© IJCIRAS | ISSN (O) - 2581-5334
May 2020 | Vol. 2 Issue. 12

FORMULATION AND DEVELOPMENT OF ANTI-ACNE SERUM USING EUPHORBIA HIRTA.

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Abstract

Cosmetic science may be real science, and it's a multidisciplinary field since it includes basic knowledge and a big selection of data from a variety of various scientific fields. Cosmetic are articles intended to be cleaned, pour, spread on, lead into, or then applied to the form or any part thereof for cleaning, enhancing, promoting, charm, or modifying the looks without affecting structure or function. A cosmetic product's intended use will be established in an exceedingly no. of ways, including claims, consumer perception of the merchandise, and therefore the history of an ingredient. Many claims commonly used today for the cosmetic product aren't recognized or employed by the Food and Drug Administration in any sense and have only limited scientific evidence behind them.

Keyword: Anti-acne, Anti-microbial, Euphorbia hirta, Quercetin.

1.INTRODUCTION

1.1. Skin

Skin is the largest sensory and calls organ within the physical body. Its area in adults is approximately 1.5-2 m The human skin consists of two main layers, namely the epidermis and dermis. Underneath the dermis, there's a 3rd layer, called the hypodermis, which consists mainly of fat cells and isn't considered a component of the skin. The skin may be a complex organ made of dead cells, epithelium, animal tissue, muscles, nerves, blood vessels, further because of the so-called appendages, including the nails, hair, and glands, like sebaceous glands, eccrine and apocrine sweat glands. it's composed of 5 main layers, corneum is formed of dead cells that continuously shed and are replaced by cells within the adjacent layer. This layer is incredibly thick compared to the others; it contains 15-30 layers of dead cells. stratum, the granulosum layer, accommodates 3-5 layers of flattened keratinocytes that begin to die. during this layer, granules are observed within the cells. Stratum spinosum, the prickle cell layers, contains 8-10 rows of cells. This layer is accountable for lipid and protein synthesis. stratum basal is formed of one layer of cells. Dermis is established below the epidermis, and its roles as a supportive structure to the epidermis, providing it with nutrients and oxygen via the blood capillary.

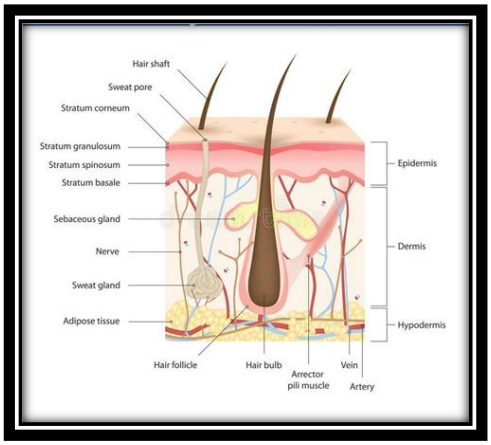

Fig.1 Structure of the skin

1.2. The normal skin flora

The word skin flora indications to the microbes which exist in on the skin, characteristically human skin. Several of them are microbes of which there are around 1000 species upon human skin from 19 phyla. Most are found within the superficial layers of the epidermis and also the upper parts of hair follicles. Skin flora is sometimes non-pathogenic, and either commensal or mutualistic

2. NEED OF WORK

Skin is a vital barrier against abrasion, chemicals, and pathogens. Proper skin hygiene is the best thanks to keeping your skin healthy. Removing dead cells, dirt, and microbes on the surface are vital to good hygiene. This definition has been expanded to any of the plants of which parts or whole are often utilized in medicinal treatments, culinary preparations, nutritional supplementation, or used as a coloring or cosmetic agents. Today's many popular active ingredients claim to cure such infections, still, new active is usually being identified, studied, and promoted, because there's always an area to experiment with new ideas. The present project is additionally a humble attempt within the same direction, where Euphorbia Hirta l. has been evaluated microbiologically to test its anti-microbial property against various infectious problems. Euphorbia Hirta l. is usually utilized in Indian traditional medicine as antibacterial, antifungal, antioxidant, wound healing, anti-inflammation. It is reported that the leaves of Euphorbia Hirta l. has antibacterial activity against some gram-negative bacteria. Therefore this study aims to judge antibacterial properties of *Euphorbia Hirta* leaves on the bacteria related to the skin

3. PLAN OF WORK

The plan of work proposed for the study as follows

- **a study of Euphorbia Hirta l. leaves for their anti-microbial activity Which include –**

  1.Collection of herbal material
  2. preparation of extract
  3. phytochemical screening test
  4. preparation of culture

- **Evaluation of the antimicrobial activity of Euphorbia Hirta l. leaves**

  1. well diffusion method

- **Formulate anti-acne serum**

- **Evaluation of serum**

4. ACTIVE PROFILE

Fig.2 Euphorbia Hirta

4.1. Botanical Calssification

Kingdom	-	Plantae
Division	-	Tracheophyta
Subdivision	-	Spermatophytina
Class	-	Magnoliopsida
Superorder	-	Rosanae
Order	-	Malpighiales
Family	-	Euphorbiaceae
Genus	-	Euphorbia
Species	-	hirta
Synonyms	-	Chamaesyce hirta (L) Millspaugh, Euphorbia pilulifera Linn.

4.2. PHYTOCHEMISTRY

Euphorbia hirta contains flavonoids, terpenoids, phenols, essential oil and other compounds.

Flavonoids:- Quercetin, quercitrin, quercitol and derivatives containing rhamnose, quercetin-rhamnoside

Terpenoids:- Triterpenoids, α-amyrin, β-amyrin, friedelin, teraxerol, and its esters-taraxerone, 11α, 12α-oxidoteraxerol, cycloartenol, 24-methylene-cycloartenol, euphorbol hexacosonate.
Tannins:- Dimeric hydrolysable dehydro ellagic tannins, euphorbins A, B, C, E and terchebin, the monomeric hydrolysable tannins geraniin,

Acids:- Ellagic, gallic, tannins, maleic, and tartaric acids.

Essential oil:- Major constituents include 3,7,11,15-tetra methyl-2-hexadecon-1-ol,6,10,14-trimethyl-2-pentadecanone,hexaecanal, phytol and n-hexadecanoic acid.

Other compounds:- Alkaloids, saponins, amino acid, and mineral.

4.3. TRADITIONAL USES

Traditionally, the plant is utilized to cure several indications: gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, bowel complaints, digestive problems), respiratory diseases (cough, cold, asthma, bronchitis, hay fever, emphysema), apparatus urogenitalis (diuretic, kidney stones), genital apparatus (metrorrhagic , agalactosis, gonorrhoea, urethritis), various ocular ailments (conjunctivitis, corneal ulcer), skin and mucous membranes problems (guinea worm, scabies, tinea, trush, aphtha) and tumor. In south India, it's used as ear drops, within the treatment of boils, score, and wounds. The latex of the plant is usually used as warts and cuts to stop pathogen infection. it's also effective in treating ulcers. The plant is additionally eaten as vegetables.

4.4. PHARMACOLOGICAL ACTIVITY

investigated chemical constituents from aerial a part of Euphorbia hirta Linn. These chemical constituents were isolated, purified by chromatographic techniques and structural elucidation supported chemical analysis. Nine compounds were isolated and identified. The 9 compounds remained scopoletin (1), scoparone (2), isoscopoletin (3), quercetin (4), isorhamnetin (5), pinocembrin (6), kaempferol (4), luteolin (8), acid (9). Among these compounds 1-3, 5-8 were identified for the primary time from this plant. insulated, selected, and characterized a microbial straining from rhizospheric soil of Euphorbia hirta. The strain was screened as a gram-positive motile rod bacteria with terminal spore. The 16srRNA gene sequence construction identified the strain as hay bacillus KC3. the most enzyme production was achieved after 48h (22.92U/ml at 400c at PH 7. The optimum temperature and pH for enzyme activity were 500c and 6.5 respectively. These properties suggest that hay bacillus KC3 may be commercially exploited for the production of α-amylase in starch and various biotechnological processes.

5. AIM- FORMULATION AND DEVELOPMENT OF ANTI ACNE SERUM USING *EUPHORBIA HIRTA.*

6. OBJECTIVE

- The main objective of anti-acne serum is that destroys acne-causing bacteria or suppresses their growth or their ability to reproduce acne-causing bacteria
- This also helps to inhibit acne-causing bacteria i.e. Propionibacterium acnes It also gives anti-inflammatory action.
- This also gives the skin conditioning effect due to the presence of amino acid.

7. METHODOLOGY

7.1 Formulation

Sr no.	Ingredients	Quantity for 100%
1.	Glycerine	5%
2.	Propylene glycol	5%
3.	Xanthum gum	0.5%
4.	Sodium EDTA	0.2%
5.	Allantion	0.1%
6.	Methylparaben	0.15%
7.	Active	10%
8.	Water	79.05%

8. EXPERIMENTAL WORK

8.1. Preparation of extract

Extracts were prepared by the soxhlet method.

8.2. Soxhlet extraction

The leaves of Euphorbia Hirta were shade dried. The leaves were coarsely ground and dried powder (50g) was extracted using soxhlet extractor with ethanol (140ml) and water (60ml). The crude extract was then filtered through No.1 filter paper and concentrated in a vacuum using a rotary evaporator. The concentrated extract was subsequently dried aseptically at room temperature.

Fig.3 soxhletion

8.3. Phytochemical screening

The concentrated extracts of the selected plant were subjected to different chemical tests for the detection of different phytoconstituents using standard methods

(i)Test for saponins
The crude extract when mixed with 5ml H2O in a very tubing then it had been shaken briskly. The creation of constant foam which points out the occurrence of saponins.

(ii) Test for flavonoids
The crude extract when mixed with 10ml distilled water, 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate solution then added 1ml concentrated sulphuric acid. Sign of yellowish color appearance the presence of flavonoids.

(iii) Test for steroids
The crude extract of the selected plant was dissolved in 0.5mL dichloromethane to organize a dilute solution then 0.5 mL of anhydride was added followed by four drops of concentrated oil of vitriol. A blue-green coloration indicated the presence of steroids.

(iv) Test for tannins
The crude extract of the plant was mixed with a minor quantity of H2O and heated on a water bath. The blend was clarified and ferric chloride was additional drop by drop to the filtrate. A dark green look which shows the presence of tannins.

(v) Test for Alkaloids
The crude extract was solidified with 2ml of 1% HCl and heated gently. Wagner and Mayers reagents were added to the blend. The turbidity of the resultant precipitate was taken as approval for the presence of alkaloids.

(vi) Test for carbohydrate
Mutually Fehling A and Fehling B solutions were varied in equivalent volume. These reagents are added in crude extract and smoothly boiled. A sepia precipitate is appeared at the underside of the tubing and indicate the presence of reducing sugar

TABLE 1: **Preliminary phytochemical analysis of flowers and leaves of Euphorbia hirta**

Phytochemical Constituents	Leaves Extract		
	Ethanol	Chloroform	Hexane
Alkaloids	+	+	-
Flavonoids	+	+	+
Terpenoids	+	+	-
Tannins	-	+	-
Saponins	+	-	-
Carbohydrate	+	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals

8.4. Microbial Assay

Microorganisms occur nearly anywhere in nature. They are carried by air currents from yhe earth's surface to the upper atmosphere. They occur most abundantly where they find food, moisture, and temperature suitable for their growth and multiplication. They can be found on the surface of our bodies, in our mouth, and on feet.
Fortunately, most organisms are harmless to us; and we have ways of resisting various by those that are potentially harmful.
The microbiological assay is important, as one of the cause for the formation of acne, bad breath, and athlete's foot is the presence of bacteria on the skin surface and make the condition worse.

8.5. Procurement of organisms

The standard bacterial culture used for this study were procured from our college.

8.6. Material required

a) **Instruments**: autoclave, incubator
b) Media: Nutrient agar
c) Reagent: Sterile distilled water
d) Apparatus: conical flask, test tube, measuring cylinder, Petri dishes, beaker, non-absorbent cotton plugs, inoculating needle, micropipette, burner.
All the apparatus was cleaned, dried, and sterilized. The sterilization was done by carefully wrapping the apparatus in a paper and autoclaving in a hot air oven at 1600C for 1.5 to 2hrs.

8.7. Preparation of media

Weighed a medium in the required quantity and added to a dry conical flask.
Added the required quantity of freshly distilled water, a little at a time with constant agitation to prevent the formation of lumps.
Heated the flask in a boiling water bath with intermittent stirring till the contents were completely dissolved.
Filled the test tubes using a funnel when the medium is hot.
Plugged the test tubs of the medium using non-absorbent cotton, covered them with paper, and tie them together using a rubber band sterilized for 15 minutes at 1210C i.e. at 15 ibs p.s.i. pressure.

8.8. Handling of culture media

1. the container was closed properly after use because the culture media were hygroscopic and forms lumps when exposed to high humidity or left open for a long time or if spatula wet.
2. Used the containers of the proper size for the preparation of media.
3. the media was melted using a water bath and media must be crystal clear.
4. Avoided overheating, incomplete mixing, prolonged sterilization, repeater melting, and prolonged storage at high temperatures.
5. Stored the prepared media in a clean, cool, and dry place to prevent drying. Used the fresh medium a few days from the date of preparation.

8.9. Aseptic transfer of microorganisms

Material required for aseptic transfer of microorganisms: Sterile test tubes, inoculating needle, gas burners, a disinfectant solution, and pure culture of microorganisms.

9. PROCEDURE

1. Wash the hand with disinfectant soap and apply the disinfectant gel.
2. The working platform was cleaned with a disinfectant solution using a cotton swab the microorganisms present on the working platform.
3. the inoculating needle was heated to redness by holding vertical in the flame and the steel rod was heated on which the needle is mounted. This was necessary to destroy the microorganisms, which were presented on the inoculating needle.
4. Holed the culture tube and media tube in the left hand near the flame and removed and holding the plugs with fingers of the right hand. The plugs were not kept on the working platform. The tube was holed in an approximately horizontal position and do not

keep them open for a long period than necessary.
5. The mouth of the test tube was pressed through the flame. This destroyed the microorganisms on and near the mouth of the test tube. Flaming of the mouth also creates outward convection currents, which decreases the chances of contamination.
6. A little growth of microorganisms was removed with sterilized and cooled loop transfer it on the fresh medium by moving the loop on the medium surface in a zigzag manner only once.
7. Sterilized the used loop in flame avoiding spurting.
8. Flamed the mouth of test tubes and plug them. The working platform and hand were cleaned as described earlier.
9. Incubated the inoculated tubes at 370C in an incubator and observed the growth after 24hrs for bacteria and 48hrs for fungi.

Observation

The growth of microorganisms on agar medium slope is observed as an opaque mass of cells.

9.1. Preparation of Petri dishes

1. The nutrient medium was melted in a hot water bath.
2. They were allowed to cool up to 45-48oC.
3. The melted nutrient agar was poured into the petri dish, the dishes were immediately covered and were allowed to solidify.

9.2. Method used for evaluation of the microbial activity of Euphorbia hirta Leaves.

1. The good diffusion method:

i. The Petri dishes prepared in the above step are used in this method.
ii. After the media solidified small well (3-4mm) bored on the media with the help of sterilized cork borer.
iii. Different concentrations of extract prepared.
iv. These well were filled with different concentration of extract of Euphorbia hirta
v. leaves, and incubated at 340C for 24hrs.
vi. Tetracycline solution used as standard.
vii. After 24 hrs., the zone of inhibition was observed10.

Table No.2 concentration of extract:

Sr. No.	Concentrations	Extract
1.	1500mg	1.5ml
2.	2000mg	2ml

Observation

Following plate No. 1&2 showing measurement of the zone of inhibition when using a good diffusion method for Propionibacterium acnes.

Result

As per the observation the zone of inhibition was found in the good diffusion method against Propionibacterium acnes.

Table No. 3 Diameter of zone of inhibition of Euphorbia hirta leaves extract

Microorganism	The diameter of zone of inhibition in(mm)	
	ethanol extract	
	1500mg/ml	2000mg/ml
Propionibacterium acnes.	14mm	15mm

10. EVALUATION TESTS FOR PRODUCT

1. physical appearance

- Colour
- Odor
- consistency
- Homogeneity

2. **Thermal Stability**
3. **pH Test**
4. **Spreadability**

10.1. Physical Appearance

- **Colour-** light green
- **Odor-** Pleasant
- **Consistency-** Liquid
- **Homogeneity-** The formulation produces a uniform distribution of extract.

10.2. Thermal Stability-

Formulation and development of a pharmaceutical product aren't complete without proper stability analysis dole out thereon to work out physical and chemical stability and thus the safety of the merchandise. A general methodology for predicting the soundness is accelerated stability analysis which subjects the fabric to elevated temperatures. the soundness studies were dole out as per ICH guidelines. Short term accelerated stability study was dole out for the amount of three months for the formulation. The samples were stored at different storage conditions of temperatures like 3-5oC, 250C RH=60%, and 40oC±2% RH=75%. Samples were withdrawn on monthly interval and analyzed

Stability	1st week	2nd week	3rd week	4th week
A	Stable	Instable	Instable	Instable
B	Stable	Stable	Instable	Instable
C	Stable	Stable	Stable	Stable
D	Stable	Stable	Stable	Instable

Conclusion- From the above test it was observed that formulation C was stable throughout the stability period, formulation A, B, and D showed instability at week 3 week 4.

10.3. pH Test

The pH meter was calibrated using a standard solution. About 1 ml of the serum was weighed and dissolved in 50.0 ml of H2O and its pH was measured
Conclusion-pH of prepared serum is 5.2.

10. 4. Spreadability-

Spread ability denotes the extent of the area to which the serum readily spreads on application to the skin or the affected part. To simulate human skin, the Fisher brand paper was chosen. Each paper weighs within milligrams of the opposite sheet of that size and sort. A Becton Dickinson &Co. 5ml rubber injects without the needle attached was used. Fluid pressed out of the needle attachment end of the B-D syringe made very uniform globules. Each drop is approximately 0.03 grams in weight. Standard tin foil is used as a base to induce the paper on for testing
Conclusion- The prepared serum has excellent spreading property.

11. RESULT

Currently, most research work has been focused on the investigation of antimicrobial potential of ***Euphorbia Hirta*** plant. The antimicrobial activity of Euphorbia hirta leaf extract was examined against Propionibacterium acnes and the extraction was carried out using ethanol, chloroform, and hexane solvents by soxhlet method. Thus, the study suggests that the plant extract exhibits antibacterial activity against the selected microorganisms.

12. CONCLUSION

From the above discussion it is concluded that ***Euphorbia Hirta*** had antimicrobial property against Propionibacterium acnes. From the above experimental work, the Euphorbia Hirta leaf extract showing good activity against Propionibacterium acnes.
Finally, it was concluded that the extract of ***Euphorbia Hirta*** shows antibacterial activity against selected microorganisms with an increase in concentration the

activity is increase therefore it can be incorporated in cosmetics products.

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