Formulation and Evaluation of Liposomal encapsulated Pedalitin Cream Cookies from *Pedalium Murex*

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ABSTRACT

Pedalium murex Linn (family: *Pedaliaceae*) (P. murex) commonly known as Large Caltrops and Gokhru (India) is a shrub found in the Southern part, Deccan region of India and in some parts of Ceylon. Different parts of the plant are used to treat various ailments like, cough, cold and as an antiseptic. In this work, the methanol extract of *Pedalium murex* stem was collected and silver nanoparticles were synthesized by green way synthesis method. The synthesized particles were characterized by UV-Visible Spectrophotometer. The peak was formed between 420 – 440 nm. The extract were analysed by HPLC and FTIR. The crude extract of *Pedalium murex Linn* contains ketones, Bromine and alkaloid functional groups. The HPLC analysis (254 nm) of crude extract for Flavonoids finds the 8 compounds. The peak 9.387 was confirms the presence of pedalitin. Commonly worldwide 95% of people are taking cookies as a snack. In this work the cookies were formulated with various concentrations by using Wheat flour, Horse gram flour, Sorghum flour and Flax seed flour and it gets optimized. The drug is encapsulated by Liposomal Encapsulation process. The cream is formulated by Soy Lecithin, sunflower oil and then our compound pedalitin is added. It gets incorporated with the Cookie. It has quantitatively analysed. The 9 point hedonic test has done.

KEYWORDS: *Pedalium Murex*, Pedalitin, Stem extract, Green synthesis, Cookie, Soy Lecithin, Quantitative analysis, Sensory Properties.

1. INTRODUCTION:

1. PLANT STUDY

Gallstones, which are small formations that develop within the human gallbladder, represent one of the most agonizing biliary tract conditions in adults, showing a notably high incidence in India.

The formation of stones within the kidneys, a painful nephrological disorder, is prevalent worldwide.

Kidney stones are solid formations, composed of aggregated crystals of dissolved minerals in urine. They can range in size from a few millimetres to several centimetres **[1].**

Often, they exit the body through the urine stream, and many are formed and expelled without causing noticeable symptoms **[2].** However, when stones exceed 3 millimetres in diameter prior to passing, they can lead to urethral obstruction. This obstruction induces dilation and stretching of the upper ureter and renal pelvis, along with spasms in the associated muscle.

Kidney stones are hard deposits of mineral and acid salts tion that stick together in concentration urine. They can be pain full when passing through the urinary tract, but usually don't cause permanent damage. Among different types of kidney stones, struvite stone is the second major type of stone is referred as an infection stone which is composed of magnesium Hydrogen phosphate tetra hydrate.

Kidney stones form in your kidneys. As stones move into your ureter-the thin tubes that allow urine to pass from your kidneys to your bladder-signs and symptoms can result. Signs and symptoms of kidney stones can include serve pain, nausea, vomiting, fever, chills and blood in your urine.

Instead of using allopathic medicine, researchers isolate the active components from medicinal plants for treating various diseases.

Based on traditional healers, the plant *Pedalium Murex* is used to dissolute and prevent the kidney stone formation. Not only for kidney stone, it is also used to treat ailments like inconvenience of urine, gonorrhoea, promote lochial discharge. The plant has an excellent cure in patients with Leucorrhoea in women. The stem part of this plant is used for treating spermatorrhoea, dysuria, ardour urinate and gonorrhoea **[3].**

Plant Name

ENGLISH NAME : Large Caltrops

TAMIL NAME : Yaanai Nerinji

BOTANICAL NAME: *Pedalium Murex*

FIG.1 *PEDALIUM MUREX*

Taxonomical Classification

Kingdom: Plantae, Plant

Phylum: Mangnoliopsida

Class: Mangnoliopsida

Subclass: Lamiidae

Order: Caryophyllales

Family: Pedaliaceae

Genus: Pedalium

Species: *P.murex L* **[4].**

1.1. Botanical Description

P. murex L. is a succulent herb found along the coastal regions of South India and certain tropical areas in the country. It typically emerges between July and September, flourishing in fertile soils and crop fields as a weed, thriving in temperatures ranging from 25 to 30 degrees Celsius. This creeper attains a length of 2 to 3 feet, adorned with an irregularly shaped array of branches. Its leaves grow in pairs of 5 to 8, while the small, yellow flowers bloom in early winters, followed by the formation of round fruits containing 5 to 12 compartments, each housing a seed rich in aromatic oil.

The roots, which exude a sweet aroma, measure about 4 to 5 inches in length and are brown in colour **[5].**

1.2. Chemical constituents

The fruit contains 3.5%–5% alkaloids, stable oil, aromatic oil, resins, glycosides, carbohydrates, saponins, and triterpenoids. The stem is rich in saponins, Herman, phytosterols, tannins, and carbohydrates. In the roots, you can find reducing sugars, phenolic compounds, saponins, xanthoproteins, alkaloids, triterpenoids, and flavonoids. Meanwhile, the leaves contain flavonoids, alkaloids, steroids, resins, saponins, and proteins.

1.3. Phytochemical studies

The preliminary chemical analysis of *P. murex* revealed a diverse array of natural compounds throughout the plant. The entire plant is rich in medicinally significant constituents. The fruits, in particular, contain alkaloids (3.5%–5%), stable oil, aromatic oil, resins, carbohydrates, saponins, glycosides, and triterpenoids, along with two important flavonoids: 2′, 4′, 5′-trihydroxy-5,

7dimethoxy flavones and triacontanyl dotriacontanoate. The leaves were found to contain notable flavonoids such as dinatin and its 7-glucuronide, diosmetin and its 7-glucuronide, pedaltin, and pedalin. Alkaloids, steroids, resins, saponins, and proteins were also identified. The root, on the other hand, contained novel phenolic compounds like phenol, 2-(5,6-dimethyl pyrazinyl) methyl. Stem components included saponins, phytosterols, tannins, and carbohydrates. Additionally, the flower was a source of quercetin, dinatin, querimctrin, and an unidentified di-glycoside of quercetin.

1.4. Pharmacological Studies

1.4.1. *Antiulcerogenic activity:*

The aqueous extract of *P. murex* leaves, administered at a dose of 200mg/kg, p.o. in a single schedule and 100mg/kg, p.o. for 15 to 30 days **[6].** It is demonstrated significant efficacy in treating gastric ulceration induced by ethanol, as assessed by parameters including total acid, acid duration, total protein, ulcer index, and glutathione levels. Famotidine was used as a reference product. This highlights the potential of *P. murex* leaf extract as an effective and relatively less toxic antiulcerogenic agent, likely attributed to its flavonoid and mucilage content **[7].**

1.4.2 *Antidermatophytic*

In India, dermatophyte infections are prevalent due to the warm, humid climate and subpar hygiene conditions. The primary causative agents, Trichophyton and Microsporum species, contribute to the widespread occurrence of dermatophytosis. In this study, various solvent extracts of *Pedalium murex L.* were assessed for their effectiveness against two dermatophyte: Trichophyton rubrum and Microsporum gypseum. Notably, the methanol and petroleum ether extracts of *Pedalium murex* exhibited a prominent inhibitory effect against Microsporum gypseum at a concentration of 200µg/ml. Additionally, the distilled water and methanol extracts demonstrated

significant activity against Trichophyton rubrum. Positive control drugs including griseofulvin, fluconazole, and ketoconazole yielded zones of inhibition ranging from 10 to 20mm. This underscores the potential antidermatophytic properties of *Pedalium murex* extracts.

1.4.3.*Anti inflammatory*

The ethanolic extract of *P.murex* fruit is concluded to have anti-inflammatory and analgesic effect **[8].** The anti-inflammatory activity of the *P.murex* plant was proved by the experiment conducted in Lambda-carrageenan induced paw oedema in Wistar albino rats at a dose of 200 and 400 mg/kg. This concluded that the given dose gives comparatively positive control **[9].**

1.4.4. *Anti-microbial activity*

Benzene, butanol and dimethylformamideextracts of the leaves of P.murex were tested against three Aspergillus species by disc diffusion assay .*P.murex* plant extract has reported to have anti-fungal activity. Based on all the observations, it was concluded that the P.murex plant can be used as the pesticide to combat blast disease of rice **[10].**

1.4.5. *Nephroprotective activity*

When compared to standard drug cystone against the renal damages caused by cisplatin and cadmium chloride in Wistar rats **[11].** In the research, the aqueous extract of fruit was reported to e against cadmium chloride induced renal damage with changes in bloods urea nitrogen, glutathione, catalase in kidney **[12].**

1.4.6. Hypolipidemic activity

The extract of *P.murex* is reported to be the excellent hypolipidemic agent .It was proved by the studied carried out on the high-fat die fed rats at dose of 200 and 400 mg/kg/P.O **[13].**

1.4.7. Antithelmintic activity

The roots of the plant were subjected to extraction using petroleum ether and ethanol. The anthelmintic activity of these extracts was evaluated using the

Indian earthworm (Pheretima posthuma) as a test organism, with albendazole and piperazine citrate as reference standards. All the extracts exhibited noteworthy anthelmintic activity. This leads to the conclusion that *Pedalium murex* roots possess significant anthelmintic properties.

1.4.8. Antipyretic activity

The antipyretic activity of *Pedalium murex* was examined using Brewer's yeast-induced pyrexia models in rats, utilizing both aqueous and ethanolic extracts. Pyrexia was induced in rats using Brewer's yeast (15%). Both extracts, at doses of 200 and 400mg/kg body weight administered orally, demonstrated a significant dose-dependent reduction in temperature elevation compared to the standard drug Paracetamol (150mg/kg body weight). The aqueous extract notably ($P<0.001$) mitigated yeast-induced pyrexia in rats at a dose of 200mg/kg body weight. These findings affirm that leaf extracts of *Pedalium murex* possess robust antipyretic properties, providing pharmacological support for its traditional use in fever management**.**

1.4.9. Anti-oxidant

Antioxidants in *Pedalium murex* play a crucial role in preventing diseases by scavenging free radicals. The ethyl acetate fraction is rich in phenolic antioxidants, exhibiting potent free radical scavenging activity. The aqueous extract shows efficient free radical scavenging, possibly due to flavonoids. In a rat study, methanol extract *of P. murex* fruits demonstrated effectiveness in regulating oxidative stress and restoring antioxidant enzyme activity in CCl4-intoxicated liver, suggesting potential therapeutic value. Further research on active compounds is warranted**.**

1.5. **Pedalitin**

Pedalitin is a tetra mono hydroxyl flavone with four hydroxyl group. It is isolated from a number of plant species including *Eremosparton Songurium*. A natural product found in *Teucrium Hircanicum, Pedalium Murex*. It is a fatty acid hydroxyl group and acetate extract that has potent antitumor activity**.**

It is traditionally used in various cultures for its potential diuretic and stone dissolving properties. While research is ongoing, some studies suggest it may help prevent the formation of certain type of kidney stone by inhibiting crystal growth and promoting the expulsion however, its crucial to consult a healthcare professional before using pedalitin, as it may interact with medications or have contraindications for specific medical conditions it should be seen as a complementary approach rather than a sole treatment for kidney stones.

1.5.1. IUPAC Name

2-(3, 4dihydroxyphenyl)-5, 6-dihydroxy-7-methoxychromen-4-one.

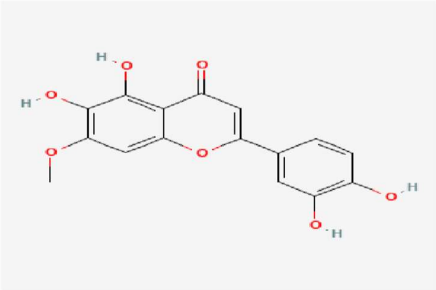

FIG.2 PEDALITIN

1.6. COOKIE

Cookies are a type of confectionery product typically dried to a low moisture content. They are popularly consumed as a snack food worldwide, with significant consumption in developing countries. Bakery products, including cookies, can be easily enriched and fortified to cater to the nutritional requirements of specific target groups, particularly those who are undernourished. Furthermore, these products can be tailored to address the therapeutic needs of consumers **[14].**

Cookies are having wider consumption base, relatively long shelf-life, more convenience and good palatability which make it attractive for protein fortification and other nutritional improvements.

1.7. ENCPSULATION FOOD

Encapsulation is an extensively used technique for designing engineered products in various food industries especially in functional and specialties food manufacturing, food processing and product innovation. It involves entrapping a functionally active core material into a matrix of an inert material **[15].**

The encapsulation process finds diverse applications within the food industry. It serves as a valuable technique for enhancing the delivery of bioactive molecules (such as antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, and lycopene) and living cells (like probiotics) into foods. Typically, encapsulation involves enveloping, covering, and safeguarding bioactive components with a physical barrier, ensuring no protrusion. Moreover, it is described as a technology for packaging solids, liquids, or gases within small capsules, enabling controlled release over extended periods and specific conditions. The resulting particles typically range in diameter from a few nanometers to a few millimeters. Originally, encapsulation found its roots in biotechnology to enhance production processes **[16].**

1.8. LIPOSOMAL ENCAPSULATION

Extensive research has explored liposomal applications in dairy products, focusing on factors such as the stability of food components against degradation and the improved delivery of antimicrobial peptides **[17].**

In cheese-making, the use of liposome-encapsulated enzymes has been shown to reduce cheese ripening time by up to 50%. This innovation not only accelerates the ripening process but also enhances economic profitability for producers by significantly shortening the ripening time compared to conventional methods, which often require more than a year to achieve optimal acceptability **[18].**

An encapsulation system is an excellent choice for protecting bioactive compounds and an additive in food applications. It facilitates site specific

controlled release of hydrophobic or lipophilic and hydrophilic or lipophobic molecules to a food preparation system **[31].**

1.9. FLAX SEEDS

Flaxseed (Linum usitatissimmum) is also known as linseed and these terms are used interchangeably **[19].**

Flaxseed is renowned for its chemical compounds that exhibit specific biological activities and functional properties, including solubility, thermal stability, emulsifying abilities, and electrostatic charge density, as well as water-holding and fat-absorption capacities. It is rich in polyunsaturated fatty acids (PUFAs) of the omega-3 family, soluble dietary fibers, lignans, proteins, and carbohydrates. Analysis of flaxseed typically reveals a composition of 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash, the latter being the mineral-rich residue remaining after burning samples **[20].**

1.9.1. Nutrient composition

Flaxseed proteins are relatively high in arginine, aspartic acid and glutamic acid, while lysine is limiting **[21].**

Flaxseed proteins exhibit antifungal properties against Alternaria solani, Candida albicans and Aspergillus flavus**[22].**

1.10. HORSE GRAM

Horse gram is an annual, photosensitive, slender, twining herb with cylindrical tomentose stems **[23].**

1.10.1. Nutrient Composition

Horse gram is recognized as an economical source of vegetable protein, with its protein content ranging between 18.5 to 28.5%. It is rich in essential amino acids like arginine, histidine, lysine, valine, leucine, etc., though it has a primary deficiency in sulfur-containing amino acids such as methionine and tryptophan.

Notably, horse gram has a higher lysine content compared to pigeon pea and chickpea, making it an excellent supplement to cereal-based diets. It is also high

in non-digestible carbohydrates, which can slow the release of glucose into the bloodstream, offering potential benefits for the dietary management of diabetes. This resistant starch acts as a Probiotic, marking a new generation of dietary fibres **[32].**

1.10.2. Medical Application

The substantial dietary fiber content in horse gram supports healthy intestine and colon physiology and aids in regulating the digestive system, eliminating worm infections, and preventing acidity and flatulence. Furthermore, the horse gram plant possesses astringent, diuretic, and antioxidant properties **[29].**

It is particularly beneficial in addressing menstrual issues, leucorrhea, and bleeding during pregnancy due to its high iron content. Horse gram seed extract contains anti-calcifying agents that inhibit crystallization, are water-soluble, polar, heat-stable, non-tannin, and non-protein, making it effective in treating kidney stones.

Given its wide range of medicinal applications, including treatments for heart disease, asthma, bronchitis, urinary discharges, and kidney stones, horse gram is celebrated for its health benefits, with various parts of the plant utilized for medicinal purposes.

Horse gram has high nutritional value comparable to other commonly grown pulses. As a nutrient-dense legume, it provides food and nutritional security to many low-income communities of developing countries **[32].**

1.11. SORGHUM SEED

Sorghum is well-known for its high agronomic productivity, or ability to grow in a variety of environments. It has the ability to grow at high elevations from the soil surface, salinity and barren soils, as well as in environments that contain rare water and high temperatures **[24].**

1.11.1. Fibers

Sorghum has high fiber content. It is mostly made up of insoluble (75%–90%) and soluble fibers (10%–25%), which are present on cell walls in the pericarp and endosperm and have an amount of about 6–15 g per 100 g of grain **[25].**

Non-starch sorghum carbohydrates consist mainly of β-glucan and arabinoxylans. Arabinoxylans are mainly glucuronoarabinoxylans and contain important sorghum phenolic acids, and bound p-coumaric and ferulic acids **[26].**

The lipids consist mainly of unsaturated fatty acids in sorghum grains, the most readily available being polyunsaturated fatty acids. Oleic, linoleic, palmitic, linolenic, and stearic acids are the principal fatty acids in sorghum; the lipid profile of maize is similar but is more unsaturated **[27].**

1.12. SOY LECITHIN

Lecithin commonly encompasses a variety of triglycerides, phospholipids such as phosphatidylinositol, phosphatidylcholine, and phosphatidyl ethanol amine, and glycolipids. However, in biochemistry, the term specifically denotes pure phosphatidylcholine phospholipids extracted from the phosphate portion of vegetables like soybeans, sunflowers, rice beans, and rape seeds. It can also be obtained from animal sources, including egg yolks, marine organisms, and milk. For liposomal formulations, lecithin from non-soybean sources poses challenges due to the high levels of polyunsaturated fatty acids in bovine or egg-derived lipids, leading to stability issues. Hence, lecithin from these sources is generally less stable than that from soybeans and other plants, which have lower polyunsaturated fatty acid content **[33].**

Research has explored the application of liposomal formulations derived from soy lecithin for delivering drugs to the skin. The studies indicate that carrier systems based on soy lecithin liposomes are particularly effective for

transporting herbal medications, such as Methanolic Neem Extract (MeNE), which is obtained from the leaves of the Azadirachta indica plant **[33].**

2. Methodology

2.1. Raw Material

The plant Pedalium Murex is chosen as raw material and it is collected from local area (seeragapadi).

2.2. Collection of plants

The whole plant of *Pedalium Murex* was collected from local area (chinna seeragapadi- Salem). Then all the parts were separated and washed thoroughly to get rid of microbes.

2.3. Conversion of fine powder

Then let it dry under shade until it gets dried. After it gets dried, the stem part is collected and it is grinded thorough mixer grinder. Converted it into fine powder and stored in the glass container.

2.4. Preparation of the extract

The powdered particles are taken around 100g and the Methanol was taken as solvent for extraction and extracted through Soxhlet extraction apparatus.

2.5. FT-IR Analysis

Commonly Potassium bromide (KBr) is the alkali halide used in the pellets. First, mix approximately 0.1 to1.0 %of the sample thoroughly with 200 to 250mg of fine alkali halide powder. Subsequently, finely pulverize the mixture and place it into a pellet-forming die. Apply a force of about 8 tons under a vacuum of several mm Hg for several minutes to form transparent pellets. To ensure optimal results, degassing is essential to remove any air and moisture from the KBr powder. It's crucial to maintain a sufficient vacuum level, as inadequate vacuum may lead to pellets that are prone to breakage and scatter light. Prior to pellet formation, pulverize the KBr powder to a maximum of 200 mesh and then dry it at approximately 110 °C for two to three hours. It's important to note that rapid heating may lead to oxidation of some KBr powder to KBrO3, resulting in a brown discoloration. After the powder has been dried, store it in a desiccator for preservation.

During measurements, the background can be determined by using an empty pellet holder placed in the sample chamber. Alternatively, conducting background measurements on a pellet holder containing solely a KBr pellet (without any sample) serves to account for losses due to infrared light scattering within the pellet and for any moisture adsorbed on the KBr.

2.5. HPLC Analysis

The methanolic extract undergone HPLC analysis using a chromatographic system. The separation occurred on an SGE protecol column at ambient temperatrure. The mobile phase comprised methanol to water(70:30v/v) with isocratic elution at a flow rate of 1ml/min. Sample run for 15mins and detection was at 254nm by the UV detector. Chromatographic data were recorded and processed with autochro software**.**

2.6. ENCAPSULATION

Nano-liposomes were prepared following the modified method by Rasti et al (2012). In summary, the liposomal formulation's ingredients (lecithin, sunflower oil) were mixed in a heating bath at 30°C until lecithin completely dissolved in the oil. Subsequently, pedalitin extract was added drop wise into the lecithin-oil mixture while stirring at 1000 rpm on a hotplate. The solution was then hydrated by adding deionized water and glycerol (final concentration 2%, v/v, preheated to 30°C) and homogenized for 10 min using a rotor-stator homogenizer. The liposomal dispersions underwent sonication (7 min) at 25°C using a probe sonicator with a nominal frequency of 20 kHz at 80% of full power before annealing. The final nano-liposomes were stored at 25°C under nitrogen for at least 1 hour after preparation to anneal and stabilize them.

2.7. RAW MATERIAL COLLECTION

The main ingredients such as Wheat, Horse Gram, Flax Seed, and Sorgham Seed are collected and converted into flour and followed by various concentrations the cookie has made. The ingredients are mixed together with butter, powdered sugar, Egg and Vanilla essence **[28].**

2.8. COOKIE FORMULATION

The cookies are made by the following concentration respectively.

28.1. CONTROL SAMPLE

Around 150g of wheat flour has taken 83g of melted butter and 2 drop of vanilla essence, 25ml of Egg and 63g of powdered sugar has weighed and mixed together in a plate. The dough was prepared and kept in the freezer for stiffness for one hour. The settled dough was taken and made in shape. The dough was kept in oven at 140C. After few minutes the cookie was prepared **[28].**

2.8.2. SAMPLE 1

Around 75g of wheat flour, 32.5g of Horse gram and Sorgham seed flour and 10g of Flax seed flour has taken 83g of melted butter and 2 drop of vanilla essence, 25ml of Egg and 63g of powdered sugar has weighed and mixed together in a plate. The dough was prepared and kept in the freezer for stiffness for one hour. The settled dough was taken and made in shape. The dough was kept in oven at 140C. After few minutes the cookie was prepared **[28].**

2.8.3. SAMPLE 2

Around 37g of wheat flour, 51.5g of Horse gram and Swargam Seed Flour and 10g of Flax Seed flour has taken 83g of melted butter and 2 drop of vanilla essence, 25ml of Egg and 63g of powdered sugar has weighed and mixed together in a plate. The dough was prepared and kept in the freezer for stiffness for one hour. The settled dough was taken and made in shape. The dough was kept in oven at 140C. After few minutes the cookie was prepared **[28].**

2.8.4. SAMPLE 3

Around 113g of wheat flour, 13.5g of Horse gram flour and Swargam Seed flour and 10g of Flax Seed flour has taken 83g of melted butter and 2 drop of vanilla essence, 25ml of Egg and 63g of powdered sugar has weighed and mixed together in a plate. The dough was prepared and kept in the freezer for stiffness for one hour. The settled dough was taken and made in shape. The

dough was kept in oven at 140C. After few minutes the cookie was prepared **[28].**

2.9. QUANTITATIVE ANALYSIS

The quantitative analysis is done for both cookies and cream.

2.9.1. +DETERMINATION OF CARBOHYDRATES

10 Test tubes were taken and 5 are labeled for standard, 1 for reference and 4 for sample. 1ml of distilled water and 3ml of H_2SO_4 were added and labeled reference.

In T1, T2, T3, T4, T5 add 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml of sucrose is added respectively and add 3ml of H_2SO_4. It serves as standard. For sample 5 test tubes were taken and label them as control, S1, S2, S3 and Cream. In each test add 1ml of sample respectively and 3ml of H_2SO_4. They can be measured with the help of a UV-Spectrophotometer and measure absorbance with 330 nm and plot the standard graph. Estimate the number of carbohydrates present in the sample from the standard graph.

2.9.2. DETERMINATION OF PROTEINS

The protein has been determined by Lowry's method. 10 Test tubes were taken and 5 are labeled for standard, 1 for reference and 4 for sample. Reagent A, B, C, D has prepared. BSA is taken for protein sample. Add 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml of working standard solution in to the series of labeled test tubes and make it up all into 1ml by adding distilled water. It serves as Standard. For reference add 1ml of distilled water. For sample 5 test tubes were taken and labeled as Control, S1, S2, S3 and Cream.In each test tube add 1ml of sample respectively. Then add 5ml of reagent C to all the test tubes including reference. Mix it and let it stand for 10 minutes in room temperature.Then add 0.5ml of reagent D rapidly and mix well and let it stand for 10 minutes in room temperature. They can be measured with the help of a UV-Spectrophotometer and measure absorbance with 660nm and plot the standard graph. Estimate the number of proteins present in the sample from the standard graph.

2.9.3. DETERMINATION OF LIPIDS

The lipids have been determined by ZAK'S method. 10 test tubes were taken and 5 are labeled for standard, 1 for reference and 4 for sample. The required reagents were prepared. Add 0.1ml of lipid in 5 standard test tubes, 0.1ml of distilled water in reference and 0.1ml of sample respectively. Then add 4ml of ferric chloride precipitating agent, mix well using a glass rod and centrifuge for 15mins. From this take 05ml filtrate is taken and add 2.5ml of ferric chloride diluting agent and 4ml of concentrated sulphuric acid with through mixing. They can be measured with the help of a UV-Spectrophotometer and measure absorbance with 560nm and plot the standard graph. Estimate the number of lipids present in the sample from the standard graph.

3. RESULTS AND DISCUSSION

3.1. Plant Extraction

The stem of *Pedalium* Murex is dried and converted into fine powder. Methanol is used to extract through soxhlet extractor.

3.2. FT-IR Spectroscopy

The isolated compounds were analysed by the IR spectral studies by KBr pellet method. Information on molecular vibration, or more specifically, the transitions between vibrational and rotational energy levels in molecules, is provided via infrared spectroscopy. Bond deformations, either stretching or bending, are excited when radiation in the infrared spectrum is absorbed; different stretching and bending vibrations happen at certain quantized frequencies. The molecule absorbs energy and increases the amplitude of its oscillation when that frequency of infrared light strikes it. When the frequency of the absorbed infrared radiation matches the frequency of molecular vibration, the infrared spectrum is produced. The atom's reduced mass and force constant determine the vibrational frequency. The mesmeric effect, inductive effect, field effect, steric effect, and hydrogen bonding are the factors that determine how little changes in molecule structure affect vibrational frequencies. The broad peak at 3444 cm^{-1} is due to -OH is stretching frequency. The Carbonyl (>C=O) stretching vibration is observed at 1743 cm^{-1}. The peak at 2925 cm^{-1} is due to aliphatic >C-H stretching frequency.

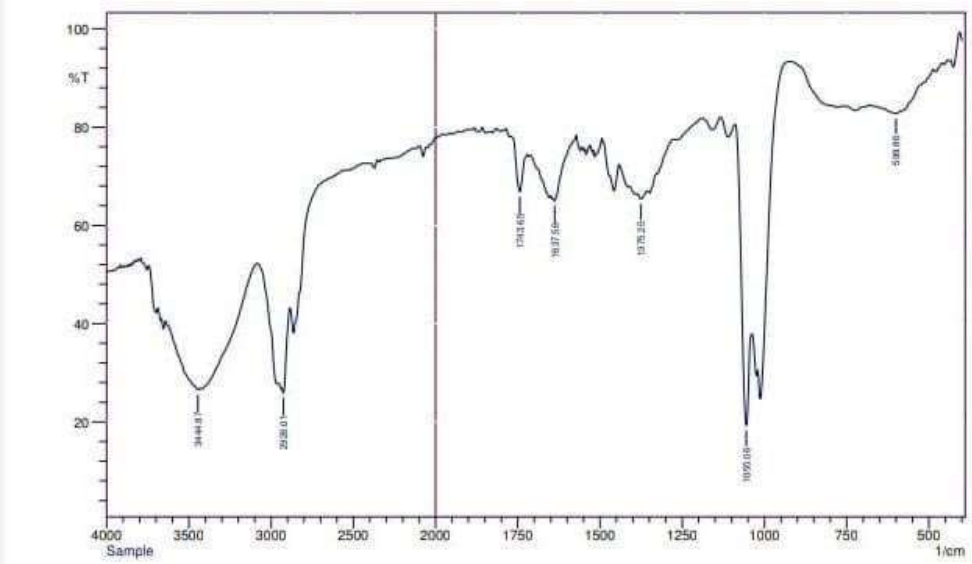

FIG.3 FTIR-ANALYSIS

Table.1 FTIR ANALYSIS

Sl.no	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr. Area
1	599.86	82.797	0.205	605.65	578.64	2.179	0.014
2	1055.06	19.327	32.813	1089.78	1037.7	20.575	7.167
3	1375.25	65.401	1.268	1386.82	1357.89	5.223	0.139
4	1637.56	65.037	1.344	1643.35	1571.99	10.13	-0.259
5	1743.65	66.868	9.232	1772.58	1720.5	7.256	1.133
6	2926.01	25.96	4.55	2937.59	2885.51	25.412	1.062
7	3444.87	26.604	0.986	3641.6	3433.29	103.597	3.172

Table.2 FTIR PEAK WITH RESPECTIVE FUNCTIONAL GROUPS

S. No	Peak	Compound
1	3444.87	N-H stretch
2	2926.01	C-H
3	1743.65	C=O
4	1637.50	Diketones
5	1375.25	C-O
6	1055.06	Polysaccharide
7	599.86	C-Br

3.3. HPLC

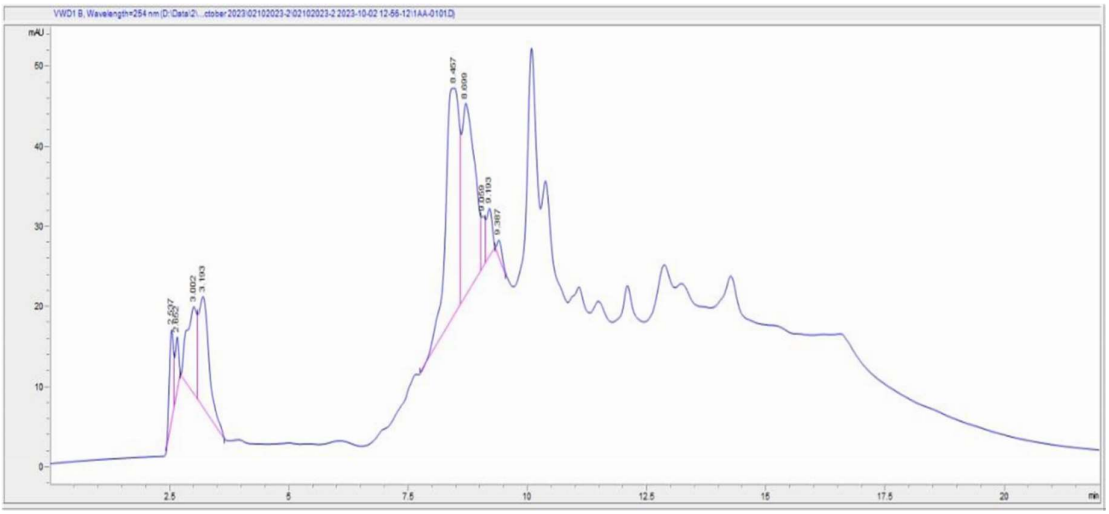

FIG.4 HPLC ANALYSIS AT-254nm

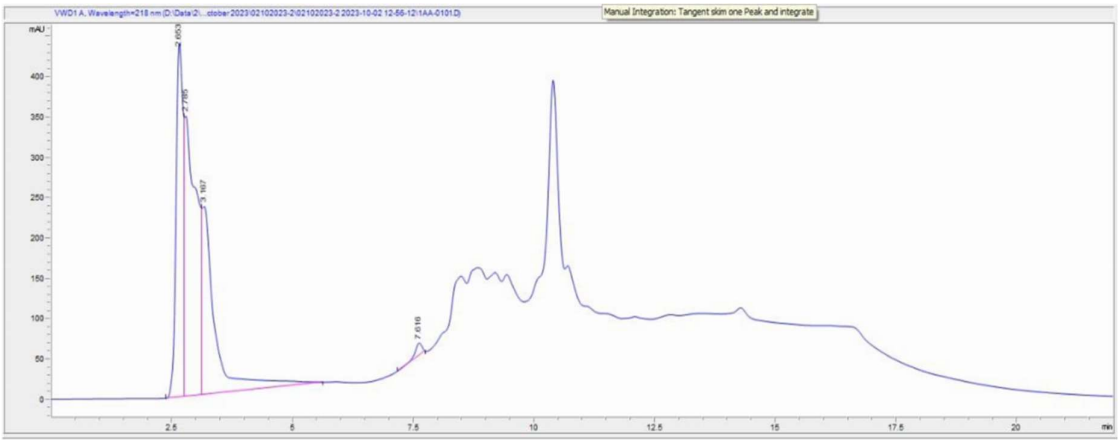

FIG.5 HPLC ANALYSIS AT -218nm

The peak 9-10 confirms the presence of the pedalitin compound. The peak 9.387 present in the graphical representation shows the presence of Flavonoid (pedalitin compound).

3.4. Encapsulation

The encapsulation process finds diverse applications within the food industry. It serves as a valuable technique for enhancing the delivery of bioactive molecules into food. An encapsulation system is an excellent choice for protecting bioactive compounds and an additive in food applications. Even the liposome-encapsulated enzymes helps to reduce cheese ripening and the liposomal encapsulation plays a vital role in food products. Commonly lecithin is used in liposomal encapsulation. Lecithin from non-soybean sources is

generally less stable than that from soybeans and other plants, which have lower polyunsaturated fatty acid content. Thus Liposomal Encapsulation with Soy Lecithin and Sunflower Oil has prepared successfully.

Fig.6. Encapsulation

3.5. COOKIE PREPARATION

The cookies in various concentrations were prepared. Wheat, flax seeds, Sorghum Seeds, Horse gram flour were used as the raw materials to bake the cookie. For taste and texture small amount of butter, sugar free powder was added. By taking all the ingredients in various concentrations the cookie were baked. Based on the aroma, texture, taste and few parameters the cookie was analyzed. The sample 3 has the best aroma, texture, taste, flavor, color than other samples. So we conclude that the sample 3(fig 3) is the best sample.

Fig.7. Control Sample

Fig.8. Sample 1

Fig.9. Sample 2

Fig.10. Sample

3.6. Quantitative Analysis

3.6.1. Determination of Carbohydrates

Using the UV visible -Spectrum technique, the carbohydrates in the cookie sample and cream were examined. The encapsulated cream, Samples A, B, and C, and the control sample are all analyzed. According to the data,

Sample C has 2.3µg/ml of carbohydrates, while the encapsulated cream has 1.53µg. Sample B and Sample A have reasonable amounts of carbohydrates. In comparison to the other samples, the control sample has fewer carbs. The outcomes are listed below.

Table No 1. Determination of Carbohydrates

Sample	Concentration(µg/ml)
Control	1.4
Sample A	1.7
Sample B	2.0
Sample C	2.3
Encapsulated Cream	1.5

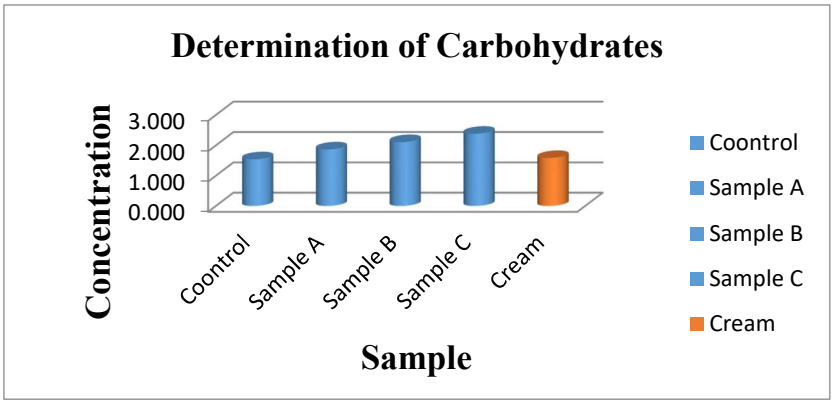

FIG.12. Determination of Carbohydrates

3.6.2. Determination of Proteins

The proteins present in the cookies sample and cream were analyzed.

The protein content of the cream and cookie samples was examined using the UV-visible Spectrum technique. The encapsulated cream, Samples A, B, and C, and the control are all analysed. The results indicated that sample A had a considerable number of proteins (3.6 µg/ml), while sample B, the encapsulated cream control sample, had a moderate level of proteins. Protein content in sample C is 1 µg/ml, which is lower compared to other samples. The outcomes are listed below.

Table No 2. Determination of Proteins

Sample	Concentration (µg/ml)
Control	2.6
Sample A	3.6
Sample B	1.3
Sample C	1
Encapsulated cream	3.1

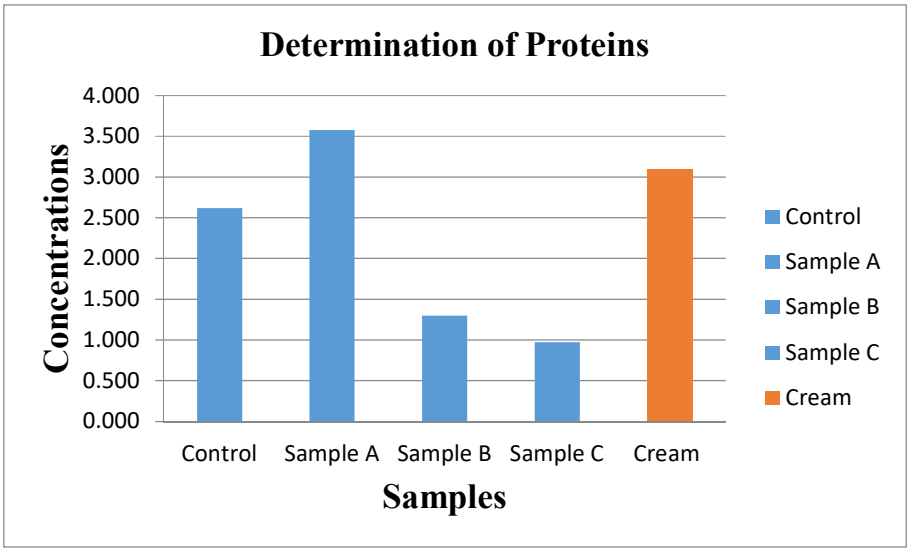

FIG.13. Determination of Proteins

3.6.3. Determination of Lipids

The cream and cookie sample's lipid contents were examined using the UV Spectrum technique. The encapsulated cream, Samples 1, 2, and 3, as well as the control sample, are all analyzed. Based on analysis, it can be observed that the encapsulated cream is liposomal, containing 1.76 µg/ml of lipids, while sample 3 has a modest amount of lipids. In comparison to the other samples, there are less lipids in the control, sample 2, and sample 1.

Table No 3. Determination of Lipids

Sample	Concentration (µg/ml)
Control	0.05
Sample 1	0.23
Sample 2	0.46
Sample 3	1.62
Encapsulated Cream	1.76

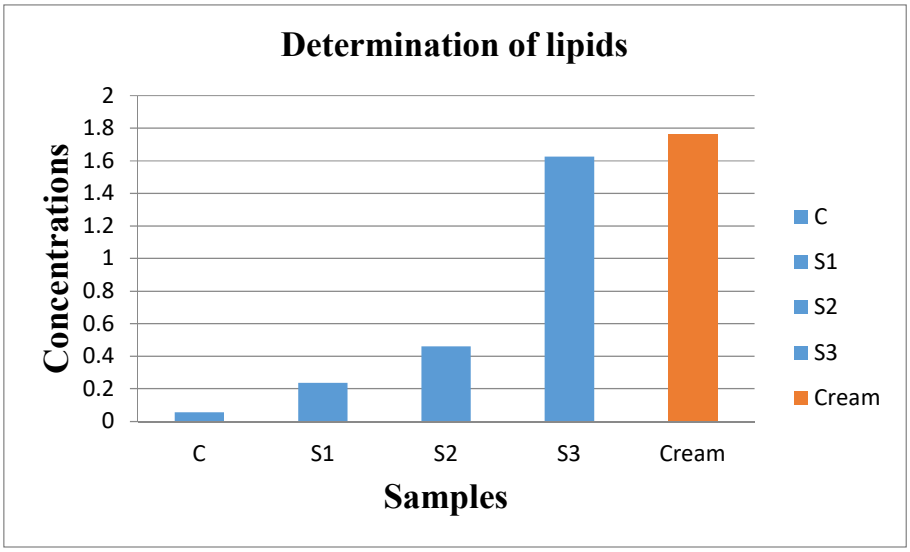

Fig.14. Determination of Lipids

3.7. SENSORY EVALUATION

Conducting sensory evaluation is crucial in assessing food products, ensuring they meet quality standards and align with consumer expectations. This process plays a vital role in research, product development, and quality control efforts **[30].** The sensory score was given as per the table.

The most common hedonic scale is the nine-point hedonic scale ranging from 1 = Dislike extremely and 9 = Like extremely. The hedonic scale assumes that participants' preferences exist on a continuum and that their responses can be categorized into like and dislike. Thus, the scale

has ruler-like and equal-interval properties. The nine-point scale is straightforward to use, and it has been widely studied. The results obtained from the hedonic scale are the scores that each consumer gives each product **[31].**

Table.1. Nine Point Hedonic Test

Parameter	Cont.	S1	S2	S3
COLOUR	7.9	7.4	8.6	8.5
TEXTURE	8.3	8.5	7.4	8.4
FLAVOUR	7.8	8.4	8	9.7
TASTE	7.6	7.1	8.1	9.9
ODOUR	8.1	8.1	7.9	8.4

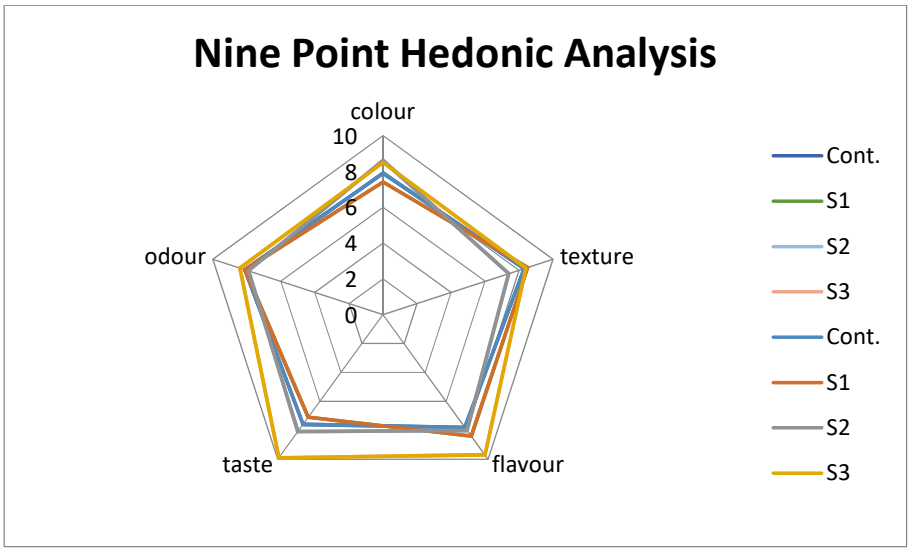

Fig.15. Nine Point Hedonic Analysis

Conclusion

The plant *Pedalium Murex* is one of the herbal plant which helps in curing kidney ailments. We have collected stem part of the plat and allowed to dry. Then converted it into fine powder and extracted it through Soxhlet extractor using methanol. We have analyzed the sample qualitatively and quantitatively. The FTIR analysis is done to find out the functional group present in the sample. The HPLC analysis is done to identify the compound present in the mixture sample. Silver nano-particle is synthesized by green synthesis method and the characterization is done. Further we have to do XRD and Nano encapsulation.

In recent years, cookies have been the foremost desirable for kids and adults in the world. It receives loads of excellent and dangerous impressions. Nowadays, customers are getting a lot of health awareness regarding the helpful worth of the food. Cookies contains flours, sugar, egg, and some type of oil and rich in fat or butter, which can lead to obesity and developing type 2 diabetes and Alzheimer's.

By considering the above aspects, the present proposal of study is designed for the development of encapsulated cream cookie by doing qualitative and quantitative analysis for carbohydrates, proteins and lipids by formulation and optimization. In this work for the preparation of cookies with different concentrations of Flax seed flour, Horsegram flour, Sorghum flour and Wheat flour. For the cream formulation, the liposomal encapsulation has done using Soy Lecithin, sunflower oil and then pedalitin has added drop by drop and then it is incorporated with cookies. The sensory evaluation has done by nine point hedonic test.

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