from Pennadam, Cuddalore, Tamilnadu, India. Plant materials were rinsed in tap and distilled water, then air-dried at room temperature in the shade before being homogenized in a planetary high-energy mill with a hardened chromium steel vial.

Sample processing and preparation

The leaves, roots, and stems were processed as described by Odeyemi with modifications. First, the samples were sorted according to appearance and condition, while those in a spoilt state were discarded. It was followed by surface disinfection of selected samples by soaking in aqueous for 1 hour and then rinsed at least five times with sterile distilled water. The samples were then oven-dried at 40 °C for 72 h until a constant weight was obtained. After that, the dried samples

were finely ground into small particle sizes (< 0.2 mm) and then transferred into sterile containers for storage in a dry condition.

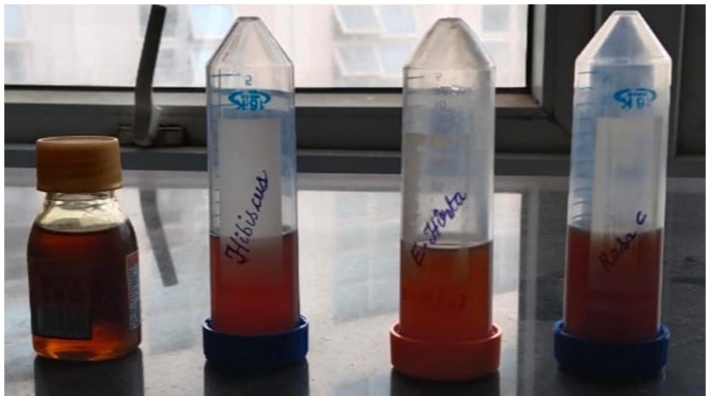


Fig. No.1. Synthesis of leaf and flower extractions

Phytochemical Analysis:

Using standard protocols stated by Harborne, the plant material extracts were subjected to a qualitative phytochemical analysis to determine the presence of tannins, saponin, flavonoids, alkaloids, and phenol[15].

1. **Alkaloids – Wagners test**

2 ml of extract added with few drops of 1% Hcl. 1ml of Wagners Reagent (Iodine + Potassium Iodide). Reddish brown colour solution indicates the presence of alkaloids[11].

2. **Flavonoids – Alkaline Reagent Test**

A 3 ml portion of 1% Aluminium chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5 ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration indicates a positive test for flavonoids[14,9].

3. **Saponins – Foam Test**

In a water bath, roughly 5 ml of the extract was boiled with 20 ml of distilled water and then filtered. For a stable, long-lasting froth, 10 milliliters of the filtrate and 5 milliliters of distilled water were combined and vigorously shaken. After adding three drops of olive oil to the foam and giving it a good shake, the presence of saponins was verified. An emulsion formed[14,9].

4. Phytosterols – Libermann Burchard Test

1 ml of extract sample added with few drops of Chloroform. Add few drops (2-3 ml) Acetic anhydride. Add 1-2 ml of concentrated Sulphuric acid. The result of dark green or brown ring results in the presence of phytosterols[34].

5. Quinones – Sulphuric Acid Test

A few drops of sulfuric acid are added to the extracts, and the formation of orange colour indicates the presence of flavonoids[11].

6. Phenol – Ferric Chloride Test

1 ml of extract sample is added with 2 ml of distilled water. Add 3-4 drops of 1% ferric chloride solution. Blue green colour or bluish black colour results in presence of phenol[25].

7. Tannins – Braymer's Test

About 1 ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride were added and observed green or a blue – black coloration which confirmed the presence of tannin[14,9].

8. Glycosides – Keller Killani Test

1ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated deoxysugar characteristics of cardenolides which confirmed the presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicated the presence of glycoside.

9. Terpenoids – Salkowski Test

Extract of 5 mg of the selected plant part is mixed with 2 mL chloroform and 3 mL concentrated sulfuric acid added carefully to form a layer. A reddish-brown colour indicates the presence of terpenoids[33].

10. Anthraquinones – Borntrager's Test

About 5ml of extract was mixed with 10 ml benzene, filtered and 5 ml of 10% NH3 solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones.

Preliminary Test – Protein and Amino Acid

1. Lead Acetate Test

2 ml of extract sample added with 2 ml of NaOH. Boil the solution for 2 min and add few drops of Lead acetate solution. The result of black precipitation states the presence of protein components[11].

2. Ninhydrin Test

1 ml of extract sample added with 0.25% Ninhydrin reagent and boil the solution. The result of blue colour formation states the presence of amino acid components.

Antimicrobial Activity:

The bacterial cultures *E. coli*, *S. aureus*, *Pseudomonas aeruginosa, Klebsiella pneumonia* were used for antimicrobial activity for the extract and nanoparticle synthesised sample. The Muller-Hinton Agar plates were swabbed with respective cultures and wells were made for control, nanoparticle, extract and nanoparticle synthesised extract. The plates were filled and incubated for 24 hrs at 37°c. The zone of inhibition is calculated to state the effectiveness in action against the bacterial cultures.

Antifungal Activity:

The fungal cultures *C. albicans*, *A. niger* were used for antifungal activity for the extract and nanoparticle synthesised sample. The Muller-Hinton Agar plates were swabbed with respective cultures and wells were made for control, nanoparticle, extract and nanoparticle synthesised extract. The plates were filled and incubated for 28 hrs at 37°c. The zone of inhibition is calculated to state the effectiveness in action against the fungal cultures.

Formulation of cream

Hydrophobic and hydrophilic bases are used in the formulation of creams to create preparations that are nearly miscible with skin secretions. The base should be smooth, inert, odorless or nearly odorless, physically and chemically stable, compatible with the skin, and compatible with integrated medications. It should also not cause irritation or sensitization of the skin or delay wound healing.

Herbal cream

The benefits of botanical ingredients are utilized for both therapeutic and cosmetic purposes in herbal creams that are intended for topical application. These formulas, which are classified as water-in-oil or oil-in-water creams, target different types of skin conditions and offer moisturization. When modern cosmetic technology is integrated with traditional medicine, safe and effective products that address a variety of skincare needs are produced.

Evaluation of cream:

pH of the Cream:

Standard buffer solution was used for pH metre calibration. The cream was weighed out to be about 0.5 g, dissolved in 50.0 ml of distilled water, and the pH was recorded.

Fig. No. 2. pH

Spreadability test

Two slides were stacked with 500 mg of the cream between them. 100 grams of weight was put on the upper slide. The excess formulation was scraped off and the weight was removed. The

apparatus's lower slide was fixed to the board, and the upper slide was secured with a non-flexible string that was subjected to a 20 g load. The amount of time the upper slide took to slip off was recorded[31].

Homogeneity:

The uniformity of the formulations was assessed by both touch and visual inspection.

Appearance:

The appearance of the cream was judged by its dark green color, opacity and smoothness.

Patch test

The skin behind the ears is one of the sensitive areas of the body, and 1-3 grams of the material to be tested was applied there using a funnel or piece of cloth. One square meter of skin was covered with the cosmetic that was going to be tested. Additionally, control patches—of a comparable cosmetic brand—were used. After a day, the patch site is examined. The test was conducted three times because there was no response. Since there was no reaction after the third application, the person might not be considered hypersensitive.

After feel:

After the cream was examined, it was applied to the face. The cream felt nice and was easily moisturising, similar to cold fresheners**.**

Type of smear:

Following cream application, the kind of film or smear that developed on the skin was examined.

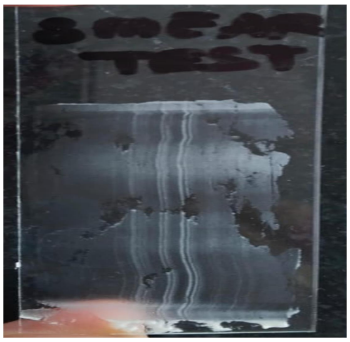


Fig.No.3. Smear test

Washability test:

By using tap water to wash the area where the cream was applied, the cream's ease of removal was assessed.

Irritancy test:

On the dorsal left-hand surface, a 1 cm^2 region was designated. After applying the cream to the designated area, the time was recorded. For a full day, irritability, erythema, and oedema were observed at regular intervals and recorded[26].

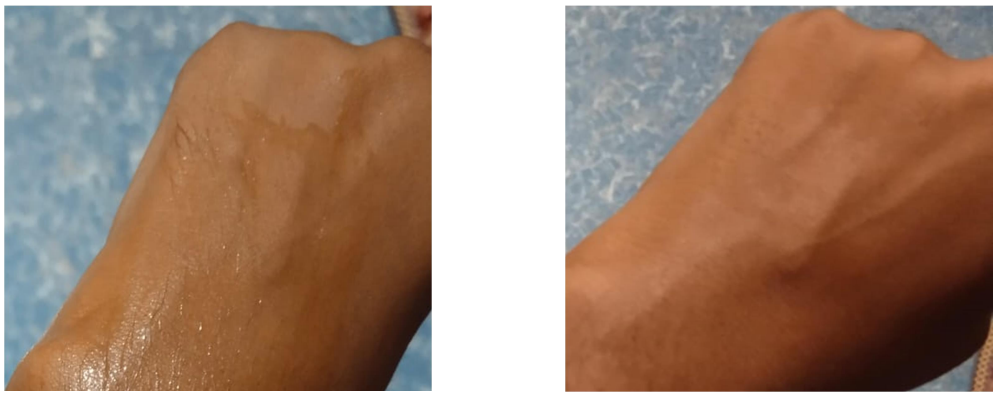


Fig. No. 4. Irritancy test

Accelerated stability studies

All of the formulations experienced accelerated stability studies, which involved constant time intervals of 20 days at room temperature. Parameters including homogeneity, viscosity, physical changes, pH, and smear type were examined during the stability studies[32].

RESULT AND DISCUSSION

Phytochemical Analysis

The extracts of the plant material were subjected to qualitative phytochemical analysis for the presence of tannins, saponin, flavonoids, alkaloids and phenol which were carried out on the extracts using standard procedures. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs.

Tests	To Detect	Appearance	Result
Wagner's test	Alkaloids	Reddish brown	Presence
Alkaline Reagent	Flavonoids	Yellow	Presence
Foam test	Saponins	Foam persistence	Presence
Libermann Burchard	Phytosterol	Dark Brown ring	Presence
H2SO4 Test	Quinones	Red colour	Presence
Ferric chloride	Phenols	Blue / green	Presence
Gelatin Test	Tannins	White precipitate	Presence
Keller Killani Test	Glycosides	Brown ring	Presence
Salkowski's Test	Terepenoids	Red/brown	Presence
Borntrager's Test	Anthraquinones	Pink layer	Absence

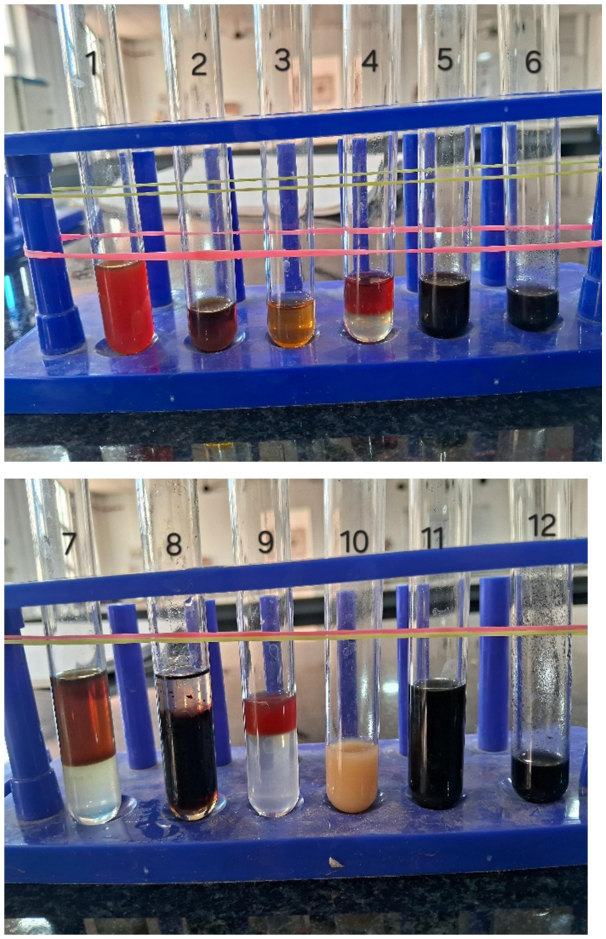
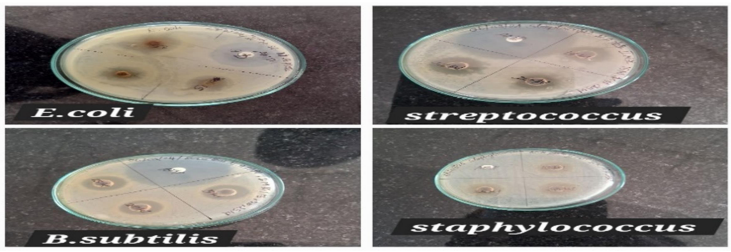


Fig. No. 5. Phytochemical analysis

Antimicrobial activity

Fig. No. 6. Zone of inhibition of *Euphorbia hirta* against antibacterial activity



From the result of successive extraction by using different compounds, the percent yield of aqueous extracts was found to be 8.57%, 6.8% and 4.41% respectively. The anti-microbial properties may be associated to the presence of secondary metabolic products of plant. So, the presence of phytoconstituents (Table 1) likes carbohydrates, proteins, alkaloids, flavonoids, tannins and phenolic compounds etc. in cream formulation were found to be effective and responsible for antimicrobial action against a wide range of micro-organisms[13].

The zones of inhibition obtained with aqueous extracts and standard drug are presented in Table 2, Figure 2. It was observed that cream extracts exhibited significant antimicrobial activity against various microorganisms. Among the three concentration 25µl exhibited maximum inhibition against the various organisms compared with other concentrations and order of inhibition was found to be *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*. *B. subtilis*. The result showed that the warts removal was active against gram negative species *E. coli, Streptococcus, B. subtilis* with the inhibition zone of diameter 11, 8, 4, 2mm respectively. However, extract has only very frail activity towards gram positive species *S. aureus*.

Table. 1 Zones of inhibition (in mm) of against *E. coli, Streptococcus aureus, Staphylococcus aureus, Bacillus subtilis*

Zone of inhibition (mm)				
Components	**Control N/A (Ampicillin disc)**	**15µl Plant Extract**	**20µl Plant Extract**	**25µlPlant Extract**
E. coli	3	2	1.5	2
Streptococcus aureus	4.5	1.5	2	2.5
Staphylococcus aureus	-	1.5	1.9	2
Bacillus subtilis	4.5	1.5	1.8	2

Fig. No. 7. Zone of inhibition of sample extract against antifungl

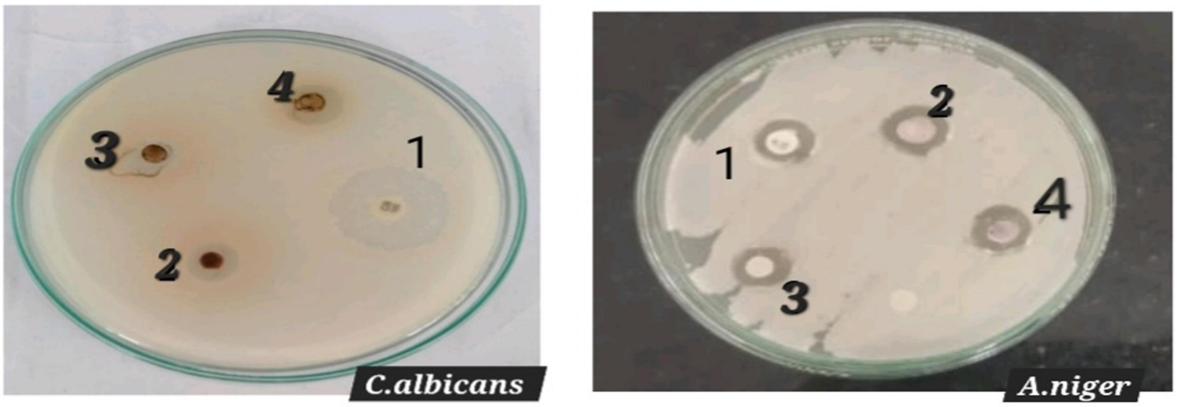


Table. 2 Zones of inhibition (in mm) of *Euphorbia hirta* against *C. albicans, A. niger*.

Zone of inhibition (mm)					
Components	**Control (SDA) Ampicillin disc**	**15µl Plant Extract**	**20 µl Plant Extract**	**25µl Plant Extract**	**30µl plant extract**
C. albicans	4.5	1.5	2	2.5	2.6
A. niger	5.5	1	1.5	2.3	2.5

Table. 3 Composition of Herbal wart removal moisturizer cream

Composition	**Quantity for 100 gm (in %)**
Euphorbia hirta	10 %
Hibiscus rosa-sinensis	10 %
Rosa damascene	5.5 %
Cassia alata	5.5 %
Aloe vera gel	5.5 %
Honey	2.5 %
Shea butter	10 %
Glycerine	6 %

Water	45 %

Fig. No. 8 Formulated Herbal warts removal cream

Table. 9 Evaluation Parameter

S.No	Parameter	Observation
1.	Colour and odour	Dark green
2.	pH	6.8
3.	Spreadability	Uniform with a value of 30 g.cm/sec
4.	Washability	Washable
5.	Consistency	Good
6.	Grittiness	No gritty particles found
7.	Homogeneity By visual By Touch	 Homogeneous Smooth and Consistent
8.	Irritancy test	No redness and oedema
9.	Patch test	Not hypersensitive
10.	Accelerated stability studies	Stable
11.	Type of Smear	Non-greasy

The papilloma virus causes warts, which are skin growths that are frequently communicable[1]. These are painful in day-to-day living, and they feel rough to the touch. The color can range from black to brown to the color of skin. With the use of contemporary technologies, these warts can be removed using a variety of techniques, including chemical peeling, laser treatment, and excision of the wart. Having treatment for any of these procedures is painful. However, no such pain was experienced during treatment when using the latex of the *Euphorbia hirta* plant[24].

Numerous studies on *Euphorbia hirta* have been conducted in the past, and it has been discovered that it contains a large number of chemically active compounds. Along with *E. hirta, Hibiscus rosa-sinensis, Rosa damascene, Cassia alata, Aloe* and Shea butter these ingredients

aid in reducing oxidative stress and treat HPV virus to shed without pain and damaging the cells. Since they are easily oxidized, antioxidants are inherently very unstable. As a result, it's critical to guarantee their stability and other qualities to make them safer for application on human skin.

Physical qualities such as viscosity, homogeneity, and grittiness were assessed for cream formulation. A small amount of product was used for the tests. The sample was physically observed and pressed between the thumb and index finger. We observed the cream's texture and consistency. To assess homogeneity and spreadability, a tiny amount of the sample was applied to the back of the hand. The formulation's (cream) pH was measured using a digital pH meter by rinsing the electrode in the samples and recording the reading.

CONCLUSION

This herbal formulation, particularly in cream form, was created with very natural, safe ingredients that are also readily available in daily life. The primary motivation for formulating it as a cream was its spreadability and ease of application. Additionally, the purpose of creating a moisturizer was to evaluate the efficacy of natural ingredients like aloe vera and shea butter on skin and to see if they could have a moisturizing effect in the form of a cream. Upon examination of the findings and observations, it was found to be satisfactory. When applied, the formulation demonstrated a very calming, purifying, and—most importantly—warts removal without pain on the skin.

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