

Research Article

Ultrasound-assisted extraction of *Nigella sativa* L essential oil using green solvent and its encapsulation in albumin nanoparticles

Ravichandran Kiruthika

Division of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore-641114, India

Jacob Anu *

Division of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore-641114, India

*Corresponding author. E-mail: anujacob@karunya.edu

Article Info<https://doi.org/10.31018/jans.v17i2.6541>

Received: January 09, 2025

Revised: June 04, 2025

Accepted: June 11, 2025

How to Cite

Kiruthika, R. and Anu, J. (2025). Ultrasound-assisted extraction of *Nigella sativa* L essential oil using green solvent and its encapsulation in albumin nanoparticles. *Journal of Applied and Natural Science*, 17(2), 894 - 902. <https://doi.org/10.31018/jans.v17i2.6541>

Abstract

The abundance of numerous bioactive compounds with a range of therapeutic benefits primarily distinguishes medicinal and aromatic plants. *Nigella sativa* (black cumin) is a medicinal plant species that has been well known for a variety of therapeutic uses. Health-conscious people, researchers, and the pharmaceutical industry are becoming more interested in black cumin, a highly valued nutraceutical herb with numerous health advantages. The present study evaluated the impact of both traditional and advanced extraction techniques on the chemical composition and bioactive properties of essential oils extracted from *Nigella sativa* L. seeds. Hexane and petroleum ether were the solvents used for conventional extraction, while natural deep eutectic solvents (NADES) were used for ultrasonic extraction. Ultrasonic extraction addresses the drawbacks of traditional solvent extraction. The hydrophobic properties of essential oils restrict their use in therapeutic settings. The essential oil extract encapsulated in albumin nanoparticles resolves this bottleneck. The results showed that NADES extraction enabled higher yields (37.1%) than essential oils obtained after Soxhlet extraction. Further, functional groups associated with the active ingredients with therapeutic activity were identified by Fourier Transform Infrared Spectroscopy (FTIR) analysis. Different chemical components with identified bioactivities were determined by the Gas Chromatography/Mass Spectrometry chromatograms (GC-MS). The yield (85 %) and extract trapping (89 %) in albumin nanoparticles were determined. The findings demonstrated that the average size of albumin nanoparticles grew from 72 nm to 102 nm for two distinct drug-loading procedures. The findings of the Scanning electron microscope (SEM) supported the spherical shape of the nanoparticles. The study will help *Nigella* extract loaded albumin nanoparticles to incorporate essential oil (EO) in food formulations and pharmaceutical preparations.

Keywords: Albumin nanoparticle, Encapsulation, Essential oil, Ultrasonic extraction**INTRODUCTION**

Nigella sativa L. is an annual herbaceous plant in the Ranunculaceae family. It is an aromatic plant with a historical and religious background, used for culinary purposes and therapeutics for decades. Black cumin, *Nigella*, or kalonji are common names for *N. sativa* (Zouirech *et al.*, 2022). The specific bioactive composition imparts the seeds with numerous pharmacological properties, such as cardioprotective (Ali *et al.*, 2022), immune-modulator (Niu *et al.*, 2021), antioxidant (Dalli *et al.*, 2021), antiviral (Maideen *et al.*, 2023), antimicrobial (Shafodino *et al.*, 2022), anti-inflammatory (Setiawatie *et al.*, 2022), and anticancer (Manjunath *et al.*, 2020). *Nigella* seed oil treats respiratory conditions,

including asthma, emphysema, and bronchitis (Saadat *et al.*, 2021). The *Nigella* seed contains mainly protein, fixed oil, alkaloids, and saponins (Telci *et al.*, 2023). The oil extracted contains thymoquinone (TQ) as one of its bioactive components (Shad *et al.*, 2021). TQ and other *N. sativa* constituents, including p-cymene, monoterpenes, thydrothymoquinone, thymol, carvacrol and α -pinene have exhibited bioactivities.

The method of seed oil extraction plays a significant role in the characteristics of the oils. According to the literature, the oil has typically been generated using a hot solvent extraction process employing the Soxhlet extractor at 40–60 °C and even at 70 °C (Liu, X. *et al.*, 2011). The hot solvent extraction process may alter oil characteristics, and most minor compounds have func-

tional, antioxidative, and pro-oxidative actions (Solati *et al.*, 2014). While organic solvents are very effective in extracting phytochemicals, they have several inherent disadvantages, including accumulation in the atmosphere (low boiling temperatures), flammability, high toxicity, non-biodegradability, and expense. Additionally, these solvents can no longer follow the trend of green chemistry from an environmental perspective. For all of these reasons, new environmentally friendly solvents and alternative technologies are of research interest to extract phytochemicals from plants.

Recently, several environment-friendly techniques have been in practice for essential oil extraction from plant sources, including supercritical fluid extraction (Xiong and Chen, 2020), microwave-assisted extraction (Yingngam *et al.*, 2021), and ultrasound-assisted extraction (Chen *et al.*, 2022). Among these methods, ultrasound-assisted extraction (UAE) offers the benefits of reduced extraction period, improved yield, enhanced bioactivity and minimum loss of oil. It involves applying high-intensity sound wave frequencies on the plant material in a solvent, resulting in cavitation phenomena that release phytochemicals. Dar and colleagues (Dar *et al.*, 2024) optimised the UAE extraction of *Nigella sativa* essential oil, resulting in a maximum yield of 34.53%. However, they used hexane as the solvent in the extraction studies.

Innovative solvents such as ionic liquids, deep eutectic solvents and natural deep eutectic solvents contribute significantly to sustainable, green and energy-efficient extraction procedures. The Natural Deep Eutectic Solvent (NADES) consists of primary metabolites (Eg: sugars, small organic acids, bases, sugar alcohols and amino acids) in distinct molar ratios. These natural compounds are combined with the proper amount of water after physical grinding at specific temperatures to form NADES (Choi *et al.*, 2011). Its properties include non-volatility, renewability with a broad spectrum of polarity, non-toxicity and biodegradability (Mehariya *et al.*, 2021). Sharma and colleagues (Sharma *et al.*, 2023) obtained a better yield of essential oil (EO) when extracted with NADES constituting choline chloride and maleic acid (2:1) compared to the conventional hydro-distillation method from the roots of *Nardostachys jatamansi* (D.Don) DC.

Nigella oil has long been used in traditional therapeutics. Nevertheless, the limited water solubility, instability, and low bioavailability constrain the application. A potential solution to these problems is encapsulating the compounds in a suitable matrix (Fatmi *et al.*, 2024). Encapsulation methods based on albumin micro- and nanoparticles deliver several therapeutic compounds to the target site. Albumin particles shield the therapeutic molecules from deterioration and better absorption. Moreover, it enhances intracellular absorption and circulation in the body and modifies the pharmacokinetic parameters (Tao *et al.*, 2019). Badri (Badri *et al.*, 2018)

prepared poly (ϵ -caprolactone) based nanoparticles to encapsulate *Nigella sativa* L. seeds essential oil and indomethacin to improve the analgesic and anti-inflammatory properties of indomethacin. They attained an essential oil encapsulation efficiency of 84%. The EO encapsulation and indomethacin improved its anti-inflammatory and analgesic effects.

The present study explored ultrasound-assisted extraction of *Nigella sativa* essential oil using different NADES compositions and analysed their chemical constituents in maximum yield extraction using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) methods. The study also attempted to encapsulate the oil in albumin nanoparticles using the coacervation-crosslinking method.

MATERIALS AND METHODS

Sample preparation and physicochemical characterization of seed

Nigella sativa L. seeds were procured from Coimbatore. The seeds were cleaned, washed with water, and dried. Seed samples were ground into a powder for further study. The following physicochemical analyses were done

Moisture content

The Association of Official Analytical Chemists (AOAC) Technique No. 984.25 (Firestone, 2005) was used to calculate the moisture content.

$$MC (\%) = (M1-M2) / (M1-M0) \times 100 \quad \dots \text{Eq. 1}$$

Where, M0 = mass of a dry, tarred petri dish, in grams, M1 = mass in grams of an undried sample in a dried, tarred petri dish

M2 = dry, tarred petri dish's mass in grams.

Fat content

Oil was extracted from 15 g of seed powder in a Soxhlet extractor using petroleum ether (40–60°C) as the solvent for 8 hours. The proportion of lipids (on a wet weight basis) was used to represent the result.

Protein content

The total protein was estimated using Lowry's method.

Ash content

Powdered seed samples weighing around 0.5 g were burned in a muffle furnace for around 12 hours at 550°C. The ash was expressed in percentage on a wet weight basis.

Carbohydrate content

The difference between the mean readings, or [100 - (protein + fats + ash + moisture)], was used to determine the amount of carbohydrates.

Extraction of the oil constituents

The oil constituents from the seeds were extracted using Solid-liquid extraction, Soxhlet extraction, and Ultrasound-assisted extraction.

Solid liquid extraction

A flask containing 50 g of *Nigella* seed powder was homogenised using 250 ml of hexane. After mixing for 4 hours at 180 rpm in a shaker, it was centrifuged at 8000 rpm for 15 min at room temperature. After that, it was filtered to obtain the extract and concentrated in a rotary evaporator for 2h.

Soxhlet extraction

In this process, 15g of powdered seeds were immersed in 50 ml of absolute petroleum ether at 80°C for 2h, and then the extract was concentrated in a rotary evaporator for 2h.

Natural deep eutectic solvent preparation and extraction

NADES consisting of Citric acid and D-(+)-glucose were prepared with different combinations as in Table 1. Since NADES are viscous, a small quantity of water is required to keep the liquid state. According to previous studies, supramolecular complex structures of NADES are retained in water with a concentration of 50 g/100 g or less and further dilution results in structural breakdown. De-ionized water was therefore, introduced to the experiment in the right quantity.

The extraction procedure in brief, in a beaker, 7.2 g of the NADES components combined with 4.8 g of de-ionized water produced a translucent liquid at 80°C water bath and 100 W ultrasonic treatments for 15 minutes. The NADES was mixed with 2 g of dried seed powder and extracted at 600 W for 15 min in an ultrasonic extraction device. (Yulianita and Monica., 2023)

Characterisation of extracted oil

The fixed oil extracted was characterised according to the AOCS technique (AOCS., 2004) to assess the Peroxide Values (PV), Saponification value (SV) and free fatty acid (FFA).

Determination of oil yield

The oil yield was determined using the following formula:

$$\text{Yield of EO (\%)} = (\text{WE/WS}) \times 100\% \quad \dots\dots \text{Eq. 2}$$

Where, WE is the weight of extracted seed oil in g, and WS is the weight of seed (g)

Characterisation of oil- Fourier Transform Infrared Spectroscopy and Gas Chromatography/Mass Spectrometry Analysis

The FTIR spectra of the sample with maximum oil yield were recorded in the region of 4000-400 cm⁻¹ on an IR

Prestige 21 Fourier Transform Infrared Spectrum (Shimadzu) housed at 25°C, 50% relative humidity. The sample with maximum oil yield was analysed by Agilent GC 7890A / MS5975C. Chromatographic separation was done with an Agilent DB5MS Capillary column (30m / 0.25mm internal dia / 0.25 micron film thickness).

Ultraviolet-Visible Spectroscopy analysis

UV double beam spectrometer was used to scan the wavelength of extract from 200 nm up to 400 nm. This was to determine the maximum wavelength of absorbance value for use as detection wavelength in the UV analysis method.

The technique's consistency, sensitivity, precision, and accuracy were all validated. The solvent used was chloroform to minimise the inconsistency incurred by the solvent volatility (Ismail *et al.*, 2015).

Preparation of the formulation

Albumin- nanoparticles were synthesized using the coacervation-crosslinking method (Rahimnejad *et al.*, 2012). Initially, 50 mg of bovine serum albumin was dissolved in 2 mL, 10 mM NaCl solution and was then agitated for 15 minutes at room temperature. After bringing the BSA solution's pH to 8.0, ethanol was introduced drop-wise (1 mL/min). To stabilise the BSA nanoparticles, glutaraldehyde aqueous solution (8%) was added after the solution had been agitated for 3 hours at room temperature. The nanoparticle suspension was purified by centrifugation (12000 rpm, 30 min) three times after stirring for 24 hours at room temperature.

Essential oil loading into the nanoparticle was carried out by two approaches: Model F1 was obtained by the incubation of albumin NPs with Essential oil (concentration of 2mg/l) under magnetic stirring for 2h at 500 rpm. For Model F2, the same concentration of essential oil was incubated with albumin-NPs while shaking for 2h at room temperature (Honary *et al.*, 2010). Albumin particles were also prepared using the same procedure without oil loading. The essential oil-loaded nanoparticles were analysed for their size, shape and morphology by Scanning Electron Microscopy.

Yield of Essential oil -albumin nanoparticle

At the end of the formulation process, the nanoparticle formulation was centrifuged, the debris was removed, washed, and centrifuged (12000 rpm) twice to produce a pellet. This pellet of nanoparticles was weighed, and the following equation was used to get the per cent yield (Jithan *et al.*, 2011).

$$\text{Percent yield (\%)} = \text{Weight of the nanoparticle/ Total weight of drug+ polymer} \times 100 \quad \dots\dots \text{Eq. 3}$$

Determination of drug entrapment

To determine the amount of extract loading in nanopar-

Table 1. Preparation of Natural deep eutectic solvents (NADES): Solvent groups, components and molar ratios

Solvent abbreviation	NADES composition (molar ratio) (D- (+)-Glucose: Citric Acid)
GluCA-1	1:1
GluCA-2	1:2
GluCA-3	2:1
GluCA-4	1:3
GluCA-5	3:1

ticles, 3 ml of essential oil-loaded nanoparticle suspension was centrifuged (20000 rpm/45 min/4°C). The amount of drug present in the supernatant (obtained at the end of preparation of nanoparticles) was determined (w) by UV-spectrophotometer. A standard calibration curve of concentration vs. absorbance was plotted for this purpose. The amount of drug in the supernatant was then subtracted from the total amount of drug added during the desolvation process (W). (W-w) will give the amount of drug entrapped in the pellet (Singh and Chaudhary, 2010).

Then percentage entrapment is given by $(W-w)/W \times 100$.
..... Eq. 4

Thin-Layer Chromatography(TLC) experiment

TLC is a quick, cost-effective method to separate the components based on polarity using a silica-coated plate and organic solvents. A TLC analysis was performed on a piece of silica sheet (2 mm), pre-coated with silica gel 60 G. In the mobile phase, a 7/3 v/v combination of dichloromethane and methanol was used. The samples examined were (a) Loaded nanoparticles method F1; (b) Loaded nanoparticles method F2; (c) Physical mixture empty nanoparticle/essential oil. The spots were detected at UV light (Honary et al., 2010).

RESULTS AND DISCUSSION

Proximate analysis of *Nigella* seed powder

The proximate analysis of *Nigella* seed powder is represented in Table 2 and follows the previous studies. The lipid content (35%) extracted with petroleum ether was a major component in the seed. The literature shows that the moisture content varied from 5.40 to 7.00%, their ether-extractable lipids from 34.80 to 37.33%, their crude protein ranged from 20.02 to 21.20%, their ash ranged from 3.70 to 6.70 %, and their carbohydrates ranged from 30 to 34% (Atta, 2003; Khoddami et al., 2011). In this instance, the ash level (5.24 %) was greater than the cases described in the literature, while the protein content (21.51%) was a little lower than the cases identified in the literature. The geographic areas where the black cumin seed grows impact the oil properties(Choudhury et al., 2023).

Comparison of the oil yield with different solvents

The oil from the seed was extracted using different methods and different solvents. Table 3 displays the oil yields from three distinct extraction techniques. Ultra-sound-assisted extraction using NADES (GluCA-3) yielded a maximum oil (37.1%) compared to other techniques. The difference in yield proves NADES's superior capacity to extract certain lipid components from *Nigella* seeds compared to other extraction techniques and its shorter extraction time (30 mins).

Table 4 shows the physicochemical parameters of the seed oil and the most significant indicators of seed oil quality are FFA and PV. The hydrolysis of oil fat produces free fatty acid. The FFA is more susceptible to oxidation and instability than the neutral oil. A lower FFA (5.26 %) value for UAE (GluCA-3) indicates better stability than the other methods. Similarly, peroxide value reflects the level of oil oxidation, and high PV is unstable. The lowest PV (8.26 meq/kg) is observed in the oil obtained by UAE (GluCA-3). The saponification value predicts the type of triacylglycerols in the oil. A high SV (185.23 mg/100g) indicates high TAGs content in oil. According to the literature, the SV of *Nigella* oil varies from 172.6 to 211 mg/100 g (Farhan et al., 2021).

Functional groups in the oil

The FTIR spectrum of the UAE (GluCA-3, GluCA-1, and GluCA-5) oil samples in the range of 400 to 4000 cm^{-1} represents the functional groups (Fig. 1 and Table 5). GluCA-1 and GluCA-3 samples showed similar peaks, while GluCA-5 showed minor differences in the peaks.

GC-MS analysis

The isolation of essential oil by GluCA-3 showed a yield of 37.1%, and Fig. 2 represents its GC-MS analysis. It resulted in about 25 peaks and Table 6 lists the major compounds found in the extract.

Preparation of extract-loaded albumin nanoparticles

Protein nanoparticles have great potential as drug carriers compared to alternative drug delivery methods, as they are simple to scale up in the production process (Karimi et al., 2016). The properties of albumin such as

Table 2. Proximate Analysis of *Nigella* seed powder

Proximate composition	Determined value (%)
Moisture	5 ± 0.23
Fat	35.97 ± 0.38
Protein	21.51 ± 0.11
Ash	5.24 ± 0.46
Carbohydrate	32.16 ± 0.32

Table 3. Characteristics of *Nigella* seed oil obtained by different extraction methods

Extraction methods	Solvent	Extraction period (min)	Yield (%)	Colour
Solid Liquid Extraction	Hexane	240	31.76±0.12	Pale yellow oil
Soxhlet Extraction	Petroleum ether	120	35.97±0.46	Brown-yellow oil
Ultrasound Assisted Extraction	GluCA-1	30	36.2 ±0.23	Pale Yellow oil
	GluCA-2		30.7 ±0.02	
	GluCA-3		37.1 ±0.38	
	GluCA-4		32.2 ±0.14	
	GluCA-5		36.6 ±0.2	

Table 4. Effects of extraction on physicochemical parameters of *Nigella* seed oil

Physicochemical parameters	Extraction methods		
	Hexane	Soxhlet	UAE (GluCA-3)
FFA (as oleic %)	10.32±0.22	7.22 ±0.12	5.26 ±0.21
PV (meq/kg)	12.62 ±0.3	9.23 ±0.34	8.26 ±0.4
SV(mg/100 g)	196.25 ±0.26	192.45 ±0.28	185.23 ±0.42

Table 5. FT-IR spectrum of UAE samples

S.NO.	Peak value	Bond	Functional group	GluCA-1	GluCA-3	GluCA-5
1.	3448/ 3450	OH	polyphenols	Yes	Yes	Yes
2.	1637	C=C Stretch	Alkene	Yes	Yes	
3.	1463	CH bonding	Alkane	Yes	Yes	Yes
4.	1548	N-O stretching	Nitro compound			Yes
5.	1039	OH bending	Alcohol	Yes	Yes	
6.	677	C-C Bending	Alkene			Yes

biodegradability, biocompatibility, metabolism *in vivo* to yield harmless products, ease in purification and drug delivery and water solvability, make it a desirable macromolecular carrier (Elzoghby *et al.*, 2012). Bovine serum albumin is a protein, and the pH of the medium influences its crosslinking with glutaraldehyde. Roughly 90% of the free amino acids in the bovine serum albumin protein could keep the protein functioning (Zhao *et al.*, 2010). In the present study, 8% of glutaraldehyde resulted in the formation of nanoparticles. The functional groups on albumin NPs make surface modification easier when utilising different methods, including conjugation, coating, or electrostatic adsorption (Tan and Ho, 2018).

Albumin nanoparticles were morphologically characterised using SEM, and the results revealed the morphology of particles. EO-loaded albumin nanoparticles made using models F1 and F2 are shown in Fig 3 as SEM micrographs. The micrographs showed that both methods resulted in nanometer scale and rough surface particles. The rough surface is due to the loading of essential oil in the nanoparticle. The morphology of particles in model F2 is mostly spherical or irregularly rounded. Meanwhile, the particles in model F1 were

spherical to semi-spherical in shape.

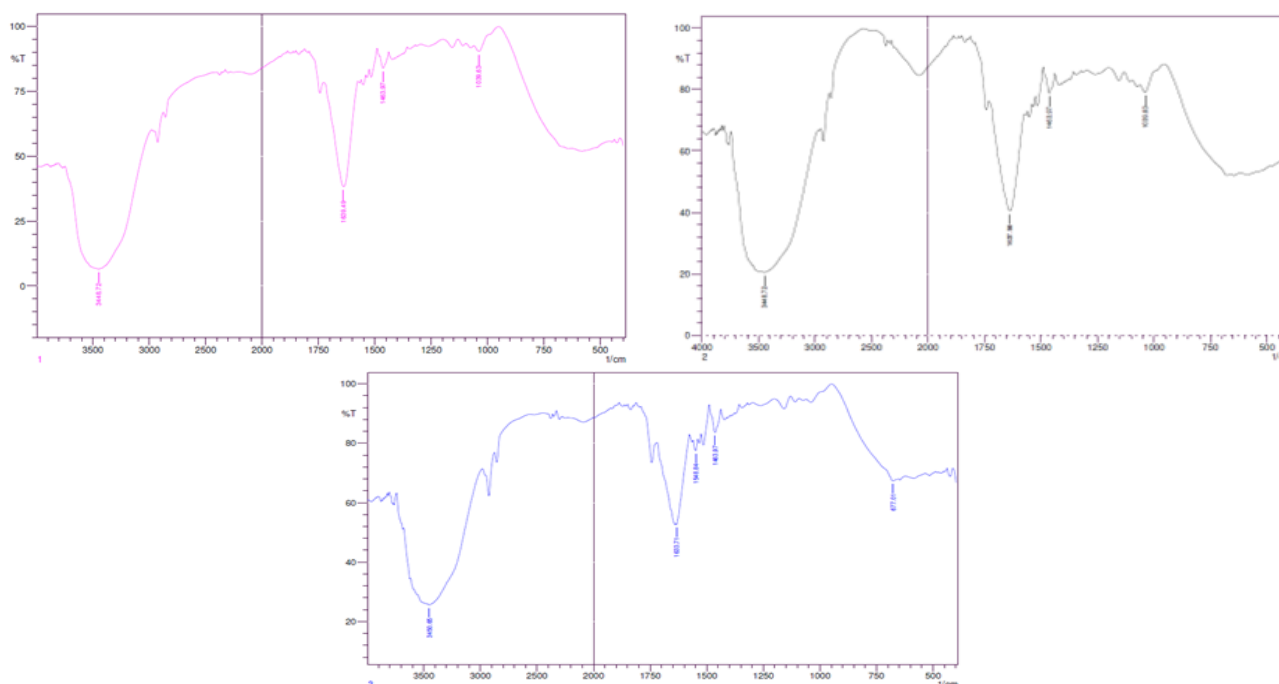
The encapsulation of palladium complex in BSA nanoparticles has been reported. The comparison of morphology by Field emission scanning electron microscopy analysis showed spherical, smooth particles for BSA-NPs, while loading the palladium complex altered the overall morphology to develop a rough surface and slightly increased in size (Karami *et al.*, 2020). Sadeghzadeh and coworkers prepared albumin nanoparticles loaded with anethole, a phytochemical for cancer treatment. They further modified the surface of the nanoparticle with chitosan attached to folate. The chitosan addition improved the mucoadhesive and permeation properties of the nanoparticle, and folate helped target the folate receptor, which overexpressed tumors. Its SEM examination demonstrated a smooth surface with diverse shapes and multiple facets displaying a heterogeneous and complex morphology (Sadeghzadeh *et al.*, 2023).

Yield and Entrapment efficiency of Essential oil (EO)-albumin nanoparticles

Table 7 shows the nanoparticle yields from two different methods, and the maximum yield is from model F1.

Table 6. Chemical composition of *Nigella* seed oil

Retention time	Area percentage	Compound	Description
7.431	56.29	Phenylethyl alcohol	A colourless liquid with a pleasant floral odor. It is found in a variety of essential oils.
8.431	5.13	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	An isomeric hydrocarbons that are classified as monoterpenes found in plant essential oils
9.309	2.43	2,5-Diethylphenol	phenolic compound
9.864	2.76	Thymol	Monoterpene derivative of cymene. It has a role as a volatile oil component. It derives from a hydride of a p-cymene.
12.675	4.28	5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)-	Monoterpenoids
15.441	2.09	2-Ethylacridine	derived from isoquinoline alkaloids
16.674	1.06	Pentacosanoic acid, 2,10-dimethyl-, methyl ester,	Methyl ester of pentacosanoic acid.
19.974	1.17	Benzo[h]quinoline, 2,4-dimethyl-	Alkaloids
22.396	4.96	Benzo[h]quinoline	Alkaloids
22.807	3.2	Benzo[h]quinoline	Alkaloids

**Fig. 1.** FTIR spectrum of UAE samples 1) GluCA-3, 2) GluCA-1, and 3) GluCA-5

It shows continuous stirring for 2 hours gives a better yield than the shaking method. A similar observation was made by (Honary *et al.*, 2010) for the encapsulation of Doxorubicin.

In the UV analysis, the maximum wavelength of absorption is 271 nm. The standard plot generated for different concentrations of EO at 271 nm was linear and used to determine the concentration of EO and calculate the entrapment efficiency.

Analysis by Thin-Layer Chromatography

Two spots were found for both drug-loading models. A

spot on the origin attributed to the drug associated with the carrier and a spot with an $R_f = 0.69$ indicated that free and/or poorly combined extract with albumin in the matrix was present in the loaded nanoparticles. As previously stated (Merodio *et al.*, 2001), EO, which was entrapped in the nanoparticles and released in a sustained way slowly over days due to extract being covalently bonded to polymer fragments, may be responsible for the spot on the origin. Due to the absence of such limitations, as shown in Fig. 4, no spot was seen on the origin for the physical mixing of extract and albumin nanoparticles. In thin-layer chromatography, phe-

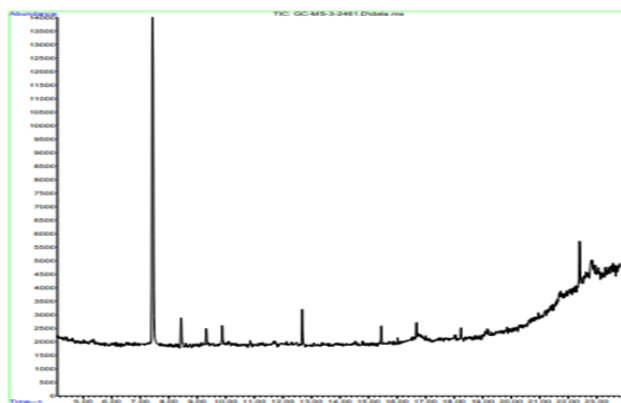


Fig. 2. GC-MS result of NADES Extracted oil of *Nigella* seeds

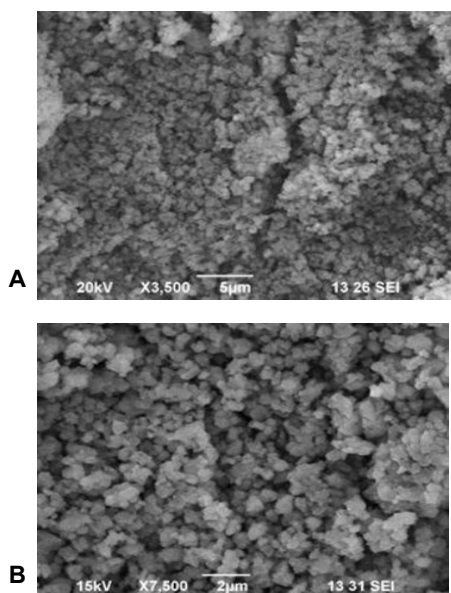


Fig. 3. Scanning electron micrograph of essential oil loaded albumin nanoparticles. (A) F1 method, (B) F2 method. F1 is heterogeneous with spherical to semi-spherical particles, and F2 is mostly spherical

nolic and other aromatic chemicals in EO and EO cause the characteristic fluorescence when observed under UV and blue light excitation (Botnick *et al.*, 2012).

Conclusion

The present study used an ultrasound-assisted method employing glucose and citric acid-based NADES to extract essential oil from *Nigella sativa*. Among the different molar compositions of NADESs used, Glucose: Citric Acid in the ratio of 2:1 resulted in maximum yield in extraction. The study also revealed enhanced extraction yield for NADES (37.1%) compared to the conventional methods, including Solid Liquid Extraction and Soxhlet Extraction. The FTIR analysis of UAE samples revealed the presence of functional groups of polyphenols and other phytochemicals. The GCMS analysis of

Table 7. Yield and entrapment efficiency of Essential oil-Albumin nanoparticles

MODELS	YIELD (%)	ENTRAPMENT EFFICIENCY (%)
F1	85.74±0.56	89.72±1.24
F2	82.28±0.68	78.26±1.78

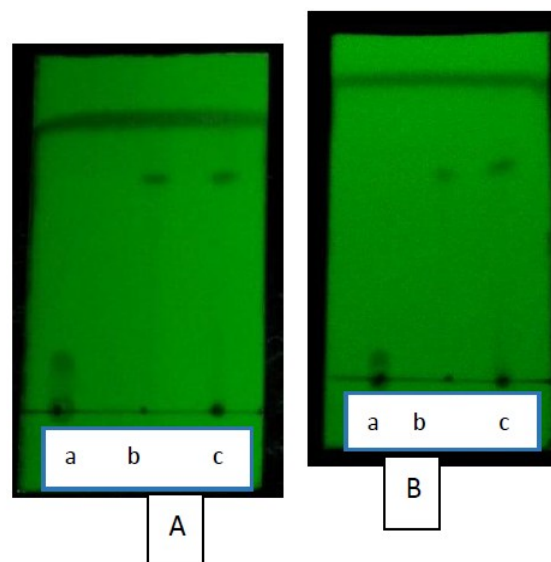


Fig. 4. TLC for determining the type of interaction between the extract and albumin nanoparticles: (A) (a) extract loaded nanoparticles (F1), (b) extract solution, (c) Physical mixture between unloaded nanoparticles and EO; (B): (a) extract loaded nanoparticles (F2), (b) Extract solution, (c) Physical mixture between unloaded nanoparticles and EO; The difference in the band retention factor for the three samples in F1 and F2 indicates successful loading of essential oil

the volatile metabolites sample revealed the presence of terpenes, phenolic compounds, and alkaloids. The present study also showed that the Coacervation method can prepare albumin nanoparticles. It also showed that The stirring method resulted in smaller and homogeneous EO-loaded albumin nanoparticles with a high entrapment efficiency of 89%. The EO-loaded nanoparticles prepared in this study demonstrate potential for therapeutic applications, necessitating further investigation to evaluate their efficacy and safety in vivo.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ali, S., Hashim, A., Shiekh, A., Majid, S., & Rehman, M. U. (2022). The cardioprotective effect of thymoquinone from *Nigella sativa*. In *Black Seeds (Nigella Sativa)* (pp. 239–252). Elsevier. <https://doi.org/10.1016/B978-0-12-824462->

- 3.00006-8
2. AOCS. (2004). *Official methods and recommended practices of the American Oil Chemists' Society*. AOCS Publishing.
3. Atta, M. B. (2003). Some characteristics of *Nigella* (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. *Food Chemistry*, 83, 63–68. [https://doi.org/10.1016/S0308-8146\(03\)00038-4](https://doi.org/10.1016/S0308-8146(03)00038-4)
4. Badri, W., El Asbahani, A., Miladi, K., Baraket, A., Agusti, G., Nazari, Q. A., & Elaissari, A. (2018). Poly (ϵ -caprolactone) nanoparticles loaded with indomethacin and *Nigella sativa* L. essential oil for the topical treatment of inflammation. *Journal of Drug Delivery Science and Technology*, 46, 234–242. <https://doi.org/10.1016/j.jddst.2018.05.022>
5. Botnick, I., Xue, W., Bar, E., Ibdah, M., Schwartz, A., Joel, D. M., Lev, E., Fait, A., & Lewinsohn, E. (2012). Distribution of primary and specialized metabolites in *Nigella sativa* seeds, a spice with vast traditional and historical uses. *Molecules*, 17(9), 10159–10177. <https://doi.org/10.3390/molecules170910159>
6. Chen, Z., Wu, K., Zhu, W., Wang, Y., Su, C., & Yi, F. (2022). Chemical compositions and bioactivities of essential oil from perilla leaf (*Perillae Folium*) obtained by ultrasonic-assisted hydro-distillation with natural deep eutectic solvents. *Food Chemistry*, 375, 131834. <https://doi.org/10.1016/j.foodchem.2021.131834>
7. Choi, Y. H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W., & Verpoorte, R. (2011). Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology*, 156(4), 1701–1705. <https://doi.org/10.1104/pp.111.178426>
8. Choudhury, M. S., Islam, M. N., Khan, M. M., Ahiduz-zaman, M., Masum, M. M. I., & Ali, M. A. (2023). Effect of extraction methods on physical and chemical properties and shelf life of black cumin (*Nigella sativa* L.) oil. *Journal of Agriculture and Food Research*, 14, 100836. <https://doi.org/10.1016/j.jafr.2023.100836>
9. Dalli, M., Azizi, S. E., Kandsi, F., & Gseyra, N. (2021). Evaluation of the in vitro antioxidant activity of different extracts of *Nigella sativa* L. seeds, and the quantification of their bioactive compounds. *Materials Today: Proceedings*, 45, 7259-7263. <https://doi.org/10.1016/j.matpr.2020.12.743>
10. Dar, I. H., Junaid, P. M., Ahmad, S., Shams, R., Dash, K. K., Shaikh, A. M., & Béla, K. (2024). Optimization of ultrasound-assisted extraction of *Nigella sativa* seed oil for enhancement of yield and antioxidant activity. *Discover Applied Sciences*, 6(3), 104. | <https://doi.org/10.1007/s42452-024-05714-7>
11. Elzoghby, A. O., Samy, W. M., & Elgindy, N. A. (2012). Albumin-based nanoparticles as potential controlled release drug delivery systems. *Journal of Controlled Release*, 157, 168–82. <https://doi.org/10.1016/j.jconrel.2011.07.031>
12. Farhan, N., Salih, N., & Salimon, J. (2021). Physicochemical properties of Saudi *Nigella sativa* L. ('Black cumin') seed oil. *OCL*, 28(11), 1–9. <https://doi.org/10.1051/ocl/2020075>
13. Fatmi, S., Taouzinet, L., Lezreg, A., Pokajewicz, K., Toutou, Z., Skiba, M., & Iguerouada, M. (2024). Advances and Trends in the Encapsulation of *Nigella sativa* Oil and Essential Oil Using Cyclodextrins and Liposomes: a Review. *BioNanoScience*, 1–18. <https://doi.org/10.1007/s12668-024-01463-4>
14. Firestone, D. (2005). AOAC. In *Association of the Official Analytical Chemists*.
15. Honary, S., Jahanshahi, M., Golbayani, P., Ebrahimi, P., & Ghajar, K. (2010). Doxorubicin-loaded albumin nanoparticles: formulation and characterization. *Journal of Nanoscience and Nanotechnology*, 10(11), 7752–7757. <https://doi.org/10.1166/jnn.2010.2832>
16. Ismail, A. F. H., Mohamed, F., Mansor, N., Shafri, M. A. M., & Yusof, F. A. (2015). Method development and validation using UV spectrophotometry for *Nigella sativa* oil microparticles quantification. *Journal of Applied Pharmaceutical Science*, 5(9), 082-088. DOI: 10.7324/JAPS.2015.50915
17. Jithan, A. V., Madhavi, K., Madhavi, M., & Prabhakar, K. (2011). Preparation and characterization of albumin nanoparticles encapsulating curcumin intended for the treatment of breast cancer. *International Journal of Pharmaceutical Investigation*, 1(2), 119–125. doi: 10.4103/2230-973X.82432
18. Karami, K., Jamshidian, N., Hajiaghasia, A., & Amirghofran, Z. (2020). BSA nanoparticles as controlled release carriers for isophthalaldoxime palladacycle complex; synthesis, characterization, in vitro evaluation, cytotoxicity and release kinetics analysis. *New Journal of Chemistry*, 44(11), 4394–4405. <https://doi.org/10.1039/C9NJ05847H>
19. Karimi, M., Bahrami, S., Ravari, S. B., Zangabad, P. S., Mirshekari, H., Bozorgomid, M., Shahreza, S., Sori, M., & Hamblin, M. R. (2016). Albumin nanostructures as advanced drug delivery systems. *Expert Opinion on Drug Delivery*, 13(11), 1609–1623. <https://doi.org/10.1080/17425247.2016.1193149>
20. Khoddami, A., Ghazali, H. M., Yassoralipour, A., Ramakrishnan, Y., & Ganjloo, A. (2011). Physicochemical characteristics of *Nigella* seed (*Nigella sativa* L.) oil as affected by different extraction methods. *Journal of the American Oil Chemists' Society*, 88, 533–540. <https://doi.org/10.1007/s11746-010-1687-6>
21. Liu, X., M., Abd El-Aty, A., & Shim, J. H. (2011). Various extraction and analytical techniques for isolation and identification of secondary metabolites from *Nigella sativa* seeds. *Mini Reviews in Medicinal Chemistry*, 11(11), 947–955. <https://doi.org/10.2174/138955711797068472>
22. Maideen, N. M. P., Hadda, T. B., Almalki, F. A., Laarousi, H., Soliman, S. S., & Kawsar, S. (2023). Black seeds (*Nigella sativa*) for the management of dengue viral disease: insight into the evidence and POM analyses for the identification of antiviral pharmacophore sites: a review. *Journal of Medicinal Herbs*, 14(1), 19–36. <https://doi.org/10.30495/medherb.2023.702425>
23. Manjunath, N. S., Rangaswamy, B. E., Hafsa, J., Ganavi, D., Sahana, J. K., & Ullas, K. (2020). Evaluation of *Nigella sativa* (Black cumin) for anticancer and anti-inflammatory activities. *International Journal of Herbal Medicine*, 8(5), 01–09.
24. Mehariya, S., Fratini, F., Lavecchia, R., & Zuurro, A. (2021). Green extraction of value-added compounds from microalgae: A short review on natural deep eutectic solvents (NaDES) and related pre-treatments. *Journal of Environmental Chemical Engineering*, 9(5), 105989.

- <https://doi.org/10.1016/j.jece.2021.105989>
25. Merodio, M., Arnedo, A., Renedo, M. J., & Irache, J. M. (2001). Ganciclovir-loaded albumin nanoparticles: characterization and in vitro release properties. *European Journal of Pharmaceutical Sciences*, 12(3), 251–259. [https://doi.org/10.1016/S0928-0987\(00\)00169-X](https://doi.org/10.1016/S0928-0987(00)00169-X)
26. Niu, Y., Wang, B., Zhou, L., Ma, C., Waterhouse, G. I., Liu, Z., & Kang, W. (2021). *Nigella sativa*: A dietary supplement as an immune-modulator on the basis of bioactive components. *Frontiers in Nutrition*, 8, 722813. <https://doi.org/10.3389/fnut.2021.722813>
27. Rahimnejad, M., Najafpour, G., & Bakeri, G. (2012). Investigation and modeling effective parameters influencing the size of BSA protein nanoparticles as colloidal carrier. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 412, 96–100. <https://doi.org/10.1016/j.colsurfa.2012.07.022>
28. Saadat, S., Aslani, M. R., Ghorani, V., Keyhanmanesh, R., & Boskabady, M. H. (2021). The effects of *Nigella sativa* on respiratory, allergic and immunologic disorders, evidence from experimental and clinical studies, a comprehensive and updated review. *Phytotherapy Research*, 35(6), 2968–2996. <https://doi.org/10.1002/ptr.7003>
29. Sadeghzadeh, F., Haghighi, H. N., Ghiyamati, M., Hajizadenadaf, F., & Tabrizi, H. M. (2023). *In vitro* and *in vivo* study on the anticancer effects of anethole-loaded bovine serum albumin nanoparticles surface decorated with chitosan and folic acid. *Cancer Nanotechnology*, 14(24), 1–15. <https://doi.org/10.1186/s12645-023-00181-y>
30. Setiawatie, E. M., Gani, M. A., Rahayu, R. P., Ulfah, N., Kurnia, S., Augustina, E. F., & Sari, D. S. (2022). *Nigella sativa* toothpaste promotes anti-inflammatory and anti-destructive effects in a rat model of periodontitis. *Archives of Oral Biology*, 137, 105396. <https://doi.org/10.1016/j.archoralbio.2022.105396>
31. Shad, K. F., Soubra, W., & Cordato, D. J. (2021). The role of thymoquinone, a major constituent of *Nigella sativa*, in the treatment of inflammatory and infectious diseases. *Clinical and Experimental Pharmacology and Physiology*, 48(11), 1445–1453. <https://doi.org/10.1111/1440-1681.13553>
32. Shafodino, F. S., Lusilao, J. M., & Mwapagha, L. M. (2022). Phytochemical characterization and antimicrobial activity of *Nigella sativa* seeds. *PloS One*, 17(8), e0272457. <https://doi.org/10.1371/journal.pone.0272457>
33. Sharma, M., Arokiyaraj, C., Rana, S., Sharma, U., & Reddy, S. E. (2023). Natural deep eutectic solvents (NADESs) assisted extraction of essential oil from *Nardostachys jatamansi* (D. Don) DC with insecticidal activities. *Industrial Crops and Products*, 202, 117040. <https://doi.org/10.1016/j.indcrop.2023.117040>
34. Singh, V., & Chaudhary, A. K. (2010). Development and Characterization of Rosiglitazone Loaded Gelatin Nanoparticles Using Two Step Desolvation Method. *International Journal of Pharmaceutical Sciences Review and Research*, 5(1), 100–103.
35. Solati, Z., Baharin, B. S., & Bagheri, H. (2014). Antioxidant property, thymoquinone content and chemical characteristics of different extracts from *Nigella sativa* L. seeds. *Journal of the American Oil Chemists' Society*, 91(2), 295–300. <https://doi.org/10.1007/s11746-013-2362-5>
36. Tan, Y. L., & Ho, H. K. (2018). Navigating albumin-based nanoparticles through various drug delivery routes. *Drug Discov Today*, 23, 1108–14. <https://doi.org/10.1016/j.drudis.2018.01.051>
37. Tao, C., Chuah, Y. J., Xu, C., & Wang, D. A. (2019). Albumin conjugates and assemblies as versatile bio-functional additives and carriers for biomedical applications. *Journal of Materials Chemistry B*, 7(3), 357–367. <https://doi.org/10.1039/C8TB02477D>
38. Telci, İ., Özek, T., Demirtaş, İ., Özek, G., Yur, S., Ersoy, S., & Karakurt, Y. (2023). Studies on black cumin genotypes of Türkiye: Agronomy, seed and thymoquinone yields. *Journal of Applied Research on Medicinal and Aromatic Plants*, 35, 100494. <https://doi.org/10.1016/j.jarmap.2023.100494>
39. Xiong, K., & Chen, Y. (2020). Supercritical carbon dioxide extraction of essential oil from tangerine peel: Experimental optimization and kinetics modelling. *Chemical Engineering Research and Design*, 164, 412–423. <https://doi.org/10.1016/j.cherd.2020.09.032>
40. Yingngam, B., Brantner, A., Treichler, M., Brugger, N., Navabhatra, A., & Nakonrat, P. (2021). Optimization of the eco-friendly solvent-free microwave extraction of *Limnophila aromatica* essential oil. *Industrial Crops and Products*, 165, 113443. <https://doi.org/10.1016/j.indcrop.2021.113443>
41. Yulianita, Z. R., & Monica, H. R. (2023). Screening and Optimization Method of Natural Deep Eutectic Solvent Extracts from *Phyllanthus niruri* by Ultrasonic-Assisted Extraction. *Research Journal of Pharmacy and Technology*, 16(11), 5213–5217. <https://doi.org/10.52711/0974-360X.2023.00845>
42. Zhao, D., Zhao, X., Zu, Y., Li, J., Zhang, Y., Jiang, R., & Zhang, Z. (2010). Preparation, characterization, and in vitro targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. *International Journal of Nanomedicine*, 669–677. <https://doi.org/10.2147/IJN.S12918>
43. Zouirech, O., Alyousef, A. A., El Barnossi, A., El Moussaoui, A., Bourhia, M., & Salamatullah, A. M., Derwich, E. (2022). Phytochemical analysis and antioxidant, antibacterial, and antifungal effects of essential oil of black caraway (*Nigella sativa* L.) seeds against drug-resistant clinically pathogenic microorganisms. *BioMed Research International*, 1(5218950). <https://doi.org/10.1155/2022/5218950>