



Formulation And Evalution Of Nanoparticlesincorporated Anti-Inflammatory Ointment From The Alcoholic Extract Of Ricinus Communis Leaves

¹ E.Aswathi,² Ayisha Jinan,³ Ansitha M,⁴ Najiya Nasrin,⁵ Mr.Babu K M

² Assistant Professor, Department of pharmacognosy

¹ Dr. Moopen's College of Pharmacy

ABSTRACT

The development and assessment of nanoparticles in anti inflammatory ointments represent a significant advancement in topical therapy. This research aimed to create an anti inflammatory ointment with nanoparticles to enhance the delivery and effectiveness of the active pharmaceutical ingredient. Characterisation of the nanoparticles involved techniques like zeta potential, scanning electron microscopy which confirmed their uniform size and morphology.

The ointment was formulated by integrating the nanoparticles into an appropriate ointment base, followed by thorough evaluation of physical properties, spreadability, viscosity and Ph. In vitro release studies indicated a sustained release profile and anti inflammatory activities. This novel formulation harnesses the benefits of nanotechnology, offering a controlled release system and enhanced skin penetration leading to better therapeutic outcome. The studies concludes that nanoparticle-infused ointments have great potential for managing inflammatory conditions effectively, necessitating further clinical investigation.

GRAPHICAL ABSTRACT



Keywords: Nanoparticles, Anti inflammatory, ointment, controlled release, skin permeation.



1. INTRODUCTION

Herbs are any plant or part of plants that are used for the medicine, food, flavor or perfume.¹ They consist of flower buds, barks, seed, leaves or any other parts of the plant. Herbal plants have been used for thousands of years in various cultures around the world for their medicinal properties, culinary uses and overall benefits to health and well-being.²

The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings. With ongoing research and increasing interest in natural remedies, herbal plants continue to play a significant role in modern healthcare.^{3,4}

Inflammation is the body's natural response to injury or infection. It is a complex biological process that involves the immune system, blood vessels, and various molecular mediators. The primary purpose of inflammation is to eliminate the initial cause of cell injury, clear out damaged cells and tissues and establish a repair process.⁵

The inflammation is of two type acute inflammation and chronic inflammation. The key components of inflammation are vascular changes (vasodilation, increased permeability), cellular events (Leukocyte Migration, Phagocytosis) and Chemical Mediators (Histamines, Prostaglandins, Cytokines).⁶

Anti-inflammatory ointments are a group of medicines that are used to ease muscle pains, sprains, strains and arthritis. When they are applied to the skin they are called topical anti-inflammatory ointment. Sometimes they are called 'topical non-steroidal anti-inflammatory drugs' (NSAIDs), or just 'topical anti-inflammatories'.⁷

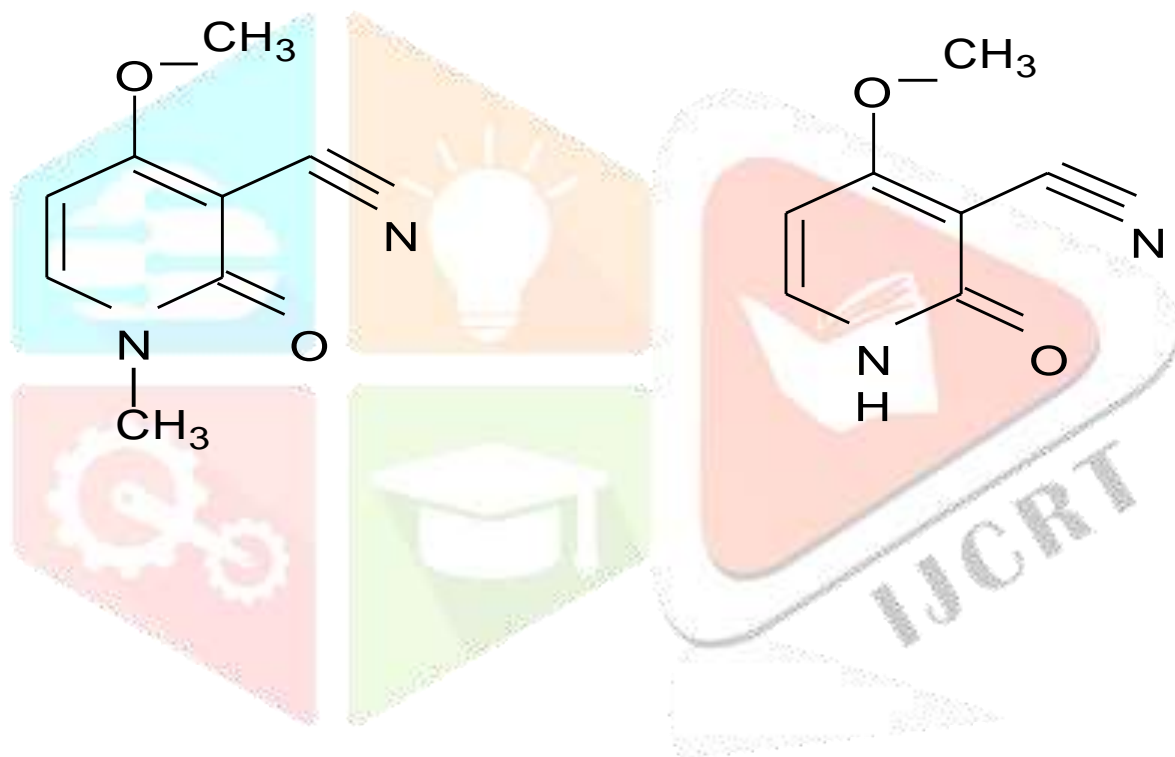
Ricinus communis Linn. is commonly known as Castor. It is also known as 'Eranda' in Sanskrit. It is a perennial shrub. Castor belongs to the family Euphorbiaceae. This plant is indigenous to the southeastern Mediterranean Basin, Eastern Africa and India.⁸ The plant is widespread throughout tropical regions as ornamental plants. The castor oil plant is a fast-growing, suckering perennial shrub or occasionally a softwood small tree up to 6 meter or more, but it is not hardy in nature.⁹

This plant was cultivated for leaf and flower colours and for oil production. Leaves are green or reddish in colour and about 30-60 cm in diameter¹⁰

The stems are varying in pigmentation. The flowers are monoecious and about 30-60 cm. long. The fruit is a three-celled thorny capsule. The capsule of fruit covered with soft spines like processes and

dehiscing in to three 2-valved cocci. The seeds are considerable differences in size and colour. They are oval, somewhat compressed, 8-18 mm long and 4-12 mm broad. The testa is very smooth, thin and brittle. Castor seeds have a warty appendage called the caruncle, which is present usually at one end from which runs the raphe to terminate in a slightly raised chalaza at the opposite end of the seed.¹¹

The medicinal properties of the plant are due to the presence of key phytochemical constituents like saponins, flavonoids, alkaloids, steroids and glucosides. Leaves of the plant have shown the presence of major phenolic compounds such as camphor, and α sesquiterpenoid (β -caryophyllene), gallic acid, quercetin, gentilic acid, rutin, epicatechin and ellagic acid.¹² Whereas roots contain Indole-3-acetic and the several forms of ester have also been identified in the form of palmitic, stearic, arachidic-hexadecenoic, oleic, linoleic, ricinoleic, and dihydroxy stearic acids through the study of castor oil.¹³



RICININE

N – DEMETHYLRICININE

Castor oil is a tasteless and odorless vegetable oil that is extracted by pressing of the seeds of the castor plant. Castor seeds are rich in triglycerides. They also contain small amounts of a toxin called ricin. It was extensively used as a purgative and, most peculiarly, as a medicine against almost all ailments in children and the elderly. These possible illnesses ranged from a cough, cold, and fever to constipation and indigestion. Castor oil was also used to treat other problems such as ingestion of any poisonous material, tapeworms and roundworms and skin diseases.¹⁴



Stomach malfunctioning was believed to be the root of all the problems. All the medicinal uses of this oil are mostly due to its germicidal, toxic, purgative, anti-inflammatory and disinfectant properties. The health benefits of castor oil include boosting immunity, skin and hair care, and relieving rheumatism, menstrual disorders, and constipation.^{xv}



Figure: 1



Figure: 2

Anti-inflammatory and free radical scavenging activities of the methanolic extract of root of *Ricinus communis* (Euphorbiaceae) Linn was studied in Wistar albino rats. The methanolic extract at doses 250 and 500 mg/kg p.o. exhibited significant anti-inflammatory activity in carrageenin induced hind paw edema model.^{xvi} The ethanolic extract at the dose of 500 mg/kg p.o. also exhibited significant ($P < 0.001$) anti-inflammatory activity in cotton pellet granuloma model. The methanolic extract showed significant free radical scavenging activity by inhibiting lipid peroxidation initiated by carbon tetrachloride and ferrous sulphate in rat liver and kidney homogenates. The extract enhanced free radical scavenging activity of stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH), nitric oxide and hydroxyl radical in in-vitro assay methods.^{xvii}

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research towards a multitude of potential uses for nanomaterials.

According to the National Nanotechnology Initiative, USA, nanotechnology is the manipulation of matter with at least one dimension sized from 1 to 100 nanometers (Drexler, 2012).^{xviii}

Nanotechnology includes various fields of science such as surface science, organic chemistry, molecular biology, semiconductor physics and micro fabrication. Nanotechnology has the potential to create many new materials and devices with a vast range of applications, such as in medicine, electronics, biomaterials and energy production etc.^{xix}

Nanoparticles are broadly classified in to two categories,

- i) Organic nanoparticles and
- ii) inorganic nanoparticles.

Organic nanoparticles include carbon nanoparticles and inorganic nanoparticles include metal nanoparticles (Ag, Au, Pt, and Pd), magnetic nanoparticles and semi-conductor nanoparticles (TiO₂, SiO₂, ZnO₂). In general, two process are used for the synthesis of nanoparticles viz. top-down process and bottom-up process. In top-down process bulk materials will be broken down into particles at nanoscale with different lithographic techniques such as grinding, milling etc. and in bottom-up approach, atoms self-accumulate to new nuclei which convert into a particle of nanoscale. Nanoparticles are prepared by centrifugation using centrifuge.^{xx}



Figure: 3

The conventional methods include ion sputtering, solvothermal synthesis, reduction and sol-gel technique. However, overall, these methods are energy demanding, expensive and are not eco-friendly. Due to the utilization of toxic chemicals and nonpolar solvents and later on synthetic additives or capping agents, their applications in clinical and biomedical fields are prohibited. Consequently, the need for the development of a clean, reliable, biocompatible, benign, and ecofriendly process to synthesize nanoparticles leads to turning researchers toward 'green' chemistry and bioprocesses.^{xxi} The green methods of synthesizing nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers and microorganisms as reductants and capping agents are proven to be more environmentally friendly and effective.^{xxii}

Plant parts such as leaf, root, latex, seed, and stem are being used for metal nanoparticles synthesis. Metal nanoparticles in particular have been widely investigated due to their ability to be functionalized with many biocompatible materials. Natural bioactive compounds have the potential to reduce metal ion into metal nanoparticles.^{xxiii}

Nanoparticles (NPs) fabricated with plant extracts are currently well-known and considered to

be environment friendly approaches to prepare nanomaterials. Phytochemicals, such as alkaloids, flavonoids, tannins, terpenoids, glycosides and phenylpropanoids present in plant extracts have been employed in these environmentally benign means of NP preparation. Plant extracts have latent biological activities, such as anti-inflammatory, antimicrobial, antioxidant, antimutagenic or antihyperglycemic activities, and these properties may also manifest themselves in the biotic functions of the subsequent colloidal nanoparticle solution; therefore, plant extracts can be used in medicinal applications.^{xxiv}

Plant extract–NP bio conjugates have displayed both in-vitro and in-vivo anti-inflammatory properties. The wound-healing properties of plant extract–NP bio conjugates may be attributed to their antimicrobial, antioxidant and anti-inflammatory activities. Furthermore, synthetic plant extract–NP nanomaterials may enhance the bioavailability and biocompatibility of the resulting NPs.^{xxv}

2. AIM AND OBJECTIVES

The aim of study is to evaluate and formulate nanoparticle incorporated anti-inflammatory ointment using the alcoholic extract of *Ricinus communis*

Specific objectives of the proposed research include:

1. To formulate nanoparticles incorporated ointment
2. To perform evaluation studies for the prepared ointment
3. To increase the availability of the traditional remedies
4. To improve stability of the formulation

MATERIAL AND METHODS

4.1 COLLECTION AND AUTHENTICATION OF THE PLANT MATERIALS

Leaves of *Ricinus communis* were collected from the surrounding areas and nearby localities. The plant part (leaves and seeds) Were authenticated by Dr. Raji Associate professor, Department of Botany, St. Mary's college, Sulthan bathery, Wayanad

PLANT PROFILE



Figure: 4

4.1.1 Synonyms

Croton spinosus L, *Ricinus africanus* Mill., *Ricinus armatus* Andr.

4.1.2 Vernacular Names

Assamese: Era-gach English: Castor, Ricin
Malayalam: aavannakku Hindi:
Arandi

Kannada: Oudla Bengali:

Veranda Manipuri: Kege

Tamil: Amanakku Sanskrit:

Eranda

4.1.3 Geographical source

Castor bean is native of tropical Africa but has naturalized in moist tropical and subtropical regions throughout the world. It may be found naturally and in cultivation as an ornamental plant throughout the tropical and subtropical areas of the United States and its territories ^{xxvi}

4.1.4 Taxonomical classification:

Kingdom: Plantae Order:

Malpighiales Family:

Euphorbiaceae

Sub Family: Acalyphoideae Tribe:

Acalypheae

Genus: Ricinus

Species: Ricinus communis Linn

Occurrence: India, Southeastern Mediterranean Basin, Eastern Africa

4.1.5 Method of propagation

Castor is propagated by sowing the seeds in the field for raising the plantation.

4.1.6 Medicinal properties

Ricinus communis are having several medicinal properties including antimicrobial, antihistaminic, antinociceptive, antifertility, anti-asthmatic, hepatoprotective, anti-inflammatory, antimicrobial, wound healing, and antidiabetic activities.

Various parts of Ricinus communis are used for eye infection, liver infection and sexually transmitted diseases ^{xxvii}

APPARATUS

Table no: 1 Apparatus

SI NO	Apparatus	Size/Volume
1.	Soxhlet extraction apparatus	1000 ml
2.	Conical flask	500ml
3.	Beaker	100ml,250 ml,500ml
4.	China dish	2
5.	Silica crucible	1
6.	Test tube	15*125ml
7.	Funnel	75mm
8.	Measuring cylinder	50ml,100 ml

CHEMICALS AND REAGENTS

Table no: 2 Chemicals and reagents

1.	Methanol	500 ml
2.	Ethanol	100 ml
4.	Silver nitrate	2g
5.	Beeswax	30g
6.	Hydrochloric acid	10ml
7.	phloroglucinol	5ml

8.	Distilled water	q. s
9.	Glycerol	5ml

INSTRUMENTS

Table no: 3 Instruments

SI NO	Equipment	Model/company
1.	Weighing balance	D-Sonic
2.	Muffle furnace	Kemi
3.	Incinerator	Kemi
4.	Heating mantle	Kemi
5.	Centrifuge	Kemi
6.	Magnetic stirrer	Kemi

INGREDIENTS

Table no:4 Ingredients

SI NO.	INGREDIENTS	QUANTITY
1.	Castor Nanoparticles	0.15 g
2.	Beeswax	15g

3.	Coconut oil	25 ml
4.	Shea butter	10 g
5.	Lavender oil	5-10 drops
6.	Salt	qs

4.2 PREPARATION:

PROCEDURE OF OINTMENT

1. Melt the Base Ingredients:

In a double boiler, melt the beeswax, coconut oil, and shea butter over low heat. Stir occasionally until fully melted.

2. Add Castor incorporated nanoparticles

Once the base ingredients are melted and well combined, remove the mixture from heat. Add the castor incorporated nanoparticles to the formulation

3. Incorporate Essential Oils and salt:

Lavender oil and salt is then added to the mixture. Stirred well to ensure even distribution.^{xxviii}

4.3 MACROSCOPIC DESCRIPTION

Leaves of *Ricinus communis* Linn subjected to macroscopical evaluation for parameters like colour, shape, size and taste.

Herbarium of the plant was prepared consisting of roots, leaves and seeds to perform the identification and authentication of *Ricinus communis*.^{xxix}

4.4 MICROSCOPIC DESCRIPTION

4.4.1 Transverse section of leaves

For microscopic evaluation leaves were sectioned in transverse direction. Leaves were cross sectioned and section was prepared, staining was done to study anatomical structure by using hydrochloric acid and phloroglucinol.^{xxx}

4.4.2 Powder microscopy

The powder microscopy was studied as per the standard procedure by capturing the images using eye capture. For performing powder microscopical evaluation the powdered leaves of *Ricinus communis* were taken and stained using hydrochloric acid and phloroglucinol. Then it was mounted on a glass slide and viewed under

microscope.^{xxxi}

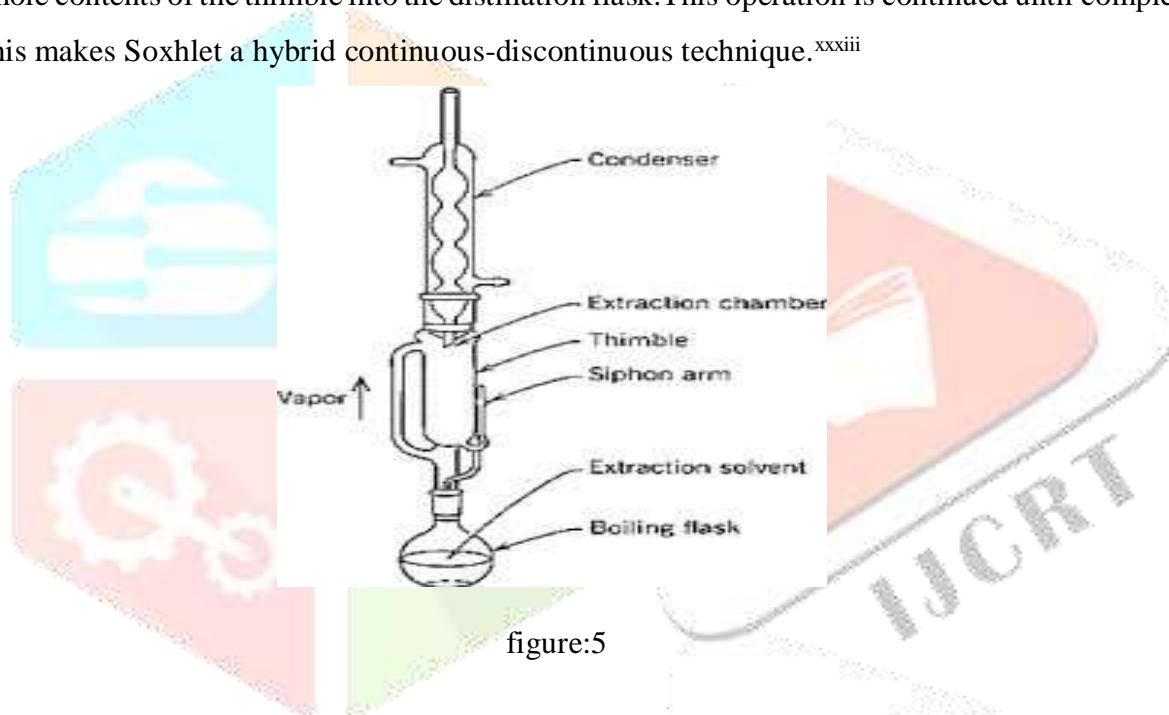
4.5 EXTRACTION

Dried plant material was extracted by using the Soxhlet extraction method. Plant leaves was shade dried and then powdered. The powder of the leaf extracted was weighted and placed andmethanol was taken as a suitable solvent. The collected extract was the taken for determining its phytochemicals.^{xxxii}

SOXHLET EXTRACTION

Soxhlet extraction is a standard technique and a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from interfering substances. Conventional Soxhlet extraction is one of the most relevant techniques of extraction.

In Soxhlet extraction the sample is placed in the thimble-holder and during operation is gradually filled with condensed fresh solvent from distillation flask. When the liquid reaches alevel to overflow a siphon aspirated the whole contents of the thimble into the distillation flask.This operation is continued until complete extraction and this makes Soxhlet a hybrid continuous-discontinuous technique.^{xxxiii}



4.6 PHYSIOCHEMICAL PARAMETERS OF RICINUS COMMUNIS LEAVES

- **Ash value**

Determination of ash value was performed using the standard procedure. The crude powder ofthe drug was taken and kept in the muffle furnace at 450 degree Celsius and ash value was determined.

- **Moisture content**

Moisture content of the powdered drug was calculated by keeping the powder on hot air oven at 105 degrees Celsius and amount of water was frequently measured until a constant value was observed.

- **Extractive value**

Extractive value of the powder of Ricinus communis was measured by taking the required quantity of powder in conical flask. Water soluble and alcohol soluble extractive value was determined.^{xxxiv}

4.7 PRELIMINARY PHYTOCHEMICAL SCREENING

4.7.1 TEST FOR ALKALOIDS

- **Mayer's test**

A test tube was filled with 0.5 g of the crude drug and solubilised by using water and another test tube with alcohol. The solution of potassium mercuric iodide was the added, and it was mixed. A white or cream coloured precipitate's appearance indicated the presence of alkaloids.

- **Wagner's test**

To the solution of drug Wagner's reagent was added and the mixture was thoroughly agitated. Alkaloids are present because of the reddish-brown colour that appeared.

- **Dragendroff's test**

To the drug solution dragendroff's reagent was added and appearance of orange red precipitate showed the presence of alkaloids

- **Hager's test**

Hagner's reagent was added to the alcoholic and water-soluble extract of the drug and presence of yellow precipitate showed the presence of alkaloids.

4.7.2 TEST FOR FLAVANOIDS

- **Alkaline reagent test**

2ml of 2.0% NaOH mixture was mixed with 0.5g of extract an intense yellow colour was generated which becomes colourless after adding 2 drops of dilute HCL. The findings indicated the presence of flavonoids.

- **Ferric chloride test**

After adding a few drops of neutral ferric chloride solution to 0.5g of extract, a blackish red hue appears, indicating the presence of flavonoids.

- **Liebermann's Burchard test**

0.5g of extract was correctly combined with 2ml of chloroform and then 2ml of acetic acid was added to the solution, which was subsequently cooled in ice. 1ml of concentrated sulfuric acid after cooling. The process of alkaloids in the extract causes the hue to shift from violet to green.

4.7.3 TEST FOR GLYCOSIDES

- **Keller- kiliani test for cardiac glycosides**

The 0.5g of plant extract and 1 ml of concentrated H₂SO₄ were combined with 1 ml of glacial acetic acid and 1 drop of 2.0% ferric chloride mixture. Between the layers, a brown ring was developed,

indicating the presence of cardiac glycosides.

- **Borntrager's test**

Treat the test extract with dilute hydrochloric acid boil on water bath for 10 min and filter. Filtrate was extracted with benzene and add equal amount of ammonia solution to filtrate and shake. Formulation of pink or red colour in the presence of anthraquinone molecule.

- **Modified Borntrager's test**

To the required quantity of drug, add dilute hydrochloric acid followed by 5ml ferric chloride. Boil for 10 min on water bath ,cool and filter, filtrate was extracted with carbon tetrachloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety.

- **Legal test**

Treat the test extract with pyridine, methanolic alkali and sodium nitroprusside. The presence of cardiac glycoside indicated by the formation of pink to red colour.

- **Baljet test**

Treat the test extract with sodium picrate. The presence of cardiac glycosides indicates the formation of orange to yellow colour.

4.7.4 TEST FOR TANNINS

- **Test for ferric chloride**

0.5 g of extract solution was combined with a 0.5ml ferric chloride solution. The presence oftannin is indicated by the formation of a black coloration.

- **Vanillin hydrochloride test**

Add drop of vanillin hydrochloride to test extract. Formation of pink colour indicates the presence of tannin.

4.7.5 TEST FOR SAPONINS

- **Froth test**

5ml of distilled water and 1 ml of extract were combined, then shaken or until there was foam after good mixing. The presence of saponins is indicated by the presence of foam.

4.7.6 TEST FOR STEROIDS

Following the addition of a few drops of approved sulfuric acid and acetic acid. Extract was combined with 1ml of chloroform. The sulfuric acid layer displays yellow with green fluorescence while the top layers display red.

- **Salkowski test**

0.5g of extract was mixed with chloroform and a few drops of concentrated sulphuric acid, shaken well and

allowed to stand for some time. Red colour appeared at the lower layer indicated the presence of steroids and formation of a yellow coloured layer indicated the presence of tri terpenoids.

4.7.7 TEST FOR PROTEINS

- **Biuret test**

0.5 g of extract was added with few drops of biuret's reagent. The obtained mixture was shaken well and obtained to warm for 10-15 min. Appearance of red or violet colour indicated the presence of protein.

- **Millon's test**

0.5 g of extract was mixed with 2ml of million's reagent. The solution was heated for 5min, a red colour precipitate turns into red colour which confirmed the presence of proteins.

- **Xanthoproteic test**

Test sample is treated with hot, concentrated nitric acid which reacted with aromatic amino acid form a yellow-coloured product.

- **Ninhydrin test**

0.5g of extract was mixed with 2ml of 0.2% solution of ninhydrin reagent. Boiled for 2 min on water bath, appearance of violet colour indicates the presence of protein.

4.7.8 TEST FOR CARBOHYDRATES

- **Molisch's test**

Add alcoholic alpha naphthol solution and concentrated sulphuric acid to the test extract, violet ring at the junction indicates the presence of carbohydrates.

- **Benedict's test**

Add benedict's reagent to the test extract and heat the mixture on a water bath, orange red precipitate indicates the presence of carbohydrates.

- **Fehling's test**

Add dilute hydrochloric acid to the test extract and heat. Add equal volume of Fehling's A and B solutions, formation of deep red colour indicates the presence of carbohydrates.

- **Barfoed's test**

Add barfoed's reagent to the test extract and heat the mixture on a water bath, formation of orange red colour precipitate indicates the presence of carbohydrates.^{xxxv}

4.8 ANGLE OF REPOSE

Angle of repose is calculated using the castor leaves powder which was dried and grinded. Angle of repose is the angle that differentiates the transition between the phases of granular materials.^{xxxvi}

According to Carr's classification

DESCRIPTION	REPOSE ANGLE
Very free flowing	<30
Free flowing	30-38
Fair	38-45
Cohesive	45-55
Very cohesive	>55



Figure:6

4.9 EVALUATION OF OINTMENT

4.9.1. ORGANOLEPTIC EVALUATION

- Colour

The prepared formulation was examined for the colour against a black and white background.

- Odour

The prepared formulation was examined for the odour.

4.9.2. DETERMINATION OF PH

The pH of the prepared formulation was checked using pH paper. A drop of the formulation was spotted on the pH paper and colour was observed. It was then compared with the standard colour on the pH paper^{xxxvii}

4.9.3. DETERMINATION OF SPREADABILITY

To evaluate the spreadability of formulations, the spreading diameter of 1 gram of ointment is measured and placed between two horizontal plates, each measuring 20 cm by 20 cm. A weight of 100 grams is placed on the upper plate to exert pressure. The spreadability is calculated using the formula $S = M \times L \times T$ where:

- **S** represents the spreadability,
- **M** is the applied weight (100 grams),
- **L** is the length of the glass plates (20 cm),
- **T** is the time required to completely separate the plates.

This formula determines how easily the gel spreads under a standard weight: higher values of **S** indicate better spreadability^{xxxviii}

4.9.4. DETERMINATION OF EXTRUDABILITY

The extrudability test measures the force needed to push a material out of a collapsible tube under a specific amount of applied weight. In this study, the percentage of ointment extruded from the tube under a given load was assessed. The extrudability of the *Ricinus communis* ointment formulations was determined using the following formula:^{xxxix}

$$\text{Ext} = \frac{\text{Amount of ointment extruded from the tube}}{\text{Total amount of ointment filled in the base}}$$

4.9.5. DETERMINATION OF VISCOSITY

The viscosity of the prepared herbal formulations was measured using a Brookfield viscometer. Test sample was taken in a clean and dry culture tube. Viscosity was determined by rotating the spindle in the sample at different Rpm (1.5, 3.0, 6.0, 12.0, 30.0, 60.0) and readings were noted. Spindle no 4 is usually used in the determination of viscosity in Brookfield viscometer.

5. RESULT AND DISCUSSION

5.1 MACROSCOPIC DESCRIPTION

Medicinal plant materials can be classified based on their macroscopic and microscopic properties.

5.1.1 LEAVES

The castor leaves are petiolate, alternate, large, palmately lobed, lobes 7-8, serrate margin and reticulate venation. The upper surface of leaf is dark green while the lower surface is light green in colour. It measures 22-23.2-24.5-25cm in length and 11-12.2-15.1-15.5cm in breadth.^{xi}



Figure:7

5.1.2 FRUITS

The fruit can be spiny and non-spiny, in case of spiny fruit there is variation in the length of spine. There are about 150 spines per fruit. The non spiny fruit can be smooth and warty wereepicarp is rugged.^{xli}

Figure: 8



5.2 EXTRACTION

15 grams of the powder were placed in a thimble, and 250 ml of ethanol were added to a round- bottom flask. Extraction was performed for 2 hours. After the extraction, the extract was collected in the flask and transferred to a beaker. The mixture was then left to allow the solvent to evaporate, leaving behind the extracted compound. The resulting extract was a dark greenish colour with a semi-solid consistency.^{xlii}



Finger :9

5.3. MICROSCOPIC DESCRIPTION

5.3.1 TRANSVERSE SECTION OF LEAF

Transverse hand cut sections of authenticated samples were taken and made permanent with suitable stains. For the measurement of cell contents like, starch grains, vessels, calcium oxalate crystals, stomata etc. ocular and stage micrometer.



Figure: 10

The leaf is characterized by single layer of epidermis covered with thin cuticle and both type of trichomes. Midrib contain 10-14 layers of collenchyma below the upper epidermis 5–6-layer, 4 vascular bundles present in centre, mesophyll differentiated into single layer palisade and 2-3 spongy parenchyma.^{xliii}

5.2.2 POWDER MICROSCOPY

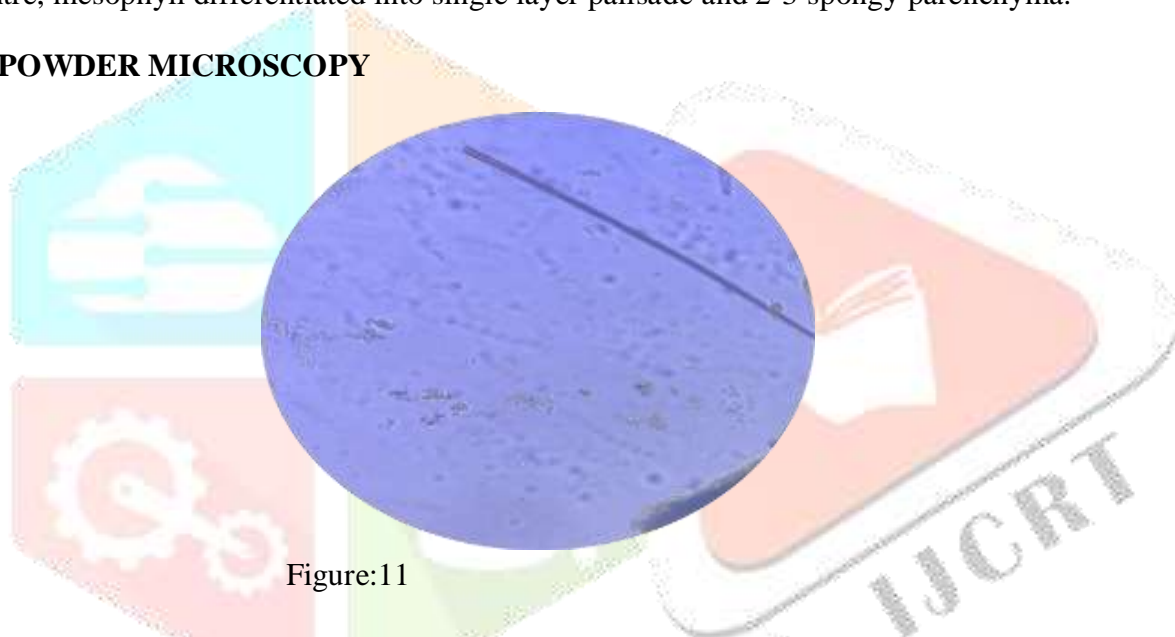


Figure:11

When analysing the powder microscopy of castor leaves different microscopic features were identified including epidermal cells that are polygonal in shapes, trichomes, calcium oxalate crystals, starch grains, and palisade cells.

5.4 PHYSICAL EVALUATION

5.4.1 ANGLE OF REPOSE

The angle of repose refers to the steepest angle at which a pile of unconsolidated material remains stable without sliding or slumping. It varies depending on factors like the size, shape, and cohesion of the particle involved.

Angle of repose of castor powder was found to be 38.65, 35.35, 36.12 and it was found to have good flow property and the powder is free flowing.

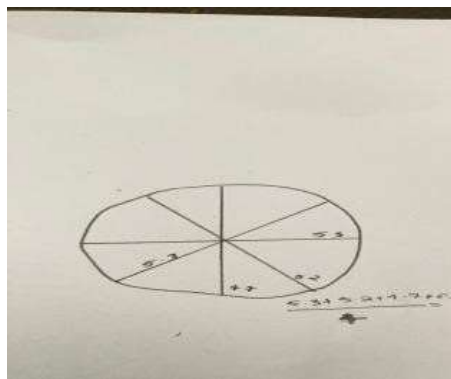


Figure: 12 Height (h)=2cm

Radius(r)=2.5cm $\theta = \tan^{-1}$ (h/r)

$$= 38.65$$

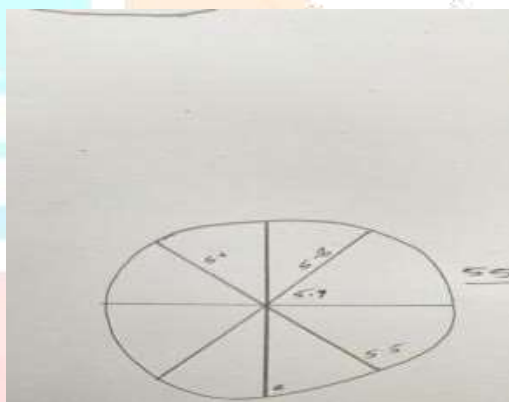


figure: 13 Height (h)=2cm

Radius (r)=2.8cm $\theta = \tan^{-1}$ (h/r)

$$= 35.35$$

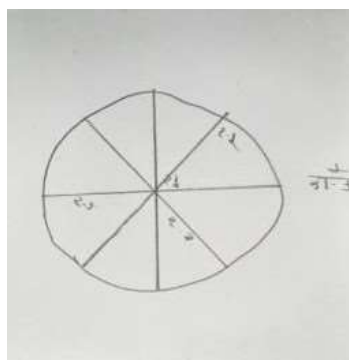


Figure:14 Height(h)=2cm

Radius(r)=2.71cm $\theta = \tan^{-1}$

(h/r)

$=36.12$

Therefore, angle of repose was found to be= $(38.65+35.35+36.12)$

3

$=36.7066$

5.4.2 EXTRACTIVE VALUE



figure:15

Extractive values	Values in %
Water soluble extractive value	Not less than 7 %
Alcohol soluble extractive value	Not less than 1.6 %

5.4.3 ASH VALUE

Ash values	Values in %
Total ash	Not more than 3.3%
Acid insoluble ash	Not more than 1 %

Water soluble ash	Not more than 8%
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Figure: 16

5.4.4 MOISTURE CONTENT

Weight of dish + sample before drying = 75.95g
 Weight of dish + sample after drying = 75.75g
 Loss on drying = 75.95g - 75.75g = 0.2g

So % moisture content = $\frac{\text{weight before} - \text{weight after}}{\text{Weight}} \times 100$
 = 0.26% w/w

5.4.5 PRELIMINARY PHYTOCHEMICAL SCREENING

Test for phytoconstituents	Water extract	Ethanol extract
starch	+	+
Terpenoids	+	+
Proteins	+	+

alkaloids	+	+
glycoside	+	+
saponins	+	-
tannins	+	+
flavonoids	+	+
phenols	+	+



Figure:17

5.5 EVALUATION OF NANOPARTICLES

5.5.1 PARTICLE SIZE

Particle size is measured in litesizer 500 in a temperature 25.0 °C with a solvent using water. The particle hydrodynamic diameter is 166.59nm

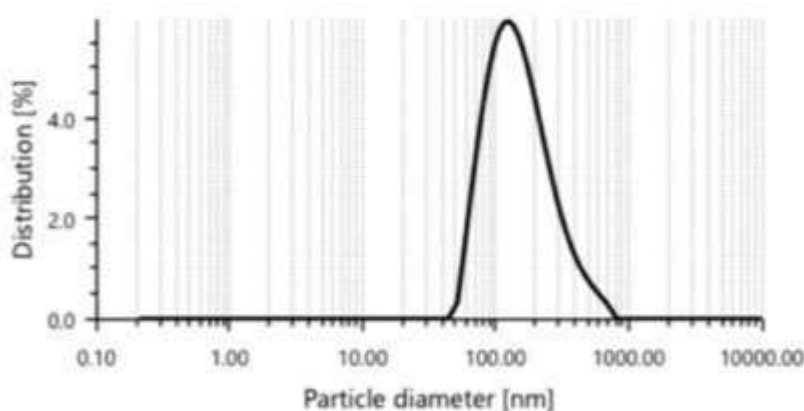


Figure: 18

A nanoparticle diameter of 166 nm indicates that the particles are in an intermediate size range (1-1000) suitable for a variety of applications, particularly in drug delivery and diagnostics. They offer a good balance between surface area and volume, enabling efficient functionalization and interaction with biological systems. Stability, biocompatibility, and targeting properties are key considerations.

5.5.2 ZETA POTENTIAL

Zeta potential is a key indicator of the stability of colloidal dispersions, including nanoparticles. It measures the magnitude of the electrostatic or charge repulsion/attraction between particles in a liquid suspension or a solution.^{xliv}

A zeta potential of -47.6 mV is generally a good indicator of a stable colloidal system with particles that are well-dispersed and unlikely to aggregate. The strong negative charge creates significant electrostatic repulsion between particles, preventing them from coming close enough to aggregate or settle out of suspension, which is important in various applications like drug delivery, where surface charge can affect cellular uptake.

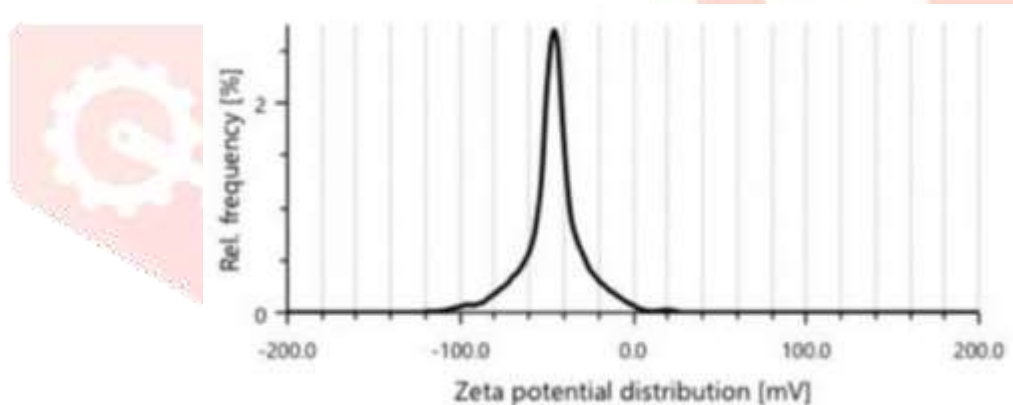


Figure:19

5.5.3 (SEM)SCANNING ELECTRON MICROSCOPY

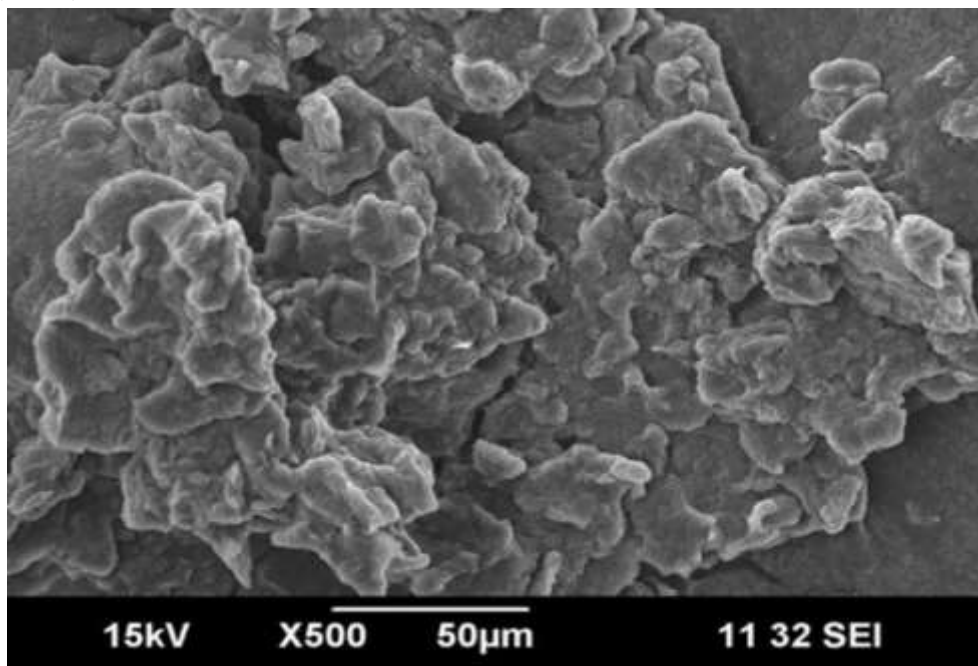


Figure :20

5.6 EVALUATION OF OINMENT

5.6.1 ORGANOLEPTIC EVALUATION

Colour: The colour of the formulation was found to be green
Odour: no characteristic odour

5.6.2 DETERMINATION OF pH



figure:21

Determination of pH was done using pH paper by placing a drop of sample in pH paper and it was found to be yellow in colour which indicates it is acidic in nature.

5.6.3 : DETERMINATION OF EXTRUDABILITY



Figure :22

Extrudability value of the given formulation was found to be 1.2, indicating moderate ease of extrusion under the specified conditions. This suggests that the ointment can be easily dispensed by users, making it user-friendly and suitable for standard packaging.

5.6.4 DETERMINATION OF SPREADABILITY



figure:23

Spreadability of the formulation was calculated and it was found to be 4.1cm, which indicate ointment spread to a 4.1 cm diameter under the test conditions correspond to a specific viscosity or a rheological index indicating moderate spreadability.

5.6.5 : DETERMINATION OF VISCOSITY



figure:24

Viscosity of the formulation was determined using Brookfield viscometer. The formulation was taken in a clean and dry culture tube and the viscosity of the test sample was determined by using the standard operating procedure using spindle no 4.^{xlv} The viscometer was rotated at 0.6, 1.5, 3.0, 6.0, 12.0, 30.0 and 60.0 Rpm respectively.

Rpm	Reading
0.6	459112
1.5	269032
3.0	126458
6.0	104871
12.0	52458
30.0	20000
60.0	10490

5.7 DISCUSSION

Ricinus communis, commonly known as castor, is a plant renowned for its therapeutic properties. The leaves contain various bioactive compounds, including flavonoids, alkaloids, saponins, and tannins, which have anti-inflammatory potential. This discussion focuses on the formulation of an ointment using nanoparticle incorporated ethanolic extracts of *Ricinus communis* leaves and the subsequent evaluation

of its efficacy in treating inflammation.

The goal of this research is to formulate a nanoparticle incorporated ointment using ethanolic extract of *Ricinus communis* leaves and evaluate its efficacy in treating inflammation. The extraction of bioactive compounds from *Ricinus communis* leaves was performed using ethanol, a solvent known for its efficiency in extracting a wide range of phytochemicals. Soxhlet extraction is used for extraction which is advantageous due to its continuous cycle of solvent percolation, ensuring thorough extraction.

Nanoparticles were selected based on their compatibility with *Ricinus communis* leaf extract and their ability to improve bioavailability and targeted delivery. The chosen nanoparticles were loaded with the ethanolic extract. These methods ensured efficient encapsulation of bioactive compounds, protecting them from degradation and facilitating controlled release within the ointment base. Physicochemical characterization involved assessing the appearance, color, texture, and homogeneity of the formulated ointment. Techniques such as scanning electron microscopy (SEM) were employed to analyze the size distribution and morphology of nanoparticles within the ointment. These analyses confirmed the successful incorporation and dispersion of nanoparticles, essential for ensuring consistent performance and efficacy.

The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage. The phytochemical screening was done for the leaf extract, nanoparticle and the ointment. The phytochemical properties were studied which shows a satisfactory result about the macroscopic and microscopic character, physical properties including the ash value, extraction value, moisture content, angle of repose of powder and the ointment's pH, spreadability, extrudability and viscosity were evaluated to ensure compatibility with skin and ease of application. The pH range (typically 4.5-6.5) ensures minimal irritation, while appropriate viscosity promotes spreadability and adherence to the skin surface.

The development of nanoparticle-incorporated anti-inflammatory ointments from *Ricinus communis* leaves represents a significant advancement in pharmaceutical science. This approach harnesses the synergistic benefits of nanotechnology and natural plant extracts to deliver targeted therapies with enhanced efficacy and safety profiles.

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