Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

DEVELOPMENT AND EVALUATION OF TOPICAL FORMULATION OF HERBAL EXTRACT OF *EHRETIA ACUMINATA*

Dr. Dipti. B. Ruikar[1], Mr. Chetan M. Jain*[2], Tejas Pande[3], Abhishek Gore[3], Shubham Amadare[3] Avishkar Ingole[3], Gajanan J Deshmukh[4]

[1] School of Pharmacy, G H Raisoni University, Amravati, 444701
[2] Dr. Rajendra Gode Institute of Pharmacy, Amravati, 444602
[3] P R Pote Patil College of Pharmacy, Amravati, 444604
4 Government Polytechnic, Pharmacy Department, Amravati, Maharashtra 444602

*** Corresponding author:Dr. Dipti B. Ruikar**
dipti21ruikar@gmail.com

Abstract
The aim of present work was to prepare and evaluate topical dosage forms such as ointment and gel from extract of *Ehretia acuminate* (Khanduchakka). Ointment can stay on the application surface for a long time before being wiped away. These ointment properties aid in the prolongation of drug delivery at the place of application. Sustained release become important to supply the skin with a drug over a prolong period of time hence, a dermatological delivery system such as gel was considered to be formulated. The preliminary phytochemical screening of extract khanduchakka was done in view to know the various class of chemical constituents such as alkaloids, glycoside etc. The formulated gel and ointment was evaluated with respect to different physicochemical parameter such as pH, viscosity, washability, speredability etc. Sustained release become important to supply the skin with a drug over the prolong period of time hence, a dermatological delivery system such as gel was considered to be formulated. In present study it is concluded that developed formulation F1 and F3 for ointment and gel respectively was found to be stable, Safe, and Efficient.

Keywords: - Semisolid dosage form, Herbal ointment, Herbal gel, Ointment Base, *Ehretiaacuminate* , Brookfield Viscometer.

INTRODUCTION:

A. Pharmaceutical Semi-Solid Dosage From

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

Advantages of semi-solid dosage form:

- It is used externally.
- The probability of side effect can be reduced.
- First-pass gut and hepatic metabolism is avoided.
- Convenient for comatose patients or patient having difficulty on oral administration.
- Convenient dosage form for bitter drugs.
- More stable than liquid dosage form.

Disadvantages of semi - solid dosage form:

There is no dosage accuracy in semi solid dosage forms.

- The base which is used in the semisolid dosage form can be easily oxidized.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

- May cause staining.
- They are bulky to handle.
- Application with finger may cause contamination.
- Physio - chemically less stable than solid dosage form.
- May cause irritation or allergy to some patient (KaushalD, et al,.2022).

Ointment:

Ointments are homogeneous, 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.

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.

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.

Characteristics of an ideal ointment

- It should be physically and chemically stable.
- In ointment base, finely divided active ingredients should be uniformly distributed.
- The base of ointment should not possess any therapeutic action.
- The ointment should be smooth and free from grittiness.

Advantages of an ointment

- They have site specific application of drug on affected area, which avoids unnecessary non target exposure of drug thereby avoiding side effect i.e., site specific action with less side effect.
- They avoid first pass metabolism of drug.
- Convenient for unconscious patients having difficulty in oral administration.
- Comparatively they are chemically more stable and easier to handle than liquid dosage forms.
- They are suitable dosage forms for bitter taste drugs

Disadvantages of an ointment: -

1) These oily semisolid preparations are staining and cosmetically less aesthetic.
2) Application with fingertip may contaminate the formulation or cause irritation when applied.
3) As compared to solid dosage forms, semisolid preparation are more bulky to handle.
4) Though semisolid allow more flexibility in dose, dose accuracy is determined by uniformity in the quantity to be applied.
5) Physio-chemically less stable than solid dosage form.

Ointment 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.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Ointment bases with example type of ointment bases (according to usp) mainly ointment bases are of following types:

- Oleaginous ointment base or hydrocarbon ointment base
- Absorption ointment bases
- Water removable bases or water washable base
- Water soluble base

Ideal Properties of Ointment Bases

- Should not retard wound healing,
- Have a low sensitization index,
- Pharmaceutically elegant,
- Release the medicament efficiently at the site of application,
- Have a low index of irritation,
- Non-dehydrating, non-greasy and neutral in reaction,
- Compatible with common medicaments and also with the skin,
- Easily washable with water,
- Have minimum number of ingredients,
- Easy to compound and remain stable on storage.

Method Of Preparation of Ointment: -

Preparation of ointment mainly depend on nature of ingredients. Ointments are mainly prepared by two general methods:

a) Incorporation

b) Fusion

a) Incorporation: - In this finely subdivided insoluble medicaments are evenly distributed by grinding with a small amount of the base followed by dilution with gradually increasing amounts of the base.

b) Fusion: - In this method the ingredients are melted together in descending order of their melting points and stirred to ensure homogeneity (Bhaskar R, et al,.2016).

Evaluation of ointment: -

Colour and Odor:-

Physical parameters like colour and odour were examined by visual examination. Consistency Smooth and no greediness is observed.

pH:-

pH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated.

Spreadability:-

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability.

Spreadability was calculated by following formula

$$S=M\times L/T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Extrudability:

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds. Diffusion study The diffusion study was carried out by preparing agar nutrient medium. A hole board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (after 60 minutes)

LOD:

LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105oC. Solubility Soluble in boiling water, miscible with alcohol, ether, chloroform.

Washability:

Formulation was applied on the skin and then ease extend of washing with water was checked. Non irritancy Test Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability study:

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2oC, 25oC and 37oC. The herbal ointment was found to be physically stable at different temperature i.e. 2oC, 25oC, 37oC within four weeks (Sawant S, et al,.2016).

Test of rate of penetration

The rate of penetration of semi solid dosage form is crucial in the onset and duration of action of the drug. Weighed the quantity and applied over the skin for a definite period of time. Then the preparation left over is collected and weighed. The difference between the initial and the final weight and the final weight of the preparation gives the amount of preparation penetrated through the skin and this when divided by the area and the time period of application give the rate of penetration of the preparation. The test should be repeated 2 to 3 times.

Test of rate of drug release:

To assess the rate of release of medicament small amount of the ointment can be placed on the surface of nutrient agar contain in a petri dish or alternately in a small cup in the agar surface. If the medicament is bactericidal the agar plate is previously seeded with a suitable organism like staphylococcus aureus. After a suitable period of incubation, the zone of inhibition is measured and correlated with the rate of release.

Test of rheological properties:

The viscosity of the preparation should be such that the product can be easily optimal from the container and easily applied over the skin. Using cone and plate viscometer the viscosity of the preparation is determine

Test of content uniformity:

The net weight of contents of ten filled ointment containers is determined. The result should match each other and with the labeled quantity(Kushal D, et al,.2022).

Gel: -

Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase.

The word ‘‘gel’’ is derived from ‘‘gelatin,’’ and both ‘‘gel’’ and ‘‘jelly’’ can be drawn back to the Latin gelu for ‘‘frost’’ and gel are, meaning ‘‘freeze’’ or ‘‘congeal.’’ This origin indicates the essential idea of a liquid setting to a solid-like material that does not flow, but is elastic and retains some liquid characteristics. Use of the term ‘‘gel’’ as a classification originated during the late 1800s as chemists attempted to classify semisolid substances according to their phenomenological characteristics rather than

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

their molecular compositions. At that time, analytical methods needed to determine chemical structures were lacking.

The USP defines gels (sometimes called jellies) as semisolid systems containing either suspensions made up of small inorganic particles, or large organic molecules interpenetrated by a liquid. Where the gel mass contains a network of small separate particles, the gel is classified as a two-phase system. In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes called as a magma. Single-phase gels consist of organic macromolecules uniformly circulated throughout a liquid in such a way that no apparent boundaries occur between the dispersed macromolecules and the liquid (Rathod H, et al,.2015).

Gels are semisolid dosage forms which are used for local or transdermal delivery of active ingredients, or emollient or/and protective action. Gels include, in addition to gelling agents of natural or synthetic origin, preservatives, stabilizers and emulsifiers, one or more active ingredients. Gelling agents can act as stabilizers for such dispersed systems as suspensions or emulsions. As a rule, gels have a viscous consistency, they are homogeneous, transparent, fluid, elastic and plastic (Zalivskaya A, et al,.2021).

Classification of gel :-

Gels are classified mainly by two methods based on:

Nature of colloid phase

a. Inorganic gels (Two phase system)
b. Organic gels (single phase system)

Based on nature of solvent

a. Hydrogel (Aqueous gels)
b. Xerogel
c. Organicgel (Non aqueous gels)

Based on rheological properties

Usually, gels exhibit non
Newtonian flow properties.
They are classified into,
a. Plastic gels
b. Pseudo plastic gels
C.Thixotropic gels

Based on physical nature

a. Elastic gel
b. rigid gel (Patil P, et al,.2019).

Evaluation of Herbal Gel

Physical Evaluation;

Physical parameters such as colour and appearance were checked.

Measurement of pH

pH of the gel was measured by using pH meter.

Spreadability

The steel blocks used to check spreadability. Spreadability was measured by this method on the basis of the slip and the drug characteristics of the gel put on the ground slide and the excess gel (approximately 2 gm) under analysis. The gel was then placed between the slides and 200 g weighted for 5 minutes was placed on the top of 2 slides to expel air to provide a uniform gel film between the slides where excess gel was scrapped off the edges. The time noted by the top slide (in seconds) to cover a distance of 7.5 cm must be noted.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

S= M.L/ T

Where,
S= Spreadability
M= Weight tide to the upper slide
L= Length of glass slide
T= Time taken to separate the slides (Dange V, et al,.2020).

Appearance and Homogeneity

All developed gels were tested for physical appearance and homogeneity by visual observation. Viscosity The measurement of viscosity of the prepared polyherbal gel was done with Brookfield viscometer (Model RVTDV II). The reading was taken at 100 rpm using spindle no. 6.

Skin irritation studies

The Wistar rats of either sex weighing 150-200 g were used for this test. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gels containing extracts were used on test animal. Gel base was applied on the back of animal taken as control. The animals were treated daily up to seven days and finally the treated skin was examined visually for erythema and edema (Misal G, et al,.20).

B. PLANT PROFILE

Ehretia acuminata is a widespread species with much variation. Chinese and Japanese plants named as *E. Thyrsiflora* are not specifically different from the Australian type, and the varieties recognized by Johnston show no geographical correlations. Attempts to subdivide the complex have not been successful, and it seems most appropriate to apply the specific epithet in a very wide sense.

Common Name: Kodowood

General Information

Kodowood is a small to large tree with a dense canopy, usually ranging in height from 3 - 15 metres, though specimens up to 30 metres have been recorded in Australia. Usually evergreen, though it can become deciduous in dry times. The bole is straight and cylindrical. The plant is harvested from the wild for local used as a food, medicine and timber source. The tree is also used for roadside plantings.

Height : 15.00 m

Growth Rate: Fast

Uses: The leaves and branch lets are used in Chinese medicine. The timber is used for building, flooring, cabinet making and furniture. The wood of var. polyantha is used in the Philippines for furniture, household utensils, carrying poles and other items requiring a strong tough wood

MATERIALS AND METHODS

Collection of plant

The leaves of the plant *Ehretiaacuminate were* collected from the outfield of Amravati city, Maharashtra, India.

Authentication of plant

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

The plant materials were identified and authenticated by Dr. G. B. Hedawoo, Head of Department of Bioinformatics, Shri Shivaji Science College, Amravati, India.

Figure 11: Herbarium of E. acuminate

Extraction of crude drug by suitable extraction techniques

Leaves of Ehretia acuminata plants were washed with distilled water to remove dirt and soil, and shade dried. The dried leaves of plant were coarsely powdered and defatted with petroleum ether (60-80^{0}C), and then extracted with Hydroalcohol. The extracts were filtered, and concentrated by distilling off the solvents and evaporated to dryness to get crude extract.

Figure 12: Soxhlet extraction of leaves of *E. acuminate.*

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Figure 13: Distillation of crude extract of *E. acuminate.*

Phytochemical screening of extract

All extracts of *Ehretia acuminata* was subjected to phytochemical screening for tannins, glycosides, steroids, flavonoids, alkaloids, carbohydrates and proteins.All extract were chemically tested to know the presence of different secondary metabolites present in them as per the following scheme:

Test for steroids

Salkowaski test

Few mg of the sample was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid and shaken. The development of red color in the chloroform layer indicates the presence of sterols/steroids.

Test for Alkaloids

Few mg of the sample was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was then tested using following reagents:

Dragendorff's test

Dragendorff's reagent is a solution of potassium bismuth iodide. It was prepared by dissolving bismuth nitrate (8 gm) in nitric acid (20 ml), and separately dissolving potassium iodide (27.2 gm) in water (50 ml), mixing the two solutions, and making up the volume to 100 ml. Take few ml of extract and add 2-3 ml of Dragendorff's reagent. Development of an orange red color indicate the presence of alkaloid.

Mayer's test

gm of mercuric chloride, and 3 gm of potassium iodide were dissolved in water to make 100 ml Mayer's reagent. To a few ml of extract taken in test tube, 1-2 drops of the reagent was added, formation of cream-colored precipitate shows the presence of alkaloid.

Hager's test

Hager's reagent is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

Tannic Acid solution test

A buff-colored precipitate was produced after the test filtrate was treated with tannic acid solution, confirming the presence of alkaloids.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Test for Tannins

Sample was taken separately in water, warmed, and filtered. Tests were carried out with the filtrate using following reagents.

Ferric chloride test

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue color shows the presence of tannins.

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate indicates the presence of tannins.

Acetic Acid test

Transfer 5.74 ml of glacial acetic acid to a measuring cylinder or volumetric flask,
then add 94.26 ml of water to bring the total volume to 100 ml.

Test for Flavonoids

Lead Acetate Solution test

A 10% w/v solution of basic lead acetate in distilled water was added to 1 ml plant extract. A yellow colour precipitate formed.

NaOH and Acid

Addition of increasing amount of sodium hydroxide to the residue shows colouration which decolourises after addition of acid.

Test for Carbohydrates

Molisch's test

About 0.1 gm of the sample was dissolved in 2 ml of water, and added 2-3 drops of 1% ethanolic solution of alpha naphthol, and then carefully poured 2 ml of concentrated sulphuric acid down the side of the test tube so that it forms a heavy layer at the bottom. A deep violet color is produced if carbohydrates are present.

Fehling's test

1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and boiled for 1 minute. Equal volume of sample was then added, heated in boiling water bath for 5-10 minutes. First a yellow then brick red color shows the presence of carbohydrates.

Benedict's test

Equal volumes of the reagent, and sample were added. Mixture was then boiled for five minutes. Depending on the reducing sugar present a range of colors develops.

Barfoed Test

Add 30.5 gm copper acetate in 1.8 ml glacial acetic acid to prepare reagent. Mix equal volume of Barfoed reagent and test solution. Heat for 1 to 2 min in boiling water bath and cool. Red ppt indicates the presence of carbohydrate.

Iodine Test

Mix 3 ml test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.

Test for Proteins

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Xanthoproteic test

Sample was mixed with 1 ml of concentrated sulphuricacid, formation of precipitate shows positive test.

Millon's test

3 ml of sample was mixed with Millon's reagent, formation of precipitate indicates the presence of proteins.

Test for Saponins glycosides

Foam test

A few mg of the sample was shaken vigorously with water. Honeycomb-like foam indicates the presence of saponins.

Test for Cardiac glycosides

Keller-kiliani test

The sample was dissolved in acetic acid containing trace amounts of ferric chloride. It was then transferred to the surface of concentrated sulphuric acid. A reddish-brown ring will be formed at the junction, and the color slowly changes to blue.

Legal's test

A few drops of pyridine, and sodium nitroprusside were added to the sample, and made alkaline. A pink or red color is obtained.

Test for Coumarin Glycosides

Alcoholic extract when made alkaline shows blue or green fluorescence.

PROTOTYPE FORMULATION DEVELOPMENT:

Formulation of ointment base:

Formulation 1:

Table No:1 Ointment base (Formulation 1)

Sr.No.	Name of Ingredient	Concentration
1.	**Cetostearyl Alcohol**	1.5gm
2.	**Lanolin**	1.5gm
3.	**Hard Paraffin**	1.5gm
4.	**Petrolatum**	25.5gm

Procedure:

Hard paraffin and cetostearyl alcohol were melted on water bath. To this lanolin (wool fat) and Petrolatum (yellow soft paraffin) were incorporated. Formulation was stirred until all the ingredients are melted. Examined the content of any foreign particle decant or strain whenever required. Mixture was thoroughly stir until cold and packed it in ointment jar and labeled.

Formulation of herbal ointment:

Table No:2 Formulation of herbal ointment

Sr. No.	Name of Ingredient	F1	F2	F3
1.	**EhretiaacyminataExtract**	0.6gm	1.2 gm	1.8gm
2.	**Ointment Base**	Upto 30 gm	Upto 30 gm	Upto 30 gm

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Procedure:

Calculated quantity of the extract was triturated with small amount of simple ointment base. Remaining quantity of simple ointment base was added with through trituration, until a homogenous mass is obtained. Mixture was thoroughly stir until cold and packed it in ointment jar and labeled.

Formulation of herbal gel:

Table No:3Formulation of Herbal Gel

Sr.no	Name of Ingredient	F1	F2	F3
1.	Extract	0.6 gm	1.2 gm	0.8 gm
2.	Water	5 gm	5 gm	40gm
3.	Methanol	15 ml	15 ml	-
4.	Carbapol	1 gm	1.5 gm	0.4 gm
5.	NaOH	2 drop	3 drop	-
6.	Triethanolamine	1 drop	1 drop	2 drops
7.	Glycerin	10 ml	10 ml	2 gm
8.	Methylparaben	0.05	0.05	0.1 gm

Procedure for F1 and F2:

Preparation of 1% NaOH:

Accurately weighed 1 gm of sodium hydroxide (NaOH) dissolved in small amount of water and volume was make the volume upto 100 ml.

Preparation of extract solution:

Accurately weighed 0.6 gm (2%) and 1.2 gm (4%) in of extracts were dissolved in 15 ml of methanol separately in two beakers.

Preparation of Gel:

Accurately weighed quantity of carbopol 934 (1 gm and 1.5 gm) of in 5 ml of water separately in two different beakers. Methyl paraben (0.5 gm) was added in previous solution. 2% and 4% solution of extract were added into carbapol solution separately. Finally 10 ml glycerin was added and pH was maintain by using few drops of 1% NaOH.

Procedure for F3

0.8g of extract was dissolved in 10 ml water (solution A). Methyl paraben (0.05) and 0.2gm of carbapol were dissolved in 30 ml of water. (Solution B). Solution B was thoroughly added in solution A with continuous stirring. Few drops of triethanolamine was added to make solution alkaline followed by glycerin (1gm) with continuous stirring. Formulation was packed in well close container and store in proper storage condition.

PHARMACEUTICAL EVALUATION OF OINTMENT AND GEL

Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Consistency

Consistency is checked by the spreading the ointment in between the fingers.

pH

pH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S=M\times L/T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Viscosity:

The viscosity of the formulations was determined at varying shear stresses using a Brookfield viscometer. The viscosity was reported in a unit of centipoise.

Wash ability:

Formulation was applied on the skin and then ease extend of washing with water was check.

RESULT AND DISCUSSION:

Extraction

The yield values for hydroalcoholic extract (EAHAE) was 20.48%.

Phytochemical screening of all solvent extracts of *Ehretia acuminata*

Table 4 depicts results of screening of different solvent extracts of plants for various phytochemical constituents. Hydroalcoholic extract showed presence of tannin, saponin glycoside, cardiac glycoside, flavonoid, steroid, protein, carbohydrate and alkaloid.

Table no. 4 Results of phytochemical investigation of *Ehretia acuminate.*

Chemical constituents	Test	Hydroalcoholic Extract
Alkaloids	Dragendorff's Test	+
	Mayer's Test	+
	Hager's Test	+
	Tannic acid Test	+
Phenolic & Tannins	$FeCl_3$ Solution Test	+
	Lead acetate Test	+
	Acetic acid Test	+
Saponin glycoside	Foam test Test	+
Cardiac glycoside	Legal's Test	+

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

	Keller-Killiani	+
Coumarin glycoside	NaOH + Acid Test	-
Flavonoid	Lead acetate Test	+
	NaOH + acid Test	-
Steroid	Salkowski Test	+
Protein	Millon's Test	+
	Xanthoproteic Test	-
Carbohydrate	Molish test Test	+
	Iodine test Test	+
	Benedict test Test	+
	Barfoed test Test	-
	Fehling test Test	+

+ indicates presence of constituents; - indicates absence of constituents.
NP - Not Performed

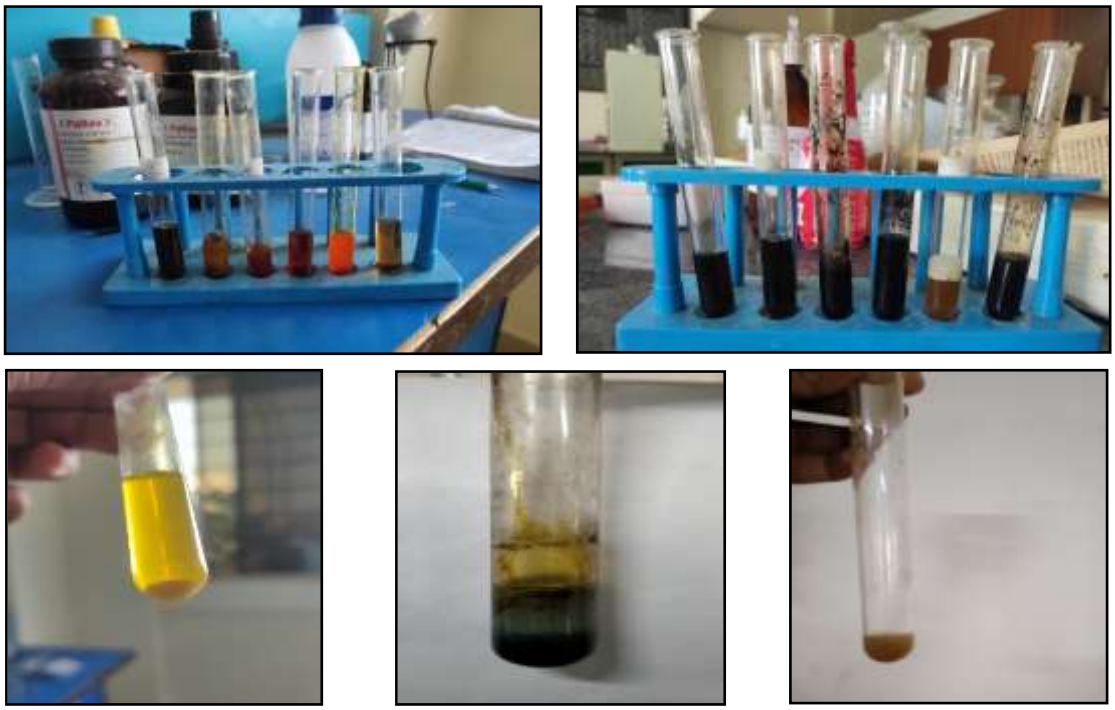

Figure 14: Phytochemical Screening results of crude extract of *E. acuminate.*

PROTOTYPE FORMULATION DEVELOPMENT:

Three formulation batches of herbal ointment were prepared viz. F1, F2 and F3. From all three batches batch F1 was found to be good in appearance and consistency. Hence only F1 batch is proceed for further evaluation and remaining batches were discarded.

PHARMACEUTICAL EVALUATION OF OINTMENT

Colour and Odour:

Colour of the formulation is slightly yellow and odour is characteristic.

Consistency:

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Batch F1 was found to be Smooth and no greediness is observed

Wash ability:

The wash ability of the formulation batch was found to be good.

pH:

The pH of batch F1 was found to be 6.4.

Fig no. 15 Digital pH meter (ointment)

Viscosity:

The viscosities for the formulated products at 100 rpm were found to be 3196 cps It is claimed that a non-Newtonian behaviour with pseudoplastic flow was observed in the F1 batch.

Fig no. 16Brookfield Viscometer(ointment)

PHARMACEUTICAL EVALUATION OF GEL

Color and Odor:

Color of the formulation is slightly brownish yellow and odor is characteristic.

Consistency:

Batch F3 was found to be Smooth and no greediness is observed.

pH: The pH of batch F3 was found to be 7.05

Fig no 17 Digital pH meter (Gel)

Viscosity:

Fig no 18 Brookfield Viscometer (Gel)

The viscosities for the formulated products at 100 rpm were found to be 3556 cps It is claimed that a non-Newtonian behavior with pseudoplastic flow was observed in the F3 batch.

Wash ability:

The wash ability of the formulation batch was found to be good.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Table No 5 Evaluation parameter of Herbal ointment and gel

Fig no.19

Sr.no	Evalution parameter	Ointment (F1 Bach)	Gel (F3 batch)
1.	**pH**	6.4	7.05
2.	**Viscosity**	3196 cp at 100 rpm	3356 cp at 100 rpm
3.	**Appearance**	Slightly yellowish	Slightly brownish yellow
4.	**Stability**	Stable at room temperature	Stable at room temperature
5.	**Consistency**	Smooth and no greediness	Smooth and no greediness
6.	**Washability**	Good	Good

Prepared ointment of *E. acuminata*

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Fig no. 20 Prepared Gel of *E. acuminata*

SUMMARY AND CONCLUSION

The aim of dissertation entitled "Development and Evaluation of Topical Formulation of Herbal Extract for Antibacterial and Antifungal Activity" is to formulate for a stable, Safe, Efficient as well as qualitative dosage form. From the literature review it is assumed that leaves of *Ehretia acuminata* don't have any microbial activity. For that purpose, topical formulation from the category of semisolid dosage form is to be prepared as the concern of easy of administration to the affected area. The results of the study indicated that the hydroalcoholic crude extract of plant showed presence of tannin, saponin glycoside, cardiac glycoside, flavonoid, steroid, protein, carbohydrate and alkaloid as secondary metabolites.

In prototype formulation development initially three ointment bases were prepared. These ointment bases were used for formulation of different batches of ointment as topical semisolid dosage. From all three batches batch F1 was found to be good in appearance and consistency. Hence only F1 batch is proceed for further evaluation and remaining batches were discarded. In further evaluation various tests like pH, Spreadability, Viscosity, and Wash ability were performed. From the results obtained from the entire evaluation test it is concluded that batch F1 was given the satisfactory results. Similarly herbal gel was prepared as topical semisolid dosage.

Development of gel was done by using carbapol 934 which was analyzed with smooth and homogenous appearance. It was easily spreadable with an acceptable mechanical property. The observation of pH revealed that all the formulations were very near to the skin pH make it suitable for application of skin. The result obtained from the present work indicate that the entire drug was uniformly distributed there was no precipitation in the formulation. From the results obtained from the entire evaluation test it was observed that F3 batch showed smooth texture, optimum pH & good spreadability hence, F3 batch was taken for further studies.

Sustained release become important to supply the skin with a drug over a prolong period of time hence, a dermatological delivery system such as gel was considered to be formulated.

In present study it is concluded that developed formula for ointment and gel was found to be stable, Safe, and Efficient dosages form.

List of abbreviations

TAT - *TryptoneAzolectic*
ml -*Milliliter*
cm - *Centimeter*
m - *Meter*
gm-*gram*
USP- *United state pharmacopoeia*
IP- *Indian pharmacopoeia*
E..-*Ehretia*
Rpm- *Rotation per minutes*

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Cps- *Centipoise*
EAHAE- *Ehretiaacuminatahydroalcoholic extrac*t
Hrs- *Hour*
Min- *Minutes*
w/v- *Weight by volume*
w/w- *Weight by weight*
v/v- *Volume by volume*
CAS-*Chemical abstract service*
BP- *British Pharmacopoeia*
JP- *Japanese Pharmacopoeia*
Ph. Eur.-*European Pharmacopoeia*
ICH-*International Council for Harmonisation*
MEEO- *methanolic extract of Emblicaofficinalis*

References:

1. Winslow LC. Herbs as medicines. Arc Int Med. 1998 Nov 9;158(20):2192-9.
2. Gossell-Williams M. The past and present use of plants for medicines. West Ind. Med. J. 2006, Sep;55(4):217-8.
3. De Smet PA. The role of plant-derived drugs and herbal medicines in healthcare. Drugs. 1997 Dec 1;54(6):801-40.
4. WHO technical report series. Guidelines for the Assessment of Herbal Medicines. 1996; 863:178-184.
5. Abhishek K. Herbal drugs-present status and efforts to promote and regulate cultivation. Pha. Rev. 2006;6:73-7.
6. Harish P. Herbal drugs. Meeting report. Curr Sci. 2001;81(1):15-6.
7. Mishra A. Topical antibiotics and semisolid dosage forms. Int J. Phar. Eru. 2014;4:33-54.
8. Bhaskar R. A review on: ointment and ointment bases.(2016). World Jour. of Pharma. Res. Volume 5, Issue 9, 335-345.
9. Kaushal D, Upadhyaya N. REVIEW ON OINTMENT.
10. Sawant SE, Tajane MD. Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. Journal of Scientific and Innovative Research. 2016;5(4):149-51.
11. Rathod HJ, Mehta DP. A review on pharmaceutical gel. International Journal of Pharmaceutical Sciences. 2015 Oct 1;1(1):33-47.
12. Zalivskaya A, Fadeeva D, Shestopalova N, Avtina N, Radyukova V, Ivanova V. Comparative characteristics of gel bases for semisolid dosage forms. InBIO Web of Conferences 2021 (Vol. 40, p. 03008). EDP Sciences.
13. Patil PB, Datir SK, Saudagar RB. A review on topical gels as drug delivery system. Journal of Drug Delivery and Therapeutics. 2019 Jun 15;9(3-s):989-94.
14. Dange V, Dinde S, Doiphode A, Dhavane S, Dudhal B, Shid S, Yadav A. Formulation and evaluation of herbal gel containing Lantana camara for management of Acne vulgaris. Journal of University of Shanghai for Science and Technology. 2020;22(11):799-809.
15. Misal G, Dixit G, Gulkari V. Formulation and evaluation of herbal gel.
16. Mishra A, Panola R, Vyas B, Marothia D, Kansara H. Topical antibiotics and semisolid dosage forms. Int. J. Pharm. Erud. 2014;4:33-54.
17. Jhawat VC, Saini V, Kamboj S, Maggon N. Transdermal drug delivery systems: approaches and advancements in drug absorption through skin. Int J Pharm Sci Rev Res. 2013 May;20(1):47-56.
18. Rowe RC, Sheskey PJ, Quinn ME, editors. Handbook of Pharmaceutical Excipients. 6th ed. London: Pharmaceutical Press; 2009.
19. Thakre R, Harne K. Comparative Antimicrobial Study Of Polar And Non Polar Extracts Of EhretiaLaevisRoxb.(KhanduChakka) Plant.
20. Doijad C, Bhagat S, Mahajan S, Barsagade AG. Development and Preparation of Pain Balm from EhretiaLeavis and it's Physiochemical Evaluation. Development.;7(17):2020.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

21. Sangode CM, Tidke PC, Umekar MJ. A general appraisal of EhretialaevisRoxb: An essential medicinal plant.
22. Sharma P, Shri R, Ntie-Kang F, Kumar S. Phytochemical and ethnopharmacological perspectives of Ehretialaevis. Molecules. 2021 Jun 8;26(12):3489.
23. Rangnathrao TS, Shanmugasundaram P. Antioxidant and Hepatoprotective activity of EhretialaevisRoxb against paracetamol induced acute Hepatotoxicityinwistar rats. Research Journal of Pharmacy and Technology. 2019;12(12):6143-8.
24. Borkar AV, Karande NA, Rathi LG. ROLE OF EHRETIA LAEVIS ROXB IN WOUND HEALING ACTIVITY–A REVIEW.
25. Jadhav S, Phatak PP. FORMULATION AND EVALUATION OF HERBAL TOPICAL DRUG DELIVERY.EPRA International Journal of Research and Development (IJRD). 2023 Jan 27;8(1):139-51.
26. Praveen T, Niroji S, Lojiny M, Thuvaragan S, Gnanakarunyan TJ, Srikaran R. Formulation and evaluation of poly herbal topical anti-bacterial ointment.
27. Kale PS, Parekar PB, Shivpuje SS, Navghare VV, Savale MM, Surwase VB, Mane-Kolpe PS. Polyherbal Gel Development And Evaluation For Antifungal Activity. European Journal of Molecular & Clinical Medicine. 2022 May 22;9(3):5409-18.
28. Gavan A, Colobatiu L, Hanganu D, Bogdan C, Olah NK, Achim M, Mirel S. Development and evaluation of hydrogel wound dressings loaded with herbal extracts. Processes. 2022 Jan 26;10(2):242.
29. Derkar S, Ingole A, Baheti JR. FORMULATION AND EVALUATION OF HERBAL ANTIBACTERIAL AND ANTIMICROBACTERIAL GEL.
30. Saritha K, Bindhu AA, Madhusudhan R, Mounika G, Parshuram K, Haritha S. FORMULATION AND EVALUATION OF ANTIMCROBIAL ACTIVITY OF POLYHERBAL FORMULATION. Journal homepage: www. ijrpr. com ISSN.;2582:7421.
31. Chandra S, Sodiyal N, Patil S. A Review on Herbal Gel Face Wash with Scrub. International Journal of Research in Engineering and Science. 2022;10(6):19-30