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Bioactive Potential of *Nigella sativa* (Black Seed): Comparative Assessment of Antioxidant, Antimicrobial, and Anticancer Activities across Different Extraction Methods

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ABSTRACT

Nigella sativa L. (black seed) is among the most commonly used medicinal plants and is well-known for its high content of bioactive ingredients and its various pharmacological activities. This work explores the antioxidant, antimicrobial, and anticancer properties of *N. sativa* seed extracts from various extraction methods: water (hydrodistillation and decoction), ethanolic and methanolic extractions, and cold-pressed oil. The methanol extracts had the highest total phenolic contents (2.93 mg GAE/g) and total flavonoid contents (1.89 mg QE/g), and this was associated with the highest protective antioxidant efficacy in the FRAP, ABTS, and 2,2-diphenyl-1-picrylhydrazyl tests. The antibacterial and antifungal activities of the extracts were evaluated with clinically significant bacterial and fungal strains. Methanol and ethanol extracts showed the most inhibitory activity with 17 mm of inhibitory zone and water and oil extracts showed the lower inhibitory activity. Cytotoxicity on PC-3 prostate cancer cells *in vitro* was also assessed by means of the MTT assay. The data obtained exhibited a significant concentration-dependent decrease in cell viability, where the methanol extract showed the greatest antiproliferative effects, reaching a percentage decrease in growth of 48.49% at the highest tested concentration. These results indicate that the choice of solvents has a substantial effect on the extraction yield and bioactivities of *N. sativa*. The better activities of methanol and ethanol extracts demonstrate the potential of them as a natural source for the development of antioxidants, antimicrobials, and anticancer that can be employed in nutraceutical, pharmaceutical, and therapeutic products.

Index Terms: *Nigella sativa,* Antioxidant Activity, Antimicrobial Activity, Anticancer Potential and Extraction Methods

1. INTRODUCTION

Nigella sativa L. (black seed) is an annual herb belonging to the Ranunculaceae family. It has been known and used since ancient times in the Middle East, South Asia, and North Africa traditional medicine systems because of its wide range of medicinal uses. Seeds of *N. sativa* have traditionally been used in natural remedies for various diseases such as asthma, bronchitis, dysentery, and skin diseases as well [1]. Scientific studies in more recent times have discovered and further elaborated many of these traditional uses, indicating the overwhelming pharmacological profile of *N. sativa*. It has been used as an antioxidant, anti-inflammatory, antidiabetic, anticancer, hepatoprotective, and neuroprotective agent such that it can be considered a "jack of all trades" in herbal therapeutics [2]. Bioactive substances from royal black cumin (*N. sativa*) have attracted considerable interest in

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recent years and are believed to function in Human Health and Disease Prevention. Recently, bioactive compounds from royal black cumin (*Nigella sativa*) have attracted a lot of attention because they help people stay healthy and avoid getting sick. The bioactivity of *Nigella sativa* is mainly due to the different bioactive compounds it contains that help with these beneficial effects. The bioactivity of *N. sativa* might be attributed to its various embedded substances including thymoquinone (TQ), which has been significantly tested for its antioxidant and antimicrobial activity [3]. It also contains p-cymene, carvacrol, nigellidine, and alpha hederin, which also play a role in its pharmacological activity [4]. These agents work together to improve oxidative stress and microbial infections both of which have a major impact on chronic inflammation and the development of certain diseases. The antioxidant activity of *N. sativa* is a direct result of its capacity to scavenge free radicals and decrease oxidative injury at the cellular level. It has been reported that *N. sativa* extracts have the capacity to improve the activities of antioxidant enzymes including glutathione peroxidase, superoxide dismutase, and catalase to decrease lipid peroxidation markers [5], [6]. Such behaviors may contribute toward retardation of the development of disorders related to oxidative stress such as cardiovascular and neurodegenerative diseases. Moreover, the essential oil of *N. sativa* is rich in phenolic compounds and flavonoids which also contributes to its potent free radical scavenging capabilities [7]. Its cytoprotective roles are also linked to the downregulation of inflammatory mediators and apoptotic pathways, which have therapeutic relevance in chronic inflammation-related diseases [8]. Meanwhile, *N. sativa* has potent antimicrobial activities against various pathogens such as Gram +Ve, Gram -Ve bacteria, fungi, and some viruses. TQ has potent bactericidal and fungicidal properties, especially against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* [9], [10]. *N. sativa* seeds are such comfortable natural, they can be less or even more adjuvant to chemical treatments, which always are not the best antibiotics because of increasing antimicrobial resistance [11]. *N. sativa* lies not only in its pharmacological effects but also in the lack of toxicity and easy accessibility. Its use in food systems, supplements, and dermal formulations indicates its adaptability for the promotion of health and reduced risk of infection [12]. Considering the increasing worldwide interest in herbal bioactives, the full assessment of the antioxidant and antimicrobial role of *N. sativa* comes both timely and necessary. This work synthesizes current knowledge on the antioxidant and antimicrobial mechanisms of *N. sativa*, some supported by recent experimental data, and discusses their potential usefulness in the context of the development of functional foods, nutraceuticals, and pharmaceutical products.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Black Seed

Black seeds were collected from a medical market in Sulaymaniyah city, Iraq. The methods of extractions were carried out at the Halabja Technical College, Sulaimani Polytechnic University. Approximately 300 g of the residues of black seed were left to air dry at ambient temperature to lose their moisture content. The desiccated seeds were subsequently pulverized into a fine powder utilizing a mechanical grinder and filtered over an 80-mesh screen to guarantee consistent particle size. The powdered black seed was preserved in sealed containers at 4°C until subsequent use. To isolate antioxidant and antibacterial compounds, three different extraction methods were employed: aqueous, ethanolic, and methanolic extraction (Fig. 1).

2.2. Methods of Extraction

2.2.1. Aqueous extraction

A sterile beaker was used to suspend 100 g of black seed powder in 1 liter of distilled water. The suspension was agitated continuously using a magnetic stirrer for 18 h at ambient temperature. Whatman No. 41 filter paper was used to filter the solution. The resultant filtrates were transferred into dry sterile petri dishes and then dried at ambient temperature to produce a solid water extract [1], [13].

2.2.2. Ethanolic extraction

100 g of black seed were grinded and mixed with 1 L 70% ethyl alcohol in a sealed conical flask and stored far from light (25–30°C) for 24 h. Subsequent to extraction, the mixture was subjected to filtration, using Whatman filter paper. Ethanol was removed at 40°C with a rotary evaporator. The resultant semi-solid residue obtained was desiccated at ambient temperature to yield the ethanolic extract powder [1].

2.2.3. Methanolic extraction

Black seed powder (100 g) was incubated in 1 L 70% methanol and incubated under identical conditions as the ethanolic extraction. Following a 24-h period, the extract was subjected to filtration, and methanol was eliminated at 40°C utilizing a rotary evaporator. The concentrate was dehydrated at ambient temperature to get the powdered methanolic extract [14]. A stock solution (1% w/v) of each

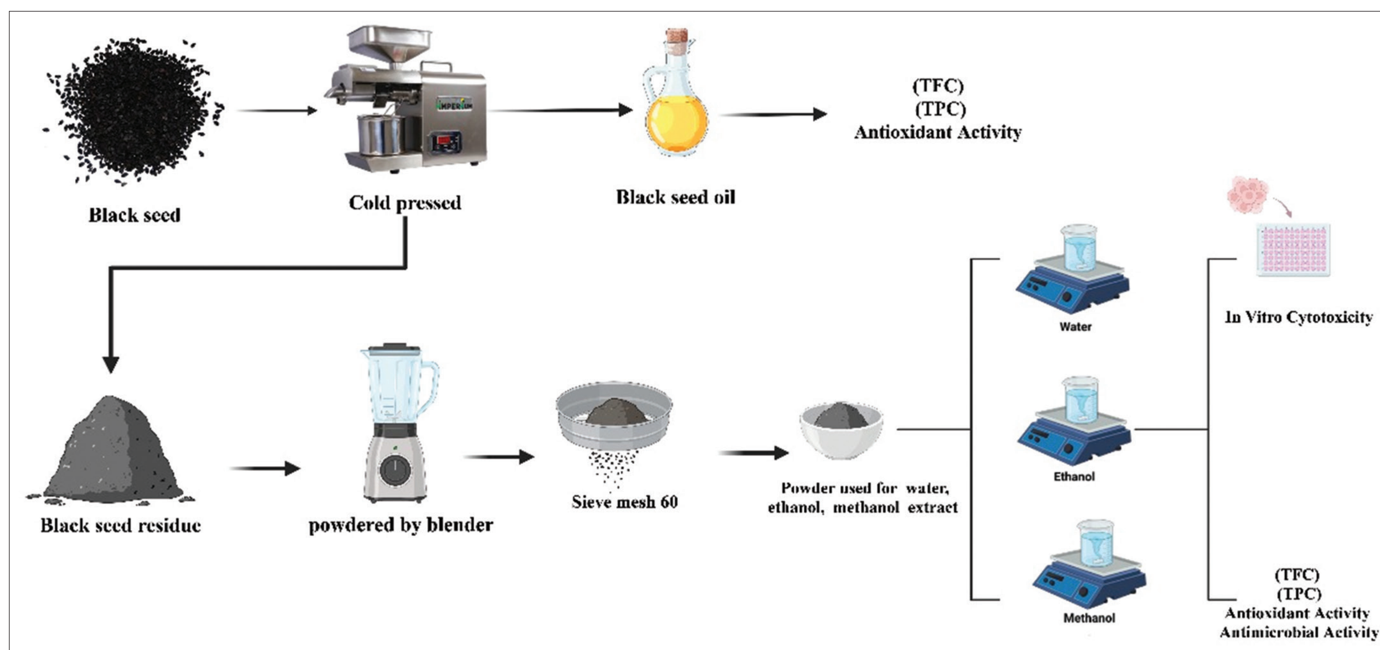

Fig. 1. Illustration of *Nigella sativa* processing and extraction workflow. Black seed oil was obtained by cold pressing, while the residual seed powder was subjected to aqueous, ethanolic, and methanolic extractions for subsequent antioxidant, antimicrobial, and cytotoxicity evaluations.

dried extract was produced by dissolving 1 g of extract in 100 mL of distilled water.

2.2.4. Oil extraction

Black seed powder residues were cold-pressed to obtain oil (Fig. 1). Extraction was carried out by a mechanical screw press without heat and chemical solvent addition, to preserve the integrity and bioactivity of thermolabile compounds. The newly extracted oil was filtered to eliminate possible suspended debris, then kept in amber glass bottles at 4°C until chemical characterization.

2.3. Assessment of Total Phenolic and Flavonoid Contents (TPC and TFC)

Measurement of (TPC) was carried out according to the Folin–Ciocalteu technique. In brief, 1.0 mL of black seed extracts were combined with 5.0 mL of 10% Folin–Ciocalteu reagent, after 5 min, 4.0 mL of 7.5% Na_2CO_3 was added. After 30 min of incubation at ambient temperature in the dark, an absorbance reading was performed at 765 nm. The data calculated were represented as mg GAE/g FW, using a calibration curve of gallic acid. TFC was determined using the aluminum chloride method, in which 0.5 mL of black seed extracts were combined with 0.1 mL of 10% $AlCl_3$, followed by adding to it a mixture of 0.1 mL (1 M) $C_2H_3KO_2$ and 4.3 mL distilled water. Absorbance was recorded at 415 nm after 30-min incubation at ambient temperature, and TFC was calculated in mg of quercetin equivalents per gram of fresh weight.

2.4. Antioxidant Assays

Three standard *in vitro* tests, including FRAP, ABTS+, and 2,2-diphenyl-1-picrylhydrazyl (DPPH)• assays, were utilized to determine the antioxidant activity of black seed residue extracts. In the DPPH assay, a 0.1 mM methanolic solution of DPPH was mixed in equal volume with black seed extract at different concentrations, kept in darkness at ambient temperature for 30 min, and optical density was determined at 517 nm. The formula $(A_0–A_1)/A_0 \times 100$ was used to calculate the scavenging activity (%), where A_0 is the absorbance of the control and A_1 is with extract. For ABTS assay, a solution of 7 mM ABTS with 2.45 mM $K_2S_2O_8$ was prepared to generate the ABTS$\bullet^+$ radical cation which was then incubated in darkness for 12–16 h. One milliliter of the solution was diluted, mixed with 1 mL of black seed extract, and incubated at ambient temperature for 6 min. The reaction was terminated with the addition of 5 N NaOH (50 μL), and absorbance was read at 734 nm. The antioxidant activities were also expressed by the same equation of DPPH assay. FRAP was determined by adding FRAP reagent (1 M acetate buffer pH 3.6, TPTZ (10 mM) solution in 40 mM HCl and $FeCl_3$ (20 mM), mixed at a ratio of 10:1:1, and incubated for 10 min at 37°C). 3 mL of pre-warmed FRAP reagent was mixed with a 100 μL aliquot of black seed extract at several

concentrations and incubated for 30 min at 37°C. Optical density was recorded at 593 nm using a Trolox calibration curve, and the result was expressed as milligrams of Trolox equivalent per gram of extract (mg TE/g) [14], [15].

2.5. *In vitro* Cytotoxicity Assay for PC3 Cell Line

The viability of the human prostate cancer cells was determined through the MTT assay, after being treated with *N. sativa* extracts for cytotoxic activity. PC-3 cells (ATCC, USA) were grown in DMEM containing 10% FBS and 1% penicillin-streptomycin, then passed onto 96-well plate at a density of 6×10^3 cells per well. After adherence for 24 h, the cells were exposed to various concentrations (10, 15, and 25 μg/mL) of methanol, ethanol, or water of *N. sativa* for inhibition determination. Unstimulated cells were used as a negative control, whereas 0.1% dimethyl sulfoxide (DMSO) was employed as a solvent control.

After incubating for 24 h, 10 μL of MTT reagent (0.5 mg/mL) was put into the wells and incubated at 37°C for another 4 h, followed by adding 100 μL of supplemented reagent to solubilize formazan crystals, and then the absorbance was recorded at a wavelength of 570 nm (reference wavelength is 650 nm) on a microplate reader (Molecular Devices LLC, USA). The percentage of cell viability was compared with the untreated control, and IC_{50} values were obtained from the concentration-response curves using GraphPad Prism version 9.0. A *P*-value below 0.05 was deemed statistically significant.

2.6. Antimicrobial Activity Assessment

Antimicrobial properties of *N. sativa* extracts were examined against 6 prevalent pathogens: fungus (*C. albicans* and *Aspergillus fumigatus*), Gram-negative (*P. aeruginosa* and *E. coli*), and Gram-positive (*Bacillus subtilis* and *S. aureus*) bacteria. All strains of microorganisms were collected from Halabja Technical College. Strains were activated in nutrient broth and incubated at 37°C overnight until they reached their logarithmic growth phase.

A 1,000 μL culture aliquot was evenly spread on Mueller–Hinton agar plates using a sterile L-shaped spreader, followed by incubation for 24 h at 37°C to obtain an even lawn. Extracts of black seed were also made at the concentration of 100 mg/ml with distilled water or a 5% DMSO solution. A volume of 4 μL of the black seed extract solution was implemented to sterile 6mm Whatman No. 1 filter paper discs using micropipette. The discs were then transferred to sterile Petri dishes for drying by laminar airflow during 24 h to allow efficient impregnation of the extract before use in testing antimicrobial tests [16]. Clear inhibition zones around each disc were measured in millimeters after being incubated at 37°C for 24 h for bacterial strains and 48 h for fungal strains.

2.7. Statistical Analysis

All experiments were performed in triplicate, and the results are represented as mean ± standard deviation. Statistical analyses were carried out with GraphPad Prism (version 9.0, GraphPad Software). Mean comparisons within extraction groups were done using Tukey's *post hoc* test following one-way analysis of variance. A *P*-value below 0.05 was deemed statistically significant.

3. RESULTS AND DISCUSSION

3.1. TFC and TPC

The TPC and TFC of black seed extracts prepared using different solvents (water, methanol, ethanol, and seed oil) were quantified and the results are illustrated in (Fig. 2) and (Table 1). The methanol extract exhibited the highest levels of both TFC (1.89 mg QE/g) and TPC (2.93 mg GAE/g), followed by the ethanol (1.82 mg QE/g; 2.89 mg GAE/g), water (1.75 mg QE/g; 2.87 mg GAE/g), and seed oil extract (0.84 mg QE/g; 0.94 mg GAE/g). The effectiveness of methanol as an extraction solvent is ascribed to its high polarity and capacity to break down plant cell walls, facilitating the solubilization of diverse polyphenolic and flavonoid compounds [17]. Similar findings were reported that methanolic extracts of *N. sativa* seeds yielded significantly higher flavonoid content than ethanol or water-based extracts [18], [19]. A study by Ibrahim *et al.* (2022) also highlighted the superior antioxidant profile of methanol extracts in phenolic

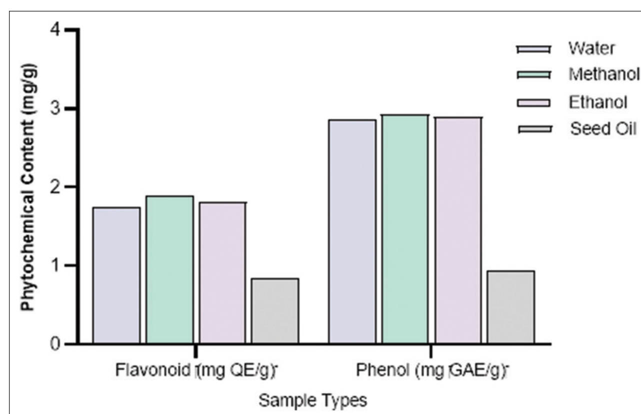

Fig. 2. Total flavonoid and phenolic content of black seed (*Nigella sativa*) extracts obtained using different solvents (water, methanol, ethanol, and seed oil).

TABLE 1: Antioxidant activity including (FRAP, ABTS, DPPH), total phenolic, and flavonoid contents of *Nigella sativa* extracts obtained using different extraction methods

Extraction type	DPPH (mg TE/g)	ABTS (mg TE/g)	Flavonoid (mg QE/g)	Phenol (mg GAE/g)	FRAP (mg TE/g)
Water extract	5.4	61.96	1.75	2.87	5.64
Ethanol extract	5.46	62.74	1.82	2.89	5.96
Methanol extract	5.53	67.18	1.89	2.93	5.78
Seed oil	2.3	50.31	0.84	0.94	3.29

DPPH: 2,2-diphenyl-1-picrylhydrazyl

recovery from *N. sativa* seeds, with strong DPPH and TPC correlations. Ethanol, with its intermediate polarity, provided slightly lower but still considerable amounts of TFC and TPC. Pharmaceutical and food-grade application usually prefers ethanol because it is less toxic than methanol [20]. Some research studies by Plaskova and Mlcek (2023) confirm the use of ethanol as a neutral solvent to get good polyphenol and flavonoid yields that is also biocompatible and safe [21]. Water extraction, generally known to be less efficient for flavonoid solubilization, unexpectedly gave a TPC (2.87mgGAE/g) almost the same as ethanol and methanol extraction. This could be attributed to water's power to dissolve highly hydrophilic phenolics, especially when using long extraction conditions (strict maceration, gentle heating has been applied in this study). A study published by Muzolf-Panek and Gliszczyńska-Świgło (2022) reported TPC (1.8–2.6 mgGAE/g) of water extracts prepared through 24h maceration of *N. sativa* to be comparable with those of 70% methanol and ethanol solvents [22]. The results showed that, with extended contact time, water can extract a considerable amount of phenolic compounds from the seed matrix and characterized the influence of extraction time and temperature in bridging the gap in efficiency between polar organic solvents and water. Seed oil, cold-pressed, had the minimal quantity of TPC (0.94 mg GAE/g) and TFC (0.84 mg QE/g). However, the existence of bioactive phenolics and flavonoid-type compounds in the oil matrix can still participate in its functional role. In a recent study of Ketenoglu, Ketenoglu, O *et al*. (2021) demonstrated that seeds of *N. sativa* and their cold-pressed oil contain antioxidant ingredients, such as TQ, that are not present in high quantities in the usual flavonoid assay, but are still major contributors to the antioxidant capacity [23]. These results highlight the importance of solvent polarity and extraction approach on the recovery of polyphenolic and flavonoid constituents. Methanol remains the most effective solvent for the analytical extraction and ethanol is a safer substitute for the nutraceuticals and food uses. Water, under optimized conditions, can also yield valuable antioxidant compounds. Seed oil, although not a rich source of flavonoids, retains unique lipophilic antioxidants contributing to its therapeutic relevance.

3.2. Antioxidant Assays (DPPH, ABTS, FRAP)

Antioxidant activities of black seed extracts, evaluated using DPPH, ABTS, and FRAP assays, showed marked variation based on the solvent used for extraction: methanol, ethanol, water, and seed oil (Fig. 3) and (Table 1). The methanolic extract of black seed were exhibited the strongest antioxidant performance with DPPH at 5.53 mg TE/g, ABTS at 67.18 mg TE/g, and FRAP 5.53 mg TE/g. This finding is consistent with previous research highlighting the high polarity of methanol and its effectiveness in extracting phenolic compounds and flavonoids with strong antioxidant potential. Kaushik and Barmanray (2022) reported that methanol extracts of *N. sativa* possessed the highest antioxidant capacity, attributed to their elevated phenolic and flavonoid content [24]. Water extracts, although generally considered less effective in extracting lipophilic antioxidants, surprisingly demonstrated considerable antioxidant activity: DPPH at 5.4 mg TE/g, ABTS at 61.96 mg TE/g, and FRAP at 5.4 mg TE/g. These results may be due to the solubility of hydrophilic phenolic acids and tannins in water, especially when aided by extended extraction times or moderate heating. Balyan and Ali (2022) and Rabiej-Kozioł and Szydłowska-Czerniak (2024) reported similar findings, showing that water, under optimal conditions, can yield antioxidant activities comparable to methanol in *N. sativa* extracts [25], [26]. The ethanol extract displayed intermediate antioxidant values DPPH at 5.46 mg TE/g, ABTS at 62.74 mg TE/g, and FRAP at 5.46 mg TE/g. This reflects ethanol's ability to dissolve both polar and some non-polar antioxidant compounds. As demonstrated by Bueraheng and Petchlert (2015), Dalli *et al*. (2021), ethanol extractions from *N. sativa,* provided balanced antioxidant properties, making ethanol a suitable choice for applications prioritizing safety alongside efficacy, such as food and pharmaceutical formulations [27], [28]. Cold-pressed seed oil showed the lowest antioxidant activity across all tests, with DPPH at 2.3 mg TE/g, ABTS at 50.31 mg TE/g, and FRAP at 3.29 mg TE/g. This lower activity is expected, as seed oils primarily consist of fatty acids with limited phenolic

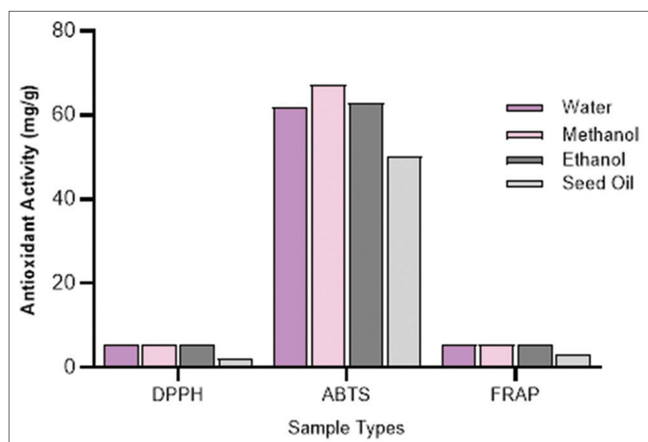

Fig. 3. Antioxidant activity of *Nigella sativa* extracts obtained using different solvents (water, methanol, ethanol, and seed oil) assessed by 2,2-diphenyl-1-picrylhydrazyl, ABTS, and FRAP assays.

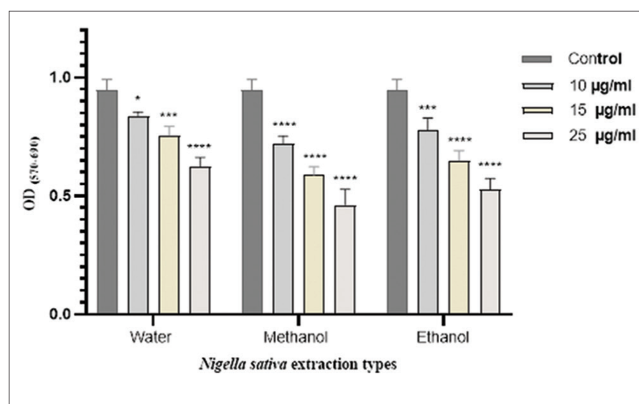

Fig. 4. Effect of different concentrations (10, 15, and 25 µg/mL) of *Nigella sativa* extracts (water, methanol, ethanol) on PC-3 prostate cancer cell proliferation assessed by MTT assay.

content. However, the presence of lipophilic antioxidants such as TQ and tocopherols contributes to measurable activity in the FRAP assay. Alrashidi *et al*. (2022) and Neunert *et al*. (2025) confirmed that *N. sativa* seed oil may lack potent hydrophilic radical scavengers, it retains valuable antioxidant properties from its lipid-soluble constituents [29], [30]. In conclusion, the antioxidant activity of *N. sativa* extracts depends heavily on the extraction solvent used. Methanol yielded the highest antioxidant capacities across all three assays, followed by water and ethanol, while seed oil showed comparatively lower activity. These findings demonstrate the significance of optimizing solvent selection depending on the desired application whether maximizing antioxidant yield for therapeutic, nutraceutical, or cosmetic uses.

3.3. *In vitro* Cytotoxicity Method for PC3 Cell Line

The present study demonstrates that *N. sativa* extracts prepared with methanol, ethanol, and water significantly inhibit PC3 prostate cancer cell proliferation in a dose-dependent manner, with statistical confirmation by Dunnett's multiple comparisons test (Fig. 4). Specifically, treatment with water extract reduced cell viability by 11.06%, 19.23%, and 32.46% at 10, 15, and 25 µg/mL respectively ($P = 0.0368$, 0.0001, <0.0001), indicating modest but significant antiproliferative effects. In comparison, the ethanol extract produced stronger reductions of 16.93%, 29.84%, and 41.93% at the same concentrations ($P = 0.0007$, <0.0001, <0.0001), highlighting its superior efficacy relative to water. However, the methanol extract exhibited the most pronounced cytotoxic effect, decreasing cell viability by 22.78%, 35.90%, and 48.49% at 10, 15, and 25 µg/mL, respectively (all $P < 0.0001$), confirming its potent antiproliferative activity. These findings are in line with extensive literature demonstrating the superior ability of methanol to extract TQ, flavonoids, and other phenolic compounds, which are primarily responsible for the anticancer properties of *N. sativa* [18], [31], [32]. In addition, these results are strongly supported by Toy *et al.* (2022), who demonstrated that methanol extract of *N. sativa* not only inhibits PC3 cell viability but also induces apoptosis through p53 and caspase-3 upregulation, modulates LC3-based autophagy, and suppresses clonogenic potential [33]. Together, these results confirm that while all *N. sativa* extracts exhibit anticancer activity, methanol extract is the most effective, likely due to its enhanced extraction of key bioactive compounds, further supporting its potential as a promising therapeutic candidate for prostate cancer management.

3.4. Antimicrobial Properties of Black Seed Extracts

Antimicrobial potential of various black seed extracts including water, methanol, and ethanol, was assessed against various Gram- negative and Gram-positive bacterial species, in addition to fungal strains (Table 2) and (Fig. 5), demonstrating that all extracts exhibited measurable inhibition, though methanol and ethanol consistently showed the strongest effects. Specifically, Gram-positive bacteria such as *B. subtilis* and *S. aureus* were notably sensitive, with methanol and ethanol extracts producing inhibition zones of 14–17 mm, corroborating findings that phenolic- and TQ -rich extracts exert potent activity against Gram-positive pathogens [34], [35]. In contrast, Gram-negative bacteria including *P. aeruginosa* and *E. coli* showed moderate susceptibility, with inhibition zones ranging from 12 to 15 mm for methanol and ethanol extracts, likely due to

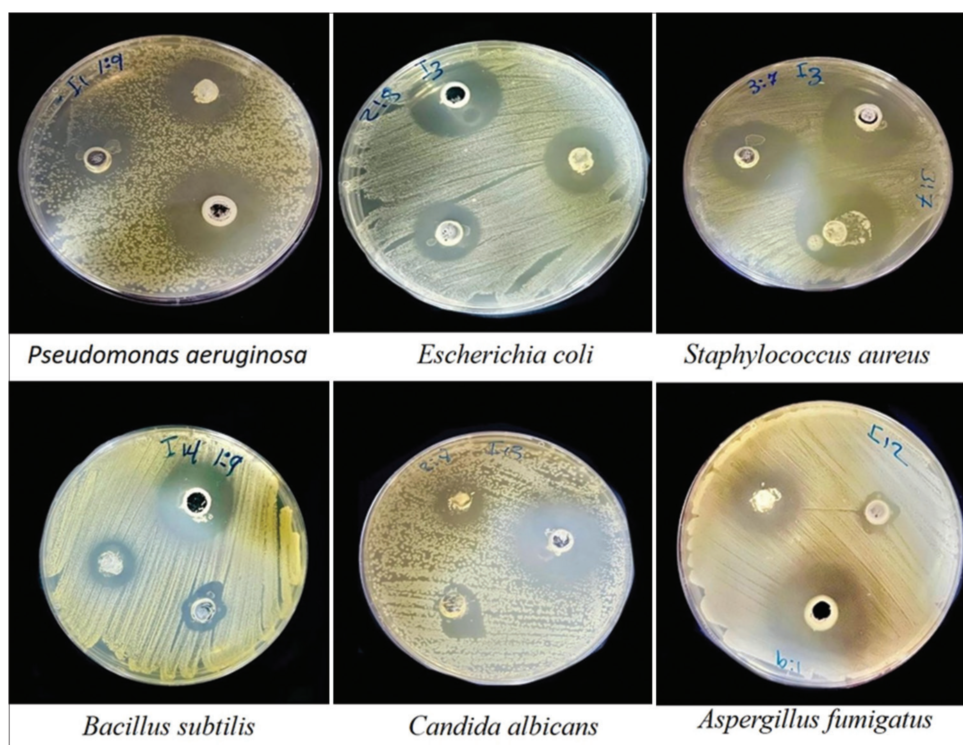

Fig. 5. Antimicrobial activity of black seed (*Nigella sativa*) extract against selected microbial strains: Using disc diffusion method.

TABLE 2: Antimicrobial activity of *Nigella sativa* extracts (water, methanol, ethanol, and seed oil) against selected microbial strains

Microbial strains	W. E. (mm)	M. E. (mm)	E. E. (mm)	Seed oil (mm)
Bacillus subtilis	12	16	17	13
Staphylococcus aureus	10	14	15	12
Escherichia coli	8	14	15	11
Pseudomonas aeruginosa	7	12	14	10
Candida albicans	10	17	16	12
Aspergillus fumigatus	9	15	16	11

their ability to extract antimicrobial alkaloids and flavonoids capable of penetrating the outer membrane of Gram-negative bacteria [4], [36]. Fungal strains, including *C. albicans* and *A. fumigatus*, were highly susceptible to methanol and ethanol extracts (zones of 15–17 mm), which is supported by data showing the antifungal efficacy of TQ, thymol, and other phenolic compounds found in these solvents [16], [35]. Although water and oil extracts exhibited lower inhibition zones (7–13 mm), they still showed noteworthy effects, particularly against *C. albicans*, *B. subtilis*, and *S. aureus*, indicating the presence of water-soluble saponins and oil-soluble bioactives such as tocopherols and TQ [35], [37]. Overall, the comparative efficacy clearly favors ethanol and methanol as extraction solvents due to their ability to isolate a broader range of antimicrobial phytochemicals, reaffirming *N. sativa*'s traditional medicinal use and its potential as a plant-based antimicrobial agent.

4. CONCLUSION

This study provides strong evidence that black seed is a rich natural source of bioactive compounds with potent antioxidant, antimicrobial, and anticancer properties. Among the extraction methods evaluated, methanolic and ethanolic extracts consistently demonstrated superior biological activity, attributed to their enhanced ability to extract TQ, flavonoids, and phenolic compounds. The methanolic extract of black seed demonstrated the highest concentration of total phenolic and flavonoids, the strongest antioxidant potential across DPPH, ABTS, and FRAP assays, and the most pronounced antimicrobial and antiproliferative effects, particularly against PC-3 prostate cancer cells. While aqueous and cold-pressed oil extracts showed comparatively lower activity, they still retained measurable bioactive potential, supporting their suitability for applications prioritizing safety and accessibility. These findings show the critical role of solvent selection in optimizing the yield and efficacy of bioactive compounds from *N. sativa*. Overall, this research highlights black seed

as a promising alternative for the production of natural antioxidant, antimicrobial, and anticancer agents applicable in pharmaceutical, nutraceutical, and functional food industries. Further studies focusing on the isolation of individual active constituents and *in vivo* validation are recommended to support its future clinical applications.

REFERENCES

[1] A. Ahmad, A. Husain, M. Mujeeb, S. A. Khan, A. K. Najmi, N. A. Siddique, Z. A. Damanhouri and F. Anwar. "A review on therapeutic potential of *Nigella sativa*: A miracle herb". *Asian Pacific Journal of Tropical Biomedicine*, vol. 3, no. 5, pp. 337-352, 2013.

[2] S. Milovanovic, D. Markovic, I. Jankovic-Castvan and I. Lukic. "Cornstarch aerogels with thymol, citronellol, carvacrol, and eugenol prepared by supercritical CO_2- assisted techniques for potential biomedical applications". *Carbohydrate Polymers*, vol. 331, p. 121874, 2024.

[3] A. H. Rahmani, M. A. Alzohairy, M. A. Khan and S. M. Aly. "Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways". *Evidence-based Complementary and Alternative Medicine*, vol. 2014, p. 724658, 2014.

[4] F. Forouzanfar, B. S. F. Bazzaz and H. Hosseinzadeh. "Black cumin (*Nigella sativa*) and its constituent (thymoquinone): A review on antimicrobial effects". *Iran Journal of Basic Medical Science*, vol. 17, no. 12, pp. 929-938, 2014.

[5] A. L. Ayed and Z. Talal. "Long-term effects of *Nigella sativa* L. Oil on some physiological parameters in normal and streptozotocin-induced diabetic rats". *Journal of Diabetes Mellitus*, vol. 1, no. 1, pp. 46-53, 2011.

[6] C. Sarkar, S. Jamaddar, T. Islam, M. Mondal, M. T. Islam and M. S. Mubarak. "Therapeutic perspectives of the black cumin component thymoquinone: A review". *Food and Function*, vol. 12, no. 14, pp. 6167-6213, 2021.

[7] M. Alagawany, S. S. Elnesr, M. R. Farag, M. E. Abd El-Hack, A. F. Khafaga and K. Sharun. "Health-promoting activities of *Nigella sativa* essential oil". In: *Black Cumin (Nigella sativa) Seeds: Chemistry, Technology, Functionality, and Applications*. Springer, Berlin, 2020, pp. 457-478.

[8] A. Thakur, K. Pathania, L. Sharma and D. Deepika. "Exploring the potential of *Nigella sativa* seeds: A modern perspective on an ancient remedy". *Journal of Advances in Medicine and Medical Research*, vol. 37, no. 1, pp. 6-16, 2025.

[9] M. Abbas, M. A. Gururani, A. Ali, S. Bajwa, R. Hassan, S. W. Batool, M. Imam and D. Wei. "Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella sativa*: A review". *Molecules*, vol. 29, no. 20, p. 4914, 2024.

[10] M. Shamim Molla, A. K. Azad, M. A. A. Al Hasib, M. M. Hossain, M. S. Ahammed, S. Rana and M. T. Islam. "A review on antiviral effects of *Nigella sativa* L". *Pharmacology Online*, vol. 2, pp. 47-53, 2019.

[11] S. N. Bakal, S. Bereswill and M. M. Heimesaat. "Finding novel antibiotic substances from medicinal plants - antimicrobial properties of *Nigella sativa* directed against multidrug-resistant bacteria". *European Journal of Microbiology and Immunology*, vol. 7, no. 1, pp. 92-98, 2017.

[12] A. Alberts, E. T. Moldoveanu, A. G. Niculescu and A. M. Grumezescu. "*Nigella sativa*: A comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications". *International Journal of Molecular Sciences*, vol. 25, no. 24, pp. 13410, 2024.

[13] E. Dušková, K. Dušek, P. Indrák and K. Smékalová. "Postharvest changes in essential oil content and quality of lavender flowers". *Industrial Crops and Products*, vol. 79, pp. 225-231, 2016.

[14] W. Elfalleh, N. Tlili, N. Nasri, Y. Yahia, H. Hannachi, N. Chaira, M. Ying and A. Ferchichi. "Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits". *Journal of Food Science*, vol. 76, no. 5, pp. C707-C713, 2011.

[15] P. Shah and H. Modi. "Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity". *International Journal for Research in Applied Science and Engineering Technology*, vol. 3, no. 6, pp. 636-641, 2015.

[16] N. A. Hasan, M. Z. Nawahwi and H. A. Malek. "Antimicrobial activity of *Nigella sativa* seed extract". *Sains Malaysiana*, vol. 42, no. 2, pp. 143-147, 2013.

[17] A. Turay, H. A. Kargbo, E. B. S. Conteh and I. A. Kamara. "Comparative analysis of methanolic and aqueous extracts of four medicinal plants in Sierra Leone". *Journal of Pharmacognosy and Phytochemistry*, vol. 14, no. 3, pp. 149-151, 2025.

[18] S. Chathoth, M. Nawaz, M. Amir, R. Ahmed, M. Aldholmi, S. Al-Mofty, C. Cyrus, C. Vatte and M. Salahuddin. "Assessment of the effect of solvent polarity on *Nigella sativa* extracts of different origin". *Journal of Pharmacy and Pharmacognosy Research*, vol. 13, no. 5, pp. 1345-1355, 2025.

[19] H. Abidat, S. Saad, S. Karoune, B. Hamida, S. Lahmadi, M. S. A. Kechebar and I. Rahmoune. "Evaluation of secondary metabolites, antioxidant and enzyme inhibitory activities of *Nigella sativa* and *Lepidium sativum* local seeds cultivated in arid environment: A comparative analysis". *Plant Biosystems*, vol. 159, no. 3, pp. 500-514, 2025.

[20] A. Oreopoulou, D. Tsimogiannis and V. Oreopoulou. "Extraction of polyphenols from aromatic and medicinal plants: An overview of the methods and the effect of extraction parameters". In: *Polyphenols in Plants*. Academic Press, United States, pp. 243-259, 2019.

[21] A. Plaskova and J. Mlcek. "New insights of the application of water or ethanol-water plant extract rich in active compounds in food". *Frontiers in Nutrition*, vol. 10, p. 1118761, 2023.

[22] M. Muzolf-Panek and A. Gliszczyńska-Świgło, "Extraction optimization for the antioxidants from *Nigella sativa* seeds using response surface methodology". *Journal of Food Measurement and Characterization*, vol. 16, no. 6, pp. 4741-4753, 2022.

[23] O. Ketenoglu, S. S. Kiralan, M. Kiralan, G. Ozkan and M. F. Ramadan, "Cold pressed black cumin (*Nigella sativa* L.) seed oil". In: *Cold Pressed Oils*. Elsevier, Netherlands, 2020, pp. 53-64.

[24] N. Kaushik and A. Barmanray. "Evaluation of flavonoids content, phenolic compounds and antioxidant properties of *Nigella sativa* seed extracts using different solvents". *International Journal of Food and Nutritional Sciences*, vol. 11, no. 3, pp. 2964-2973, 2022.

[25] P. Balyan and A. Ali. "Comparative analysis of the biological activities of different extracts of *Nigella sativa* L. seeds". *Annals of Phytomedicine: An International Journal*, vol. 11, pp. 577-587, 2022.

[26] D. Rabiej-Kozioł and A. Szydłowska-Czerniak, "Antioxidant potential evaluation at various stages of black cumin oil production". *Foods*, vol. 13, no. 21, p. 3518, 2024.

[27] S. Bueraheng and C. Petchlert, Eds. "Antioxidant Activity of Ethanol Extracts from *Nigella sativa* and *Cuminum cyminum* L". In: *Proceedings. The 7th National Science Research Conference*, 2015, pp. 30-31.

[28] M. Dalli, S. E. Azizi, F. Kandsi and N. Gseyra, "Evaluation of the *in vitro* antioxidant activity of different extracts of *Nigella sativa* L. seeds, and the quantification of their bioactive compounds". *Matererials: Today Proceeding*, vol. 45, pp. 7259-7263, 2021.

[29] M. Alrashidi, D. Derawi, J. Salimon and M. F. Yusoff. "The effects of different extraction solvents on the yield and antioxidant properties of *Nigella sativa* oil from Saudi Arabia". *Journal of Taibah University for Science*, vol. 16, no. 1, pp. 330-336, 2022.

[30] G. Neunert, W. Kamińska and J. Nowak-Karnowska. "Evaluating the thymoquinone content and antioxidant properties of black cumin (*Nigella sativa* L.) seed oil during storage at different thermal treatments". *Applied Science*, vol. 15, no. 1, p. 377, 2025.

[31] B. Ali and G. Blunden. "Pharmacological and toxicological properties of *Nigella sativa*". *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, vol. 17, no. 4, pp. 299-305, 2003.

[32] S. Çetinkaya, İ. Ç. Ayan, H. G. Dursun, İ. Süntar, K. Taban and H. N. Gök. "Enhanced apoptosis in pancreatic cancer cells through thymoquinone-rich *Nigella sativa* L. methanol extract: Targeting NRF2/HO-1 and TNF-α pathways". *Anti-Cancer Agents in Medicinal Chemistry*, vol. 25, pp. 1607-1621, 2025.

[33] Y. Toy, M. Kivrik, M. H. Alkhafaji, A. M. Faraj, I. H. Gecibesler and C. A. Ağca. "*Nigella sativa* methanol extract inhibits PC-3 cell line colonization, induced apoptosis, and modulated LC3-based autophagy". *Karbala International Journal of Modern Science*, vol. 8, no. 2, pp. 44-51, 2022.

[34] A. S. Kabbashi, M. I. Garbi, E. E. Osman, M. M. Dahab, W. S. Koko and N. Abuzeid. "*In vitro* antimicrobial activity of ethanolic seeds extract of *Nigella sativa* (Linn) in Sudan". *African Journal of Microbiology Research*, vol. 9, pp. 788-792, 2015.

[35] S. Tiji, Y. Rokni, O. Benayad, N. Laaraj, A. Asehraou and M. Mimouni. "Chemical composition related to antimicrobial activity of Moroccan *Nigella sativa* L. Extracts and isolated fractions". *Evidence-based Complementary and Alternative Medicine*, vol. 2021, p. 8308050, 2021.

[36] N. M. Morsi. "Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria". *Acta Microbiologica Polonica*, vol. 49, no. 1, pp. 63-74, 2000.

[37] A. Jabbar, F. A. Shah, A. Ashraf, G. Hassan, E. Rehman, S. A. Bangash, R. M. K. Shabbir, N. Naeem and I. Ullah. "Evaluation of *in vitro* antibacterial activity and phytochemical screening of *Nigella sativa* leaves extracts against human pathogenic bacteria". *International Journal of Health Science*, vol. 6, no. S9, pp. 4434-4445, 2022.