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ORIGINAL PAPER

Characterization of polyphenols profile, antioxidant, and in vitro activities of *Nigella sativa L.* (black cumin) seed oleoresin

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Abstract

The medicinal importance of *Nigella sativa L.* (black seeds) is portrayed by their traditional use against various ailments. The oleoresins obtained from spices are generally semi-solid concentrated whole extracts containing essential oil. This study deals with the extraction of oleoresins from black seeds by solvent extraction method using ethanol 70% (v/v), methanol & acetonitrile, and its characterization. Total polyphenols and flavonoids were estimated while DPPH and Fe^{2+} ion chelation assay was done to assess the antioxidant activity. Solvent extraction by 70% ethanol, methanol, and acetonitrile yielded 36.8, 34.9, and 22.1% oleoresin, respectively. High polyphenol and moderate flavonoid content were found in all three samples. 70% ethanolic oleoresin showed the highest polyphenol content 67.05 ± 1.48 µg GAE/mg and better antioxidant activity with the lowest IC_{50} of 0.86 ± 0.22 mg/ml for DPPH radical scavenging. The oleoresin was further characterized by HPLC and GC–MS techniques detecting the presence of various volatile compounds, thymoquinone showed well-resolved peaks with the highest percent area in the chromatograms. GC–MS chromatograms also showed that 40–45% of oleoresin consisted of essential fatty acids. In vitro enzyme inhibition assays against three digestive enzymes, pancreatic lipase (PL), α-amylase, and α-glucosidase were also done to check the anti-obese activity of the oleoresins. Results showed 50–60% inhibition of PL and α-amylase, while 20–30% inhibition of α-glucosidase activity. The study suggested that the oleoresins obtained from the spice *N. sativa* have nutraceutical properties and can be used as therapeutics for the prevention or treatment of various lifestyle disorders.

Keywords *Nigella sativa* · Oleoresins · Antioxidant activity · Polyphenols · Enzyme inhibition · Thymoquinone

Introduction

The increasing trend of exploring herbal plants and spices as medicine is currently of considerable significance due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Possible side effects and toxicity of synthetic drugs have drawn attention to finding alternative treatments for various diseases using natural food components. The natural volatile oils from different spices have been explored since ancient times and are now predominant in the pharmaceutical and food industry for their health benefits and flavoring properties [1]. One such promising spice abundantly found in the Mediterranean, middle eastern and South Asian region is *Nigella sativa L.*, also known as black cumin or black seeds, or Kalonji. *N. sativa* (black cumin) is a dicotyledon flowering plant belonging to the *Runuculaceae* family. The seeds of this plant are of main interest and have been traditionally used in food recipes as well as to cure multiple health conditions [2]. The most common therapeutic treatments from black cumin seeds include cough, asthma, headache, bronchitis, inflammation, fever, etc. [3]. Scientifically, the most explored and proven properties include anti-inflammatory, anti-cancerous, antihypertensive, antidiabetic [4], anti-obese [5], antioxidant properties and antimicrobial activity [6]. Oleoresin is the concentrated form of whole essential oil from spices, containing a mixture of volatile and non-volatile components. These can be extracted by solvent extraction methods using different solvents such as ethanol, methanol, ethyl acetate and n-hexane [7, 8]. Although

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black cumin volatile oil has been studied extensively there are very few reports on the activity of its oleoresin which has the potential to be explored as nutraceuticals due to the increased concentration of the volatile compounds in therapeutic deliveries. Essential oils reportedly have antioxidant properties due to it being rich in polyphenols and flavonoids. The importance of polyphenols and flavonoids present in natural food resources have been explored widely as they act as free radical scavengers relieving oxidative stress [9]. The formation of free radicals that occur during several biological processes is the underlying cause of many diseases such as cancer, obesity, liver cirrhosis, diabetes mellitus, hypertension, Alzheimer's [10]. According to several GC–MS profiles of the black cumin, its seed extract consists of many bioactive compounds such as thymoquinone, p-simene, α-thujen, carvacrol, thymol, longifolene etc. [11, 12]. The major phytochemical present is thymoquinone, which has been proved to possess most of the antioxidant and anti-inflammatory properties of black cumin. Preliminary experiments also showed that thymoquinone inhibits non-enzymatic lipid peroxidation in liposomes, preventing the oxidative damage [13]. Along with volatile compounds, the oils from black cumin seeds also contain diverse range of polyunsaturated fatty acids including omega-6 and omega-3, and some others like myristic acid, palmitic acid, sterols and tocopherols [7]. Recently the importance of unsaturated fatty acids in diets is well established due to their potential anti-inflammatory activity, increasing HDL levels in the body and strengthening of immune system [14]. The only drawback of phytochemicals is the unstable nature, low concentration and loss during processing of these compounds; hence we developed an oleoresin form of extract to check its nutraceutical properties. Very few studies have explored the inhibitory effects of black cumin seed oleoresin on PL, the main enzyme involved in lipid digestion, breakdown and absorption of dietary fats in the intestine into triglycerides and short chain fatty acids [15]. One of them is by [16], who reported inhibitory effects of the isolated essential fatty acids from black cumin seeds extract on PL. The inhibitory effects of black cumin oleoresin on digestive enzymes such as α-amylase and α-glucosidase is also very essential in order to assess its anti-obese and anti-diabetic properties. These enzymes function in the breakdown of complex carbohydrates by hydrolysing the α-1,4-glycosidic linkage in glycogen, starch, amylose and amylopectin into simple molecules and generate hyperglycemia [17]. Hyperglycemia is further linked with insulin resistance, which forms a vicious cycle of high blood glucose levels and more resistance to insulin, signalling the body to store excess blood sugar as fat called glycogenesis leading to subsequent weight gain progressing into obesity [18]. The role of these enzymes in digestion and metabolic pathway makes them a great therapeutic target of nutraceuticals against obesity. The purpose of the present study is to extract the concentrated oleoresins from black cumin seeds using three different solvents (70% ethanol, methanol, acetonitrile), and assessment of their phytochemical profiles as well as the antioxidant and anti-obese activities using in vitro assays.

Materials and methods

Plant material

The black cumin seeds were procured from RiTrue superfoods, Namo Organics, Delhi, India.

Chemicals and reagents

Thymoquinone standard, *p*-nitrophenyl butyrate (*p*-NPB), *p*-nitrophenyl-α-D-glucopyranoside (pNPG) and ferrozine iron reagent were purchased from Sigma-Aldrich Bangalore, India. The solvents methanol, ethanol, acetonitrile, and isopropanol were of HPLC grade and were procured from SRL, Mumbai, India, along with potassium phosphate monobasic and sodium phosphate dibasic. Pancreatic lipase, α-amylase, α-glucosidase, EDTA, phosphate buffer saline (PBS), and acarbose were procured from HiMedia, Bangalore, India. 2,2'-diphenyl-1-picrylhydrazyl (DPPH), and $FeCl_2$, were all the standard analytical grades and were purchased from standard chemical suppliers.

Extraction of oleoresin from black cumin seeds

Oleoresins were extracted by the methodology earlier used in our laboratory with some slight modifications [8]. Seeds were washed with tap water for 5 min, rinsed with distilled water twice, and air dried for 2 h in shade at room temperature. Dried seeds were grounded using an electric grinder (Philips, India). 25 g of powdered seeds were mixed with 100 ml each of 70% ethanol (v/v), methanol, and acetonitrile (1:4 w/v) and kept in the separate glass columns. The extracts were collected three times and filtered through Whatman filter paper no.1. The filtrates obtained were concentrated under reduced pressure at 37°C using a rotary evaporator (Heidolph Laborota 4000 Efficient, Triad Scientific, Inc. Manasquan) to obtain solvent free concentrated oleoresin. The weight of different oleoresins was measured and percent extract yield was calculated using the formula below.

$$\%\ \text{Extract yield} = \frac{\text{Weight of oleoresin extracted (g)}}{\text{Weight of original sample (g)}} \times 100$$

Estimation of phenolic compounds

Determination of polyphenol content

The total polyphenol content was estimated using the Folin-Ciocalteu method [19]. 100 µl of each oleoresin extract with different concentrations (40–200 µg) were mixed with 1 ml Folin-Ciocalteu reagent (1:10 diluted) and allowed to react for 5 min. Then 0.8 ml of Na_2CO_3 was added and the reaction mixture was incubated in dark for 60 min at room temperature. The absorbance was measured by the microplate reader (Tecan, Switzerland) at 760 nm.

Determination of total flavonoid content

The total flavonoids were assessed on the principle of formation of flavonoid-aluminium complex, which has an absorption maximum at 430 nm. The modified methodology by Chen et al., was adopted. 1 ml of Increasing concentration of oleoresins (40–200 µg) was mixed with 300 µL of sodium nitrate (5% w/v in methanol) and the reaction was allowed to occur by incubating at room temperature for 6 min. 300 µL of aluminium chloride (10% w/v in methanol) was added and again incubated for 5 min under the same conditions. The reaction was stopped by adding 1 ml of 1 M NaOH. Absorbance was measured at 430 nm against methanol as blank. Quercetin was used for standard curve and flavonoid content was expressed as µg QE/mg of oleoresin. All the experiments were carried out in triplicates and mean values were calculated [20].

Estimation of antioxidant activity

In this study, we confirmed and quantified the antioxidant potential of black cumin oleoresin extracts by DPPH and Fe^{2+} ion chelating assay.

DPPH method

The antioxidant activity was determined on the principle of DPPH radical scavenging by the natural compounds reflecting their hydrogen donating capacity and reducing DPPH to corresponding hydrazine resulting in color change from purple to yellow followed by a decrease in absorbance at 517 nm. The degree of decrease in absorbance is measured and is directly proportional to the concentration of antioxidant A slightly modified protocol according to Manzocco et al., was followed [21]. Serial dilution of 1 mg/ml of ascorbic acid standard and 20 µl of test samples with different concentrations (20, 40, 60, 80, 100 µg) were taken from the stock and added to 150 µl of the DPPH solution (0.5 mM in methanol). The plate was shaken gently and kept in dark at 25 °C for 30 min. The absorbance of resulting solution was taken at 517 nm against control consisting only methanol. The percentage of DPPH radical scavenging was calculated using the equation below

$$\%\ Radicle\ scavenging\ activity\ = \frac{A_{517}\ (control) - A_{517}\ (sample)}{A_{517}\ (control)}$$

Ferrous ion chelation activity

Ferrous ion chelating activity was measured by the ability of test material to inhibit the Fe^{2+} ion generation and formation of iron (II)-ferrozine complex [22]. Different concentrations of oleoresins were made (20, 40, 60, 80, 100 µg) using 1 mg/ml stock solution and 500 µl of each was added to 50 µl of 2 mM $FeCl_2$. The reaction was initiated by adding 0.1 ml of ferrozine (5 mM), mixed by vigorous shaking and left for incubation at room temperature for 10 min. The absorbance was measured at 562 nm. EDTA was used as standard and the assay was done in triplicates and IC_{50} values were calculated by plotting the graph of concentration against percent chelating activity.

The percentage inhibition of ferrozine-Fe^{2+} was calculated using the following formula:

$$\%\ \text{Chelating activity} = \frac{A_{562}\ (\text{control}) - A_{562}\ (\text{sample})}{A_{562}\ (\text{control})}$$

HPLC analysis of the bioactive compounds in extracted oleoresin

The analysis was performed on a HPLC system (Shimadzu, Japan) equipped with LC-10AT pump and D-M10AVP UV detector for the visual confirmation of the marker compound. The Shodex C18-4E column (250×4.6 mm, 5 µm) was used at 30 °C. The isocratic mobile phase filtered with 0.45 µm filter (MF-Millipore, Bangalore, India) was composed of methanol: water: 2-propanol (50:45:5, v/v) and was de-aerated before use. 10 µl of diluted oleoresin (1:50 dilution with methanol) was injected in each run of 45 min. The analysis by photodiode array detector was carried out at 254 nm and flow rate was 0.5 ml/min. 100 µg/mL standard thymoquinone was prepared in methanol and run through the same chromatographic conditions. Chromatogram of standard was used to identify the retention time of thymoquinone and the peaks were identified in the sample accordingly [23].

GC–MS analysis

The volatile components present in oleoresin were analyzed by GC–MS. gho Oleoresins were diluted (1:50) with 100% ethanol, filtered with 0.22 µm syringe filter (MF-Millipore, Bangalore, India) and subjected to Autosystem XL Gas chromatography, Gold Mass Spectrometer (Turbomass, Perkin Elmer Instruments, Norwalk CT 06859, USA), equipped with silica column (30.0 m × 250 µm). The initial temperature of the oven was kept at 70 °C for 1 min and then increased up to 250 °C for 10 min at the rate of 5 °C/min. Helium flow rate of 1 ml/min with 19:1 split ratio was used as the carrier gas for 1 µL sample injection and mass spectra analysis was taken at 70 eV ionization voltage. The volatile compounds were identified by matching their MS spectra and retention index data with the standard spectra and fragmentation pattern using mass library [24].

In vitro enzyme inhibition assays for anti-obese activity

Lipase inhibitory activity

The inhibitory activity of the oleoresin against pancreatic lipase enzyme was measured by following the protocol described by El-shiekh et al., using substrate *p*-nitrophenyl butyrate (*p*-NPB) [25]. 10 µL of extract (prepared at the concentration of 50, 100, 200, 400, 600, 800, 1000 µg/mL), positive control (orlistat) was preincubated in 96 well plate with 20 µL of pancreatic lipase enzyme (1 mg/ml prepared in 0.1 M potassium phosphate buffer) and 20 µL of phosphate buffer (pH 6.0) for 1 h at 30 °C. 20 µL of substrate (20 mM *p*-NPB in acetonitrile) was subsequently added and again incubated for 5 min at 30 °C. Absorbance of 2,4-dinitrophenol produced was measured at 405 nm in a microplate reader (Tecan, USA). Enzyme inhibitory activity was calculated as follows:

$$\text{Inhibiory activity}\,(\%) = \frac{A_{405}\,(\text{control}) - A_{405}(\text{sample})}{A_{405}\,(\text{control})}$$

α- amylase inhibitory activity

The α-amylase inhibitory activity of the oleoresins was done using starch as the substrate following the methodology by Unuofin et al., with slight modifications [26]. 50 µL of varying oleoresin concentrations, acarbose (100 µg/ml) as positive control and phosphate buffer, pH 6.9 (enzyme control) mixed with 20 µL of porcine pancreatic amylase solution (0.1 mg/mL) were added to 96 well plate and preincubated at 37 °C for 15 min. Then 40 µL starch solution was added and the reaction was incubation was done at 37 °C for 30 min followed by addition of 20 µL of 1 M HCL and 75 µL of iodine reagent. The absorbance was measured at 580 nm in a microplate reader (Tecan, USA).

$$\text{Inhibiory activity}\,(\%) = \frac{A_{580}(\text{control}) - A_{580}(\text{sample})}{A_{580}(\text{control})}$$

α-glucosidase inhibitory activity

The assay uses the hydrolysing activity of α-glucosidase enzyme on *p*-nitrophenyl-α-D-glucopyranoside (pNPG) substrate, forming a coloured compound p-nitrophenol which can be detected at 405 nm. A calorimetric assay based on same principle was done following the protocol by You et al., with slight modifications [27]. The reaction mixture containing 20 µL of varying concentrations of oleoresin or acarbose (positive control), 20 µL phosphate buffer (50 mM, pH 6.9) and 50 µL α-glucosidase solution (1 U/mL in 0.1 M phosphate buffer, pH 6.9) was incubated for 6 min at 37 °C. Then, 20 µL of 5 Mm PNPG was added as the substrate and incubated for 20 min at 37 °C. *p*-nitrophenol was released and absorbance was measured at 405 nm using a microplate reader (Tecan, USA). All the experiments were done in triplicates and IC_{50} values were calculated by plotting concentration versus percent inhibition graph.

$$\text{Inhibiory activity}\,(\%) = \frac{A_{405}(\text{control}) - A_{405}(\text{sample})}{A_{405}(\text{control})}$$

Results

Yield of oleoresin from black cumin seeds

The extracts obtained from black cumin seeds were yellow in colour with oily consistency and a characteristic pungent odour. The percentage yield was calculated as 36.8, 34.9 and 22.1% when extracted with 70% ethanol (v/v), methanol and acetonitrile respectively. The weight of each sample was measured and is presented in Table 1.

Total polyphenols and flavonoids

Many studies focus on the importance of polyphenols and flavonoids which reportedly act as antioxidants and free radical scavengers. The results of polyphenol and flavonoid content in the *N. sativa* oleoresin extracted with different solvents presented in Table 1 showed that the highest amounts of polyphenols were present in oleoresin extracted using 70% ethanol from black cumin seeds followed by methanol and acetonitrile extracted oleoresin. The highest amounts of flavonoids were also reported in 70% ethanol extract followed by acetonitrile extract and methanol extract.

Table 1 Extractable compounds, total polyphenols, total flavonoids, DPPH IC_{50} (mg/ml), and FRAP IC_{50} (mg/ml) of the *N. sativa* oleoresin extracted with different solvents

Parameters	Type of oleoresin		
	Ethanol (70%)	Methanol	Acetonitrile
Oleoresin yield (g)	8.74	9.20	5.52
Total polyphenols (µg GAE/mg) ± SD	67.05 ± 1.48	55.53 ± 1.67	48.20 ± 4.65
Total flavonoids (µg QE/mg) ± SD	29.76 ± 0.36	15.74 ± 0.30	26.81 ± 0.58
DPPH scavenging activity IC_{50} (mg/ml) ± SD	0.86 ± 0.22	0.93 ± 0.04	2.18 ± 0.76
Ion chelating activity IC_{50} (mg/ml) ± SD	1.44 ± 0.25	1.07 ± 0.10	1.95 ± 0.25

[Mean ± SEM (n = 3), $p < 0.01$]

Antioxidant activities

DDPH assay

The scavenging of DPPH radical by oleoresin was concentration dependent. The percent inhibition increased from 25.4 to 57.1% for oleoresin extracted using 70% ethanol, 22.3–52.7% for methanol and 10.2–38.6% for acetonitrile, for sample concentration ranging 0.2–1 mg/ml. Whereas, the ascorbic acid standard showed the percent inhibition range from 40 to 90.21% and 0.39 ± 0.10 IC_{50} value. As presented in Table 1, the ethanolic fraction showed the lowest IC_{50} value while the acetonitrile had the highest IC_{50}, hence the lowest activity.

Ferrous ion chelating activity

The principle of chelating free radical Fe^{2+} by antioxidant molecules was used to estimate the antioxidant efficiency of oleoresins. The percent chelating activity was calculated and showed better activity in methanol extracted oleoresin with 67.15% chelation than the ethanol and acetonitrile with 60.52 and 51.56% chelation of Fe^{2+} ion at 1 mg/ml concentration. The activity showed by all the oleoresins was less than the standard EDTA which exhibited 88.37% chelation having IC_{50} value of 0.47 ± 0.89 mg/ml. The mean IC_{50} values of all the samples were calculated and are reported in Table 1.

HPLC chromatograms

The major pharmacologically active compound found in *N. sativa* is thymoquinone (TQ) with other isomeric components like dithymoquinone (DTQ), thymohydroquinone (THQ), carvacrol, and thymol (THY) [23]. HPLC analysis of *N. sativa* oleoresins was done and the chromatograms showed a well-resolved peak for thymoquinone when compared with the standard as shown in Fig. 1. The run time for the analysis was 45 min. The retention times for TQ eluted were 36.199, 35.981, and 36.273 min for 70% ethanol, methanol, and acetonitrile-extracted oleoresin, respectively. The retention time of standard thymoquinone run through the same chromatographic conditions was 36.051 min.

GC–MS

The volatile compounds in the *N. sativa* oleoresin were analysed by GC–MS which detected 20 components. The chromatogram showed well-resolved peaks for volatile components in all three oleoresin samples as depicted in Fig. 2**.** The major peaks were identified by mass spec. library as thymoquinone (32.97, 34.76, 27.35%) along with carvacrol (1.45, 1.51, 0.69%), limonene (0.96, 1.55, 0.33%) and 4-carene, p-cymene, longifolene in trace amounts. The oleoresin also consisted of the lipid content mainly consisting of unsaturated fatty acids and their different esters. The major fatty acid present in all three oleoresins was linoleic acid ethyl ester (26.98, 10.84, 14.32%) followed by oleic acid (19.61, 8.37, 17.50%), octadecanoic acid, and hexadecenoic acid. The complete list of components identified is provided in TableS S1–S3**.**

In vitro enzyme inhibition assays

Lipase inhibitory activity

The oleoresin made using all three solvents exhibited inhibitory effect on the activity of pancreatic lipase enzyme in a concentration dependent manner. The percentage inhibition of standard drug orlistat was 96.87 ± 1.01% at 1 mg/ml, while it ranged from 28.29 ± 1.03 to 68.63 ± 1.93, 27.96 ± 0.09 to 66.41 ± 1.04 and 22.78 ± 2.07 to 63.04 ± 0.04% for 70% ethanol, methanol, and acetonitrile extract respectively. The IC_{50} values for all the samples and standard were calculated and are presented in Table 2. Ethanol-extracted oleoresin had a better lipase inhibitory activity when compared to methanol

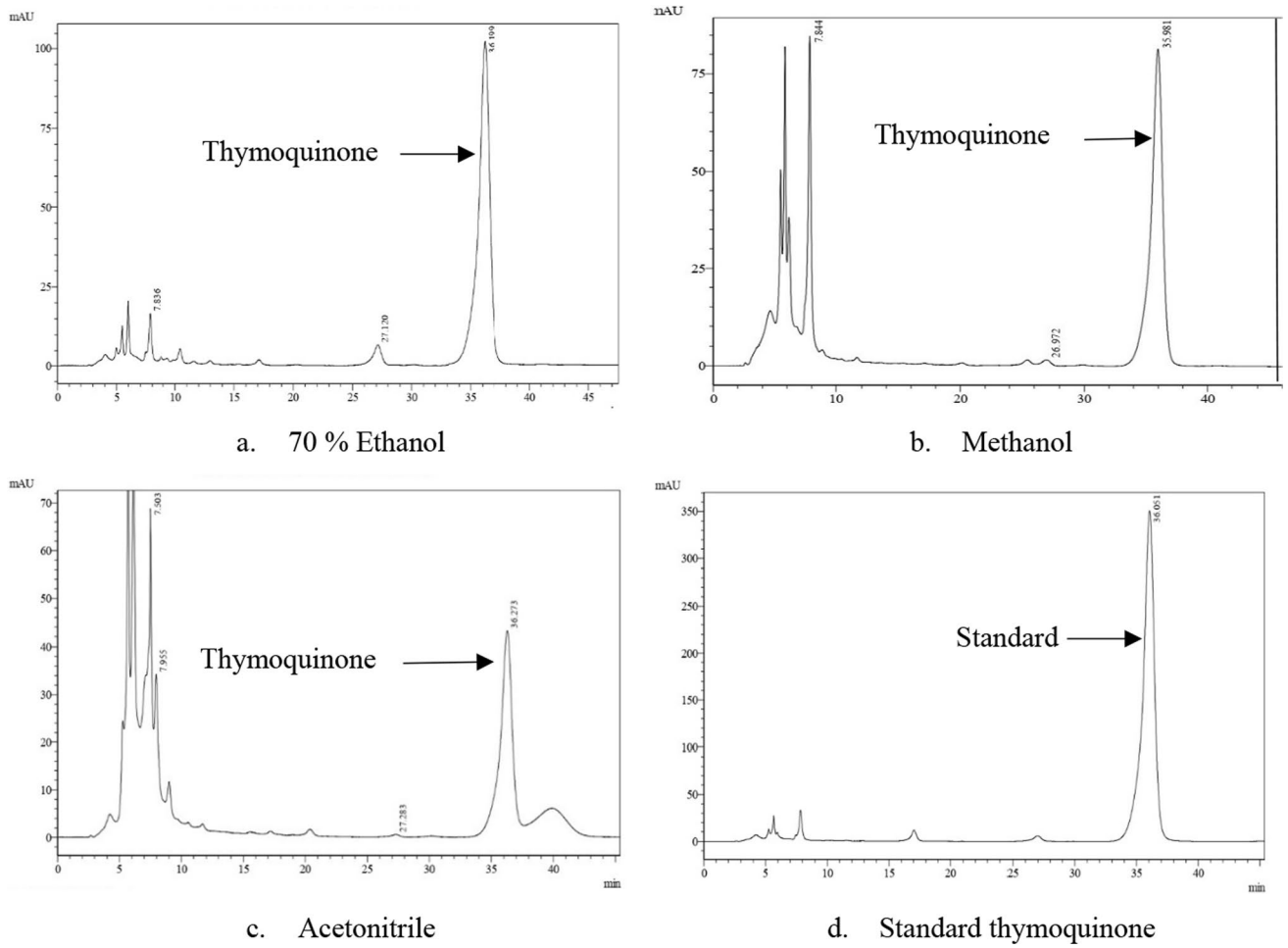

Fig. 1 HPLC chromatogram of a 10µL injection of oleoresin extracted from *N. sativa* seeds using **a** ethanol **b** methanol **c** acetonitrile and **d** standard thymoquinone

and acetonitrile extracted oleoresin, whereas the standard drug orlistat showed highest activity.

α-amylase inhibitory activity

Another target for anti-obesity therapeutics could be to inhibit the absorption of glucose through the inhibition of enzymes involved in carbohydrate metabolism, such as α-amylase and α-glucosidase. All three oleoresin samples depicted moderate activity (> 40%) against α-amylase, whereas the standard acarbose showed higher enzyme inhibition of 89.81 ± 1.7%. It is noticeable here re that 70% ethanol-extracted oleoresin showed better activity with 54.23 ± 4.13% inhibition followed by methanol and acetonitrile extract with 50.22 ± 1.57% and 43.84 ± 0.34% inhibition respectively at 1 mg/ml concentration.

α-glucosidase inhibitory activity

The final phase of carbohydrate digestion is catalysed by α-glucosidase or maltase, which breaks α-1,4 bonds to produce glucose monomer [28]. The inhibition was seen by all the oleoresin samples though a weaker inhibition was observed than α-amylase inhibition. The inhibitory activity at 1 mg/ml by 70% ethanol, methanol, and acetonitrile oleoresin extract was 22.49 ± 1.64, 21.64 ± 0.87 and 21.94 ± 0.72% respectively, while the percentage inhibition by standard acarbose was 67.98 ± 0.52% as shown in Fig. 3. Notably, there was no significant difference between the three samples inhibiting α-glucosidase enzyme activity whereas there was a lot of variation when compared to standard acarbose, which had a much lower IC_{50}, when compared to the oleoresins as presented in Table 2.

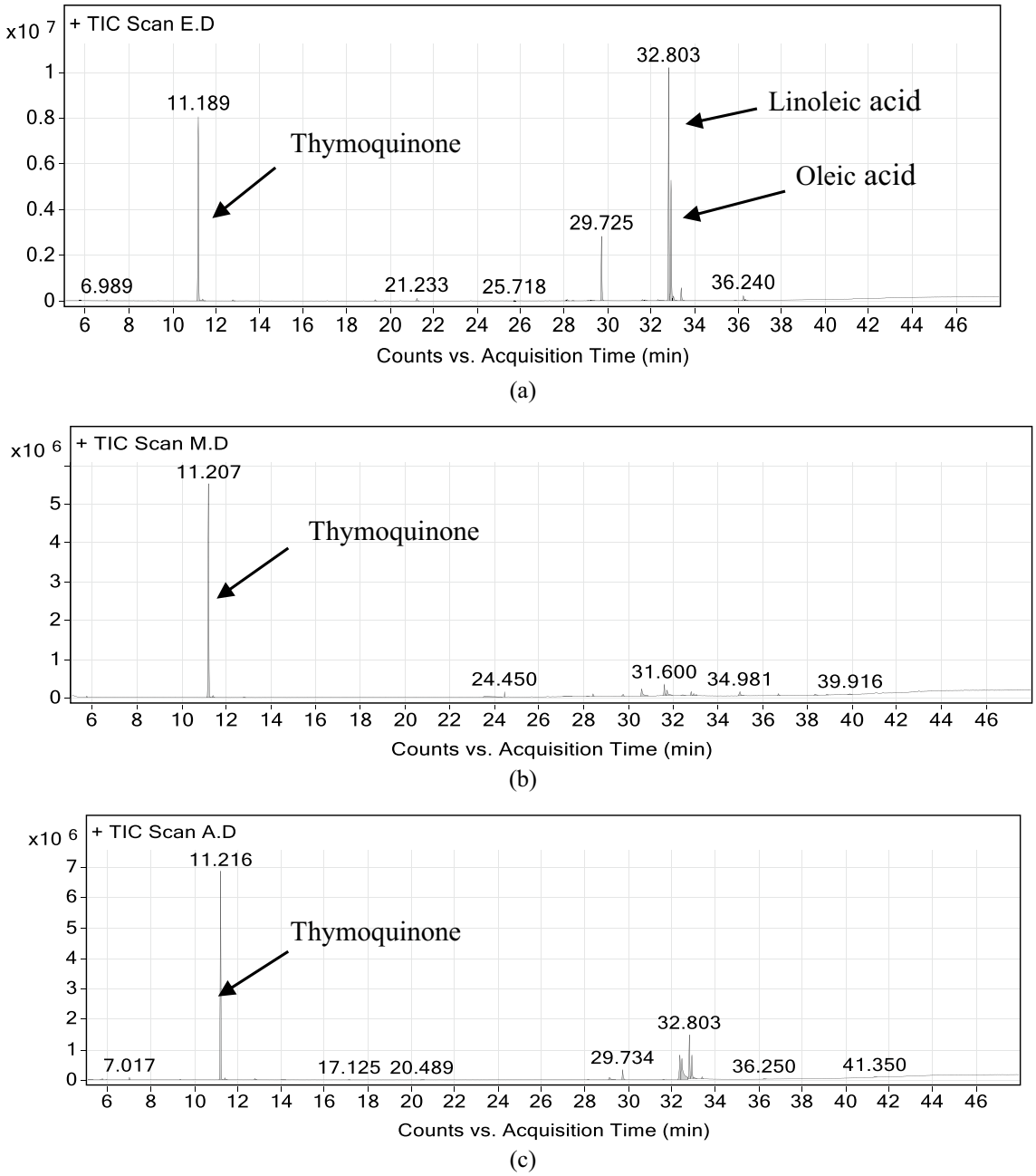

Fig. 2 GC–MS chromatogram of *N. sativa* oleoresin extracted with **a** 70% ethanol **b** methanol and **c** acetonitrile

Table 2 IC_{50} values for pancreatic lipase, α-amylase and α-glucosidase inhibition activity of *N. sativa* oleoresin

Enzymes	IC_{50} value of different oleoresins (mg/ml)				
	Ethanol (70%)	Methanol	Acetonitrile	Orlistat	Acarbose
Pancreatic lipase	0.49 ± 1.83	0.52 ± 1.64	0.54 ± 0.83	0.09 ± 2.64	–
α-amylase	0.85 ± 2.38	0.88 ± 2.38	0.90. ± 3.22	–	0.42 ± 0.53
β-glucosidase	2.39 ± 1.77	2.59 ± 3.65	2.51 ± 5.34	–	0.67 ± 4.84

[Mean ± SEM (n = 3), $p < 0.01$]

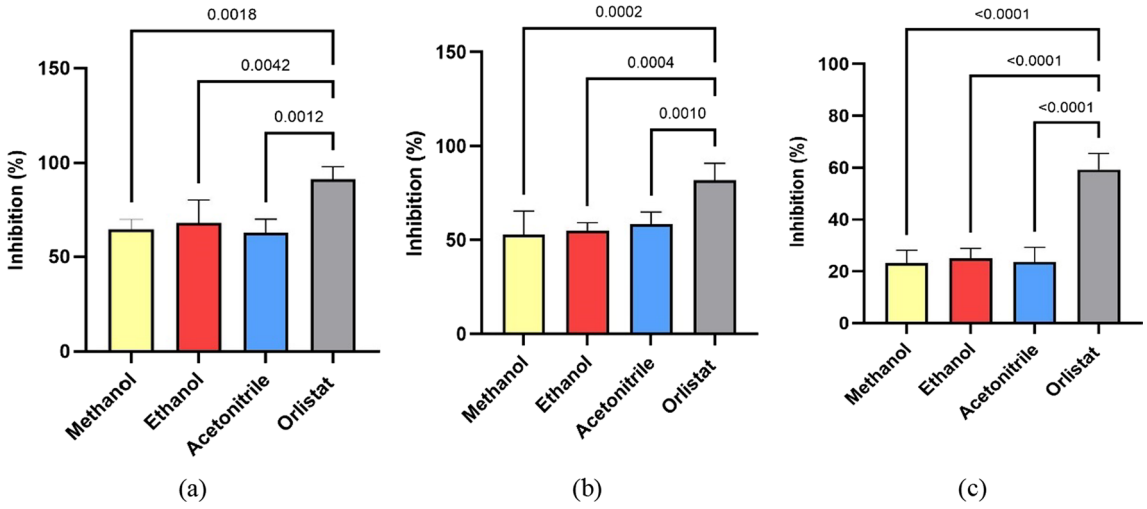

Fig. 3 Percentage inhibition by different black cumin seed oleoresins of **a** pancreatic lipase enzyme **b** α-amylase enzyme and **c** α-glucosidase enzyme Values are mean ± SEM (n = 3), $p < 0.01$

Discussion

The current study was conducted to efficiently extract oleoresins from seeds of a traditional spice *N. sativa* and evaluate its nutraceutical properties. As experimentally explained in literature, the black cumin seeds have higher amounts of thymoquinone, as compared to the aerial parts of the plant. Other than thymoquinone, it also consists of dithymoquinone, p-cymene, carvacrol, 4-terpineol and longifenone [7]. The extraction of oleoresin from black cumin seeds evidently showed that the total percentage of extractible oleoresins majorly depend on the type of solvent as well as the extraction technique. Out of the three solvents used, 70% ethanol gave maximum yield followed by methanol and then acetonitrile while the amount extracted was almost similar to the results obtained by Matthaus and Ozcan, reporting 28–34.7% yield from different varieties of *N. sativa* [29]. The yield evidently decreased with the polarity of the solvent as the 70% ethanol showed the highest values in our report. These results are in agreement with the earlier study which stated that the amount of total extractible compounds decreased with decreasing solvent polarity [30]. None of the reports have established the extraction of oleoresin using 70% ethanol which was done here in our study. The presence of polyphenols and volatile compounds attribute to the antioxidant activity of the extract and has wide spectrum of beneficial effects such as reproduction, growth, and protection against harmful pathogens [31]. As presented in the Table 1, it was experimentally found that among the samples the ethanolic extract had high amounts of polyphenols and flavonoid content. These results are in agreement with the previous studies where ethanolic extract of *N.sativa* was highly charged with polyphenols [32]. Another study by Dalli in 2021 showed that 39.82 ± 1.25 and 18.4 ± 0.44 µg QE/mg of flavonoids were present in the ethanolic and methanolic fraction, which are reportedly more than the amount found in our study [33]. The contradictory results were also found in some studies showing highest polyphenol concentration in aqueous, ethyl acetate and chloroform extract of black cumin seeds [34]. The phenolic content also correlates with the antioxidant properties. Our findings depicted that the scavenging of DPPH radical was concentration dependent and 70% ethanol extracted oleoresin had highest activity followed by methanol and acetonitrile, establishing a significant linear correlation between the polyphenol content and DPPH radical scavenging. Another study on soyabean seed extract found that the DPPH radical scavenging activity increased linearly with the polyphenolic constituents present in the samples [35]. The scavenging effect was lower in ethanol extract than the methanolic extract in the study by Dalli et al., but it corresponds to the lower polyphenol content in their ethanolic fraction. The slight deflection in the results might be due to the different genotype, climatic and environmental conditions, extraction techniques and solvents used. It is also reported that the presence of different active molecules in each extract such as thymoquinone, carvacrol, 4-terpineol cause efficient reduction of DPPH [2]. To better understand the antioxidant properties of black cumin seeds oleoresin, further metal chelation assays against Fe^{2+} ion was done. Methanolic extract showed the highest activity followed by ethanol and acetonitrile. It is noticeable that there is no significant correlation between chelation activity and phenolic constituents' concentration, hence making it a probability that the polyphenol content might not be the only reason for antioxidant activities. Similar results have been reported in other works, for example during the study

of fourteen varieties of barley, a low correlation was found in the metal chelation activity of these extracts and the phenolic content [36]. Another study established that high phenolic content of chloroform extract of black cumin seeds had lowest metal chelation activity with 4.10 ± 0.10 IC_{50} whereas water extract with low total phenolics, depicted high metal chelation activity with 0.169 ± 0.00 IC_{50} (Meziti et al., 2012). High chelation activity in methanol extract was also observed (0.31 ± 0.03 IC_{50}), similar to what reported in our findings. A detailed HPLC analysis of the three oleoresins detected thymoquinone in all the samples along with some other minor peaks, which refer to the other phytochemicals present in the oleoresins. Other than thymoquinone, carvacrol, thymol, dithymoquinone has also been detected in the already reported studies (Ghosheh et al., 1999). There has been a noticeable difference in the elution time of thymoquinone in our sample when compared with the literature for example in the study done by Ghosheh et al., the peak for thymoquinone was identified at 6.8 min while in some other studies it was 12.3 and 22.1 min [23, 37, 38]. The difference in elution time could be the result of what column and mobile phase was used or due to the reduced flow rate in our experiment. These claims are in agreement from the previously reported experiment where use of < 40–45% methanol in the mobile phase results in the separation of thymoquinone with long retention time [12]. Along with the volatile polyphenols, the black cumin seeds oleoresin has been found to be rich of unsaturated fatty acids such as linoleic acid, oleic acid etc. [7]. These essential fatty acids are proved to possess anti-inflammatory and antimicrobial activities, making them an essential contributor in the therapeutic properties of the oleoresin. According to a previous analysis reported in literature, the black cumin seed oleoresin consisted of 2.5% volatile oil while the lipid content was 38.7% [39]. As depicted by our GC–MS results, all three oleoresins were found to be rich of unsaturated fatty acids such as linoleic acid, oleic acid etc. along with the volatile polyphenols. These results were correlated with the previously reported data and variable concentrations of volatiles were found. It also further establishes the beneficial effects of unsaturated fatty acids in diet against obesity. In a study by Edris et al., 37.61% thymoquinone was detected in the oleoresin extracted with hexane at room temperature [40]. Similarly another data showed 5.7% thymoquinone in ethanol extracted oleoresin as well as 33% linoleic acid and traces of glyceryl linoleate, glyceryl palmitate and oleic acid [7]. Burits and Bucar characterized many volatile compounds in different varieties and found 30.4–55.2% thymoquinone with higher concentration of p-cymene (7.2–15%) and carvacrol (5.8–10.4%) as compared to our results [2]. These variations might arise due to different varieties of the plant, solvent used for extraction and different chromatographic conditions.

The gastrointestinal enzymes have been explored as the therapeutic targets for many phytochemicals as they are the main catalysts for the digestion and absorption of the nutrients [41]. This was the unique study which tested the inhibitory effect of whole black cumin seeds oleoresin on PL enzyme. Although orlistat has the PL inhibitory action it can also cause various side effects, hence a natural alternative is required. Our results showed concentration dependent inhibition of the enzyme reaching up to > 60%. A study conducted by isolating the fatty acid fraction of black cumin seeds ethanol extract consisting of linoleic acid, oleic acid, linolenic acid and palmitic acid depicted more than 80% pancreatic lipase inhibition [16]. Along with that, the bioactive components present in the oleoresin of different medicinal herbs also showed a positive correlation with the pancreatic lipase inhibition [15]. The in vitro inhibition assays against anti-diabetic enzymes, α-amylase and α-glucosidase and found concentration dependent inhibitory effect. The *N. sativa* extract have been tested for in vitro inhibition before and showed 20–30% inhibition of α-amylase and 30–45% inhibition of α-glucosidase enzyme showing ethanol and aqueous extract performing better than the rest [28]. Although the better activity of 70% ethanol extracted oleoresin in our study correlates with the literature but the overall results which depicted approximately 40–55% inhibition of α-amylase and 20–30% inhibition of α-glucosidase are slightly different from already reported data. The possible explanation for this is the polyphenol and flavonoid content present in different varieties. As stated in earlier studies the high levels of polyphenol have been shown to reduce the potency of α-amylase [42] and around 44 flavonoids including quercetin, catechin, luteolin, flavone., all aided to α-glucosidase inhibition [43, 44]. Hence, the inhibitory activity of oleoresins against metabolic enzymes is in correlation with the high polyphenols and low flavonoids concentration recorded in our samples.

Conclusion

The seeds of black cumin seem to possess magical properties and this study revealed that the whole oleoresin extract of black cumin seeds constitute a good source of polyphenols and various bioactive components. The better solvent was 70% ethanol when compared with methanol and acetonitrile, which exhibited better yield and biological properties. The study also revealed that spice oleoresins possess good amount of biologically active volatile compounds such as thymoquinone, carvacrol, p-cymene etc. as well as essential fatty acids such as omega-6, omega-3, tocopherol making them a potent antioxidant capable of scavenging reactive oxygen species. In this study, we for the first time tested the

N. sativa whole oleoresin, well known for its anti-inflammatory and anti-cancerous property for its anti-obese activity through an in vitro assay including inhibition of digestive enzymes involves in lipid and glucose metabolism. The inhibition was 50- 60% against pancreatic lipase as well as α-amylase enzyme, and hence the black cumin spice oleoresins can be explored as potential therapeutic agent against obesity and metabolic disorder.

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Author contributions All authors contributed to the study design. Establishing of methodology, experiments, writing original draft and editing was done by Isha Gupta. Conceptualization, resources, supervision was provided by Muthukumar S.P. along with reviewing the final draft of manuscript.

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Data Availability The data that support the findings of this study are available in the form of laboratory records and is accessible from the corresponding author upon reasonable request. The data are not publicly available due to privacy restrictions or ethical issues.

Declarations

Conflict of interest The authors have no conflict of interests to declare that are relevant to the content of this article.

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