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EVALUATION OF IN VITRO ANTI-INFLAMMATORY ACTIVITY AND ENCAPSULATION OF EXTRACTED SEED KERNEL OIL OF BUCHANANIA LANCEOLATA WIGHT

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ABSTRACT

Buchanania lanceolata Wight. is a tree species from Anacardiaceae family. It is classified as Vulnerable under IUCN Red List of Threatened Species. In Indian traditional medicine, *B. lanceolata* has various medicinal and therapeutic properties. The present study focuses on the extraction of oil from the seed kernels of *B. lanceolata* for testing its *in vitro* anti-inflammatory property followed by the encapsulation of oil in calcium alginate beads. The preliminary qualitative phytochemical analysis of the extracted seed kernel oil shows the presence of alkaloids, terpenoids, fatty acids and flavonoids. The total of 13 volatile bioactive compounds in the oil were identified by GC-MS analysis. The functional groups and bonds of the active phytoconstituents were detected by FTIR analysis. The *in vitro* anti-inflammatory activity, assessed by protein denaturation assay revealed that the sample as well as the reference medication, Diclofenac inhibited the protein denaturation in a concentration dependent manner throughout a range of 6.25 to 100 µl. The extract at the dose of 6.25µg/ml and 12.5µg/ml exhibited better anti-inflammatory activity with the percentage inhibition of 14.42 % and 22.39 % respectively. Essential oil requires encapsulation to prevent its degradation due to environmental factors. For increasing its shelf life, the oil was encapsulated in calcium alginate beads and stored for further analysis. The overall result demonstrated that the seed kernel oil of *B.lanceolata* have various bioactive compounds and exhibits good anti-inflammatory activity.

KEYWORDS: *B. lanceolata*, Essential oil, Inflammation, Protein denaturation assay, Encapsulation.

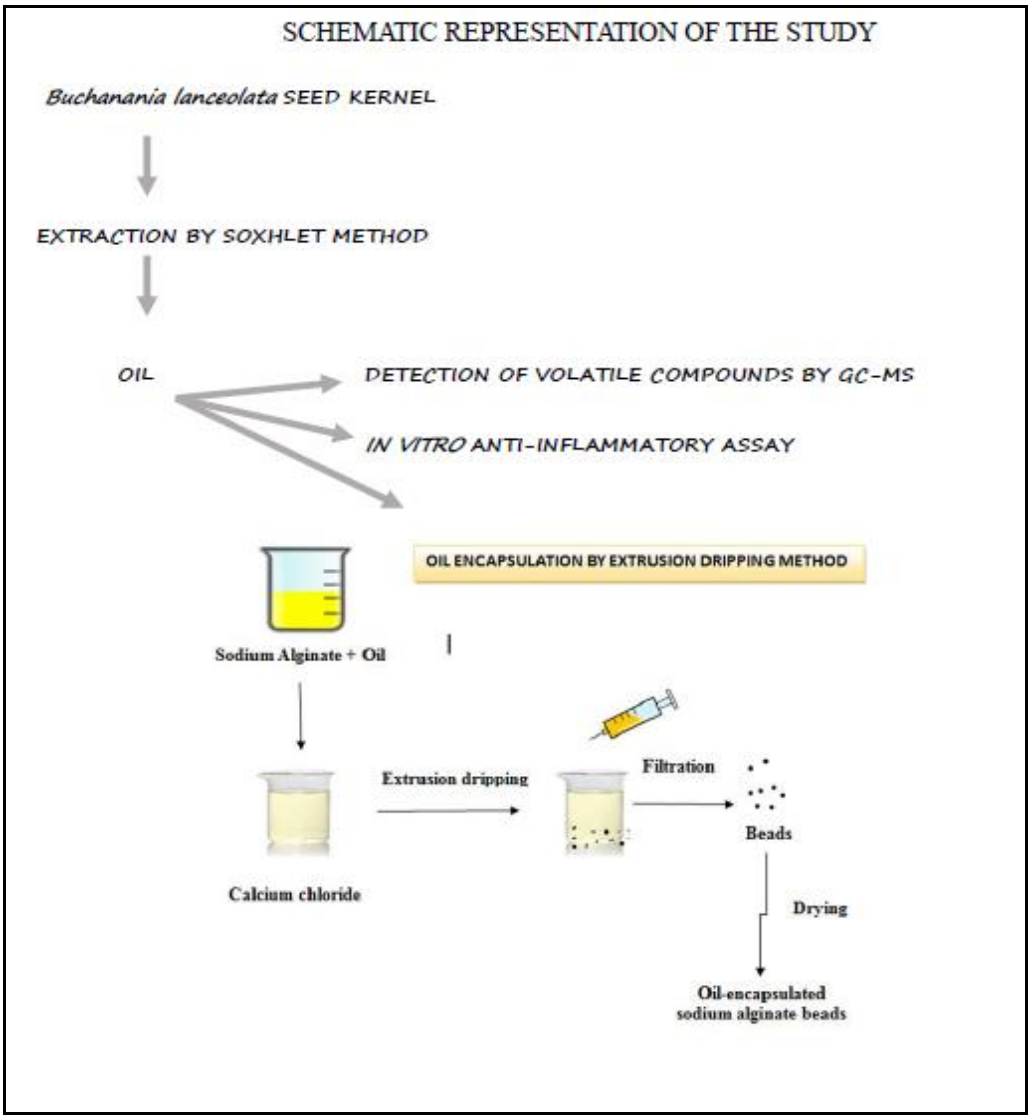
INTRODUCTION

Inflammation is indeed a complex biological response that is crucial for the body's defense against harmful stimuli such as pathogens, damaged cells or irritants. The body reacts to inflammation in a number of ways, including pain, swelling, redness, heat and loss of function in the affected area (Modi et al., 2019). The first response, known as acute inflammation, is defined by an increase in the supply of blood plasma and innate immune system cells, such as macrophages and neutrophils into the wounded tissues. Chronic inflammation is defined by the simultaneous destruction and healing of the wounded tissue and is associated with a progressive change in the kind of cells present at the location of the inflammatory reaction (C Recio et al., 2012).

Although the body uses inflammation as a defense mechanism to get rid of harmful stimuli, anti-inflammatory drugs can also be a useful therapeutic tool to reduce tissue damage and improve patient comfort by interfering with the mechanism of inflammation (de Cássia da Silveira e Sá et al., 2013). Glucocorticoids and non-steroidal anti-inflammatory medicines are the two main families of anti-inflammatory agents, which differs in their mode of function. To put it briefly, glucocorticoids work by blocking prostaglandins and proteins that are involved in inflammatory processes, such corticosteroids, which are used to treat autoimmune inflammatory response and asthma. Non-steroidal medications work by inhibiting the cyclooxygenase enzyme and are recommended for the treatment of moderate to mild pain as well as the regulation of body temperature. They are the most often prescribed medications, used to treat both acute and chronic pain that is caused by inflammation (Nunes et al., 2020). Non-steroidal anti-inflammatory medications are frequently used to treat inflammatory diseases and are linked to adverse effects including gastrointestinal bleeding and immune system suppression. So the use of medicinal plants as a significant source of chemicals with therapeutic potential, has long been acknowledged (Adebayo et al., 2015).

Plants are used to extract oils from their leaves, flowers, stems, seeds and roots. As proposed by Schilcher, Hegnauer, and Cohn Richter, ― Essential oil is a biochemical product or a combination of similar products, which are generated in cytoplasmic fluid and are located in intercellular space in the form of minute droplets. They are highly odorous and volatile in nature‖ (Naeem et al., 2018). Due to their unique fatty acid composition and other advantageous bioactive components, recent studies have indicated that certain plant seed oils may be used as specialty oils for disease prevention and health promotion. The potential health benefits of plant seed oil are associated with their high concentration of unique polyunsaturated fatty acids and other bioactive substances with pharmaceutical properties like antibacterial, antioxidant, anti-inflammatory, antiatherosclerotic, anticancer, antimutagenic, and antiviral activities (Keskin, 2019).

Over time, essential oils have attracted a number of fields because of its therapeutic qualities and biocidal activity (fungicides, viricides, and bactericides). Due to their instability, essential oils are easily affected by changes in light, temperature, oxygen content and humidity. Its high volatility and reactivity provide difficulties for the use of essential oils in various sectors. Hence, the technology of microencapsulation is frequently used to control the release of these chemicals while preserving their biological and functional properties. The technique of microencapsulation involves coating solid, liquid or gaseous particles with an encapsulating substance that functions as a barrier to fully isolate the core material from the surrounding environment (Sousa et al., 2022) (Chaabane et al., 2022).



MATERIALS AND METHODS

IDENTIFICATION OF THE PLANT

The twigs with the leaves and fruits of *Buchanania lanceolata* were collected in the month of April from Alappuzha district of Kerala **(Fig.1).** The plant material was identified using Flora of the Presidency of Madras by J.S. Gamble and authenticated by experts.

Fig. 1: Habitat of *B.lanceolata.*

COLLECTION OF SEEDS AND EXTRACTION OF SEED KERNEL

Matured fruits were picked and soaked in water for easy removal of the epicarp and mesocarp, for obtaining the seeds. The seeds were then dried under sunlight. Stone pounding was used to release the kernels from the dried nuts. The separated seed kernels were again dried, stored in air tight polythene bags and kept at 4ºC.

EXTRACTION OF OIL BY SOXHLET METHOD

The dried seeds kernels were grinded into fine powder using an electronic grinder. 10 g of the powdered sample was measured, packed in a fabric bag, sealed and placed inside an extraction tube. The extraction process was carried out using a Soxhlet extractor with hexane as the solvent, maintained at 68°C for 16 hours. The resulting extract was filtered through Whatman No. 1 filter paper and stored at 4°C (Mohd-Setapar et al., 2014) After evaporating the solvent in the filtrate, the percentage yield was determined using the equation given below,

$$\text{Percentage yield of oil} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample taken for extraction (g)}} \times 100$$

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical tests of the oil extract of B. lanceolata were carried out as follows (Soni and Sosa, 2013) (Shaikh & Patil, 2020).

- Test for alkaloids (Mayer's test)
  Add few drops of Mayer's reagent in to 1 ml of extract, the presence of a creamy orwhite precipitate indicates the presence of alkaloids.
- Test for terpenoids (Salkowski test)
  After taking 3 ml of the extract, 1.5 ml of con. H_2SO_4 and 1 ml of chloroform were poured along the edges of the test tube. The reddish-brown color at the interface indicates the presence of terpenoids.
- Test for saponins
  Mix a few millilitres of distilled water with 0.5 mg of extract. The formation of frothing is positive for saponins.
- Test for flavonoids (Alkaline reagent test)
  A couple of drops of sodium hydroxide solution are applied to the sample. When diluted acid is added, the formation of a bright yellow colour that turns colourless suggests the presence of flavonoids.
- Test for carbohydrate (Molisch's test)
  In a test tube, 1ml of the filtrate solution is treated with two drops of the alcoholic naphthol solution. Concentrated sulfuric acid (2ml) is poured throughthe test tube's walls. The formation of violet ring at the junction indicates the presence of carbohydrate.
- Test for steroid (Liebermann-Burchard test)
  Mix 2 ml of acetic acid with 0.2 g of extract, and the mixture was then thoroughly chilled in ice before the cautious addition of con. H_2SO_4. When the color changed from violet to blue or bluish- green, steroids were present.
- Test for fatty acid
  5 ml of ether and 1 ml of the extract were combined. On a filter paper, the extracts were allowed to evaporate before the paper was dried. The presence of fatty acids is indicated by the appearance of transparency.
- Test for oil and resins
  Filter paper was sprayed with the test solution. When it becomes clear on the filter paper, it means that oils and resins are present.

GC-MS ANALYSIS

The analysis was performed on GC-MS equipment (Shimadzu Nexis GC- 2030). Experimental parameters of GC-MS system were, column oven temperature: 70°C; injection temperature: 260°C; injection mode: split; flow control mode: linear velocity; pressure: 62.1 kPa; total flow: 14.1 ml/min; column flow: 1.01 ml/min; linear velocity: 36.8 cm/s; purge flow: 3.0 ml/min; split ratio: 10.0. Oven temperature program was 70°C hold time for 2 min, at 200°C hold time for 5 min and 280°C hold time for 15 min. The GC program has ion-source temperature at 210°C, interface temperature at 280°C, solvent cut time: 6.50 min, detector gain: 0.70 kV+0.00 kV and threshold of 0. The chemical components from the ethanolic extract was identified by comparing the retention times of chromatographic peaks using NIST 20 Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areasfrom the GC-MS.

FTIR ANALYSIS

The technology known as Fourier Transform Infrared (FTIR) waves employed to discern various functional group present in every extract. With FTIR, the infrared spectroscopy (IR) spectrum was acquired using Japan's Prestige-21 Shimadzu. The FTIR spectroscope was equipped with the sample and set to scan between 600 and 4000 cm^{-1} (Lewis et al., 2010)

IN VITRO ANTI-INFLAMMATORY ACTIVITY

Protein Denaturation Inhibition Assay

The reaction mixture, totaling 0.5 ml, consisted of 0.4 ml of 3% aqueous solution of bovine serum albumin combined with 0.1 ml of the test sample. This mixture was incubated at 37°C for 20 minutes. After incubation, 2.5 ml of phosphate buffered saline (pH 6.3) was introduced into each tube, which was then subjected to heating at 80°C for 10 minutes. Absorbance measurements were recorded at 660 nm using a spectrophotometer (Mizushima and Kobayashi, 1968; Sakat et al., 2010). The percentage of inhibition was calculated using the following formula:

[(Absorbance of control – Absorbance of Test) / Absorbance of Control] x 100.

ENCAPSULATION OF OIL

Preparation of oil encapsulated calcium alginate beads

The oil encapsulated calcium alginate beads were synthesized by emulsion gelation method. 5 % sodium alginate was prepared by dissolving 5 g sodium alginate in 100 ml distilled water with vigorous mechanical stirring. After the stirring, 10 ml of oil sample was added in to the polymer solution and mix well. The mixture was then loaded in to a syringe and excluded drop by drop in to 100 ml $CaCl_2$ (0.1M) gelling solution. The spherical uniform beads were allowed to harden in the gelling solution for 15 min, then filtered and washed with distilled water. The beads were allowed to dry in room temperature until constant weight was achieved. The plain beads synthesized without the addition of oil were used as the control (Durante et al., 2012).

Physical properties of beads

The colour change of the oil encapsulated beads were compared with the plain sodium alginate beads. The dry weight of the prepared beads were calculated by measuring the weight at regular intervals. After complete removal of the moisture content by air drying at room temperature for 12 hours, the constant weight attained was noted.

Morphology of the beads

The morphological examination and the surface appearance of the dried beads were observed under a scanning electron microscope (SEM; JEOL 6390LV). The beads were prepared for SEM analysis by attaching them to a stub using a two-sided adhesive tape and coating them with gold as a conductive material using an ion sputter instrument. The coated samples were observed under SEM with a vacuum condition. The particle diameter of the beads was determined by a laser diffraction-based particle size analyzer.

RESULT AND DISCUSSION

SEED KERNEL OIL EXTRACTION

Using Soxhlet extraction, seed kernel oil was extracted with 99 % Hexane as the solvent. The effectiveness of a specific solvent in extracting the constituents of a plant was determined by calculating the percentage yield. A yield of 12% was obtained from the crude extract of hexane **(Fig. 2).**

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF SEED KERNEL OIL

In the preliminary phytochemical screening, the presence of phytoconstituents such as alkaloids, terpenoids, fatty acids, flavonoids, oil and resin were detected.

GC-MS ANALYSIS

By using GC-MS analysis, the volatile phytoconstituents in the oil extract were identified. A total of 13 compounds were detected **(Fig. 3).** The various compounds present in the extract with their retention time (RT), molecular formula and its peak area (%) were represented in **Table 1**.

Among the compounds identified, methyl hexadeconate, pentadecoanoic acid, methyl-10 octadecenoate, cis-Vaccenic acid, (E)-9-Octadecenoic acid ethyl ester, Squalene 7-Hexylicosane, Heneicosane, Tetratetracontane and 2-methyloctacosane exhibits bioactivity as shown in **Table 2.**

FTIR ANALYSIS

The FTIR spectrum identified the functional group of the active components in the seed kernel oil extract of *B. lanceolata* is displayed in **Fig. 4**. The FTIR gave broad spectrum at 2954.31cm^{-1} which indicates the presence of saturated aliphatic C-H stretching; 2917.10 cm^{-1} and 2850.79 cm^{-1} represents the saturated methylene C-H stretching; 1743.90 cm^{-1} for the presence of alkyl carbonate; 1279.23 cm^{-1} for organic nitrates; 1378.18 cm^{-1} is assigned due to the bending of C-H groups The FTIR spectrum contains a total of more than 5 bands indicating the complex nature of the sample (Nandiyanto et al., 2019).

ANTI-INFLAMMATORY ANALYSIS

The protein denaturation assay is based on the idea that substances with anti-inflammatory properties may be able to stabilize protein structures and prevent denaturation, which is frequently linked to inflammation and tissue damage (Esho et al., 2021).

Results shows that the sample as well as the reference medication, Diclofenac inhibited protein denaturation in a concentration dependent manner throughout a range of 6.25 to 100 µl. The extract at the dose of 6.25 µg/ml and 12.5 µg/ml exhibited better anti-inflammatory activity with the percentage of inhibition of 14.42 % and 22.39 %, when compared with the standard drug Diclofenac showing 7.88 % and 16.01 % respectively. The IC50 value indicates that the standard drug Diclofenac is potent than the oil extract. The IC50 value of Diclofenac is 49.68 µg /ml while that of the oil extract is 88.29 µg/ml **(Fig. 5 & Table 3).**

OIL ENCAPSULATED SODIUM ALGINATE BEADS

At alginate concentration of 5 % w/v, the prepared oil encapsulated beads were spherical and were off white in colour, while the sodium alginate beads without oil (control) were white in colour as shown in **Fig. 6**. The dry weight of the beads after complete removal of the moisture content was 0.43g.

In extrusion technique, a liquid droplet grows at the capillary tip until the surface tension force no longer supports the weight of the droplet. This results in the liquid droplet to detach from the tip and falls into the gelling bath to form a bead. The concentration of sodium alginate used for entrapment also affects the shape of beads (Lee et al., 2013).

Morphology of oil encapsulated sodium alginatebeads

The surface morphology of the beads observed under SEM exhibits a spherical structure with a diameter of 2.10 mm and less visible cracks as shown in **Fig. 7.** The spherical shape of beads has been reported to have greater gel bead strength than non- spherical shapes. The microspheres containing seed kernel oil displayed a crinkled surface characterized by prominent lumps. This phenomenon can be attributed to the accumulation of oil droplets on either the outer or inner surfaces of the external layer of the synthesized beads. The presence of oil components on the alginate surfaces may result in the plasticization of their structure, thereby contributing to the formation of these lumps (Soliman et al., 2013).

Fig. 2: (A) Seeds collected (B) Seed kernel extracted (C) Oil extracted.

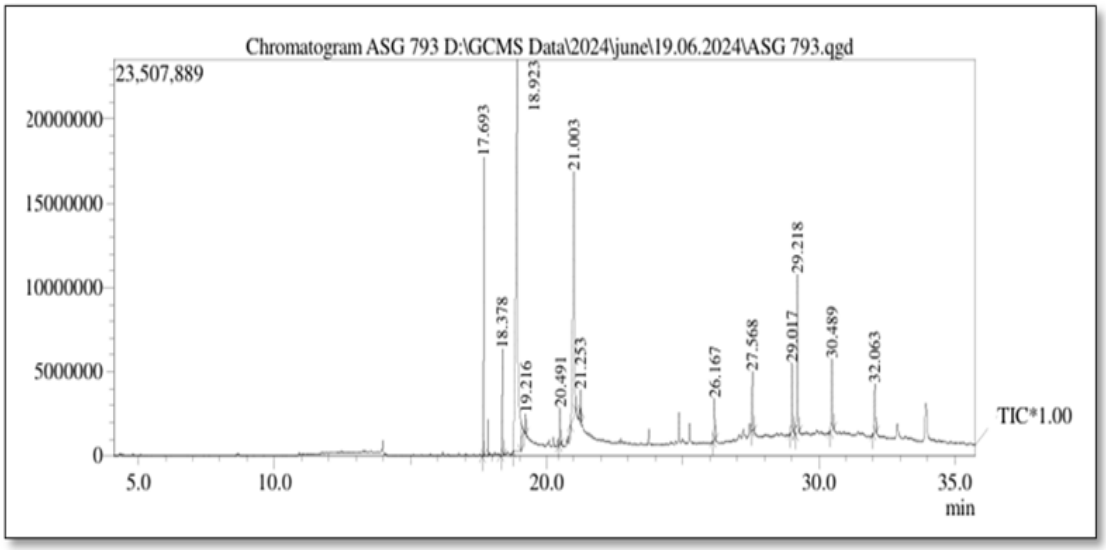


Fig. 3: GC-MS chromatogram of *B. lanceolata* Seed Kernel Oil.

Table 1: Compounds detected in GC-MS with its retention time (RT) in min, peak area (%) and molecular formula.

Sl. No.	Name of the compound	R.T.	Peak area (%)	Molecular formula
1	1-methyl-4-(tetra-pentyloxy) benzene	17.693	5.27	$C_{12}H_{18}O$
2	Methyl hexadecanoate	18.378	3.84	$C_{17}H_{34}O_2$
3	Pentadecanoic acid	18.923	42.71	$C_{15}H_{30}O_2$
4	Methyl 10-octadecenoate	20.491	1.24	$C_{19}H_{36}O_2$
5	cis-Vaccenic acid	21.003	19.56	$C_{18}H_{34}O_2$
6	(E)-9-Octadecenoic acid ethyl ester	21.253	0.77	$C_{20}H_{38}O_2$
7	10-Methylicosane	26.167	2.00	$C_{21}H_{44}$
8	7-Hexylicosane	27.568	3.10	$C_{26}H_{54}$
9	Tetratetracontane	29.017	3.79	$C_{44}H_{90}$
10	Squalene	29.218	9.58	$C_{30}H_{50}$
11	Heneicosane	30.489	4.23	$C_{21}H_{44}$
12	2-methyloctacosane	32.063	3.32	$C_{29}H_{60}$

Table 2: Bioactivity of the compounds detected.

Sl. No.	Name of the compound	Biological activity	References
1	Methyl hexadecanoate	Antioxidant, hypocholesterolemic, nematicide & anti-androgenic	(Ayoola et al., 2020)
2	Pentadecanoic acid	Antifungal &Antimicrobial	(Godwin et al., 2015)
3	Methyl 10-octadecenoate	Antioxidant and antimicrobial	(Elaiyaraja & Chandramohan 2018)
4	cis-Vaccenic acid	Antibacterial	(Qadir et al., 2020)
5	(E)-9-Octadecenoic acid ethyl ester	Antioxidant &Antiinflammatory	(Ayoola et al., 2020)
6	10-Methylicosane	Antioxidant	(Ali et al., 2022)
7	7-Hexylicosane	Antioxidant	(Vinjamuri,2017)
8	Tetratetracontane	Antioxidant & Cytoprotective	(Amudha et al., 2018)
9	Squalene	Anti-oxidant, Anti-inflammatory & Antitumour	(Sonia & Singh, 2019).
10	Heneicosane	Antimicrobial activity	(Rhetso et al., 2020), (Vanitha et al., 2020)
11	2-methyloctacosane	Anti-microbial activity	(Rhetso et al., 2020)

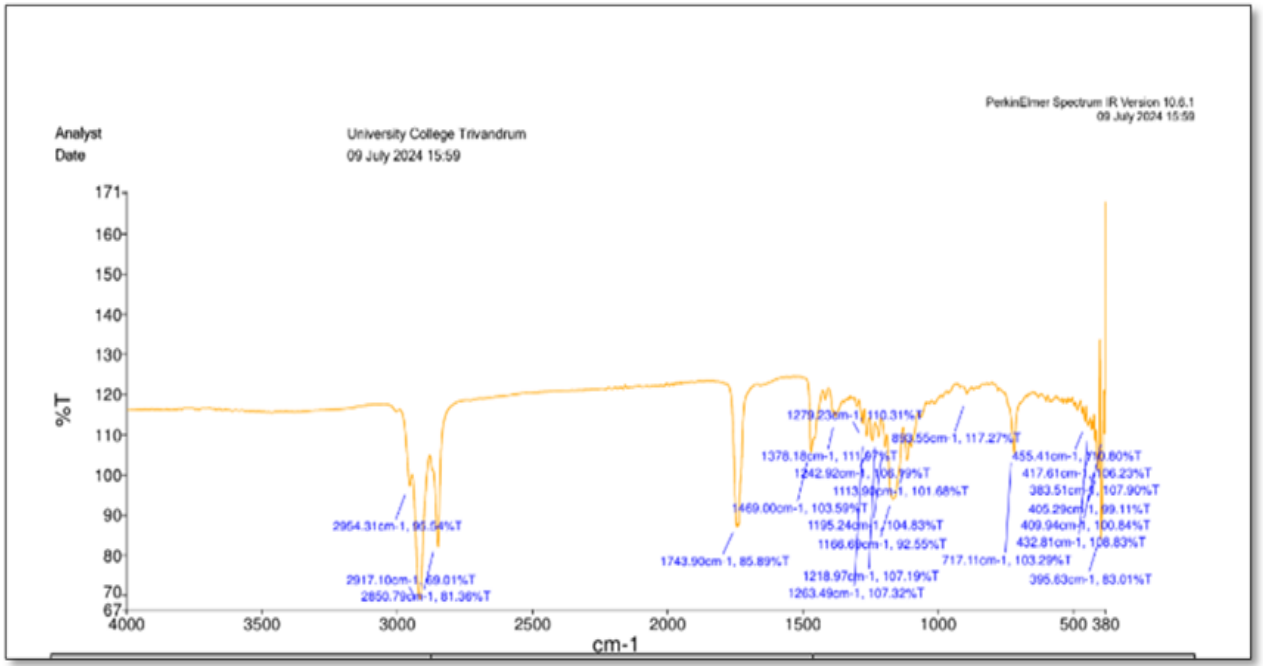


Fig. 4: FTIR spectrum of seed kernel oil extract of *B. Lanceolata*

(A) (B)

Fig. 5: Experimental setup for protein denaturation assay (A) Sample (B) Control

Table 3: Effect of (A) Seed kernel oil extract and (B) Standard drug, on the protein denaturation.

(A)

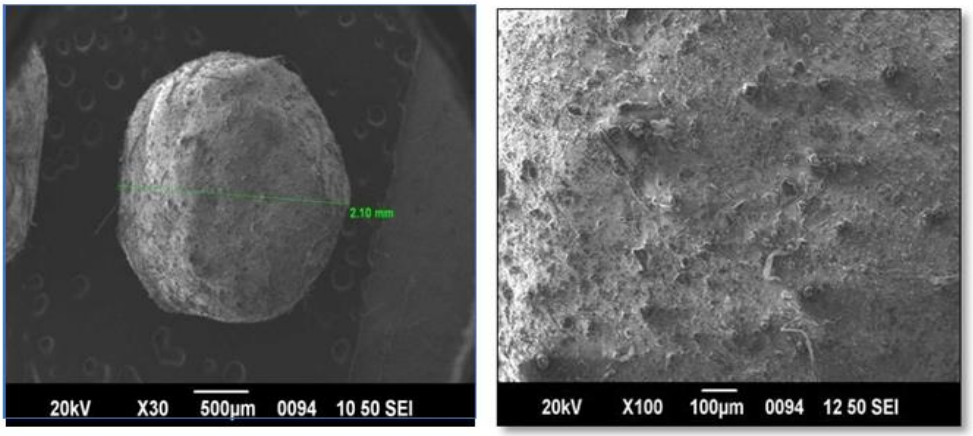
Sample	Volume of sample(µg/ml)	Absorbance at660nm	% of inhibition
Control		0.728	-
Hexane extract of seed kernel	6.25	0.623	14.42
	12.5	0.565	22.39
	25	0.508	30.22
	50	0.449	38.32
	100	0.338	53.57
IC 50	**88.29µg/ml**		

(B)

Standard	Concentration (µg/ml)	Absorbance at 660nm	% of inhibition
Control	-	0.787	
Diclofenac	6.25	0.725	7.88
	12.5	0.661	16.01
	25	0.545	30.75
	50	0.368	53.24
	100	0.125	84.12
IC50	**49.68µg/ml**		

(A) (B)

Fig. 6: (A) Sodium alginate beads loaded with seed kernel oil (B) Control- Sodium alginate gel beads,



(A) (B)

Fig. 7: SEM photographs of oil encapsulated sodium alginate beads.

CONCLUSION

In conclusion, this study successfully extracted seed kernel oil from *B. lanceolata*, with significant volatile bioactive compounds. *In vitro* anti-inflammatory property of the seed kernel oil tested through the protein denaturation assay revealed a concentration-dependent inhibition of protein denaturation highlighting the potential therapeutic benefits of the oil, particularly at lower doses. To enhance the stability and efficacy of the extracted oil, encapsulation in calcium alginate beads proved effective, safeguarding its active components from environmental degradation. Overall, the findings suggest that *B. lanceolata* seed kernel oil could be a valuable addition to the field of further research and development of therapeutic products.

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