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Formulation and Evaluation of Euphorbia Hirat Based on Herbal Ointment to Treat Wart

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Abstract: Warts are benign skin growths caused by infection with the Human papillomavirus (HPV), which leads to excessive keratin formation in the epidermis. The present study aimed to formulate and evaluate an herbal ointment containing *Euphorbia hirta* leaf extract for its keratolytic activity. The ointment was prepared using the fusion method and evaluated for its physicochemical properties, hemolytic assay, and keratin degradation ability. The formulation showed acceptable physical characteristics, good spread ability, and was found to be non-irritant and suitable for topical application. The results suggest that the *Euphorbia hirta* herbal ointment may be beneficial in the management of hyperkeratotic skin conditions such as warts.

Keywords: *Euphorbia hirta*, Herbal ointment, Keratolytic activity, Warts, Human papillomavirus (HPV), Keratin degradation, Fusion method.

1.1 INTRODUCTION

Warts are benign skin growths caused by infection with the Human papillomavirus (HPV), which leads to excessive keratin production in the epidermis. They commonly occur on the hands, feet, and other parts of the body and are more prevalent among children and adolescents. Conventional treatments such as salicylic acid and cryotherapy are widely used but may cause irritation and recurrence. Keratolytic agents play an important role in wart treatment by breaking down keratin and promoting the removal of infected skin layers. Herbal medicines have gained attention as safer alternatives due to their natural bioactive compounds and fewer side effects. The medicinal plant *Euphorbia hirta* is traditionally used for treating various skin disorders, and this study focuses on formulating and evaluating its herbal ointment for keratolytic activity.

1.2 WARTS

Human papillomavirus (HPV) infection of the epidermis is the cause of warts. Based on their DNA sequence, HPVs are classified into distinct genotypes. Both uncornified mucous membranes and the cornified stratified squamous epithelium of the skin can be selectively infected by different HPV strains. In addition to the type of virus, host and environmental factors can affect the lesion's appearance.[1]

Common warts, or Verruca vulgaris, are a serious issue for dermatology patients. Children are often affected. Children that are affected could have trouble interacting with others and might be stigmatized in their neighborhood. Because caustic remedies are sometimes applied

repeatedly over a long period of time, treatments are resource-intensive.[2]

A wart is a rough, microscopic growth that resembles a solid blister that usually appears on human hands or feet, though it can also appear anywhere on the body. Common warts, which usually appear on the hands but can appear anywhere on the body, are among the many varieties of warts. Flat warts are more common in children, less common in teenagers, and uncommon in adults. Genital warts typically appear on the pubic area, the inter-thigh region, and possibly the vagina and anal canal.[3,4]

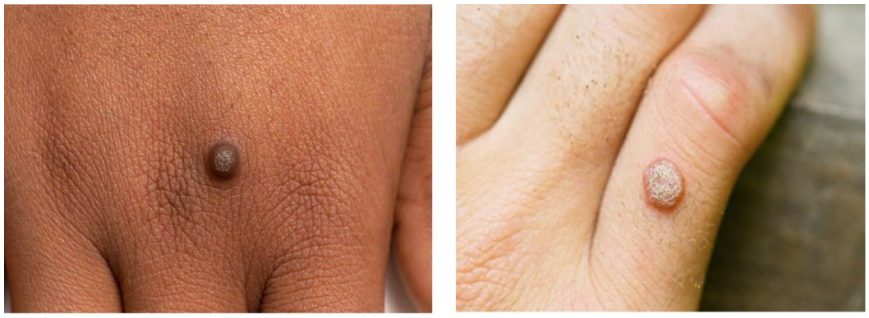


IMAGE OF WARTS

TYPES: Several types of warts have been described in dermatology, including common warts (Verruca vulgaris), plantar warts (Verruca plantaris), flat warts (Verruca plana), filiform warts, and periungual warts. Common warts typically appear on the hands and fingers and have a rough, cauliflower-like surface. Plantar warts develop on the soles of the feet and may become painful due to pressure from standing or walking. Flat warts are small, smooth, and slightly raised lesions that frequently occur on the face, neck, or dorsal surface of the hands, especially in children and young adults. Filiform warts are characterized by long, thread-like projections and commonly occur around the mouth, nose, and eyes. Periungual warts appear around the fingernails or toenails and may lead to nail deformity if untreated[5].

DIAGNOSIS: The diagnosis of warts is usually based on clinical examination and characteristic appearance, although dermoscopy or histopatho-logical examination may be used in uncertain cases. While warts are generally harmless and may resolve spontaneously, they can cause discomfort, cosmetic concerns, and social stigma. Therefore, effective treatment methods are often required to remove the lesions and prevent further spread of the virus.

TREATMENT: Warts are commonly treated to remove the lesion and prevent the spread of the Human papillomavirus (HPV). One of the most widely used treatments is the topical application of keratolytic agents such as salicylic acid, which helps soften and dissolve the thick keratin layer of the wart. Cryotherapy is another common method in which liquid nitrogen is used to freeze and destroy the infected tissue. Chemical cauterizing agents like trichloroacetic acid or silver nitrate may also be used to remove wart tissue. In some cases, surgical removal or laser therapy is performed for large or persistent warts. Recently, herbal treatments and plant-based formulations have gained attention as safer alternatives due to their natural bioactive compounds and fewer side effects.

1.3 KERATOLYTIC ACTIVITY

Keratolytic activity refers to the ability of certain substances to break down keratin, a tough structural protein found in the outer layer of the skin (stratum corneum), hair, and nails. Keratolytic agents work by softening and dissolving the keratin layer, which helps remove dead skin cells and promotes the shedding of the outer skin layer.

Keratolytic compounds are widely used in **dermatology** to treat skin conditions that involve **excess keratin buildup**, such as **acne, calluses, corns, dandruff, and warts**. For example, the medication Salicylic acid is commonly used to treat warts caused by Human papillomavirus by gradually breaking down the thickened skin tissue.

The mechanism of keratolytic agents involves **disrupting the bonds between**

keratinocytes, which leads to **desquamation (shedding) of the outer skin layer**. This process helps remove abnormal or infected tissue and allows **healthy skin to regenerate**. Because of these properties, keratolytic activity is an important factor in the **development of dermatological treatments and cosmetic for-mulations** aimed at improving skin health and treating hyperkeratotic disorders.

1.4 OINTMENT

An ointments are homogenous, viscous semisolid preparation, most commonly a greasy, oily (Oil-80%, Water-20%) with high viscosity that is intended for external application to skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired. Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membrane of the eye (an eye ointment), chest, vulva, anus and nose[6,7]

Ointment have very moisturizing characteristic and are effective for dry skin. They have very low risk of sensitization due to having few ingredients beyond the base oil or fat and also low irritation risk. They have more greasiness so mostly disliked by patients[8,9]

Pharmaceutical semi-solid dosage form include - ointments, gels, pastes, cream, plasters and foams. They contain one or more active ingredient dissolved or uniformly dispersed in a suitable base and any suitable excipients such as emulsifier, viscosity increasing agents and microbial agents and anti microbial agents antioxidants or stabilizing agent etc. Semisolid dosage form is a topical dosage form used for the therapeutic protective or cosmetic functions they may be applied to the skin or used nasally, vaginally, rectally[10].

TYPES OF OINTMENT

Ointment may be medicated or non-medicated.

a) Medicated ointment: For the application of API to skin for protective, therapeutic, or prophylactic purpose.
b) Non-medicated ointment: These are used for physical effect. They are use as protectant, emollients, or lubricants[11]

OINMENT BASES

The vehicle or carrier of an ointment is known as ointment base. The choice of ointment base depends upon the nature of medicament, stability of ointment and clinical indication of the ointment[12]

TYPE OF OINTMENT BASE[13,14]

Mainly ointment bases are of following types:

1) Oleaginous ointment base or hydrocarbon ointment base
2) Absorption ointment bases
3) Water removable bases or water washable base
4) Water soluble base

Preparation of Ointments by Fusion Method:

When associate degree ointment base contains many solid ingredients like white beeswax, acetyl alcohol, stearyl alcohol, saturated fatty acid, exhausting paraffin, etc. as elements of the bottom, it's needed to soften them.

The melting is often tired 2 methods:

Method-I

The elements square measure dissolved within the decreasing order of their temperature i.e. the upper melting point substance ought to be dissolved 1st, the substances with ensuing temperature so on.

The drug is extra slowly within the dissolved ingredients and stirred totally till the mass cools down and a same product is made.

Advantage:

This will avoid over-heating of gear having a coffee temperature.

Method – II

All the elements square measure taken in a very divided state and dissolved along.

Advantage:

The maximum temperature reached is less than Method-I, and fewer time was taken presumably thanks to the solvent action of the lower temperature substances on the remainder of the ingredients.

1.5 PLANT PROFILE

Euphorbia hirta has the characteristic of allomorphic pistillate blooms and fruits. These are soft-woody, bushy, annual plant. The plant has a thin brownish gray bark, palmately or serrated,

lobed leaves, terminally arranged male flowers on the upper half of the inflorescence, and a globe-like, dehiscent fruit covered in fleshy prickles. The seed is oblong, smooth, hard, and mottled, with a white caruncle at the top enclosing an oily endosperm.

The popular names for *Euphorbia hirta*, which is found all throughout the world, are milk weed and asthma weed. Its regional name "Boro Keruie" is used in Bangladesh and West Bengal. It can be found in lowland areas, paddy fields, gardens, waste sites, and by the sides of roads in temperate and tropical regions of India, Asia, Australia, and Africa.[15] It is native to central america. It is normally erect, growing up to a height of 40cm tall and it can also be observed laying down. The stem is reddish-colored, thin, and coated in yellowish bristles. Leaves – simple, arranged oppositely, distichous, leaf blades are lanceolate, unequal base, cuneate one side, round other side, sharp apex, finally toothed margins, dark green above, pale underside, purple bloth in middle, measures around 1-2.5 cm long.[16]

Fig 1: Euphorbia hirta

TAXONOMICAL CLASSIFICATION

Kingdom	:	Plantae
Subkingdom	:	Viridaeplantae
Division	:	Tracheophyte
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Euphorbiaceae
Genus	:	Euphorbia
Species	:	*Euphorbia hirta*
Botanical Name	:	*Euphorbia hirta Linn*

SYNONYM

Tamil	:	Amampatcharishi
English	:	Asthuma weed
Hindi	:	Dudhi
Telugu	:	Reddinanabrolu
Gujarat	:	Dudeli
Malayalam	:	Chittirappula,
Bengali	:	Barokheruie

CHEMICAL CONSTITUENT: *Euphorbia hirta*has been studied by various workers and a number of active constituents have been isolated. Afzelin (I), quercitrin (II), and myricitrin (III) have been isolated from the methanolic extract of *Euphorbia hirta*. The chemical investigation of *Euphorbia hirta*has led to the isolation of rutin (IV), quercitin (V), euphorbin-A (VI), euphorbin-B (VII), euphorbin-C (VIII), euphorbin-D (IX), 2, 4, 6-tri-O-galloylβ-d-glucose, 1, 3, 4, 6-tetra-O-galloyl-β-d-glucose, kaempferol, gallic acid, and protocatechuic acid. *Euphorbia hirta*also contains β-amyrin, 24-methylenecycloartenol, βsitosterol, heptacosane, nonacosane, shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose and chtolphenolic acid.

TRADITIONAL USES: Plant is employed to cure several indications: gastro intestinal disorders (diarrhea, dysentery, intestinal parasitosis, bowel complaints, digestive problems), respiratory diseases (cough, cold, asthma, bronchitis, hay fever, emphysema), urinary apparatus (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 is used as ear drops, in the treatment of boils, score and wounds. The latex of the plant is often used as warts and cuts to prevent pathogen infection. A decoction of leaves induces milk flow and the leaf chewed with palm kernel for restoration of virility. It is also effective in treating ulcers. The plant is also eaten as vegetables.

MORPHOLOGY OF PLANT

LEAVES: Stipules are linear, whereas leaves are opposite, distichously, and simple. The long, serrated, lanceolate-oblong leaf blades elliptic, obovate, or ovate-lanceolate; it has a sharp apex, measures 3–4 cm in length and 1-1.4 cm in breadth; its base is highly different or uneven; one side is cuneate, the other is obliquely rounded [17].

Fig 2: leaves *Euphorbiahirta*

FLOWER: *Euphorbia hirta'*sinflorescence contains a monoecious, terminal or axillary cluster of flowers known as "cyathium," and with many cyathia grouped in a delicate cyme. Both the male and female blooms are appetising and condensed in a single involucre[17].

Fig 3: flower *Euphorbiahirta*

STEM: It is round and has a thickness of 1.7 mm. It is made up of tiny epidermal cells with tangential walls that are papillate. The outer ground tissue is parenchymatous, homogeneous, and the cells are compact and round. The width of the outer ground tissue is 180 micrometers. The pith, which is made up of somewhat large, circular parenchyma cells with thin walls, is the central ground tissue[18].

Fig 4: Stem of *Euphorbia hirta*

ROOT: The root of *E. hirta*is a typical taproot system, growing positively geotropic. The primary root is dominant, distinct and enlarged at the base of the stem. Generally, the primary root is cylindrical in shape by which lateral roots (as the secondary and tertiary roots) are arising and spreading below the ground and laterally[19].

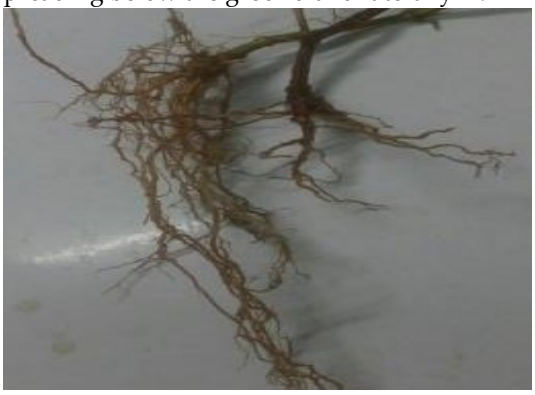


Fig 5: Root of Euphorbia hirta

2. MATERIALS AND METHOD

MATERIALS REQUIRED

FORMULATION

- Wool fat – 1 g
- Ceto stearyl alcohol – 1 g
- Hard paraffin – 1 g
- White soft paraffin – 17 g
- Sodium benzoate – 0.02 g
- Leaf extract of *Euphorbia hirta*– 2 g
- Distilled water – 1 Ml

KERATOLYTIC ACTIVITY: The materials required for this experiment include both chemicals and laboratory equipment. The chemicals used are keratin powder as the substrate, 0.05 M Tris-HCl buffer (pH 8.0), phosphate buffer, 10% trichloroacetic acid (TCA), and distilled water. The test sample used is an

ointment containing *Euphorbia hirta* extract. Essential laboratory equipment includes a water bath, incubator, centrifuge, and spectrophotometer. Additional items such as test tubes, micropipettes, and pipette tips are also required for accurate measurement and handling of solutions. These materials allow proper preparation, incubation, and analysis of the keratin degradation reaction.

HEMOLYTIC ASSAY: Sodium citrate, SDS, Nacl was purchased from SRL (India), 1X PBS was from Himedia, (India). 96 well plates were from Tarson (India).

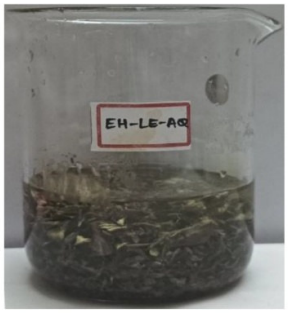
METHODS

96 WELL PLATE ASSAY: A 0.1% keratin suspension was prepared by dispersing keratin powder in 0.05 M Tris-HCl buffer (pH 8.0) with gentle heating and continuous stirring until a uniform substrate was obtained. The prepared substrate was cooled and stored at 4 °C until use. The test sample was prepared by dispersing 1 g of ointment containing *Euphorbia hirta* extract in 10 mL phosphate buffer to obtain a test solution. The reaction mixture consisted of 1 mL keratin substrate, 1 mL test solution, and 1 mL Tris-HCl buffer. For the control, the test solution was replaced with buffer solution. The mixtures were then incubated at 37 °C for a specific period to allow keratin degradation.

EXTRACTION (COLD MACERATION)

Preparation of cold extraction from the sample: 10 gm of the sample (EH-LE-AQ) was added in 100 ml of ethanol and incubated at 4°C in the refrigerator for overnight. The material was immersed in the ethanol was separated and filtered by Muslin cloth, Filter paper, and Whatman No.1 paper and dried by evaporator.

EH-LE-AQ Sample



EH-LE-AQ Sample + Solvent

Extracted sample

Heated at 70^{O}C

Final product of EH-LE-AQ sample

3. FORMULATION

Preparation of Herbal Ointment Containing *Euphorbia hirta*

MATERIALS

- Wool fat – 1 g
- Ceto stearyl alcohol – 1 g
- Hard paraffin – 1 g
- White soft paraffin – 17 g
- Sodium benzoate – 0.02 g
- Leaf extract of *Euphorbia hirta*– 2 g
- Distilled water – 1 mL

METHOD OF PREPARATION

FUSION METHOD

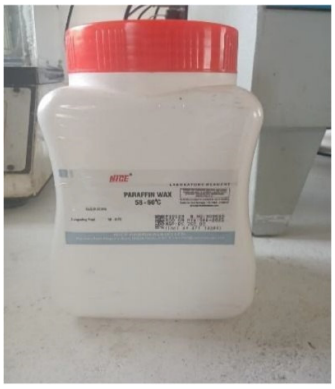
PROCEDURE: The ointment was prepared by the **fusion method**. Initially, **white soft paraffin (17 g)** was placed in a clean china dish and melted in a **water bath maintained at approximately 70 °C**. After complete melting, **hard paraffin (1 g)** was added and allowed to melt completely with continuous stirring. Subsequently, **wool fat (1 g)** was incorporated into the melted mixture and mixed thoroughly to obtain a uniform base.

In a separate china dish, **cetostearyl alcohol (1 g)** was heated at the same temperature (70 °C) until it melted. The molten cetostearyl alcohol was then **slowly added to the primary paraffin mixture with constant stirring** to ensure uniform distribution.

Meanwhile, **sodium benzoate (0.02 g)** was dissolved in **1 mL of distilled water** to prepare a preservative solution. This solution was added slowly to the molten ointment base with continuous stirring to achieve proper incorporation.

After the ointment base was formed and slightly cooled, **2 g of the leaf extract of *Euphorbia hirta*** was gradually added and mixed thoroughly to obtain a **smooth and homogeneous ointment without lumps**.

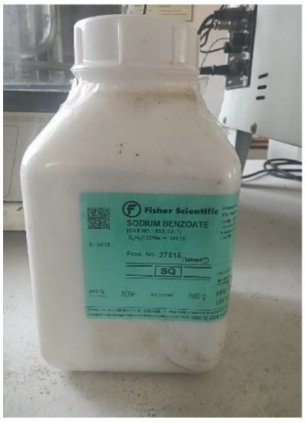
The prepared ointment was allowed to cool completely, then transferred into a **clean, airtight container**, properly labeled, and stored in a **refrigerator for further study and evaluation**.



PARAFFIN WAX

WOOL FAT

WHITE SOFT PARAFFIN



SODIUM BENZOATE

CETOSTEARYL ALCOHOL

OINTMENT 20g

4. HEMOLYTIC ASSAY[24]

Procedure

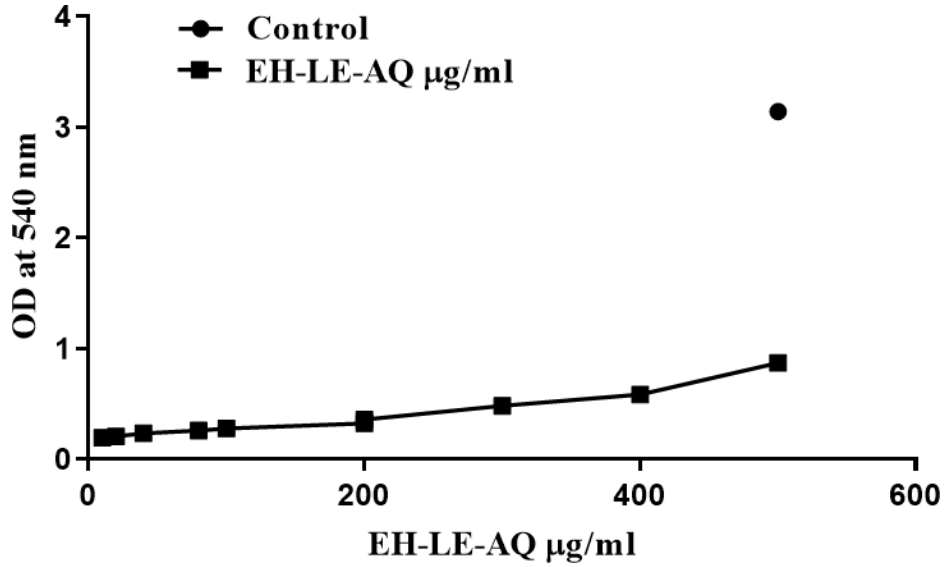
Human blood 5mL was collected from healthy volunteers in the tubes containing 3.8 % sodium citrate to prevent coagulation and washed three times in 9 volumes of sterile 0.9 % NaCl saline solution. After each washing, cells were pelleted by centrifugation at 1500 rpm for 5 min and the supernatant was discarded. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Erythrocytes suspension was prepared by adding 100 µL of erythrocytes in 900 µL 1XPBS. Each 100 µL erythrocytes suspension was mixed with 100 µL of test sample EH-LE-AQ (500, 400, 300, 200, 100, 80, 60, 40, 20 and 10 µg/mL) and 100 µL of 1XPBS was used as negative control and 100 µL of 1% SDS as positive controls. Reaction mixture was incubated at 37°C water bath for 60 min. The volume of reaction mixture was made up to 300 µl by adding 1X PBS. Finally, it was centrifuged at 1500 rpm for 5 min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The average value was calculated from triplicate assays. Hemolysis percentage for each sample was calculated by dividing sample's absorbance on positive control absorbance (complete hemolysis) multiplied by 100.

RESULT

A. OD Value at 540 nm

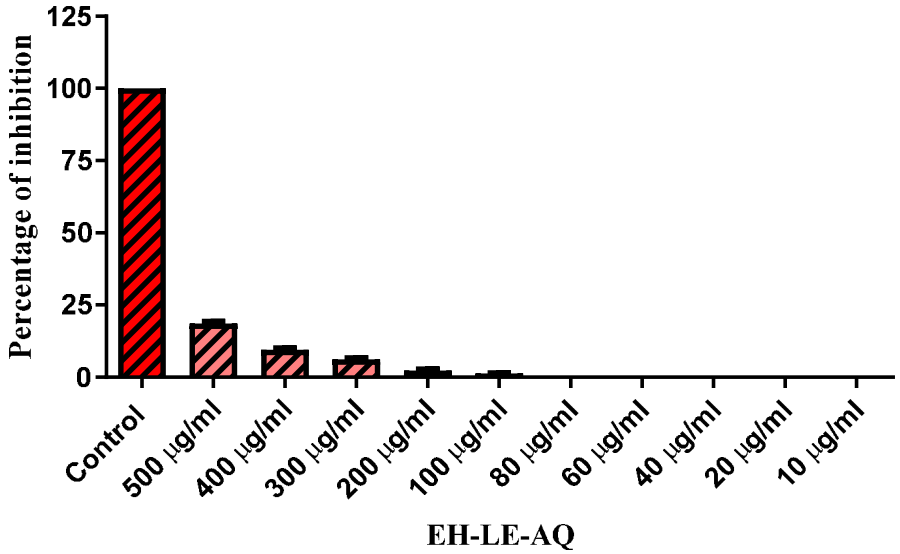
Hemolysis (%) Formula: Test OD/Control OD X 100 – Negative control

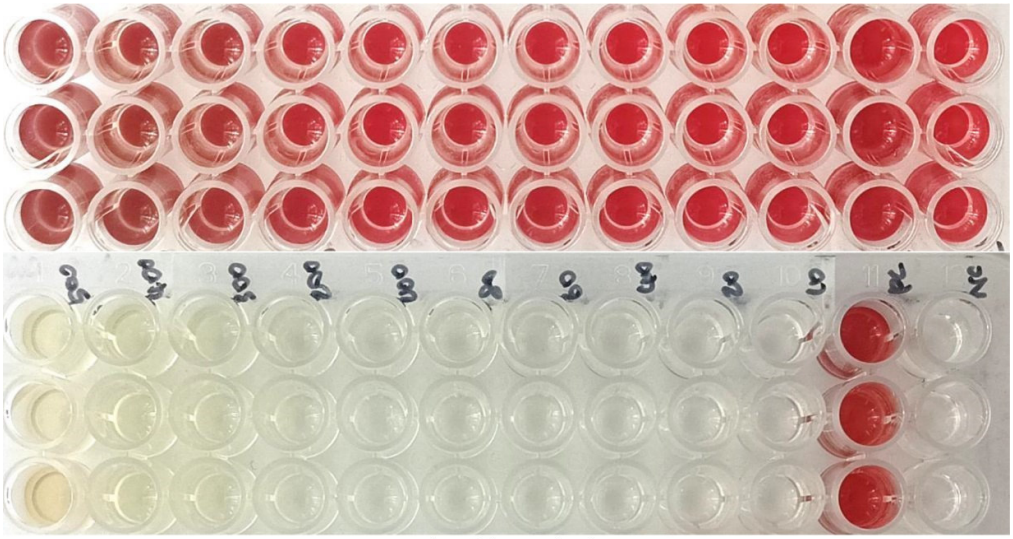
S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)		
1	Positive control	3.135	3.164	3.122
2	Negative control	0.282	0.289	0.287
3	500 µg/ml	0.855	0.898	0.854
4	400 µg/ml	0.564	0.59	0.603
5	300 µg/ml	0.483	0.464	0.496
6	200 µg/ml	0.343	0.375	0.361
7	100 µg/ml	0.325	0.332	0.319
8	80 µg/ml	0.278	0.278	0.274
9	60 µg/ml	0.256	0.256	0.264
10	40 µg/ml	0.238	0.235	0.232
11	20 µg/ml	0.206	0.207	0.206
12	10 µg/ml	0.178	0.202	0.202



B. Hemolysis (%)

S. No	Tested sample concentration (µg/ml)	Hemolysis (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	18.119	19.488	18.087	18.565
3.	400 µg/ml	8.853	9.681	10.094	9.543
4.	300 µg/ml	6.273	5.668	6.687	6.210
5.	200 µg/ml	1.815	2.834	2.388	2.346
6.	100 µg/ml	1.242	1.465	1.051	1.253
7.	80 µg/ml	0	0	0	0
8.	60 µg/ml	0	0	0	0
9.	40 µg/ml	0	0	0	0
10.	20 µg/ml	0	0	0	0
11.	10 µg/ml	0	0	0	0





Result of hemolytic assay

5. KERATOLYTIC ACTIVITY[20-23]

96 WELL PLATE ASSAY

PROCEDURE: The prepared reaction mixtures were incubated in a water bath at 37 °C for 30 minutes to facilitate keratin breakdown. After incubation, the reaction was terminated by adding 2 mL of 10% trichloroacetic acid, which precipitates undegraded proteins. The mixtures were then centrifuged at 8000–10,000 rpm for 10–15 minutes to separate the precipitated proteins. The clear supernatant containing soluble keratin degradation products was carefully collected. The absorbance of the supernatant was measured using a spectrophotometer at 280 nm. An increase in absorbance compared with the control indicates the release of soluble peptides and amino acids, confirming the keratolytic activity of the formulation.

OBESERVATION

Raw absorbance data (triplicates)

well	sample	Absorbance (280 nm)
A1	Control	0.12
A2	Control	0.11
A3	Control	0.13
B1	2% Euphorbia hirta ointment	0.40
B2	2% Euphorbia hirta ointment	0.42
B3	2% Euphorbia hirta ointment	0.41

RESULT TABLE

Sample	Mean Absorbance (280 nm)	Keratin degradation	Keratolytic Activity
Control	0.12	—	—
2% *Euphorbia hirta* ointment	0.41	70.7%	0.97

Sample	Absorbance (280 nm)	Keratolytic Activity
Control	0.12	—
2% *Euphorbia hirta* ointment	0.41	Significant activity

INTERPRETATION

A higher absorbance value indicates **greater degradation of keratin substrate**. This suggests that the ointment formulation containing *Euphorbia hirta*possesses **keratolytic activity**, which may help in **softening and removing keratinized tissues such as warts and hyperkeratotic lesions**.

96 well plate method for keratolytic activity

6. RESULT AND DISCUSSION

The herbal ointment containing *Euphorbia hirta* leaf extract was successfully prepared using the fusion method and showed acceptable physical properties such as smooth consistency, uniform texture, good spread ability, and pleasant odour. The formulation had a suitable pH for skin and was easily washable, indicating its suitability for topical application. The non-irritancy test showed no signs of redness or itching, confirming that the ointment is safe for external use. In the keratolytic study, the 2% ointment showed higher absorbance (0.41) compared to the control (0.12), indicating significant keratin degradation. The formulation exhibited 70.7% keratin degradation with keratolytic activity of 0.97 U/ml, which may be due to phytoconstituents like flavonoids, tannins, and phenolic compounds present in *Euphorbia hirta*.

7. CONCLUSION

The study concludes that a herbal ointment containing *Euphorbia hirta* leaf extract can be successfully formulated using the fusion method. The prepared ointment showed satisfactory physicochemical properties, good spread ability, acceptable pH, and non-irritant nature, making it suitable for topical application. The formulation demonstrated significant keratolytic activity with 70.7% keratin degradation and 0.97 U/ml activity. These results indicate its potential usefulness in the treatment of hyperkeratotic skin conditions such as warts. However, further studies including stability studies and clinical trials are required to confirm its therapeutic effectiveness and safety.

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