



FORMULATION AND EVALUATION OF NANO-ENCAPSULATED ANTI-INFLAMMATORY OINTMENT CONTAINING VOLATILE OIL OF *Allium cepa L. aggregatum*

Mr. Arun Ramachandran¹, Ms. Farhana Abdul Majeed², Ms. Fathimath Mansoor³, Ms. Nafeesath Misriya⁴

¹ Associate Professor, Malik Deenar College of Pharmacy, Kasaragod.

² Student, Malik Deenar College of Pharmacy, Kasaragod.

³ Student, Malik Deenar College of Pharmacy, Kasaragod.

⁴ Student, Malik Deenar College of Pharmacy, Kasaragod.

ABSTRACT :

Allium cepa L. aggregatum belonging to Amaryllidaceae family is a bulb found in South-East Asia and some African countries. It is consumed across the world for a variety of purposes that include food and medicine.

The present study highlights the formulation and evaluation of nano-encapsulated anti-inflammatory ointment containing volatile oil of *Allium cepa L. aggregatum*. Extraction of volatile oil of *Allium cepa L. aggregatum* was carried out through hydrodistillation by using clevenger apparatus. The volatile oil of *Allium cepa L. aggregatum* was incorporated into calcium alginate beads and it was successfully formulated into anti-inflammatory ointment. It was subjected to various studies like GC-MS, *in-vitro* drug release studies like diffusion study was conducted. The formulation was also evaluated for various physico chemical parameters such as homogeneity, pH, spreadability, extrudability and *In-vitro* anti-inflammatory activity was also carried out.

LIST OF ABBREVIATIONS

SI NO.	ABBREVIATIONS	DESCRIPTIONS
1.	GC-MS	Gas chromatography-mass spectroscopy
2.	°C	Degree Celcius
3.	Hrs	Hours
4.	Min	Minutes
5.	ml	Milliliter
6.	Mg	Milligram
7.	G	Gram
8.	Mg/ml	Milligram/milliliter
9.	%	Percentage
10.	Sec	Seconds
11.	UV	Ultraviolet
12.	Sl.No	Serial number
13.	HCl	Hydrochloric acid
14.	Mm	Millimeter
15.	M	Meter
16.	eV	Electron Volt

17.	µg	Microgram
18.	µL	Microliter
19.	Temp	Temperature

INTRODUCTION

Herbal medicine is one of the oldest and most widely used health-care systems. The advancement in the field of herbal drug delivery began recently with the goal of efficiently managing human diseases. According to the World Health Organization (WHO), 80 percent of the world's populations currently use herbal medicine for primary health care. Herbal medicines consist of plant or its part to treat injuries, disease or illnesses and are used to prevent, treat and promote health and healing.¹

Herbal formulations have reached widespread acceptability as therapeutic properties like anti-microbial, anti-diabetic, anti-ageing, anti-arthritic, anti-depressant, anti-anxiety, anti-inflammatory activities.²

Novel herbal drug delivery system opens new way for delivery of herbal drugs at right place, at right concentration, for right period of time and also gives scientific evidence to verify the standardization of herbal drug. In the past decades considerable attention has been focused on the development of novel drug delivery systems for herbal drugs. Herbal drugs are becoming more popular in the modern world for their ability to cure various diseases with less toxic effects and better therapeutic effects.³

Allium cepa

Allium cepa L. aggregatum is a perennial plant, often grown as cultivated annual, with linear, hollow leaves and pink or red flowers. This group, commonly known as shallots, has been selected for milder and sweeter flavour. Bulbs usually grown in a cluster and are up to 5cm large. Many named cultivars of shallots are available, varying in colour, shape and size. Shallots are widely cultivated for both culinary and medicinal uses, offering benefits like antioxidant, antimicrobial, and anti-inflammatory properties. *Allium cepa L. aggregatum* is one of the horticultural crops extensively grown and consumed in southern states of India, predominantly in Tamil Nadu. It is an indispensable bulb vegetable due to the presence of essential phytonutrients, which increases strong flavor and taste in food. It has high active nutrients which include proteins, carbohydrates, sugars, vitamins, minerals with certain flavonoids and polyphenols constituents. The phenolic acids reported in shallots are ellagic, caffeic, sinapic and p-coumaric acids. It also has sterols, saponins, and volatile oils.⁴ Moreover the bioactive compounds in shallots have anti-inflammatory, antioxidant, anti-obesity, anti-diabetic, anticancer, anti-allergic, cardiovascular protective, neuroprotective, respiratory protective and bacteriostatic properties.⁵

Encapsulation of volatile oil

Encapsulation of volatile oil is a process that involves enclosing the oil in a protective matrix. The release speeds of volatile essential oils are usually affected by different application environment conditions. Thus released too little and causes ineffectiveness, or released excessively and causes the uncomfortable feelings. Therefore how to control these volatile essential oils to have constant release in various application environment conditions is a good subject quite worth investigation and discussion. Utilizing encapsulation technology to achieve the goal of constant release is one of the most effective methods.⁶

Benefits of encapsulation of volatile oil

Encapsulation is a process of confining active compounds within a matrix in particulate form to achieve one or more desirable effects. Oils are encapsulated for various reasons, such as improved stability, reduced volatility, controlled release, enhanced bioavailability, increased shelf-life, improved safety, enhanced efficacy, for conversion of liquid to solid form to facilitate handling, transportation or incorporation into other components. Other reasons include taste/smell masking, protection from evaporation or oxidation, and controlled-release applications.^{7,8}

Topical drug delivery system

Topical drug delivery systems are gaining increase in popularity and several drugs have been successfully delivered by this route for both local and systemic action. Topical delivery can be defined as the application of a drug-containing formulation to the skin, to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.

Anti-inflammatory activity

Inflammation is a complex process, which is frequently associated with pain and involves occurrence such as: the increase of vascular permeability, increase of protein, denaturation and membrane alteration. Inflammation of tissue is due to response to stress. It is defensive response that is characterized

by redness, pain, heat and swelling and loss of function in the injured area. The most common causes of inflammation are infections, burns and trauma, and it many types of immune reactions.¹⁰

Inflammation is initiated by migration of immune cells from blood vessels and a release of mediators at the site of damage. This process is followed by recruitment of inflammatory cells, release of ROS, RNS, and pro-inflammatory cytokines to eliminate foreign pathogens, and repairing injured tissues.¹¹

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and it is achieved by the increase the movement of plasma and leucocytes (Especially granulocytes) From the blood into the injury tissues. A cascade of biochemical, events, propagates, and matures the inflammatory response, Involving the local vascular system, the Immune system, and various cells within the injured tissue¹². Chronic inflammation is characterized by an overproduction of several inflammatory mediators (e.g., Reactive species and interleukins-IL) That play a central role in numerous diseases.¹³

Protein denaturation is defined as a process where due to external factors, such as heat, a strong acid or strong base; and organic solvent or a concentrated inorganic salt causes the protein to denature that means the proteins tertiary structure and the secondary structure is disoriented¹⁴. Also disruption of the electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure occurs. It is, therefore deduced that, Compounds which are able to prevent these changes and it inhibit thermally or heat induced protein Denaturation, have potential therapeutic value as anti-inflammatory agents.¹⁵

Anti-inflammatory drugs are considered important because of their wide. Therapeutic potential ended their utility in a number of disease such as arthritis, lupus

Erythematous, pemphigus and rheumatic fever and in a number of other disorders Associated with pain, pyrexia and inflammation.¹⁶

However these drugs suffer some disadvantages like gastric irritation and renal dysfunction. Therefore, it is required to develop some naturally originated drugs as anti-inflammatory agents which can overcome adverse effects of classical NSAIDs.¹⁷ Herbal medicine showed safety, efficacy, cultural acceptability and lesser side effects than the synthetic drugs. Different phytoconstituents like alkaloids, flavonoids, xanthone, coumarin, sterols, withaferin-A, andrographolide etc., are also proved effective as anti-inflammatory agent.¹⁸ Hence the natural products today symbolize safer in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. Hence, people are returning to the natural products with the hope of safety and security.¹⁹

Extraction

Extraction involves the separation of medicinally active portions of plant or animal tissues by using selective solvents and standard procedures. The extraction of natural products progresses through the following stages.

- The solvent penetrates into the solid matrix.
- The solute dissolves in the solvents.
- The solute is diffused out of the solid matrix.
- The extracted solutes are collected.²⁰

The extraction methods, include maceration, percolation, super critical fluid extraction (SFC), pressurized liquid extraction (PLE) and microwave assisted extraction (MAE). The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ratio, the extraction temperature, as well as extraction duration will affect the extraction efficiency.²¹

Clevenger apparatus

The clevenger apparatus is a laboratory setup used for the determination of volatile organic compounds in a sample. It is a type of distillation apparatus that is commonly

used in the analysis of essential oils, perfumes, and other volatile organic compounds. The apparatus typically consists of a distillation flask, a condenser, a collection flask, and a heating source.

The distillation flask holds the sample to be analyzed, the condenser cools and condenses the vapor produced during distillation, the collection flask collects the purified sample, and the heating source provides heat for the distillation process. The clevenger apparatus works by heating the sample, causing the volatile organic compounds to vaporize and be carried away by the vapor. The condenser then cools the vapor, causing it to condense into a purified form, which is collected in the collection flask. The clevenger apparatus is a fast and efficient way to determine the composition of volatile organic compounds in a sample and is commonly used in the fragrance and flavor industries.²²

Plant profile²³



Fig no.1 *Allium cepa L aggregatum* bulb and plant

Taxonomical representation

KINGDOM: Plantae

DIVISION: Angiosperms

CLASS: Liliopsida

ORDER: Asparagales

FAMILY: Amaryllidaceae

GENUS: *Allium*

SPECIES: *Allium Cepa*

Distribution: South-East Asia and some African countries

Morphological description

Bulb: Similar to a common onion bulb, but with bladeless "true scales" and a compressed stem axis. Shallot bulbs also contain laterals in the axils of the inner leaves.

Foliage and inflorescence: Usually smaller than those of a bulb onion. **Root morphology:** Similar to a common onion.

Leaves: Uni facial, hollow, and slightly flattened tubular. **Scape:** Hollow.

Flower: Terminal inflorescence.

Flower stalk: Usually only 50 mm in length.

Common names

- Common names: scallion, eschalot, multiplier onion
- Malayalam name: cheriya ulli, chuvanna ulli, kochulli
- Hindi name: gandhana pyaaz, chote pyaaz

Chemical constituents

- **Flavonoids:** Such as apigenin, catechin, kaempferol, quercetin, and tannic acid
- **Glycosides:** Such as quercetin 3,4 -diglucoside, isorhamnetin 3,4 -diglucoside, quercetin 3-glucoside, quercetin 4 -glucoside, and isorhamnetin 4 -glucoside
- **Saponins:** Such as ascalonicoside A1/A2, ascalonicoside B, and furostanol saponins
- **Other compounds:** Such as allicin, ajoene, gallic acid, thiosulphinates, and enzymes
- **Minerals:** Such as calcium, magnesium, potassium, sodium, iron, and zinc
- **Vitamins:** Such as A, B, and C
- **Amino acids:** Several types of amino acids
- **Sugars:** Several types of sugars
- **Phytohormones:** Such as gibberellin and auxin
- **Sulfur components:** Sulfur components

Medicinal uses

- Anti-inflammatory.^{24,25}
- Anti-oxidant.

- Anti-fungal²⁶
- Anti-diabetic.
- Improves blood circulation
- Improves brain health and functioning
- Antibacterial and antiviral.²⁷

Gas chromatography- Mass spectroscopy (GC-MS) Analysis²⁸

Perkin Elmer was used for the GC-MS analysis of chloroform extract. Helium was used, as carrier gas at a constant flow rate of 1 ml per minute to separate the components of a fused silica column packed with Elite - 5 MS (5% by phenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm ID x 250 micrometer df) that was utilized in the Clarus 680 GC analysis. 260⁰ C was the injector temperature set for the chromatographic run. The oven temp for the 1 μ L of the extract sample that was injected into the device was set to 60⁰ C for 2 minutes, 300⁰ C at rate of 10⁰ C per minute, and 300⁰ C for 6 minutes. Conditions for the mass detector were a 240⁰ C transfer line, a 240⁰ C ion source, an ionization mode electron impact at 70 eV, a 0.2 s scan time, and a 0.1 s scan interval. The pieces spanning 40-600 Dalton. The component spectra were compared to the data base of known component spectra kept in the GC-MS NIST (2008) library.

MATERIALS AND METHOD

Plant collection

The bulb of *Allium cepa L aggregatum* was collected from Kasaragod town. The collected plant material was identified and authenticated by Dr. Biju. P, Associate Professor Department of Botany, Government College Kasaragod.

Extraction



Fig no 2: Extraction of volatile oil of *Allium cepa L aggregatum*

The Clevenger apparatus is a laboratory setup used for the determination of volatile organic compounds in a sample. The apparatus typically consists of a distillation flask, a condenser, a collection flask, and a heating source. The distillation flask holds the sample to be analyzed, the condenser cools and condenses the vapor produced during distillation, the collection flask collects the purified sample, and the heating source provides heat for the distillation process. The Clevenger apparatus works by heating the sample, causing the volatile organic compounds to vaporize and be carried away by the vapor.⁵⁰

The condenser then cools the vapor, causing it to condense into a purified form, which is collected in the collection flask. The Clevenger apparatus is a fast and efficient way to determine the composition of volatile organic compounds in a sample and is commonly used in the fragrance and flavor industries.⁵¹

First, the shallots were peeled and chopped into small pieces to increase the surface area and facilitate oil extraction. The chopped shallots were then placed into the RB flask, and enough distilled water was added to cover the onion pieces. The flask was heated gently until the water began to boil, vaporizing the essential oils from the shallots. As the vapor rose, it passed through the condenser, where it cooled and condensed into liquid form. The distillate, consisting of both essential oil and water, was collected in the receiving flask. Once the process was complete, the mixture of oil and water was separated. Finally, the extracted volatile oil of *Allium cepa L aggregatum* was stored in an airtight container, preferably amber-colored glass, to protect it from light degradation.⁵²

Standardization of volatile oil

GC-MS

Perkin Elmer was used for the GC-MS analysis of chloroform extract. Helium was used, as carrier gas at a constant flow rate of 1ml per minute to separate the components of a fused silica column packed with Elite - 5 MS (5% by phenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm ID x 250 micrometer df) that was utilized in the Clarus 680 GC analysis. 260⁰ C was the injector temperature set for the chromatographic run. The oven temperature for the 1µL of the extract sample that was injected into the device was set to 60⁰ C for 2 minutes, 300⁰ C at rate of 10⁰ C per minute, and 300⁰ C for 6 minutes. Conditions for the mass detector were a 240⁰ C transfer line, a 240⁰ C ion source, an ionization mode electron impact at 70 eV, a 0.2 s scan time, and a 0.1s scan interval. The pieces spanning 40-600 Dalton. The component spectrum were compared to the data base of known component spectrum kept in the GC-MS NIST (2008) library.⁵³

Phytochemical screening⁵⁴

- Chemical tests for alkaloids:

A small portion of sample was shaken (acidified) with dilute hydrochloric acid and filtered. The acidified filtrate was tested with the following reagents.

- a. Mayer's test: To two milliliters of the sample, 1 ml of Mayer's reagent (potassium mercuric iodide) was added, shaken and noted for the presence of a creamy precipitate.
 - b. Wagner's test : The sample(two milliliter) was treated with a few ml of Wagner's reagent (solution of Iodine in potassium iodide) and observed for the presence of reddish brown precipitate.
 - c. Hager's Test To two milliliters of the sample, 1 ml of Hager's reagent (saturated picric acid solution) was added and observed for the presence of yellow precipitate.
 - d. Dragendorff's test The sample(two milliliters) was treated with a few ml of Dragendorff's reagent (Potassium bismuth iodide) and observed for the presence of orange red precipitate.
- Chemical tests for glycosides

A small portion of the sample was hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was later subjected to following tests to detect the presence of different glycosides.

- a. Legal's Test: The sample was dissolved in a few milliliters of pyridine. Two milliliters of freshly prepared sodium nitro prusside solution was added to it and then made alkaline with sodium hydroxide solution. It was observed for the formation of pink red colour.
 - b. Baljet's test: To a few ml of the sample, 1ml sodium picrate solution was added, an yellow to orange colour reveals the presence of cardiac glycosides.
 - c. Liebermann-Burchard's Test: Five milliliters of the sample taken in a test-tube and mixed with chloroform (one milliliter) and then it was mixed with two milliliters of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube. It was then observed for the development of a deep red color in the lower portion and green color in the upper portion which changes to blue and violet.
 - d. Borntrager's test: A little of the sample was mixed with water and shaken with equal volume of chloroform. The chloroform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink color was present in the ammoniacal layer.
 - e. Modified Borntrager's test: To the sample: ferric chloride and dilute HCl was added and mixed with chloroform. The chloroform layer was separated and dilute ammonia solution was added and shaken. The ammoniacal layer was observed for pink colour.
- Chemical tests for phenolic compounds and tannins
- a. Ferric chloride test :A small quantity of the sample diluted with water was treated with dilute ferric chloride solution (5%) and observed for the presence of blue colour.
 - b. Gelatin test:The sample was dissolved in water, filtered. To the filtrate, 2% solution of gelatin containing 10% sodium chloride was added. Noted for the presence of milky white precipitate.
 - c. Lead acetate test: The sample dissolved in water was treated with 10% lead acetate solution. Noted for the presence of bulky white precipitate.
 - d. Decolourisation test::The sample dissolved in water was treated with dilute potassium permanganate solution. Noted for the decolourisation of potassium permanganate.

- Chemical tests for flavanones and flavanoids

- aqueous sodium hydroxide test: To a few milliliters of the sample, aqueous sodium hydroxide solution was added. Presence of yellow colouration of the solution was noted.
- Ammonia vapour test: Filter paper was wetted with gel and the filter paper was exposed to ammonia vapour, noted the yellow colour on the filter paper

- Chemical tests for carbohydrates

- Molisch's Test: Two milliliter of sample was treated with a few drops of Molisch's reagent and two milliliter of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of violet ring at the junction of two solutions.
- Fehling's Test: One milliliter of sample was boiled on a water bath for half an hour with 1 milliliter each of Fehling's solution A and B. Observed for the presence of red residue at the bottom of test tube.
- Benedict's Test: To two ml of Benedict's reagent, a few drops of the sample was added. The mixture was heated on a boiling water bath for two minutes. Presence of red precipitate was noted.

- Chemical tests for proteins and amino acids

- Million's Test: To 2 ml of sample, few drops of Million's reagent (1g of mercury + 9ml of fuming nitric acid) was added. Observed for the presence of white precipitate, which on warming gave a red coloured solution.
- Biuret Test: Two ml of the sample was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by excess of potassium hydroxide solution. Observed the presence of violet coloured solution.
- Ninhydrin Test: To few milliliter of the sample two drops of ninhydrin solution was added and heated on a water bath. The presence of violet color was noted.

- Chemical test for terpenoids Salkowski's Test

Few milliliter of sample was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red colour in the chloroform layer and greenish yellow fluorescence in the acid layer.

- Chemical tests for sterols

A little amount of the alcoholic sample was refluxed with solution of alcoholic potassium hydroxide until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated and the residue subjected to Liebermann-Burchard's and Salkowski's tests.

- Liebermann - Burchard's test: The sample was taken with dry chloroform (one milliliter) and then it was mixed with two milliliters of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube and observed for the development of a deep red color in the lower portion and green color in the upper portion which changes to blue and violet
- Salkowski's Test: The sample was dissolved in chloroform and equal volume of concentrated sulphuric acid was added to it. Observed for the red colour at lower layer.

- Chemical tests for saponins

- Foam or Froth Test: A small quantity of sample was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed.
- Hemolysis test: A drop of blood was placed in a slide and mixed with few drops of sample. Hemolysis were observed, if saponin present.

- Chemical tests for gums and mucilages.

To 10 ml sample, 25ml of absolute alcohol was added with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

- Chemical test for sulphur.

- Sodium Nitroprusside Test: Add 1-2 ml of sodium Nitroprusside solution to a test tube. Add 1-2 ml of the test substance to the test tube. - Mix the contents of the test tube using a stirring rod. - Observed for the formation of a purple color.

- b. Silver Nitrate test: Add a few drops of silver nitrate solution to the test substance and observed for yellow or brown precipitate of silver sulfide.
- c. Turbidity Test : Add a few drops of hydrogen peroxide solution to the test substance and observed for cloudy or turbid solution.

Solubility Test

- **Water**

Add 1-2 ml of volatile oil to a test tube. Add 5-10 ml of distilled water to the test tube. Mix the contents of the test tube using a stirring rod and observed

- **Ethanol**

Add 1-2 ml of volatile oil to a test tube. Add 5-10 ml of ethanol to the test tube. Mix the contents of the test tube using a stirring rod and observed.

- **Ether**

Add 1-2 ml of volatile oil to a test tube. Add 5-10 ml of diethyl ether to the test tube. Mix the contents of the test tube using a stirring rod and observed.

Preparation of calcium alginate beads

For bead formation, 50 ml of a 2% w/v aqueous solution of sodium alginate was introduced drop wise from a glass syringe with a size-22 needle into 100 ml of an aqueous calcium chloride solution being stirred at 1000 rpm. The concentration of CaCl_2 in the solution ranged from 3% w/v. In the simultaneous method, the gelation of beads by calcium ions occurred simultaneously with the drug loading into the beads. The stirring was continued for one hour and the calcium alginate beads were harvested by filtration, washed with distilled water, and air dried overnight.⁵⁵

Characterization of beads

Optical microscopy

- **Standardization of eyepiece micrometer**

Standard stage micrometer is used to calibrate the eyepiece micrometer. Stage micrometer is a glass slide which has scale engraved on it. Length of the scale is 1 mm. It has 100 divisions. Each small division is 0.01 mm or 10 μm in length. Eyepiece micrometer is a small disc of glass with a linear scale of 10 mm divided into 100 divisions.⁵⁶

- **Calibration of eyepiece micrometer**

Place the stage micrometer on the stage and focus it under low power first and then under high power. Remove the eyepiece from the microscope and screw the lens. Place the eyepiece micrometer on the ridge inside. Replace the lens and put the eyepiece into the body tube of the microscope. Then superimpose eyepiece micrometer scale over the stage micrometer.

No. of divisions of eyepiece micrometer = 100 Length of stage micrometer = 1 mm No. of divisions of stage micrometer = 100 One division of stage micrometer

Least count (LC) = Length of stage micrometer / No. of divisions of stage micrometer

$$= 1\text{mm}/100 = 0.01\text{mm} = 10\mu\text{m}$$

Note the division of eyepiece micrometer which coincide with a division of the stage micrometer and then record a few (5-6) successive coinciding.

1 eye-piece division = $\frac{\text{No. of stage micrometer divisions}}{\text{No. of eye-piece division}} \times \text{least count}$

No. of eye-piece division

The value obtained has to be multiplied by least count.⁵⁷

- **Determination of particle size**

Transfer a small portion of the given sample on a to a clean slide. Add few drops of liquid paraffin. Disperse the sample uniformly with the help of a brush. The particles should be independent and their distribution should be uniform. Place the cover slip carefully. Avoid entrapment of air bubbles. Drain the excess liquid with a blotting paper. Place the slide on a microscope. Focus on low magnification observes the presence of particles. Shift to higher power and focus the slide. Measure the size of particles in terms of EM divisions. Measure at least 150 particles. Arrange the data in the table. Calculate the average particle size.⁵⁸

Drug Diffusion study

Diffusion studies were performed by diffusion tube using egg membrane. 1 g ointment was applied evenly on the surface of the membrane. The prepared membrane was mounted on the diffusion tube. The whole assembly was maintained at 37°C , and the speed of stirring was kept constant. The 5ml aliquot of drug sample

was taken at time intervals of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 hours was replaced by the fresh solvent. Samples were analyzed by UV spectrophotometer.⁵⁹

Preparation of ointment formulation:

SI NO.	INGREDIENTS	QUANTITY
1	<i>Allium cepa L aggregatum</i> oil encapsulated beads	2g
2	Wool fat	0.6g
3	Hard paraffin	0.6g
4	Cetosteryl alcohol	0.6g
5	Yellow soft paraffin	8.2g

Table no.1 Formula of ointment

Wool fat 0.6 g, hard paraffin 0.6g, cetostearyl alcohol 0.6 g, white soft paraffin 8.2g was weighed in beaker and kept in a water bath and stirred continuously with glass rod until melted. Prepared calcium alginate beads containing volatile oil of *Allium cepa L aggregatum* were incorporated into ointment base and transferred into ointment container and capped.⁶⁰

Evaluation of ointment:**1. Homogeneity**

All developed ointment were tested for homogeneity by visual inspection after the ointment have been set in the container. They were tested by their appearance and presence of any aggregates.⁶¹

2. Measurement of pH

The pH of the ointment were measured with the help of digital pH meter. 0.5 g of ointment was dissolved in 25 ml of distilled water die was kept for two hrs. Then the pH of each formulation was determined.⁶²

3. Rheological study

The viscosities of prepared ointments were carried out by using Brookfield viscometer (spindle type T-95). 50 g of ointment was filled in a 100 ml beaker and viscosity was measured at 2.5, 4, 5 and 10 rpm. The readings of each formulation were taken.⁶³

4. Spreadability

Spreadability was determined by the multimer apparatus which consists of a wooden block, which was provided by a pulley at one end. Spreadability was measured on the basis of slip and drag characteristics of ointment. An excess of ointment under study was placed on this ground slide. The ointment was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. 500g weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the ointment between the slides. Excess of the ointment was scrapped off from the edges. The top plate was then subjected to pull by 60 gms weight. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.⁶⁴

Spreadability was calculated using the following formula:

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

Where, S = Spreadability M = Weight tied to the upper plate

5. Extrudability

In the present study, extrudability was determined by measuring the weigh required to extrude at least 0.5cm ointment from collapsible tube in 10 secs. The extrudability was then calculated by using the following equation:⁶⁵

$$\text{Extrudability} = \frac{\text{Applied weight to extrude ointment from tube (in gram)}}{\text{Area (in cm}^2\text{)}}$$

In-vitro anti inflammatory activity**1.1.1 Inhibition of Protein denaturation**

Protein denaturation method The reaction mixture (0.5ml) consists of 0.45 ml bovine serum albumin and 0.05ml of plant extracts of (50, 100, 150, 200 µg/ml

concentrations. The pH was adjusted to 6.3 using 1N HCl. The samples were incubated at 37 °C for 20 min. and then heated at 57 °C for 3min. Diclofenac ointment used as standard drug (50, 100, 150, 200 µg/ml) After cooling the samples 2.5ml phosphate buffer saline (pH 6.3) was added to each test tube. Absorbance was measured spectrophotometrically at 660nm.⁶⁶

The percentage inhibition of protein denaturation was calculated as follows;

$$\text{Percentage inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where; A₀ = Absorbance of the control A₁ = Absorbance of the sample

RESULT AND DISCUSSION

Plant collection and authentication

The bulb of *Allium cepa* L *aggregatum* (Amaryllidaceae) was collected from Kasaragod town, the plant material was taxonomically identified by the botanist Dr. Biju P, Dept of Botany, Govt College, Kasaragod.

Gas chromatography mass spectroscopy(GC-MS) analysis

After conducting a GC-MS study on volatile oil of *Allium cepa* L *aggregatum*, 20 components were found. To determine the chemical components contained in the volatile oil, the spectrum were compared to spectra found in the NIST (2008) spectral database. The identification was further aided by the peak area percentage, molecular weight and retention time. The component details are listed below

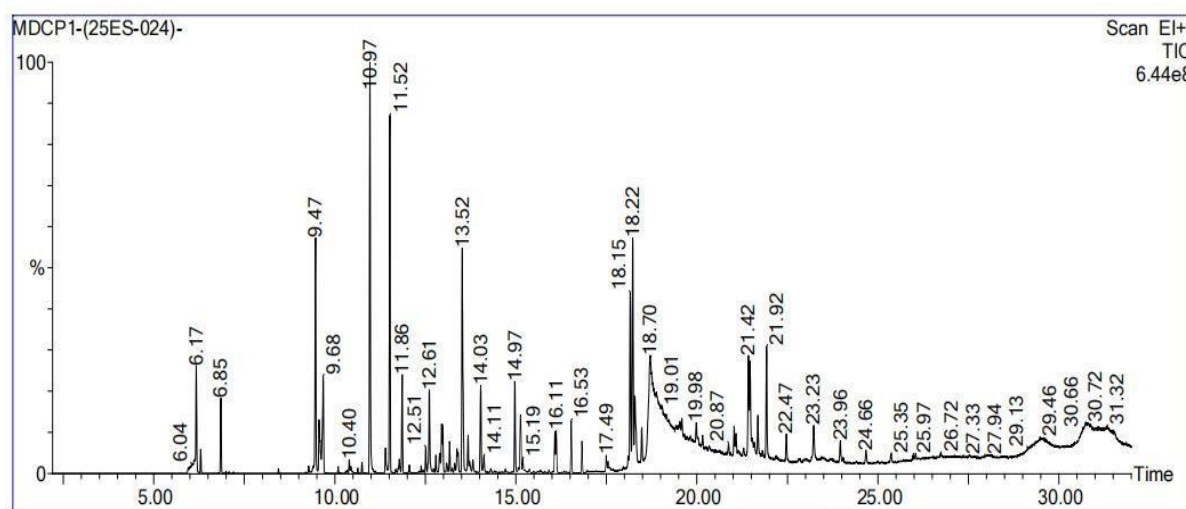


Fig.No.3 GC-MS report showing the presence of compound in chloroform extract

SL NO	RETENTION TIME	NAME OF THE MOLECULE	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE PEAK AREA	BIOLOGICAL ACTIVITY EXPECTED
1	6.170	disulfide, dipropyl	C ₅ H ₇ ON	97	3.120	Anti-inflammatory, anti-microbial, anti-oxidant

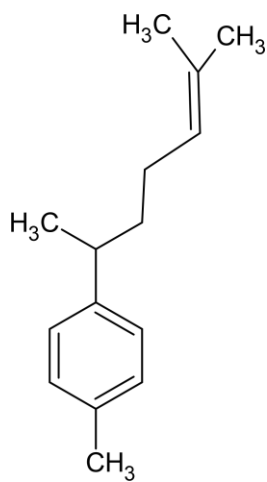
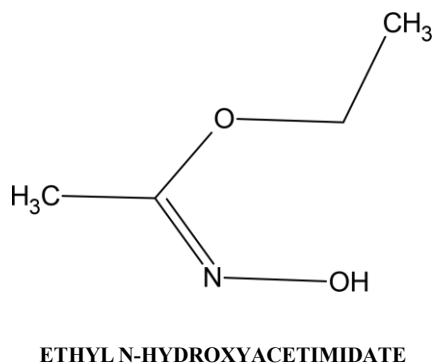
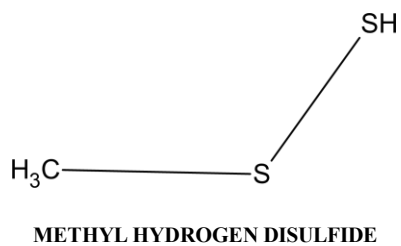
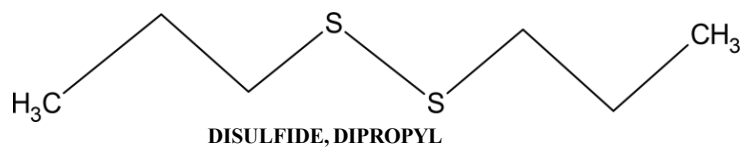
2	6.850	methyl hydrogen disulfide	C ₁₅ H ₂₂	202	1.654	Anti-inflammatory, anti-oxidant, anti-microbial
3	9.466	ethyl n-hydroxyacetimidate	C ₈ H ₁₂	108	6.005	Anti-inflammatory, anti-fungal
4	9.676	1,2,4-trithiolane,3,5-diethyl-allyl n-propyl sulphide	C ₈ H ₁₄ O ₄ N ₂	202	2.851	Anti-inflammatory, anti-microbial, anti-oxidant
5	10.967	2h-pyrrol-2-one, 1,5-dihydro-1-	C ₇ H ₁₂ N ₈	207	10.200	Anti-inflammatory,

		methyl				anti-microbial,anti-fungal
6	11.517	benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	C11H18O	166	8.065	Anti-inflammatory, anti-microbial
7	11.857	3-cyclopentyl-1-propyne	C10H18O	154	2.296	Anti-inflammatory, anti-oxidant,anti-microbial
8	12.608	diisopropyl azodicarboxylate	C6H12S	116	2.387	Anti-inflammatory, anti-oxidant,anti-microbial
9	13.518	5,5'-propylenebis[(1h-1,2,4-triazol-3-yl)amine]	C18H34 O	266	6.919	Anti-inflammatory, anti-oxidant,anti-microbial

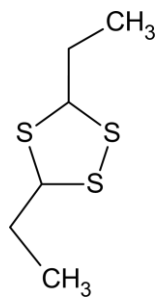
10	14.028	cyclopropanecarboxal dehyde, 2-methyl-2-(4-methyl-3-pentenyl)	C19H36 O2	296	2.468	Anti-inflammatory, anti-microbial,anti-oxidant
11	14.969	1,3-nonadien-6-ol, 8-methyl	C6H12S	268	2.317	Anti-inflammatory, anti-oxidant,skin and wound healing
12	15.124	1-propene, 3-[(1-methylethyl)thio	C6H12S	268	1.913	Anti-inflammatory, anti-microbial,anti-oxidant
13	18.155	9,12-octadecadien-1-ol, (z,z	C14H24 O	208	4.279	Anti-inflammatory, anti-microbial,anti-oxidant
14	18.225	6-octadecenoic acid, methyl ester	C18H34 O	266	6.166	Anti-inflammatory, anti-microbial,anti-oxidant
15	18.285	e-2-octadecadecen-1-ol	C10H21 Br	220	2.304	Anti-inflammatory, anti-microbial,anesthetic
16	18.705	e-2-octadecadecen-1-ol	C20H36 O4	340	27.391	Anti-inflammatory, anti-bacterial,anti-oxidant
17	21.421	13-tetradecene-11-yn-1-	C6H14S2	150	2.424	Anti-inflammatory,

		ol				anti-microbial,anti-oxidant
18	21466	9-octadecenal	CH4S2	80	2.342	Anti-inflammatory, anti-bacterial
19	21.921	1-bromo-3,7-dimethyloctane	C4H9O2 N	103	3.217	Anti-inflammatory, neuro protective activity
20	23.227	oxalic acid, allyl pentadecyl ester	C6H12S3	180	1.684	Anti-inflammatory, anti-microbial

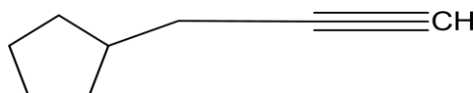
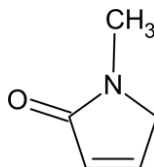
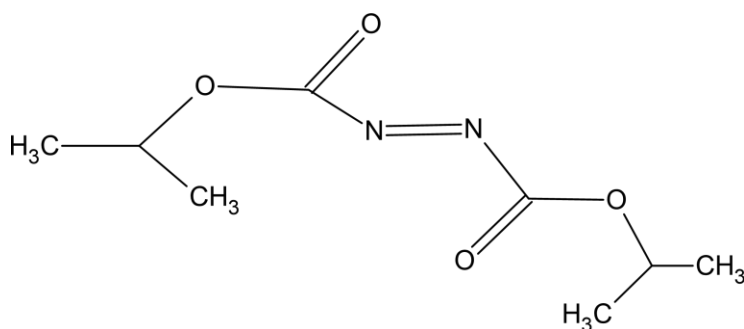
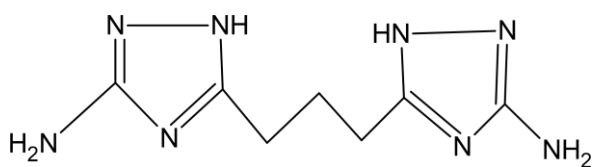
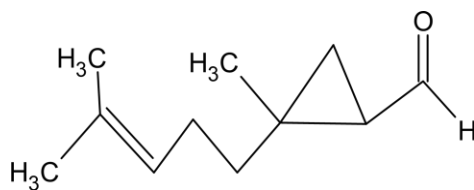
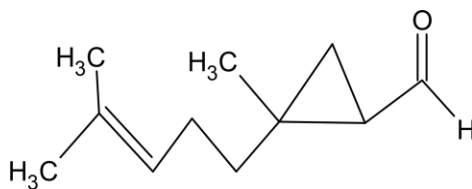
Table No.2 Components identified in volatile oil of *Allium cepa L aggregatum* using GC-MS

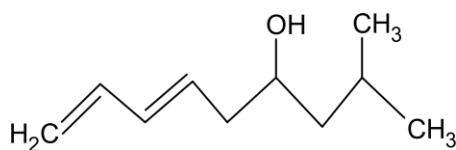
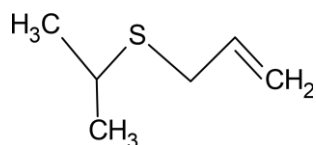
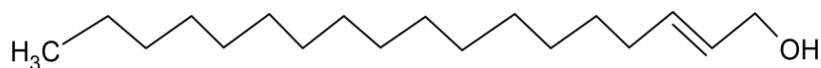
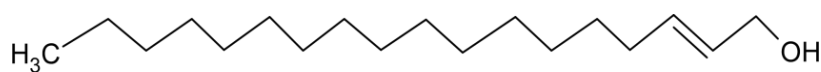
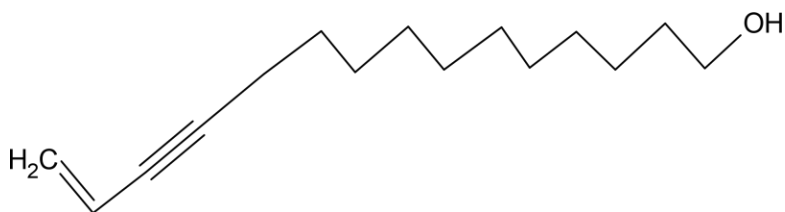
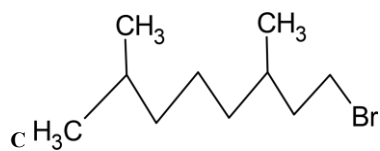


1,2,4-TRITHIOLANE,3,5-DIETHYL-ALLYL N-PROPYL SULPHIDE



2H-PYRROL-2-ONE, 1,5-DIHYDRO-1-METHYL

**BENZENE, 1-(1,5-DIMETHYL-4-HEXENYL)-4-METHYL****3-CYCLOPENTYL-1-PROPYNE****DIISOPROPYL AZODICARBOXYLATE****5,5'-PROPYLENEBIS[(1H-1,2,4-TRIAZOL-3-YL)AMINE]****CYCLOPROPANECARBOXALDEHYDE, 2-METHYL-2-(4-METHYL-3-PENTENYL)****1,3-NONADIEN-6-OL, 8-METHYL**

**1-PROPENE, 3-[(1-METHYLETHYL)THIO]****9,12-OCTADECADIEN-1-OL, (Z,Z)****6-OCTADECENOIC ACID, METHYL ESTER****E-2-OCTADECADECEN-1-OL****E-2-OCTADECADECEN-1-OL****13-TETRADECE-11-YN-1-OL****13-TETRADECE-11-YN-1-OL****1-BROMO-3,7-DIMETHYLOCTANE**



OXALIC ACID, ALLYL PENTADECYL ESTER

20 compounds were identified from GC-MS. Among the listed compounds, E-2-octadecadecen-1-ol has the most potential anti-inflammatory activity along with anti-bacterial and anti-oxidant activity. E-2-octadecenedecen-1-ol, in particular, seems to be a compound of interest, given its broad spectrum of biological activities. All other compounds obtained also shows anti-inflammatory activity.

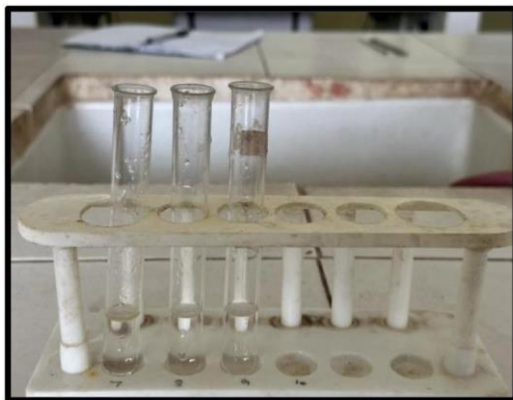
Phytochemical screening

SI NO.	CHEMICAL TEST	RESULT
1	ALKALOIDS	
	Mayer's test	-
	Wagner's test	-
	Hager's	-
	Dragondroff's test	-
2	GLYCOSIDES	
	Legal test	-
	Baljet test	-
	Borntrager's test	-
	Modified borntrager's test	-
3	PHENOLIC COMPOUNDS	
	Ferric chloride test	+
	Gelatin test	+
	Lead acetate test	+
4	FLAVONES AND FLAVANOIDS	
	Aq. Sodium hydroxide text	+
	Ammonia vapour test	+
5	CARBOHYDRATE	
	Molisch test	-
	Fehling's test	-
	Benedict's test	-
6	PROTEIN AND AMINO ACIDS	
	Million's test	-
	Biuret test	-

	Ninhydrin test	-
7	TERPINOIDS	
	Salkowski test	-
8	STEROLS	
	Liebermann- buchard test	-
	Salkowski test	-
9	SAPONINS	
	Foam or froth test	-
	Hemolysis test	-
10	GUMS AND MUCILAGE	-
	SULPHUR	
	Sodium nitroprusside test	+
	Silver nitrate test	+
	Turbidity test	+

Table.3 Phytochemical screening**Solubility test**

SI NO.	SOLVENT	RESULT
1	water	Insoluble
2	Alcohol (ethanol)	Soluble
3	Ether	Soluble

Table no.4 Test for solubility**Fig no.4 Solubility test****Fig no.5 Test for sulphur****Fig no.6 Test for flavanoids****Fig no.7 Test for phenols**

Formulation of ointment

Wool fat 0.6 g, hard paraffin 0.6g, cetostearyl alcohol 0.6 g, white soft paraffin 8.2g was weighed in beaker and kept in a water bath and stirred continuously with glass rod until melted. Prepared calcium alginate beads containing volatile oil of *Allium cepa L aggregatum* were incorporated into ointment base and transferred into ointment container and capped.

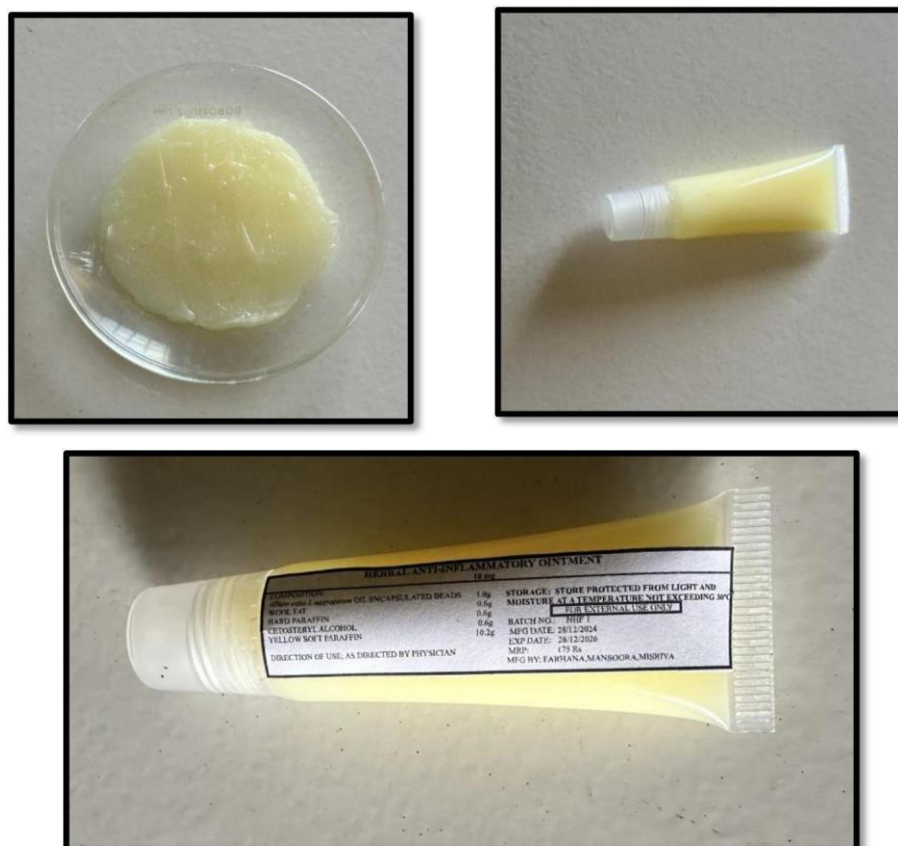


Fig no.8 Formulation of ointment

Evaluation of ointment

SL NO.	PARAMETER	
1.	homogeneity	good
2.	pH	6.21
3.	Rheological study	3.9cP
4.	Spreadability	3.8gcm/sec
5.	extrudability	9.3cm/sec

Table no.5 Results showing evaluation of ointment



Fig no.9 pH meter



Fig no.10 Extrudability



Fig no.11 Viscometer



Fig no.12 Spreadability

Optical microscopy

Calibration of eye piece micrometer

SI NO.	NO. OF DIVISION OF EYE PIECE MICROMETER	NO. OF DIVISIONS OF STAGE MICROMETER
1.	10	4
2.	15	2
3.	23	7
4.	40	12
5.	17	18

Table no.6 Result showing calibration of eye piece micrometer

Calibration factor = $\frac{\text{No of divisions of stage micrometer}}{\text{least count No. Division of eye piece micrometer}}$

$$= \frac{2}{15} \times 10 = 1.3$$

Determination of particle size

SL NO	DIAMETER (MICROMETER)	CALLIBRATION FACTOR	ACTUAL DIAMETER
1	2		2.6
2	3		3.9
3	2		2.6
4	2		2.6
5	3		3.9
6	3		3.9

7	2	1.3	2.6
8	2		2.6
9	2		2.6
10	3		3.9

Table no.7 Result showing particle size

Determination of average particle size

CALCIUM ALGINATE BEADS	MAXIMUM	MINIMUM	AVERAGE
diameter	3.9micrometer	2.6 micrometer	3.25 micrometer

Table no.8 Result showing average particle size

Invitro drug release study**Diffusion study**

TIME (HR)	DRUG RELEASE
0	0
1	13.86
2	28.54
3	40.13
4	62.05
5	70.3

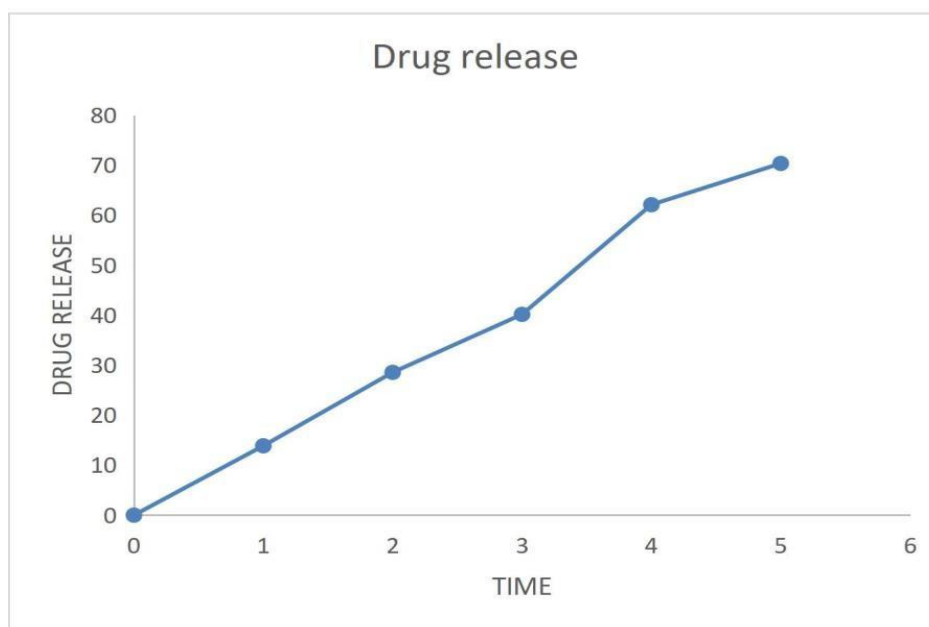
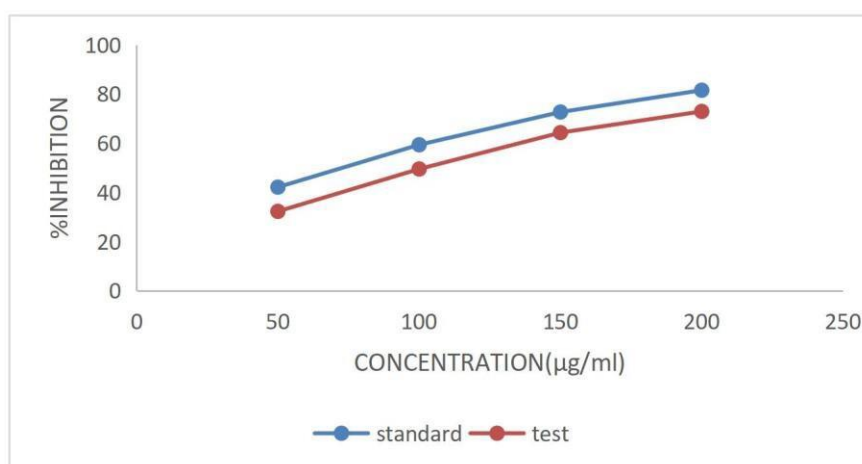
Table no.9 Result showing *invitro* drug release study

Fig no.13 Time v/s drug release graph

Protein denaturation 5.8.1Extract

SI no.	SAMPLE	CONCENTRATION	ABSORBANCE (660 nm)	PERCENTAGE INHIBITION
1.	control	-	0.812	-
2.	test	50	0.550	32.26
		100	0.410	49.50
		150	0.290	64.28
		200	0.220	72.90
3.	Standard	50	0.470	42.11
		100	0.330	59.35
		150	0.222	72.66
		200	0.150	81.52

Table no.10 Results showing percentage inhibition of protein denaturation of extraction**Fig no.14 Percentage inhibition v/s concentration graph****5.8.2 Beads**

SI NO.	SAMPLE	CONCENTRATION	ABSORBANCE	PERCENTAGE INHIBITION
1	Control	-	0.812	-
2	Standard	50	0.480	40.88
		100	0.342	57.88
		150	0.236	70.93
		200	0.150	81.52
3	Test 1	50	0.578	28.81
		100	0.435	46.42
		150	0.318	60.83
		200	0.258	68.96
4	Test 2	50	0.565	30.41
		100	0.423	47.90
		150	0.306	62.31
		200	0.241	70.32

5	Test 3	50	0.553	31.89
		100	0.413	49.13
		150	0.292	64.03
		200	0.229	71.79

Table no.11 Result showing percentage inhibition of protein denaturation of calcium alginate beads

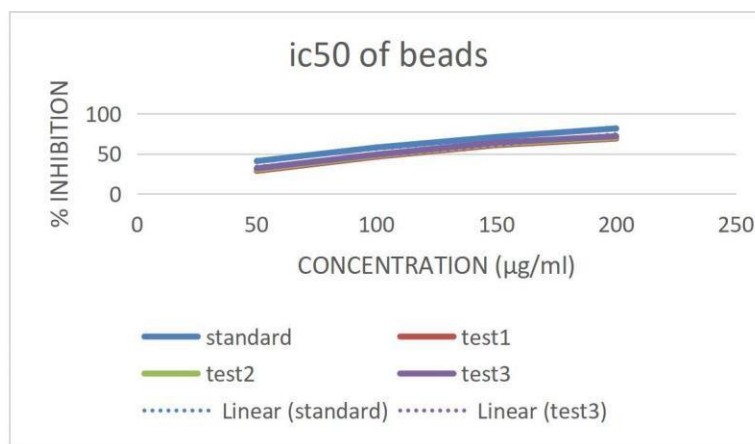


Fig no.15 Percentage v/s concentration graph

5.8.3 Formulation

SL.NO	SAMPLE	CONCENTRATION	ABSORBANCE (660 nm)	PERCENTAGE INHIBITION
1	Control	-	0.812	-
2	Test 1	50	0.556	31.52
		100	0.413	49.13
		150	0.295	63.66
		200	0.224	72.41
3	Test 2	50	0.553	31.89
		100	0.416	48.76
		150	0.293	63.91
		200	0.226	72.16
4	Test 3	50	0.540	33.49
		100	0.405	50.12
		150	0.283	65.14
		200	0.214	73.64
5	Standard	50	0.476	41.37

		100	0.335	58.74
		150	0.227	72.04
		200	0.154	81.03

Table no.12 Result showing percentage inhibition of protein denaturation of formulation

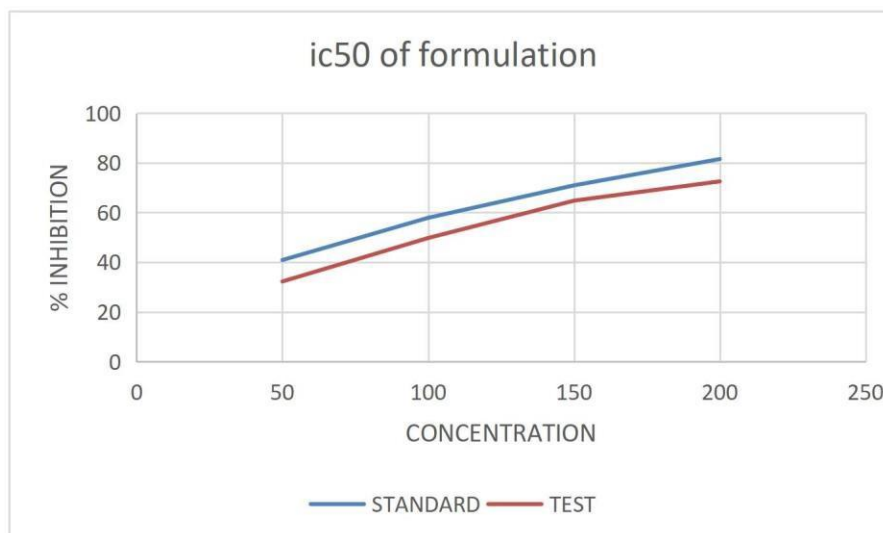


Fig no.16 Percentage inhibition v/s concentration graph



Fig no.17 Protein denaturation

SUMMARY AND CONCLUSION

Allium cepa L aggregatum is a herb belonging to the family Amaryllidaceae it was collected from Kasaragod town Scientific investigation reported its Anti-inflammatory, Anti-oxidant, Antibacterial, antiviral and anti-diabetic activities.

Extraction of volatile oil from *Allium cepa L aggregatum* was carried out by hydrodistillation using cleverger apparatus. Encapsulation of volatile oil of *Allium cepa L aggregatum* was done through calcium alginate beads and it was incorporated into prepared ointment base.

The volatile oil was subjected to GC-MS and presence of allyl sulphur compounds were found which indicates presence of anti-inflammatory activity. The physio chemical parameters such as homogeneity, pH, viscosity, spreadability and extrudability were carried out. *In-vitro* drug release study such as dissolution and diffusion study were carried out.

Thereafter, the preparation was also evaluated for *in-vitro* anti-inflammatory activity by protein denaturation method. On observation and analysis of obtained result, the formulation was found to possess anti-inflammatory activity.

As per our studies and obtained results, the volatile oil of *Allium cepa L aggregatum* can be successfully incorporated into calcium alginate beads and it is formulated into anti-inflammatory ointment which possesses significant anti-inflammatory properties.

REFERENCE

1. Manisha Yadav, Vidhi J, Bhatia, Gaurav Doshi. Novel techniques in herbal drug delivery system, International Journal of Pharmaceutical Science.2014; 1(6): 70-83.
2. Jamadar M.J, Shaikh R.H. Preparation and evaluation of herbal gel formulation. Journal of Pharmaceutical Research and Education, 2017; 1(2):201-204.
3. Anju Dhiman, Arun Nanda, Sayeed Ahmed. Novel herbal drug delivery system, International Conference on Environmental Chemistry and Biology. 2012;49(34):171-172
4. Asili A, Behravan J, Naghavi RM, Asili J (2010) Genetic diversity of Persian shallot (*Allium hirtifolium*) ecotypes based on morphological traits allacin content and RAPD markers. Open Access J Med Arom Plants 1:1–6
5. Hajian N, Rezayatmand Z, Shahanipur K (2018). Preventive effects of *Allium hirtifolium* Boiss methanolic and aqueous extracts on renal injury induced by lead in rats. Journal of Herbmmed Pharmacology 7(3):155-159.
6. C.P. Chang. Preparation of alginate complex capsules containing eucalyptus essential oil and its controlled release 2003;1(2)132-135
7. Almeida, A. P., Rodríguez-Rojo, S., Serra, A. T., Vila-Real, H., Simplicio, A. L., Delgadillo, I., Beirão Da Costa, S., Beirão Da Costa, L., Nogueira, I. D., & Duarte, C. M. M. (2013). Microencapsulation of oregano essential oil in starch- based materials using supercritical fluid technology. *Innovative Food Science and Emerging Technologies*, 20, 140–145.
8. Asbahani, A. E., Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., Mousadik, A. E., Hartmann, D., Jilale, A., Renaud, F. N. R., Elaissari, A., & Lyon, C. B. (2015). Essential oils : From extraction to encapsulation. *International Journal of Pharmaceutics*, 483(1–2),
9. Joshi M, Butola BS, Saha K. advances in topical drug delivery system: micro to nanofibrous Joshi M, Butola BS, Saha K. advances in topical drug delivery system: micro to nanofibrous particles.
10. Mahendra AG, Rasika DB. Formulation and evaluation of topical anti-inflammatory herbal gel. Asian J Pharm Clin Res. 2019;12:252-5.
11. Gyawali R, Ghimire A, Khatiwada A, Niraula P, Sharma U, Thapa R. Anti-inflammatory, Anxiolytic and Antioxidant Property of *Lactuca sativa* L and Formulation of Microspheres Loaded Sustained Release Anti-inflammatory Gel. Journal of Nepal Chemical Society. 2020 Jul 29;41(1):8-15.
12. Leelaprakash G, Dass SM. Invitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International journal of drug development and research. 2011 Jul;3(3):189-96.
13. Vieira SF, Ferreira H, Neves NM. Antioxidant and anti-inflammatory activities of cytocompatible *Salvia officinalis* extracts: A comparison between traditional and Soxhlet extraction. Antioxidants. 2020 Nov;9(11):1157.
14. Dharmadeva S, Galgamuwa LS, Prasadine C, Kumarasinghe N. In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayu. 2018 Oct;39(4):239.
15. Bailey-Shaw YA, Williams LA, Green CE, Rodney S, Smith AM. In-vitro evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. International Journal of Pharmaceutical Sciences Review and Research. 2017;47(1):145-53.
16. Gomase PV, Shire PS, Nazim S, Choudhari AB. Development and evaluation of polyherbal formulation for anti-inflammatory activity. J Nat Prod Plant Resource. 2011;1:85-90.
17. Singh H, Sarkar BK, Arya JC, Pal S, Kumar R, Gupta V, Singh R, Verma SC. Phytochemical and Anti-Inflammatory Evaluation of Herbal Gel Prepared from Bark Extract of *Mesua Ferrea* Linn. Journal of Drug Delivery and Therapeutics. 2019 Oct 15;9(5-s):53-6.
18. Sen S, Chakraborty R, De B, Ganesh T, Raghavendra HG, Debnath S. Analgesic and anti-inflammatory herbs: a potential source of modern medicine. International Journal of Pharmaceutical Sciences and Research. 2010 Nov 1;1(11):32.
19. Banerjee S, Chanda A, Adhikari A, Das AK, Biswas S. Evaluation of phytochemical screening and anti-inflammatory activity of leaves and stem of *Mikania scandens* (L.)
20. Elyemni M., Louaste B., Nechad I., Elkamli T., Bouia A., Taleb M., and Eloutassi N., Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: hydrodistillation and microwave assisted hydrodistillation, *Science World Journal*. 2019;(2):22-30
21. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine. 2018; 13(1):1-26.
22. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal Aromatic Plants. 2015; 4(196):2167- 2188
23. F.R.A. Basundari, E. Sulistyaningsih, R.H. Murti, T.R. Nuringtyas Metabolite profile of two *Allium cepa* L. Aggregatum group cultivars.2021;2(1):214-222
24. Tariq HA, Kandil O, Elkadi A, Carter J. Garlic revisited: therapeutic for the major diseases of our times? J Natl Med Assoc 1988; 80:439-445.

25. Appel E, Vallon-Eberhard A, Rabinkov A, Brenner O, Shin I, Sasson K, et al. Therapy of murine pulmonary aspergillosis with antibody-alliinase conjugates and alliin. *Antimicrob Agents Chemother* 2010; 54:898-906.
26. Rahman K, Lowe GM. Garlic and cardiovascular disease: a critical review. *J Nutr* 2006; 136:736S-740S.
27. Ban JO, Lee DH, Kim EJ, Kang JW, Kim MS, Cho MC, et al. Anti-obesity effects of a sulfur compound thiocremonone mediated via down-regulation of serum triglyceride and glucose levels and lipid accumulation in the liver of db/db mice. *Phytother Res* 2012; 10:3729.
28. Karasek FW, Clement RE. *Basic gas chromatography-mass spectrometry: principles and techniques*. Elsevier, 2012 Dec 2
29. J. Saravanan, W.R. Sheriff, N. Joshi. R. Varatharajan, V.G. Joshi, A.A. Karigar, *Research Journal of Pharmacognosy and Phytochemistry*, 2010, 2(1), 15-17
30. Nair, A.; Mallya, R.; Suvarna, V.; Khan, T.A.; Momin, M.; Omri, A. Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics* 2022, 11, 108
31. Azzazy, H. M. E., Fahmy, S. A., Mahdy, N. K., Meselhy, M. R., & Bakowsky, U. 2021. Chitosan-Coated PLGA Nanoparticles Loaded with Peganum harmala Alkaloids with Promising Antibacterial and Wound Healing Activities. *Nanomaterials*, 11(9)
32. Mansour A, Montazeri E, Mashhadizadeh M, Sheikh A. Characterization of shallot, *Pakistan J Med Sci.* (2009) 25:948-52
33. Hasmila, I., Natsir, H., & Soekarno, N. H. (2019). Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.). In *Journal of Physics: Conference Series* (Vol. 1341, No. 3, p. 032027)
34. Maroon JC, Bost JW, Maroon A. Natural anti-inflammatory agents for pain relief *Surg Neurol Int.* 2010;1:80
35. Hong X, Wei L, Ma L, Chen Y, Liu Z, Yuan W. Novel preparation method for sustained-release PLGA microspheres using water-in-oil-in-hydrophilic-oil-in-water emulsion. *Int J Nanomedicine* 2013;8:2433-41
36. Sami, R.; Elhakem, A.; Alharbi, M.; Benajiba, N.; Almatrafi, M.; Helal, M. Nutritional Values of Onion Bulbs with some Essential Structural Parameters for Packaging Process. *Appl. Sci.* 2021, 11, 2317
37. HR Mohammadi-Motlagh, A Mostafaie and K Mansouri. Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract. *Arch. Med. Sci. AMS* 2011;7, 38-44.
38. Kakadia, P.G.; Conway, B.R. Solid Lipid Nanoparticles: A Potential Approach for Dermal Drug Delivery. *Am. J. Pharmacol. Sci.* 2014, 2, 1-7. [Google Scholar] [Cross Ref] [Green Version]
39. F.J. Barba, Processing, bioaccessibility and bioavailability of bioactive sulfur compounds: Facts and gaps *Journal of Food Composition and Analysis* (2017)
40. Gilmore MA. Phases of wound healing. *Dimens Oncol Nurs*, 1991; 5: 32-4.
41. Harsh Mohan. 5th ed. Vol. 6. New Delhi: Jaypee Brothers Medical Publishers(P) Ltd; 2005. Inflammation and healing, *Textbook of pathology*; pp. 133-134.
42. Ansel's "Pharmaceutical Dosage Form & Drug Delivery System" Indian edition, 1981; 277-293,371
43. Nair, A.; Mallya, R.; Suvarna, V.; Khan, T.A.; Momin, M.; Omri, A. Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics* 2022, 11, 108
44. Chiang, E.P.I.; Chiu, S.C.; Pai, M.H.; Wang, Y.C.; Wang, F.Y.; Kuo, Y.H.; Tang, F.Y. Organosulfur garlic compounds induce neovasclogenesis in human endothelial progenitor cells through a modulation of microRNA 221 and the P13-K/Akt signaling pathways. *J. Agric. Food Chem.* 2013, 61, 4839-4849
45. Goh, C. H., Heng, P. W. S., & Chan, L. W. (2012). Alginates as a useful natural polymer for microencapsulation and therapeutic applications. *Carbohydrate Polymers*, 88, 1-12
46. Nakkala, J. R., Li, Z., Ahmad, W., Wang, K., & Gao, C. (2021). Immunomodulatory biomaterials and their application in therapies for chronic inflammation-related diseases. *Acta Biomaterialia*, 123, 1-30
47. H. Fessi et al. Nanocapsule formation by interfacial polymer deposition following solvent displacement *Int J Phar* (1989)
48. Roshan KB, Nikitha I, Sharma S, Nishikant D, Rishu T, Nanosuspension: A Review, Research and Reviews: *Journal of pharmaceutics and nanotechnology*, 2016.
49. In-vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method *W. Windig, J. Guilment, *Anal. Chem.* 63 (1991) 1425
50. Alara O. R., Abdurahman N. H., Ukaegbu C. I., and Kabbashi N. A., Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave assisted extraction techniques, *Journal of Taibah University for Science.* (2019) 13, no. 1, 414-422)
51. Elyemni M., Louaste B., Nechad I., Elkamli T., Bouia A., Taleb M., and Eloutassi N., Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: hydrodistillation and microwave assisted hydrodistillation, *Science World Journal.* (2019)
52. . Windig, J. Guilment, *Anal. Chem.* 63 (1991) 1425
53. F.Cuesta Sanchez, M.S. Khots, D.L. Massart, J.O. De Beer, *Anal. Chim. Acta* 285 (1994) 181.
54. Njoku OV, Obi C. Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry.* 2009; 3(11):228-233.
55. E.S.Chan et al, Prediction models for shape and size of calcium-alginate macrobeads produced through extrusion technique, *Journal of Colloid and Interface Science* (2009)
56. *Martin's Physical Pharmacy and Pharmaceutical Sciences*; Patrick J Sinko, 5th edition, 533-542
57. *Text Book of Physical Pharmaceutics*; C VS Subrahmanyam, 180-201
58. J.B. Coty et al. Characterization of nanomedicines: a reflection on a field under construction needed for clinical translation success *J. Control. Release* (2018)

-
59. A. Olejnik et al. Active compounds release from semisolid dosage forms J. Pharm. Sci. (2012)
 60. Dr Ak Seth. Systematic approach to practical pharmaceutics semisolid dosage forms 2010; 263.
 61. Jamadar M.J, Shaikh R.H, Preparation and evaluation of herbal gel formulation Journal of Pharmaceutical Research and Education 2017;1(2):201-204
 62. Jyothi D, Koland M. Formulation and evaluation of herbal anti-inflammatory gel containing *Trigonella foenum*, *greacum* seed extract. International Journal of Pharmaceutical Science. 2016;8(1):41-44
 63. Pattanayak S, Nayack SS, Dinda SC, Panda D, Naval KP. Evaluation of herbal ointments formulated with methanolic extract of *Cajanus scarabaeoides*. J Pharm Allied Health Sci 2011;2:47-59
 64. Multimer M. Spreadability determination by an apparatus. J Am Pharm Assoc 1956;45:212-4
 65. Swarna SK, Nivedhitha MS, Gayathri R, Selvaraj J. evaluation of anti-inflammatory ointment of ethanolic extract of leaf, bark and flower of *Tecoma stans* with ibuprofen in vitro analysis. Pharmacognosy Journal. 2019;11(5):109-115
 66. Leelaprakash G, Das S.M. In vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Science. 2011;3(3):189-199 International Journal of Pharmace