Research Article

In vitro Antioxidant, Anti-inflammatory and Antibacterial Activities of Microencapsulated *Elaeocarpus tectorius* (Lour.) Poir Leaf Extracts

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ABSTRACT

The genus *Elaeocarpus* comprises nearly three-fifty flowering plant species native to the Tropical and Subtropical Asia and Pacific. *Elaeocarpus tectorius* (Lour.) Poir. is one of the least explored plants of this genus. The present study was aimed to explore the phytochemicals present in the leaves of the plant *Elaeocarpus tectorius* (Lour.) Poir. and to formulate chitosan-coated alginate microcapsules loaded with the leaf extracts. The antioxidant, anti-inflammatory and antibacterial activities of the leaf extracts and microcapsules were then assessed. The leaf extract was found to possess a spectrum of phytochemicals in good quantities, including phenolics, flavonoids and tannins. The formulated chitosan-alginate microcapsules were found to be oval, spherical in shape and they exhibited good scavenging activities against nitric oxide and hydrogen peroxide radicals. The leaf extracts and microcapsules also exhibited good anti-inflammatory potential through inhibition of albumin denaturation and antiproteinase action. A prominent zone of inhibition was observed in the agar well diffusion assay suggesting the antibacterial potential of the microcapsules. The current study showed that the ethanolic extract of *E. tectorius* leaves has significant antioxidant, anti-inflammatory and antibacterial activities. Many biologically active secondary metabolites were present in the leaf extract which could be responsible for its therapeutic potential. Microencapsulation has protected and enhanced the biological properties of the leaf extracts as indicated by the increased scavenging, anti-inflammatory and antibacterial potential of the microcapsules.

Key words: Microencapsulation, Medicinal plants, Antioxidants, Inflammation, Antibiotics, Sodium alginate, Chitosan, Phytochemicals.

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INTRODUCTION

Medicinal plants are used as a resource for healing by mankind around the world for centuries. It remains of considerable importance as a primary healthcare approach for about 85% of the world's population.[1] *Elaeocarpus tectorius* (Lour.) Poir. belongs to the family of Elaeocarpaceae and it is a tall tree that grows up to 40 meters. The tree is primarily distributed in eastern Asia and is found in the mountainous regions of India, Myanmar, Thailand, Cambodia and Malaysia. The tree produces small fruits that are green in color and sweet to taste. Traditionally, it is commonly used against microbial infections and to treat several health issues including rheumatism and piles. This wild tree is not known by most people for its edible fruits.[2] Many biologically active molecules such as indolizilidine alkaloids, triterpenes, tannins, ellagic acid derivatives have been identified to be present in the trees of Elaeocarpus species. The fruits of *Elaeocarpus tectorius* are used in the treatment of rheumatism, leprosy, pneumonia, ulcer, piles and skin allergies.[3]

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The bioactive compounds present in the plant extracts are biologically unstable in nature and are easily oxidized. Several novel strategies are established in recent years to overcome these limitations and to improve their stability, delivery and bioavailability.[4] Microencapsulation is one such technique that entraps the active constituents in polymeric materials and creates a physical barrier against environmental conditions. Encapsulation also helps in sustained release, dosage, stability and masks unpleasant taste.[5] The process of ionotropic gelation of sodium alginate with calcium chloride to form calcium alginate beads has been extensively used in the process of encapsulation of different bioactive substances. The mechanism of this process involves guluronic residues of alginate with the specific chelation of Ca^{2+} *ions forming the "egg-box" structure.*[6] Sodium alginate is a water-soluble polyanionic polymer derived from marine algae and brown seaweeds. Though, alginate is an excellent carrier owing to its biocompatibility, biodegradability and non-toxicity, the macroporous structure of calcium alginate beads can cause rapid dissolution of the microcapsules, low encapsulation efficiency and sudden release of entrapped substances.[7,8] Chitosan is extensively employed as a coating material for calcium alginate beads to increase stability and improve permeability. Chitosan is derived from the partial deacetylation of chitin found in the shells of crustaceans such as crabs and lobsters.[9] It forms strong electrostatic interactions with the calcium alginate beads through its amino residues and carboxyl residues of alginate and makes the beads stable.[10] Hence, the focus of the present study is to formulate chitosan-alginate microcapsules loaded with ethanolic extracts of *Elaeocarpus tectorius* (Lour.) Poir. The surface and morphology of the microcapsules were studied using a field-emission scanning electron microscope (FESEM). The microcapsules and the crude leaf extracts were then investigated for their antioxidant, anti-inflammatory and antibacterial activities.

MATERIALS AND METHODS

Chemicals

Sodium alginate, chitosan, acetic acid, calcium chloride, ethanol, folin-ciocalteau reagent, sodium carbonate, tripyridyltriazine, hydrochloric acid, ferric chloride, sodium nitroprusside, sulfanilamide, naphthylethylene diamine dihydrochloride, hydrogen peroxide, bovine serum albumin, hydrochloric acid, trypsin, casein, perchloric acid, Muller Hilton agar (MHA) media were purchased from Hi-media (Mumbai) and Sigma Aldrich (USA). All the chemicals and solvents used were of analytical grade.

Collection and identification of plant materials

Plant materials were collected from the Nilgiri Hills of Tamil Nadu, India during March. The leaves were dried under shade and powdered. The plant material was identified and authenticated by Dr. Arumugam, a botanist from the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore, Tamil Nadu (Reference no. BSI/SRC/5/23/2021/Tech./319).

Extraction of plant materials

The dried leaf material was finely powdered and a sample of about 10 g was macerated with 100 mL of ethanol. It was then incubated for 48 hr in a shaker incubator at 40°C after which extract was filtered and the solvent was evaporated to get the dry extract. The amount of crude extract recovered after drying was weighed and the percentage of extraction yield was calculated.[3] The dried extract was stored at -4°C for further use.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of plant sample used for extraction (g)}} \times 100$$

Qualitative phytochemical analysis

Preliminary phytochemical screening was performed to identify the presence of different phytochemical constituents such as alkaloids, terpenoids, phenolic compounds, saponins, flavonoids, steroids and glycosides.

Ferric chloride test for phenolics

About 10 mg of plant extract was dissolved in 2 mL of ethanol and added 20 µL of 1% ferric chloride. The appearance of a bluish-black precipitate indicated the presence of phenolics.[11]

Shinoda test for flavonoids

The plant extract was dissolved in ethanol and a few fragments of magnesium ribbon, and few drops of concentrated hydrochloric acid were added. The development of pink color indicated the presence of flavonoids.[12]

Test for terpenoids

The plant extract was added to a few drops of concentrated sulphuric acid and heated in a boiling water bath for 2-4 min. A grey color formed indicated the presence of terpenoids.[12]

Test for tannins

10 mg of extract was dissolved in 1 mL of ethanol, and then 2 mL of distilled water was added followed by 4 drops of 10% ferric chloride aqueous solution. The formation of a blue or green color indicated the presence of phenols.[13]

Mayer's test for alkaloids

About 0.5 g of plant extract was mixed with 2 mL of Mayer's reagent. A positive result was indicated by the formation of a white creamy precipitate which demonstrated the presence of alkaloids.[14]

Test for glycosides

The plant extract was dissolved in ethanol and concentrated sulphuric acid was added to the test tube. The appearance of reddish color indicated the presence of glycosides.[15]

Salkowski test for steroids

The plant extract was dissolved in ethanol and an equal volume of chloroform was added followed by few drops of concentrated sulphuric acid along the sides of the test tube. The appearance of a brown ring indicated the presence of steroids.[13]

Foam test for saponins

Foam test was used to identify the presence of saponins. The plant extract was shaken vigorously with distilled water in a test tube. The formation of foam that persists on warming in a water bath for 5 min showed the presence of saponins.[13]

Quantitative phytochemical analysis

Estimation of total phenolics

Polyphenolic compounds form a blue complex with some specific redox reagents that can be quantified spectrophotometrically. The blue complex formed is made up of phosphotungstic acid and phosphomolybdenum whose maximum absorption depends on the alkaline solution and the concentration of phenolic compounds. 0.1mL of the extract (5mg/mL) was taken in test tubes and made up to 1 mL with distilled water. 0.5 mL of Folin- Ciocalteau phenol reagent (1:1) and 2.5 mL of 20% sodium carbonate solution were then added sequentially in each tube. The test tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm using a UV–VIS spectrophotometer against a reagent blank. The total phenolic content of the extract was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.[16]

Estimation of total flavonoids

The total flavonoid content of the extracts was measured using aluminium chloride method with slight modifications.[17] The principle of this test is that aluminium chloride forms a stable acid complex with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavonoid compounds. 10mg of the plant extract was diluted with 2mL of methanol. 0.5mL of the extract (5mg/mL) was taken in a test tube and added 0.1 mL of 10% aluminium chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was then measured at 415 nm using a UV–VIS spectrophotometer against a reagent blank. The total flavonoid content of the extract was expressed as milligrams of quercetin equivalent (QE) per gram of extract.

Estimation of total tannins

The estimation of tannins was done by treating the extracts with polyvinyl polypyrolidone (PVPP). 500 µL of distilled water and 500 µL of the sample extracts were added to 100 mg of PVPP. The content was then vortexed and kept in an Eppendorf tube at 4°C for 10-15 min. Then the sample was centrifuged at 4000 rpm for 10 min at room temperature. The supernatant collected consists of only simple phenolics, whereas the tannins would have been in a precipitated state along with PVPP. The phenolic content of the supernatant was then measured and expressed as the content of non-tannin phenolics. The amount of tannins was then calculated by subtracting the non-tannin phenolics from the total phenolics. The total tannin content of the extract was expressed as milligrams of tannic acid equivalent (TAE) per gram of extract.[18]

Estimation of Terpenoids

Total terpenoids were estimated in the leaf sample by the successful extraction of leaves with ethanol followed by petroleum ether since terpenoids are lipid-soluble. 2 g of plant samples were taken and soaked in 50 mL of 95% ethanol for 24 hr. The extract was then filtered and the filtrate was subsequently extracted with petroleum ether and concentrated to dryness. The dried ether extract was calculated as total terpenoids.[19]

Preparation of chitosan– alginate microcapsules

The internal gelation technique was used to form chitosan– alginate microcapsules. Sodium alginate was dissolved in distilled water at a concentration of 4% (w/v), and about 4 g of plant extract in 1% dimethyl sulfoxide (DMSO) was added. The solution was stirred thoroughly to ensure the complete mixing of the plant extract. The gelation medium was prepared by dissolving chitosan (1% w/v) in 1% acetic acid, followed by the addition of calcium chloride at the concentration of 4% (w/v). The sodium alginate solution was added drop wise into the gelation medium under stirring with the speed of 1000 r/min. After suspending for half an hour, the microspheres were rinsed with distilled water, filtered, and dried in the oven at 60°C.[20]

Morphological characterization of microcapsules

The shape, morphology, and elemental mapping of microcapsules were studied using field emission scanning electron microscopy (FESEM) (MIRA3 TESCAN). For this purpose, the lyophilized sample was sonicated for a sufficient amount of time, the smear was made on a platinum grid, and allowed to dry overnight under vacuum. The grid was then coated with a thin film of palladium and finally subjected to FESEM analysis.

Encapsulation efficiency

The encapsulation efficiency of the chitosan-alginate microcapsules was determined by total phenolic content analysis using Folin-Ciocalteu assay.[21] 10 mg of microcapsules were suspended in 5 mL of 95% methanol in water, mixed well, and left in the dark for 1 hr at room temperature. The sample then was filtered, and 0.25 mL of the sample was mixed with 0.25 mL Folin-Ciocalteau reagent, 4 mL of water, and 0.5 mL of 20% sodium carbonate. The solutions were then allowed to stand for 2 hr at room temperature in dark and the absorbance was measured at 765 nm. A standard curve was prepared using gallic acid to quantify the total phenolic expressed as gallic acid equivalents. The encapsulation efficiency was calculated according to the formula:

$$\text{Encapsulation efficiency} = \frac{\text{Amount of active compound entrapped}}{\text{Amount of initial active compound}} \times 100$$

In vitro antioxidant assays

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power assay is based on the principle of reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by the antioxidants of the sample at low pH. The product (Fe^{2+}-TPTZ) has a blue color with an absorption maximum at 593 nm and the change in absorbance is related to the antioxidant capacity of the plant extracts. The FRAP reagent was made up of 2.5 mL of a 10 mM tripyridyltriazine (TPTZ) solution in 40 mM hydrochloric acid, 2.5 mL of 20 mM ferric chloride and 25 mL of 300 mM acetate buffer (pH 3.6). 900 µL FRAP reagent was mixed with 90 µL water and 10 µL of the plant samples. The test tubes were incubated at 37°C for 30 min and the absorbance was read at 593 nm using a UV-VIS spectrophotometer. The analysis was performed in triplicates. The results were calculated based on the calibration curve prepared by plotting the absorbance at 593 nm *versus* different concentrations of $FeSO_4$.[22]

Nitric oxide radical scavenging activity

In vitro neutralization of nitric oxide radicals can be used to determine the antioxidant activity of plant extracts. This assay is based on the principle that sodium nitroprusside at physiological pH can spontaneously generate nitric oxide radicals that interact with oxygen to produce nitrite ions which are determined using Griess reagent. Scavengers of nitric oxide radical compete with oxygen, reducing the production of nitrite ions. 3 mL of 10mM sodium nitroprusside in 0.2 M phosphate-buffered saline (pH 7.4) was mixed with different concentrations (200 - 1000µg) of plant extracts and microcapsules respectively and incubated at room temperature for 150 min. After incubation time, 0.5 mL of Griess reagent was added. The absorbance formed was read at 546 nm using a UV-VIS spectrophotometer. Rutin was used as a standard.[23] The analysis was performed in triplicates. The percentage radical scavenging activity of the sample was calculated using the following formula:

$$\%\ \text{NO radical scavenging activity} = \frac{\text{Abs(control)} - \text{Abs(test)}}{\text{Abs(control)}} \times 100$$

Hydrogen peroxide scavenging activity

This assay is based on the ability of the plant extracts to scavenge hydrogen peroxide in an incubation system containing H_2O_2. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). 1.0 mL of the sample at different concentrations (50-250 µg/mL) in methanol was added to 0.6 mL of 40mM hydrogen peroxide. The solution was incubated for 10 min at room temperature and the absorbance was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard.[24] The analysis was performed in triplicates. The percentage of hydrogen peroxide scavenging is calculated using the following formula:

$$\%\ H_2O\ \text{scavenged} = \frac{\text{Abs(control)} - \text{Abs(control)}}{\text{Abs(control)}} \times 100$$

In vitro anti-inflammatory assays

Inhibition of albumin denaturation assay

This method is based on the ability of the substances to inhibit protein denaturation. Protein denaturation is induced by incubating the reaction mixture at 70°C in a water bath for 10 min. The resulting turbidity was measured spectrophotometrically at 660nm. The reaction mixture consisted of 1 mL test extracts at different concentrations (200-1000µg) and 1% aqueous solution of bovine albumin fraction. The pH of the

reaction mixture was adjusted to 6.5 using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 20 min. After cooling down the samples, the turbidity was measured spectrophotometrically at 660 nm. The readings were taken in triplicates.[25] The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ of Inhibition} = \frac{\text{Abs(control)} - \text{Abs(test)}}{\text{Abs(control)}} \times 100$$

Antiproteinase action

This assay is based on the ability of the plant extracts to inhibit the enzyme, proteinase. This enzyme plays an important role in the development of tissue injury during inflammatory reactions. The reaction mixture (2 mL) had about 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4) and 1 mL plant sample at different concentrations (200 - 1000 µg/ml). The mixture was incubated at 37°C for 5 min and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min after which 2 mL of 70% perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was read at 280 nm against buffer as blank.[26] The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Abs(control)} - \text{Abs(test)}}{\text{Abs(control)}} \times 100$$

Antibacterial assay

Antibacterial activity of the plant extracts and the chitosan-alginate microcapsules was tested with different bacterial strains by the agar gel well-diffusion method.[27] The bacterial strains, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus cereus* were used for the study. The bacterial culture (70µL) was spread in plates with Muller Hilton agar (MHA) media with a sterile cotton swab. Wells of 6-mm diameter were pinched off into the medium with sterile cork-borer (6 mm) and filled with 10µL (1mg/mL) of plant extracts and microcapsules by using a micropipette in each well respectively. Then the plates were aerobically incubated at 37°C for 24 hr. Cefixime (5mcg/disc) was used as a positive control. Distilled water was used as a negative control. After the required period of incubation, the zones of inhibition (ZOI) (mm) of each sample were measured.

RESULTS

Extraction Yield

The extraction of *Elaeocarpus tectorius* leaves was performed by maceration technique using ethanol. The yield found with the ethanolic extract was about 14.7%.

Qualitative phytochemical analysis

The secondary metabolites present in the ethanol extracts of the plant are shown in Table 1. All the phytochemicals tested were present in the leaf extracts. This shows that ethanol as a solvent has solubilized and extracted all the secondary metabolites present in the leaf material.

Quantitative phytochemical analysis

The ethanolic leaf extract was analyzed to estimate the total amount of phenols, flavonoids, tannin and terpenoid contents. The amount of these phytochemicals present in the leaf extract is given in Table 2.

The ethanolic leaf extract contained a phenolic content of 341.47 ± 3.39 mg gallic acid equivalents/gram of extract. The flavonoid content of the leaves was found to be about 245.77 ± 1.58 mg quercetin equivalents/gram of extract. The amount of tannins found in the ethanol leaf extracts of *E. tectorius* was about 302.5 ± 4.3 mg tannic acid equivalents/g extract and the amount of terpenoids present in a gram of leaves were found to be 54.6 ± 1.17 mg.

Table 1: Qualitative phytochemical analysis of leaves of *Elaeocarpus tectorius* (Lour.) Poir.

Phytochemical	Ethanolic leaf extract
Phenolics	+
Flavonoids	+
Terpenoids	+
Tannins	+
Alkaloids	+
Glycosides	+
Steroids	+
Saponins	+

Table 2: Qualitative phytochemical analysis of leaves of *Elaeocarpus tectorius* (Lour.) Poir.

Phytochemical	Ethanolic leaf extract
Phenols (mg GAE/g extract)	341.47 ± 3.39
Flavonoids (mg QE/g extract)	245.77 ± 1.58
Tannins (mg TAE/g extract)	302.5 ± 4.3
Terpenoids (mg/g)	54.6 ± 1.17

The morphology of microcapsules and encapsulation efficiency

The encapsulation efficiency of the chitosan-alginate microcapsules loaded with *E. tectorius* leaf extracts was found to be 83.2 ± 0.89%. Field-emission scanning electron micrograph (FESEM) analysis of chitosan-sodium alginate microcapsules containing *E. tectorius* leaf extracts clearly showed that they were uniform with an average diameter of 150-200nm. The microcapsules were oval, spherical in shape. Most of the microcapsules were individual, and few aggregated particles were also observed (Figure 1).

In vitro antioxidant assays

Ferric reducing antioxidant power (FRAP) assay

The chitosan-alginate microcapsules showed higher FRAP activity when compared with crude extracts. FRAP activity of the plant extracts, microcapsules and standard ascorbic acid are given in Table 3.

Nitric oxide radical scavenging activity

The plant extracts and chitosan-alginate microcapsules showed a concentration-dependent increase in the nitric oxide radical scavenging activity. The scavenging potential of the microcapsules was higher than the crude plant extracts and standard rutin. Nitric oxide radical scavenging activity of the plant extracts at different concentrations is given in the graph (Figure 2). The nitric oxide scavenging potential of chitosan-alginate microcapsules, crude plant extracts and standard rutin was found to be 82.33 ± 1.52%, 66.42 ± 0.72% and 77.12 ± 1.18% at 1000µg/ml concentration respectively. The IC_{50} values were also calculated and are given in Table 4.

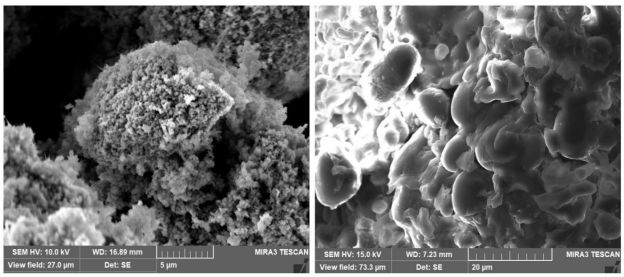

Figure 1: Field-emission scanning electron microscope analysis of chitosan-alginate microcapsules loaded with *Elaeocarpus tectorius* leaf extracts

Table 3: Ferric reducing antioxidant power assay of the plant extracts and Chitosan-alginate microcapsules

Plant extracts	FRAP (mmol (Fe(II) /g sample)
Elaeocarpus tectorius leaf extract	110.27 ± 1.5
Chitosan-sodium alginate microcapsules containing *E. tectorius* leaf extracts	134.22 ± 2.1
Ascorbic acid	197.1 ± 1.65

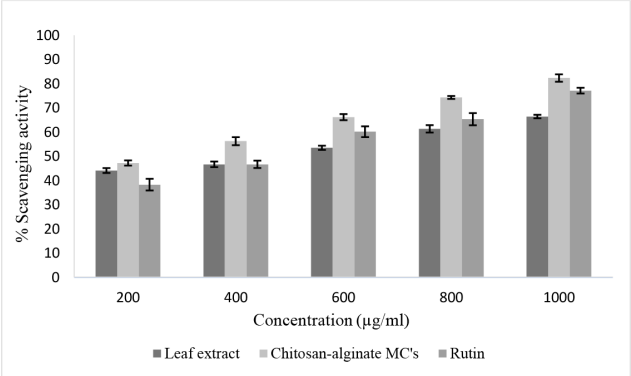

Figure 2: Nitric oxide radical scavenging activity of the plant extracts and microcapsules

Table 4: IC_{50} values of the plant extracts and microcapsules for antioxidant assays.

Plant extracts	IC_{50} *values (µg/mL)*	
	Nitric oxide radical scavenging activity	H_2O_2 radical scavenging activity
E. tectorius leaf extract	452.5	610.96
Chitosan-alginate microcapsules	443.98	575
Rutin	254.8	-
Ascorbic acid	-	500

Hydrogen peroxide radical scavenging activity.

H_2O_2 present in the reaction mixture was scavenged by the antioxidant compounds present in the plant extracts. The scavenging potential was found to be concentration-dependent and higher in the Chitosan-alginate microcapsules as shown in Figure 3. The hydrogen peroxide scavenging activity of chitosan-alginate microcapsules, crude plant extracts and standard ascorbic acid was found to be 75.37 ± 2.07%, 73.44 ± 1.7% and 79.34 ± 1.93% at 250µg/ml concentration respectively. The IC_{50} values were also calculated and are given in Table 4.

In vitro anti-inflammatory assays

Inhibition of albumin denaturation assay

The inhibition exhibited by the plant extracts on the denaturation of bovine serum albumin was analyzed and the results are presented in Figure 4. The microencapsulated extracts exhibited a slightly highest

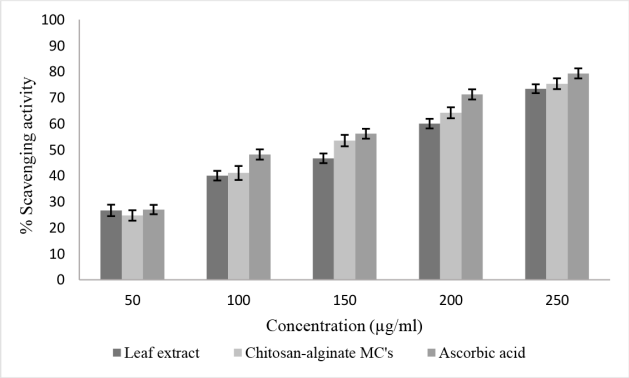

Figure 3: Hydrogen peroxide radical scavenging activity of the plant extracts and microcapsules.

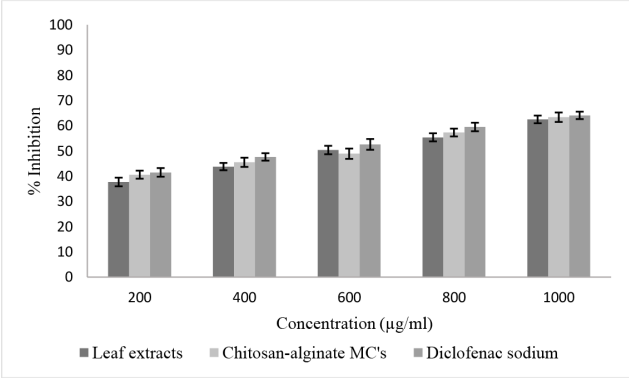

Figure 5: Antiproteinase action of the plant extracts and microcapsules Antibacterial activity.

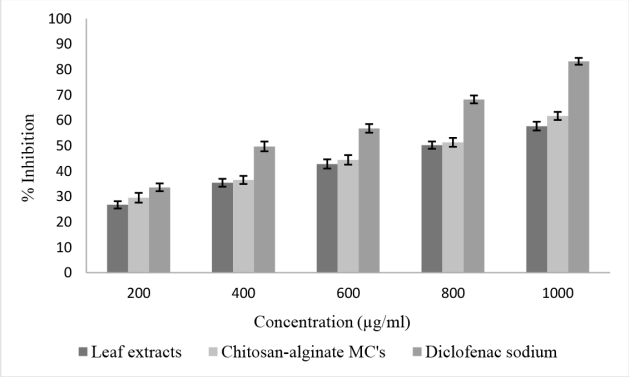

Figure 4: Inhibition of albumin denaturation activity of the plant extracts and microcapsules.

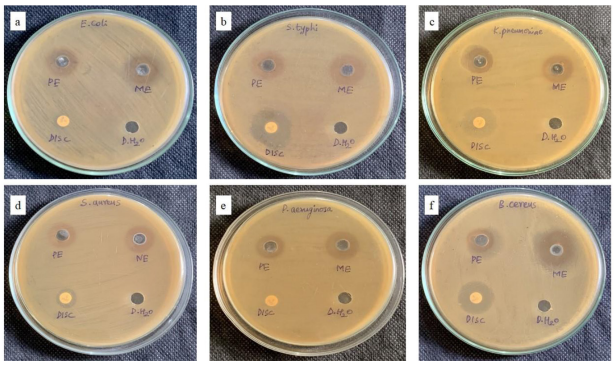

Figure 6: Antibacterial activity of the plant extracts and microcapsules.
([PE- Crude plant extract; ME- Microencapsulated plant extracts] a) *Escherichia coli* b) *Salmonella typhi* c) *Klebsiella pneumoniae* d) *Staphylococcus aureus* e) *Pseudomonas aeruginosa* f) *Bacillus cereus*)

inhibition on the protein denaturation compared with the crude extracts. The inhibition of protein denaturation activity of the chitosan-alginate microcapsules and crude plant extracts was 61.7 ± 1.59% and 57.7 ± 1.7% at 1000µg/ml respectively. Standard diclofenac sodium exhibited inhibition of protein denaturation activity of 83.2 ± 1.35% at 1000µg/ml.

Antiproteinase action

The ability of the plant extracts and microcapsules to inhibit the enzyme proteinase was estimated and the results are presented in Figure 5. Though the antiproteinase action of the microcapsules and leaf extracts were similar, the microcapsules comparatively exhibited slightly higher inhibition. Antiproteinase action exhibited by the microcapsules and crude extracts were estimated to be 63.37 ± 1.88% and 62.5 ± 1.52% at 1000µg/ml respectively while standard diclofenac exhibited inhibition of 64.1 ± 1.47% at 1000µg/ml.

Antibacterial activity

Antibacterial activities of plant extracts and microcapsules were tested using the clinical strains of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The plates showed a well-developed zone of inhibition by both the crude plant extract and microcapsules (Figure 6). The antibacterial activity of microcapsules was comparatively higher than the crude plant extracts. The zones of inhibition exhibited by plant extracts and microcapsules were measured and given in Table 5.

DISCUSSION

The analysis of medicinal plants has a long history, and especially regarding assessing a plant's quality. Extraction is the first and important step to isolate the bioactive compounds from the plant materials. The extraction technique should be able to obtain extracts with high yield and bioactive compounds with minimal changes.[28] The leaves of *E. tectorius* were extracted using ethanol as a solvent. A yield of about 14.7% was obtained after the successful extraction of the plant materials. Maceration is a simple extraction method to achieve maximum

Table 5: Antibacterial activity of plant extracts and microcapsules.

Bacterial Strains	Zone of Inhibition (mm)		
	Chitosan-sodium alginate microcapsules containing *E. tectorius* leaf extracts	*Elaeocarpus tectorius* leaf extract	Positive Control (Cefixime)
Escherichia coli	5	4	3
Salmonella typhi	5	4	10
Staphylococcus aureus	9	8	8
Klebsiella pneumoniae	5	4	6
Pseudomonas aeruginosa	4	2	0
Bacillus cereus	12	11	7

bioactive compounds in less time and energy with less solvent consumption.[29]

The extract was screened for the presence of phenols, flavonoids, tannins, terpenoids, alkaloids, glycosides, steroids and saponins. All these phytochemicals were found to be present in the plant analyzed. The phytochemical analysis is a prerequisite to ascertain the biomarker compounds in order to identify and determine the quality of plant materials.[30] In another study on the phytochemical screening of the fruits of *E. tectorius,* it was indicated that all these phytochemicals were present in the fruit powder.[3] These phytochemicals are reported to have multiple health benefits hence the presence of biologically important secondary metabolites highlights the importance of this plant species.

The quantification of total phenolics, flavonoids, tannins and terpenoids was carried out in the next step. Plant phenolic compounds including flavonoids and tannins are known for their antioxidant power and their free radical scavenging activities help in the prevention of oxidative stress-related disorders such as cancer and neurodegenerative diseases. They also possess good antimicrobial, anti-inflammatory and cardio-protective activities.[31,32] Terpenoids are one of the most important groups of secondary metabolites since they possess anticancer, antimicrobial, anti-inflammatory, hypoglycemic and antioxidant activities.[33] The plant extracts possessed high and very good quantities of these important phytochemicals. Other plants belonging to the genus Elaeocarpus were also found to exhibit high content of phenolic compounds which accounts for their biological importance.

The ethanol leaf extracts were then loaded into chitosan-alginate microcapsules. The encapsulation efficiency and the surface morphology of the microcapsules were then studied. The microcapsules exhibited maximum encapsulation efficiency. The encapsulation efficiency of a microcapsule will be affected by different parameters. The factors that influence the encapsulation efficiency include the solubility of the polymer in an organic solvent, concentration of polymer and speed of solidification of microcapsules.[34] FESEM analysis of the microcapsules clearly showed the oval, spherical nature of the microcapsules. Encapsulation of plant materials offers an array of advantages including protection of the encapsulated active agent, the possibility to control the release rate and easy administration.[35] Studies have shown that nanoencapsulation improves the antioxidant activity of various plant extracts and edible oils.[36] The chitosan-alginate microcapsules and crude plant extracts were evaluated for their *in vitro* antioxidant, anti-inflammatory and antibacterial activities.

Uncontrolled production of reactive oxygen species (ROS) leads to a phenomenon called oxidative stress which is implicated in numerous diseases and disorders. This could be due to the impairment in the endogenous antioxidant mechanism or tissue damage. Antioxidants help in scavenging the free radicals before they attack the cellular components.[37] The ability of the plant extracts and microcapsules to scavenge the free radicals was examined using FRAP, NO and H_2O_2 radical scavenging assays. The extracts and microcapsules exhibited a concentration-dependant increase in their antioxidant activity. The antioxidant potential of the chitosan-alginate microcapsules was significantly higher than the crude plant extracts in all the methods tested. A positive correlation was observed between the total phenolic content and the antioxidant capacity of the plant extracts since plants with high antioxidant potential often have a high amount of polyphenolic compounds. The antioxidant activities of polyphenolics are mainly attributed to their metal-chelating potential and redox properties that allow them to act as reducing agents and single oxygen quenchers.[38] FRAP assay measures the reducing potential of a compound reacting with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to produce ferrous tripyridyltriazine (Fe^{2+}-TPTZ), a colored complex. The reducing property of the antioxidant compounds is linked to their ability to break a free radical chain reaction by donation of a hydrogen atom.[22] In the present study, as shown in Table 3, the plant extract and microcapsules exhibited a good ferric reducing antioxidant power which shows their ability to donate electrons to free radicals in order to stabilize them. The microcapsules exhibited a higher

FRAP activity than the crude plant extracts which could be attributed to their protective shell that protected the volatile constituents of extracts against environmental conditions.

Nitric oxide (NO) is generated from the amino acid L-arginine in phagocytes and vascular endothelial cells. NO has an important role in the pathogenesis of various inflammatory processes and is highly reactive with other free radicals and certain types of proteins. The toxicity of NO increases when it forms a highly reactive peroxynitrite anion ($ONOO^-$) on reaction with superoxide radical.[39] Therefore the ability of the plant extracts and microcapsules to scavenge the nitric oxide radicals at different concentrations was studied. NO generated from sodium nitroprusside reacts with oxygen to form nitrite ions which react with sulphanilamide-naphthyl ethylenediamine forming a pink color. The intensity of the color is reduced as the antioxidant compounds present in plant extracts donate protons to the nitrite radicals.[40] The plant extract and microcapsules exhibited a dose-dependent increase in the scavenging potential where, the microcapsules were more potent in scavenging nitric oxide radicals than the crude plant extract. Hydrogen peroxide (H_2O_2) is formed *in vivo* by various enzymes including superoxide dismutase. It is capable of crossing cell membranes rapidly, oxidizing several compounds.[41] Hydrogen peroxide can deactivate critical cellular enzymes involved in the production of energy. Scavenging of H_2O_2 is therefore considered important and plant polyphenolics are known to scavenge it by electron donation.[42] The tested extracts exhibited good hydrogen peroxide scavenging potential when compared with the standard ascorbic acid.

In vitro anti-inflammatory activity of the ethanolic extract and microcapsules of *E. tectorius* leaves were studied using inhibition of albumin denaturation and anti-proteinase assays. Both the crude extract and microcapsules exhibited a concentration-dependent inhibition in both the assays where the activity increased with an increase in their concentration. The chitosan-alginate microcapsules exhibited higher anti-inflammatory activity than the crude extracts which clearly shows that encapsulation has protected and enhanced the biological activity of the leaf extract. Denaturation of cellular proteins is a well-documented cause of inflammatory reactions. Inhibition of the denaturation of proteins by plant extracts plays an important role in protecting the tissue damage during inflammatory responses.[43] The tested extracts showed good inhibitory potential on protein denaturation which might be due to their ability to stabilize the tertiary and quaternary structure of proteins. Proteinases are associated with inflammatory responses due to their ability to break down many critical proteins. Proteinase inhibitors play a major role in protecting many pathological conditions associated with inflammation.[44]

Antibiotic resistance is a major health threat to the community. There is a risk of losing the effectiveness of common antibiotics in a short period due to the rapid development of microbial resistance against antibiotics. This situation has necessitated the need to screen effective, safe and cheap antimicrobial compounds from medicinal plants.[45] The leaf extract and chitosan-alginate microcapsules were studied for their antibacterial activity using the agar well diffusion method. The extracts and microcapsules showed very good inhibitory potential against all the microorganisms tested viz, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Bacillus cereus* which were comparable to the standard antibiotic, cefixime. The highest inhibition was recorded against *B. cereus* and the lowest was observed against *P. aeruginosa.* Correlation between the phenolic, tannin content and antibacterial properties of plants have been reported earlier.[46] The present study supports this correlation since the leaf extract was found to contain high phenol and tannin contents. This might be due to their interaction with the microbial enzyme system, nucleic acid and cellular membrane. This study has clearly established the effect of microencapsulation on the biological activities of the *Elaeocarpus tectorius* leaf extract.

CONCLUSION

The study demonstrates the importance of the plant *Elaeocarpus tectorius* (Loir.) Poir. and established its phytochemical constituents. The antioxidant, anti-inflammatory and antibacterial activities of the plant species are appreciable and the technique of microencapsulation using sodium alginate and chitosan has greatly protected and enhanced the biological activities of the extract. As a first report on the anti-inflammatory activity of this plant species, this study could be an important contribution in the field of herbal medicine aiding in the identification and further research on this plant species. Further studies are in place to ascertain the *in vivo* activities of the plant extracts and their microcapsules.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

Ca^{2+}: calcium ions; **FESEM**: Field-emission scanning electron microscope; **BSI**: Botanical Survey of India; **TNAU**: Tamil Nadu Agricultural University; **MHA**: Muller Hilton agar; **GAE**: Gallic acid equivalents; **QE**: Quercetin equivalents; **TAE**: Tannic acid equivalents; **DMSO**: Dimethyl sulfoxide; **FRAP**: Ferric reducing antioxidant power; **TPTZ**: Tripyridyltriazine; **H_2O_2**: Hydrogen peroxide; **NO**: Nitric oxide; **ROS**: Reactive oxygen species; **ZOI**: Zone of inhibition.

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