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Comparative Extraction and Evaluation of Phenolic Compounds and Antioxidant Activity Using Soxhlet, Ultrasonic, and Maceration Methods with Black Cumin (Nigella Sativa)

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Highlights

•Ultrasonic extraction at 45°C using ethanol yielded the highest phenolic content and antioxidant activity.
•Soxhlet, ultrasonic, and maceration methods were compared to determine optimal extraction conditions.
•Nigella sativa (black cumin) exhibits strong bioactivity, supporting its use as a natural therapeutic agent.

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Abstract

Nigella sativa, commonly known as black cumin, is renowned for its healing properties and diverse bioactive compounds. This study investigates the extraction of black cumin using various techniques, including Soxhlet, ultrasonic, and maceration methods, examining the effects of different solvents and extraction temperatures. The total phenolic content and antioxidant capacity of the extracts obtained from each method were determined and evaluated, with antioxidant contents measured as DPPH equivalents. Extracts obtained from the Nigella sativa plant were examined using three different methods (Soxhlet, ultrasonic, maceration) and three solvents (acetone, DI water, ethanol), with the best results achieved using ethanol as a solvent and the ultrasonic method at 45 °C. The ultrasonic method was observed to be the most effective in terms of antiradical and phenolic content. These findings highlight the potential of black cumin as a natural therapeutic agent. Total phenolic content, a crucial metric for assessing phenolic compounds, offers insights into the potential health benefits of these extracts due to their antioxidant and anti-inflammatory properties. Evaluating the antioxidant properties is equally important, as antioxidants neutralize harmful free radicals, reducing oxidative stress and cellular damage. Rigorous analytical procedures ensured accuracy and reliability in quantifying both total phenolic content and antioxidant capacity. The findings identify the most suitable extraction conditions, optimizing the bioactive potential of black cumin. This comprehensive analysis provides a foundation for utilizing black cumin as a potent source of natural therapeutic agents with significant phenolic and antioxidant properties.

1. INTRODUCTION

Nigella sativa, commonly known as black cumin, has garnered widespread attention for its extraordinary therapeutic potential and is a focal point within the realm of natural medicine [1-6]. Known for its versatile bioactive profile, Nigella sativa has been extensively studied for a range of health-promoting properties, including antioxidant, anti-inflammatory, antibacterial, and antifungal effects [7-10]. The active compounds in black cumin, such as thymoquinone, phenolic acids, and flavonoids, contribute significantly to these health benefits and have shown promising applications in managing oxidative stress, inflammation, and metabolic disorders [11-14]. Given this therapeutic diversity, black cumin is considered a valuable candidate for the development of natural therapeutic agents.

This study aims to meticulously extract these bioactive constituents from the black cumin plant using a comparative approach with three different techniques: Soxhlet, ultrasonic, and maceration methods. By varying extraction methods, solvents, and temperature parameters, this research seeks to identify the

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optimal conditions for maximizing the yield and efficacy of bioactive compounds. Evaluating total phenolic content and antioxidant capacity will provide key insights into the effectiveness of each extraction method and help determine the most efficient way to harness Nigella sativa's therapeutic potential.

Historically, plants have played a significant role across multiple domains, ranging from culinary and cosmetic applications to pharmaceuticals, with their healing properties being well-documented in various traditional systems [5, 15-17]. Early extraction methods involved rudimentary techniques like grinding and boiling; however, modern technology has allowed for the development of more efficient and targeted methods for isolating plant compounds [18-20]. Extraction serves as a separation method distinct from purification, focusing on isolating specific organic or inorganic components from plants [10-14]. Such advancements have made it possible to refine and analyze bioactive components with higher accuracy, enhancing our understanding of plant-based compounds and their health implications.

Nigella sativa, an annual plant characterized by an upright, herbaceous stem, grows up to 40-80 centimeters in height. Of the approximately 20 species within the genus Nigella, 14 are found in our region's indigenous flora. In regions like Afyon, Burdur, and Isparta, black cumin is cultivated not only for culinary purposes but also for traditional medicinal uses, such as improving milk production, stimulating appetite, and aiding menstrual regulation [21-23].

The black cumin plant is renowned for its therapeutic properties, including antiviral, antitumor, antidiabetic, antioxidant, antifungal, antibacterial, antiulcer, and even antidepressant activities. These benefits are attributed to the plant's diverse array of chemical compounds, particularly phytochemicals, which are organic compounds located within the roots, stems, and leaves and are known for their substantial biological and medicinal impacts [24-26]. Studies have shown that Nigella sativa's phytochemical profile holds potential for applications in various health contexts, such as managing inflammation, reducing oxidative stress, and supporting metabolic health, which underscores the need for efficient extraction techniques [7, 27].

This study undertakes the extraction of bioactive compounds from Nigella sativa through a multi-faceted approach, using Soxhlet, ultrasonic, and maceration methods to obtain high-quality extracts. Subsequent analysis of total phenolic content and antioxidant properties will provide a comparative view of each extraction method, enabling the identification of the most effective approach for optimizing black cumin's bioactive potential. The outcomes of this research are expected to contribute to the field of natural therapeutics by offering valuable insights into the applications of black cumin extracts in health and wellness [28-30].

In this study, three different extraction methods (Soxhlet, ultrasonic, and maceration) were used for the extraction of black cumin seeds. The best solvent and method were determined, followed by experiments conducted at various temperatures using the identified method and solvent. Detailed information on the experimental studies is elaborated in the following sections. The extraction methods and conditions used in this period are listed below. An ethanol solvent with an organic structure and possessing antiseptic properties against fungi, bacteria, and viruses was employed using all three different methods. Ethanol is easily preferred in experimental studies due to the absence of toxic gas emissions. Analyzes were performed using the GENESYS 150 UV-Visible Spectrophotometer, which has a spectral bandwidth of 2 nm and a wavelength range of 190 to 1100 nm. Each of these methodologies Soxhlet extraction, ultrasonic extraction, and maceration offers a distinct approach to liberating the potent phytochemical constituents embedded within the plant's botanical matrix.

Soxhlet extraction involves continuous cycling of solvent through the sample, maximizing the extraction efficiency of lipophilic compounds. Ultrasonic extraction utilizes high-frequency sound waves to disrupt cell walls and release intracellular compounds, while maceration relies on the diffusion of solvents through the plant material over time. The combined application of these techniques promises to yield a comprehensive and nuanced understanding of the diverse phytochemical profile present in the black cumin plant. This includes phenolic compounds, flavonoids, and other antioxidants known for their health benefits, such as anti-inflammatory and antioxidant properties.

In the study, three different extraction techniques were used. The Soxhlet method, developed by Franz von Soxhlet in 1879, is a cornerstone technique in the field of botanical extraction. It provides a versatile approach for obtaining extracts, the composition and quality of which are intricately tied to the choice of solvent used. This method is particularly well-suited for extracting bioactive compounds from solid or semi-solid samples.

The Maceration method hinges on allowing the solvent to intimately interact with the plant material to facilitate the extraction of bioactive compounds. Controlled agitation promotes the gradual dissolution and diffusion of desired constituents into the solvent, resulting in an enriched extract.

The Ultrasonic method harnesses the vibrational energy of sound waves, operating at frequencies exceeding 20 kHz. This high-frequency oscillation induces dynamic changes within the liquid medium, creating microscopic voids and forming minuscule bubbles. This phenomenon greatly enhances the diffusion of the solvent, thereby facilitating the extraction process.

2. MATERIAL METHOD

2.1. Materials

Dried Nigella sativa (black cumin) seeds were obtained from Herbal Vital. The solvents used in the extraction methods included ethanol, acetone, and distilled water, each chosen for its specific properties relevant to phenolic extraction and antioxidant activity. Dimethyl sulfoxide (DMSO) was supplied by Merck (Merck KGaA, Darmstadt, Germany) for preparing solutions in the DPPH assay, while the DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent itself was obtained from Sigma Aldrich (Sigma-Aldrich Pty Ltd, affiliated with Merck KGaA, Darmstadt, Germany). Additional materials include sodium carbonate for the Folin-Ciocalteu assay, prepared as a 1% solution, and Folin reagent, also used in the phenolic quantification assay. The UV-Visible spectrophotometer (GENESYS 150) was used for absorbance measurements with high sensitivity across a broad wavelength spectrum, ensuring accuracy in assessing phenolic content and antioxidant capacity.

2.2. Soxhlet Extraction

The apparatus consists of a cartridge in which the solid sample, in this case 40 grams of black cumin, is placed. At the base of the device is a round-bottomed flask known as a balloon flask to which the selected solvent is added. The device starts working by heating the solvent in the balloon flask. As the temperature increases, vapor is formed and moves upwards through the device. When it reaches the condenser, the vapor condenses back into liquid form. The condensed liquid then drips onto the solid sample in the cartridge and starts the extraction process. Over time, the solvent is continuously drained from the sample and the bioactive compounds are gradually removed.

In this study, the Soxhlet extraction parameters as so: 40 grams of black cumin, 120 mL of ethanol, acetone and distilled water were used in experimental studies (Figure 1). Operational Temperature was 50 ºC, extraction duration was 60 minutes. After extraction, the obtained extract undergone additional refinement: The extract was separated using a rotary device set at 130 rpm, the rotary device was maintained at 50 ºC, the extract was processed for 90 minutes to further refine and concentrate the extracted bioactive compounds.

Figure 1. *Extract obtained by Soxhlet method with (a) ethanol, (b) acetone and (c) DI water solvent*

2.3. Maceration Technique

In this study, the maceration method is executed as so: 15 grams of black cumin, 190 mL of solvent (ethanol, acetone, and distilled water), Conducted at a controlled temperature of 50 ºC for a duration of 60 minutes, this carefully orchestrated procedure ensures optimal extraction of bioactive compounds from the black cumin (Figure 2).

Following the maceration phase, the resultant solution undergone additional refinement: The solution is processed using a rotary device set at 130 rpm, the rotary device is maintained at 50 ºC, the process continues for 90 minutes to further separate and concentrate the bioactive compounds obtained from the maceration process.

Figure 2. *Extract obtained by Maceration method with (a) ethanol, (b) acetone and (c) DI water solvent*

2.4. Ultrasonic Extraction

In this study, the ultrasonic extraction parameters are as so: 10 grams of black cumin, 200 mL of solvent (ethanol, acetone, and distilled water), Conducted at a controlled temperature of 50 ºC for a duration of 60 minutes (Figure 3).

Following the ultrasonic extraction phase, the resulting solution undergone additional refinement: Processed using a rotary device set at 130 rpm, the rotary device is maintained at 50 ºC, continued for 90 minutes to further isolate and concentrate the bioactive compounds obtained from the ultrasonic extraction.

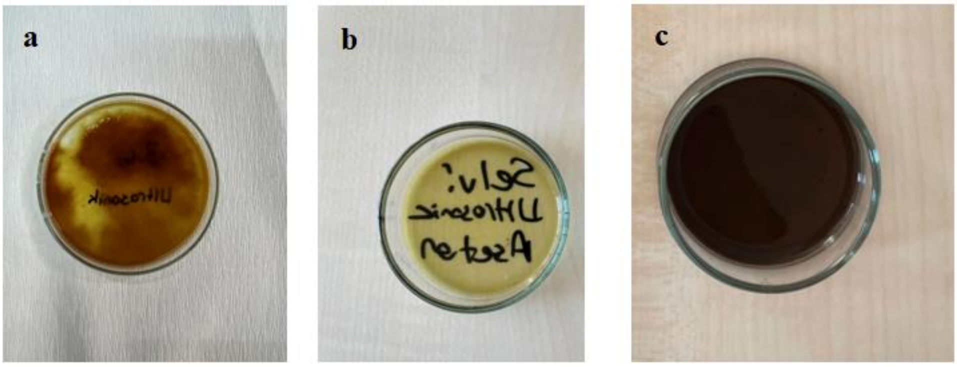

Figure 3. *Extract obtained by Ultrasonic method with (a) ethanol, (b) acetone and (c) DI water solvent*

2.5. Quantification of Phenolic Content in Black Cumin Extracts: A Detailed Methodology

The quantification of phenolic content in black cumin extracts was conducted using the Folin-Ciocalteu method, following a systematic and precise procedure [18-21]. The process began with the preparation of essential solutions: a sodium carbonate solution was made by dissolving 1 gram of sodium carbonate in 50 mL of distilled water, and a Folin-Ciocalteu reagent solution was prepared by mixing 5 mL of Folin reagent with 5 mL of distilled water. These solutions, along with additional distilled water, were combined with the black cumin extracts obtained through Soxhlet, Ultrasonic, and Maceration methods in predetermined ratios.

The prepared mixtures were transferred into falcon tubes, which were then wrapped in aluminum foil to prevent light-induced alterations. After a 45-minute incubation period to ensure reactions reached equilibrium, absorbance measurements were taken using a UV-Visible Spectrophotometer (GENESYS 150 Thermo Scientific) at a wavelength of 760 nm.

For calibration, a standard curve was generated using known concentrations of gallic acid, allowing phenolic content to be expressed as gallic acid equivalents (GAE). Measurements were conducted in groups of four to ensure accuracy and data robustness, providing greater clarity in method application and calibration.

2.6. Measurement of Antioxidant Activity in Black Cumin Extracts using DPPH Assay

The antioxidant activity of black cumin extracts obtained by Soxhlet, ultrasonic, and maceration methods was assessed using the DPPH assay [17]. This section details the experimental procedure and results obtained. A DPPH solution was prepared by dissolving 1 mg of DPPH in 25 mL of methanol, resulting in a purple-colored solution. The solution was then wrapped in aluminum foil to protect it from sunlight, as DPPH is light-sensitive. For each extract, 1 mg of plant extract was dissolved in 1 mL of DMSO solvent.

3. RESULTS AND DISCUSSION

The study involved a comprehensive analysis of total phenolic content and antioxidant properties in extracts derived from black cumin, employing various extraction methodologies. Total phenolic content serves as a pivotal metric in quantifying the concentration of phenolic compounds within a given extract. Phenolic compounds are renowned for their potential health benefits, including antioxidant and anti-inflammatory properties. Therefore, assessing the total phenolic content provides valuable insights into the potential therapeutic value of the black cumin extracts. Simultaneously, the evaluation of antioxidant properties is of paramount importance. Antioxidants play a critical role in neutralizing harmful free radicals within the body, thereby mitigating oxidative stress and reducing the risk of cellular damage. This assessment aids in understanding the capacity of the black cumin extracts to confer protective effects against oxidative stress-related disorders. To facilitate these assessments, black cumin extracts obtained through different extraction methods were subjected to rigorous analytical procedures. The specific techniques and methodologies employed in these measurements were carefully selected to ensure accuracy and reliability in quantifying both total phenolic content and antioxidant capacity. The results of these analyses will be instrumental in discerning the most effective extraction method for harnessing the bioactive potential of black cumin. This, in turn, will provide a solid foundation for further exploration and utilization of black cumin as a potent source of natural therapeutic agents with notable phenolic and antioxidant properties.

3.1. Determination of Phenolic Content

The measurement results obtained from the quantification of phenolic content in black cumin extracts are meticulously compiled and presented in Figure 4. The total phenolic measurements were performed using a UV-Visible Spectrophotometer at a wavelength of 765 nm. The results were expressed as milligrams per gram of gallic acid equivalent (GAE) extract from the gallic acid standard curve plot ($y = 0,0063x - 0,0031$ $R^2 = 0,9986$).

The data in Figure 4 demonstrate that the choice of solvent and extraction method significantly impacts the phenolic content in black cumin extracts. Ethanol is identified as the most effective solvent for extracting phenolic compounds, followed by DI water and acetone. Among the extraction methods, Soxhlet extraction consistently yields the highest phenolic content across all solvents, indicating its superior efficiency. Ultrasonic extraction is the second most effective, while maceration is the least effective method. The overall ranking of phenolic content from highest to lowest is: Ethanol Soxhlet, Ethanol Ultrasonic, Ethanol Maceration, DI Water Soxhlet, DI Water Ultrasonic, Acetone Soxhlet, DI Water Maceration, Acetone Ultrasonic, and Acetone Maceration. Thus, using ethanol with Soxhlet extraction is the most effective combination for maximizing the phenolic content in black cumin extracts. The total phenolic content is expressed as mg (GAE)/g of extract.

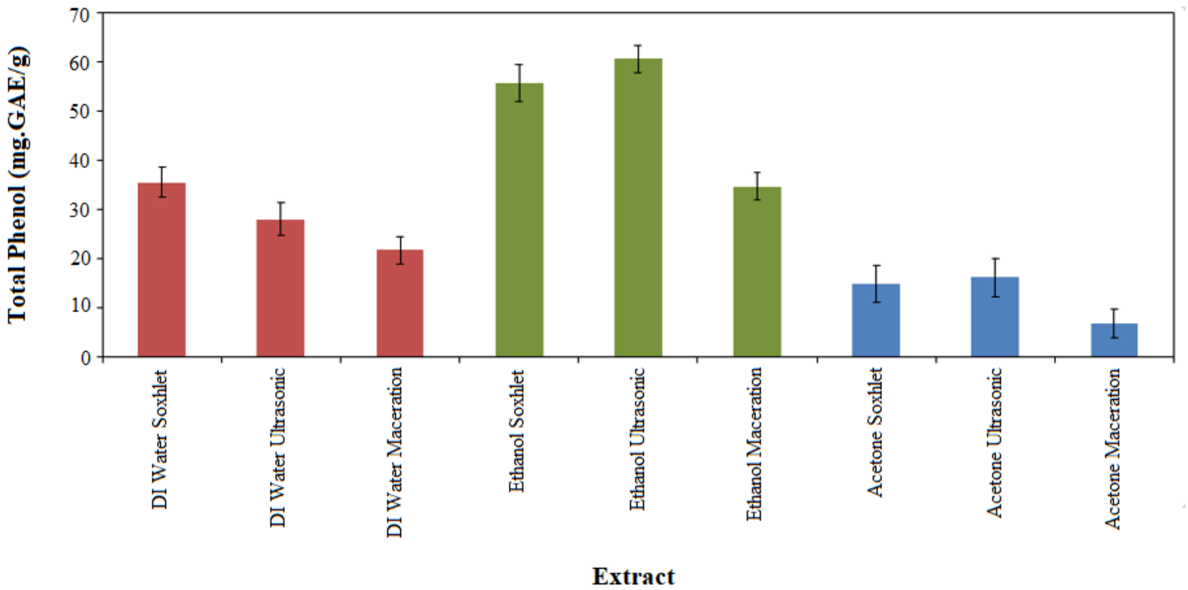

Figure 4. *Comprehensive Analysis of Phenolic Content in Black Cumin Extracts*

3.2. Antioxidant Activity Measurement

The antioxidant activity was evaluated by measuring the color change of the DPPH solution after addition of the plant extract solutions. The more the solution turns yellow, the higher the antioxidant effect. The solution of the Soxhlet extract predominantly turned yellow, indicating high antioxidant activity. Antioxidant measurements were performed using a UV-Visible Spectrophotometer at a wavelength of 517 nm, which is specific for DPPH. The measurement values were expected to be lower than the control solution (0.57 absorbance units). A decrease in absorbance indicates increased antioxidant activity, as the free radicals in the DPPH solution are scavenged by antioxidants present in the plant extracts. As the concentration of the plant extract increased, the measured values decreased, indicating enhanced antioxidant activity. This suggests that the plant extracts have the ability to neutralize free radicals, as evidenced by the reduction in absorbance values. The DPPH assay provided valuable insights into the antioxidant potential of black cumin extracts obtained through different extraction methods. The results demonstrate that the Soxhlet extract exhibited significant antioxidant activity, as indicated by the yellow color formation and reduced absorbance values. These findings underscore the potential of black cumin as a natural source of antioxidants with implications for various health applications. The DPPH radical scavenging activity values were calculated using the following Equation (1) and the results are presented in Table 1

$$\text{DPPH Radical Scavenging Activity}(\%) = \frac{\text{A(control)} - \text{A(sample)}}{\text{A(control)}} * 100 \quad (1)$$

A(control): It is the absorbance value of the control sample (DPPH solution).

A(sample): It is the absorbance value of the mixture of the compound at a specific concentration and DPPH solution.

Table 1. *DPPH Radical Scavenging Activity (%) Values*

Solvent / Technique	Ethanol			Acetone			DI Water		
Soxhlet	49.1	45.6	42.1	84.2	80.7	87.7	66.6	61.4	65.9
Ultrasonic	43.8	40.3	38.5	85.9	84.2	78.9	85.9	68.9	71.9
Maceration	68.4	64.9	63.1	91.2	89.4	94.7	80.7	77.1	75.4

Upon a detailed examination of the results, it has been observed that the best outcome for the black seed plant is obtained through the ultrasonic method using ethanol solvent [5]. Utilizing this information, the studies have continued with experiments at different temperatures. Extracts were prepared using the ultrasonic method and ethanol solvent at three different temperatures (40°C, 45°C, 50°C). Since the experiment with ethanol solvent and the ultrasonic method was conducted at 50°C, experimental studies were repeated for 40°C and 45°C (Figure 5).

Figure 5. *Extract obtained by Ultrasonic method with ethanol at 40°C and 45°C*

Total phenolic measurements were conducted with the obtained extracts and the results are presented in the following graphs.

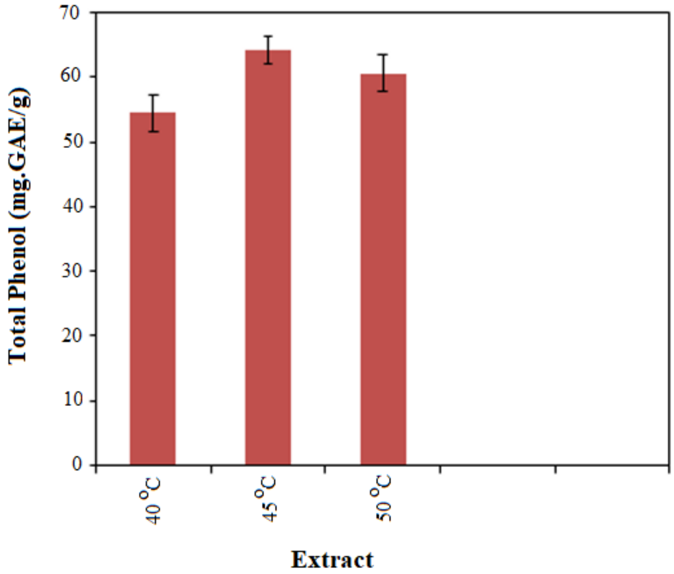

Figure 6. *Total Phenolic content of extracts at 40°C, 45°C, 50°C*

When all the collected data from the studies are examined it is observed that the optimal extraction method for the black seed plant is obtained through the ultrasonic method with ethanol solvent at 45°C (Figure 6). When we examine the 'Phenolics From Defatted Black Cumin Seeds (Nigella Sativa L.): Ultrasound-Assisted Extraction Optimization Comparison And Antioxidant Activity' article. We will see the results that support our studies. In this article it will be observed that the best extract was obtained using the ultrasonic method with ethanol solvent at a temperature of 44.6 °C and in 32.5 minutes.

3.3. Position in the Literature and Scientific Contributions

The therapeutic properties of Nigella sativa (black cumin) are well-documented, particularly regarding its bioactive compounds—such as phenolics and antioxidants—that contribute to health benefits, including anti-inflammatory and antioxidant effects. Numerous studies have investigated extraction methods for obtaining oil from Nigella sativa; however, these are often focused on optimizing oil yield, evaluating physicochemical stability, or enhancing applications in food products. For instance, prior research has examined the effect of different extraction methods on the shelf-life stability and quality of black cumin oil [31], as well as alternative green solvents for safer lipid extraction [32]. Additionally, recent studies have explored the integration of black cumin extracts into functional food matrices to improve the bioactive properties of products like yoğurt [33]. However, there is limited research that systematically evaluates multiple extraction methods to maximize both phenolic content and antioxidant activity. This study addresses this gap by directly comparing Soxhlet, ultrasonic, and maceration extraction techniques under standardized conditions to identify optimal parameters for phenolic yield and antioxidant efficacy. Unlike studies that focus on shelf life or food fortification, our research takes a targeted approach to enhance bioactivity, specifically for nutraceutical and pharmaceutical applications where antioxidant potency is critical.

Furthermore, by using food-grade solvents like ethanol, this study aligns with the growing trend towards safer and more sustainable extraction processes. The optimized parameters identified not only enhance the therapeutic potential of black cumin extracts but also present scalable solutions for industrial applications. In this way, our study contributes to the literature by providing essential insights and practical data that support both research and implementation in health-focused product development.

4. CONCLUSION

In this study, extracts from Nigella sativa were obtained using three different extraction methods (Soxhlet, ultrasonic, and maceration) and three different solvents (acetone, DI water, and ethanol). The total phenolic content was measured in the obtained extracts, as detailed in previous sections. Among the methods and solvents tested, the ultrasonic extraction method using ethanol was identified as the most effective. This method was further investigated through experimental studies at different temperatures to optimize the extraction conditions. The results indicated that the best outcomes were achieved using ultrasonic extraction with ethanol at 45°C, both in terms of extract yield and bioactivity. The antioxidant properties of Nigella sativa were also examined, and the ultrasonic extract exhibited the strongest antioxidant activity. The total phenolic values and antioxidant test results demonstrated that ethanol, especially when used in ultrasonic extraction, was the most effective solvent for maximizing phenolic content and antioxidant potential. Therefore, ultrasonic extraction with ethanol at 45°C is recommended as the optimal method due to its high efficiency and significant bioactivity, making it suitable for practical applications in large-scale production.

In terms of extraction efficiency, ultrasonic extraction was the most effective method, achieving the highest extract yield of 20%, followed by maceration at 13%, and Soxhlet at 12.5%. The ultrasonic method is not only more efficient but also faster and uses less solvent compared to Soxhlet, making it more cost-effective. Maceration, although effective, requires the longest extraction time to achieve similar yields. While Soxhlet is a widely used method for extracting a broad range of compounds, it is more resource-intensive and slower than ultrasonic extraction.

These findings highlight the potential of Nigella sativa (black cumin) as a source of bioactive compounds with strong antioxidant properties. The study demonstrates the importance of extraction methods in maximizing the bioactivity of the extracts. Given the efficiency of ultrasonic extraction with ethanol, particularly at 45°C, this method is recommended for large-scale production of antioxidant-rich extracts. Future research and optimization efforts can further enhance the use of Nigella sativa extracts in various health-related applications, such as nutraceuticals and pharmaceuticals aimed at combating oxidative stress.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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