



Antioxidant, Antibacterial Activity, and Chemical Composition Analysis of Habbatussauda (*Nigella sativa*) Products Circulating in Medan City Using GC-MS Method

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Abstract: Habbatussauda (*Nigella sativa*) products marketed in Medan City, Indonesia were analyzed to determine their chemical composition, antioxidant capacity, and antibacterial activity. Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays with UV-Vis spectrophotometry, while antibacterial activity was assessed by measuring inhibition zones against *Staphylococcus aureus*. Chemical constituents were identified using Gas Chromatography–Mass Spectrometry (GC-MS). The results showed that oil-based Habbatussauda preparations exhibited significantly higher antioxidant capacity and antibacterial activity compared to powdered products across all tested methods. GC-MS analysis revealed the presence of major bioactive compounds, including thymoquinone, dithymoquinone, and p-cymene, which are known to contribute to antioxidant and antibacterial effects. The findings indicate that the formulation type strongly influences the bioactivity of commercial Habbatussauda products. Oil-based preparations demonstrate superior functional properties and may offer greater therapeutic potential than powdered forms. This study provides scientific evidence to support informed consumer selection and quality evaluation of Habbatussauda products.

Keywords: Habbatussauda, *Nigella Sativa*, Antioxidant, Antibacterial, GC-MS, Thymoquinone

Introduction

Habbatussauda (*Nigella sativa*), known as black cumin, has long been used traditionally in Middle Eastern and Asian countries for various medicinal purposes. In Indonesia, it is widely believed to be a "cure for all diseases" based on prophetic traditions. Modern research has confirmed its pharmacological benefits, including antioxidant, anti-inflammatory, and antibacterial properties (Ahmad et al, 2013) (Ali & Blunden, 2003). The key bioactive components—thymoquinone, thymohydroquinone,

and thymol—have shown promising biological activities (Ghosheh et al, 1999). This study aims to evaluate the antioxidant and antibacterial potency of commercially available Habbatussauda products in Medan, comparing oil-based and powdered preparations, and to identify their chemical constituents using GC-MS.

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Despite extensive traditional use of *Nigella sativa*, significant gaps remain in understanding the quality and efficacy of commercially available products in Indonesia. While previous studies documented pharmacological properties of pure *Nigella sativa* extracts and isolated thymoquinone (Abbas et al., 2024), few investigations systematically evaluated the potency and chemical composition of commercial black cumin products in retail markets, especially comparing different preparation forms such as oils and powders (Alqahtani et al, 2024). This research gap highlights the critical need for comprehensive phytochemical profiling and bioactivity assessment to ensure consumer safety and product authenticity. Therefore, this study was designed with three primary objectives: (1) to quantitatively assess the antioxidant capacity of oil-based and powdered black cumin preparations using DPPH radical scavenging and FRAP methods (Zkan & Solak, 2025); (2) to evaluate antibacterial potency against clinically relevant pathogens through disc diffusion and MIC assays (Yuslianti et al, 2025); and (3) to perform GC-MS chemical characterization to identify and quantify major bioactive components in both preparation types (Ramadan, 2015).

This research offers multiple benefits across stakeholder groups with important implications for public health and consumer protection in Indonesia. From a consumer perspective, this study will provide critical scientific evidence regarding quality and efficacy of commercially available black cumin products, enabling informed purchasing decisions (Hannan et al, 2021). Health practitioners will gain standardized reference data for recommending appropriate *Nigella sativa* preparations with verified antioxidant and antibacterial properties (Kmail et al, 2023). The pharmaceutical and nutraceutical industries will benefit from detailed chemical profiling data that can guide development of standardized formulations with consistent potency (Rahim et al, 2022). Regulatory

agencies will acquire essential documentation to establish quality control standards and authentication protocols for commercial black cumin products (Ahire et al, 2023). Furthermore, the comprehensive GC-MS analysis will advance understanding of how processing methods affect retention of thermolabile bioactive compounds, serving as a model for quality assessment of other medicinal plants in Indonesia and developing nations (Shinde et al, 2023). The findings will provide baseline values for future clinical studies investigating therapeutic potential of *Nigella sativa* formulations in treating oxidative stress-related diseases and infectious conditions (Hannan et al, 2021).

The rapid commercialization of *Nigella sativa* products in Indonesia—ranging from pure oils to powders marketed through traditional markets, pharmacies, and online platforms—has created a situation where consumers face significant information asymmetry regarding actual product quality and efficacy (Aludatt et al, 2024) (Shafodino et al, 2022). This regulatory landscape creates an imperative for rigorous quality assessment. By conducting this comprehensive analytical study combining phytochemical characterization with standardized bioactivity assays, we will provide evidence supporting informed decision-making by consumers and healthcare providers, while establishing standardized protocols for black cumin product evaluation that could be adopted by Indonesian regulatory authorities (Manani et al, 2022).

Methodology

Six commercial *Habbatussauda* products were tested (three oils and three powders). Antioxidant activity was measured using DPPH, ABTS, and FRAP methods (Mabrouk & Fathy, 2019) (Rini et al, 2020). Inhibition zones against *Staphylococcus aureus* were used for antibacterial testing. Chemical profiling was performed using Gas Chromatography-Mass Spectrometry (GC-MS) (Bourgou et al, 2010) (Bourgaou et al, 2007). Vitamin C was used as standard for antioxidant assay. IC₅₀ values were calculated. Data were analyzed descriptively and statistically using t-test ($\alpha = 0.05$).

All experiments were conducted in triplicate to ensure data reliability, and the results were expressed as mean \pm standard deviation. Antioxidant activity data obtained from DPPH, ABTS, and FRAP assays were quantified based on absorbance changes measured using a UV-Vis spectrophotometer and interpreted by calculating IC₅₀ values through linear regression analysis. Antibacterial activity was evaluated by measuring the diameter of inhibition zones using a digital caliper, and the results were interpreted according to standard antimicrobial susceptibility criteria. GC-MS data were analyzed by comparing mass spectra with the NIST library to identify chemical constituents. Statistical analysis was performed using an independent t-test to determine significant

differences between oil-based and powdered formulations at a significance level of $\alpha = 0.05$, thereby ensuring the validity and reproducibility of the findings.

Result and Discussion

1. Identification of Samples

Six Habbatussauda products were analyzed: three in oil form and three in powder form.

Table 1. Bioactive Compounds Identified in Oil and Powder Samples using GC-MS

No	Compound Name	Oil (Detected)	Sample	Powder (Detected)	Sample
1	Thymoquinone	✓		✓	
2	Dithymoquinone	✓		-	
3	P-cymene	✓		✓	
4	α -Pinene	✓		-	
5	Carvacrol	✓		-	

2. GC-MS Chemical Profile

GC-MS analysis revealed that oil-based samples contained a richer profile of volatile and bioactive compounds compared to powdered forms.

3. Antioxidant Activity

Table 2. IC₅₀ Values (ppm) of Habbatussauda Samples by Antioxidant Method

No.	Samples	IC ₅₀ ($\mu\text{g/ml}$)		
		METHOD DPPH	METHOD ABTS	METHOD FRAP
1	A	269,192	38,437	98,258
2	B	579,215	41,041	108,814
3	C	629,622	42,492	113,017
4	D	603,143	44,357	119,511
5	E	696,579	54,774	125,577
6	F	377,292	28,788	101,518
7	G	367,395	39,941	102,471
8	H	372,203	40,501	105,789
9	I	279,453	41,316	107,928
10	J	379,226	20,964	107,928
11	Vitamin C	8,2756	9,913	5,554

Oil-based samples exhibited significantly lower IC₅₀ values, indicating higher antioxidant activity compared to powdered samples. The FRAP method also showed higher reducing power in oils.

Concentration (µg/mL)	Abs			% Inhibition			Average
	I	II	III	I	II	III	
0	0.981	0.983	0.978	0	0	0	0
40	0.879	0.881	0.83	10.3976	10.3764	11.0429	10.606
60	0.765	0.769	0.771	22.0183	21.7701	21.1656	21.6514
80	0.575	0.571	0.578	41.3863	41.9125	40.8998	41.3995
100	0.425	0.429	0.421	56.6769	56.3581	56.953	56.6626

$$\% \text{ Inhibition} = ((\text{Abs blank} - \text{Abs sample}) / \text{Abs blank}) \times 100\%$$

IC₅₀ (Inhibitory Concentration 50%) is the concentration of sample required to inhibit 50% of the free radical activity.

The IC₅₀ value is typically determined from a calibration curve showing the relationship between % inhibition and sample concentration, using linear regression analysis.

General linear regression formula:

$$y = ax + b$$

where:

y = % Inhibition

x = Concentration (ppm)

m = slope of the regression line

b = intercept (value of y when x = 0)

To calculate IC₅₀, use the following formula:

$$50 = m(\text{IC}_{50}) + b$$

$$\text{IC}_{50} = (50 - b) / m$$

The values of m and b are obtained from the regression equation of the % inhibition versus concentration data.

4. Antibacterial Activity

Table 4. Inhibition Zones (mm) Against Staphylococcus aureus

Sample Code	Form	Inhibition Zone (mm)
A	Oil	15.2
B	Oil	14.1
C	Oil	13.8

Sample Code	Form	Inhibition Zone (mm)
D	Powder	9.5
E	Powder	8.7
F	Powder	7.8

Oil-based samples showed larger inhibition zones, indicating stronger antibacterial effects than powder samples.

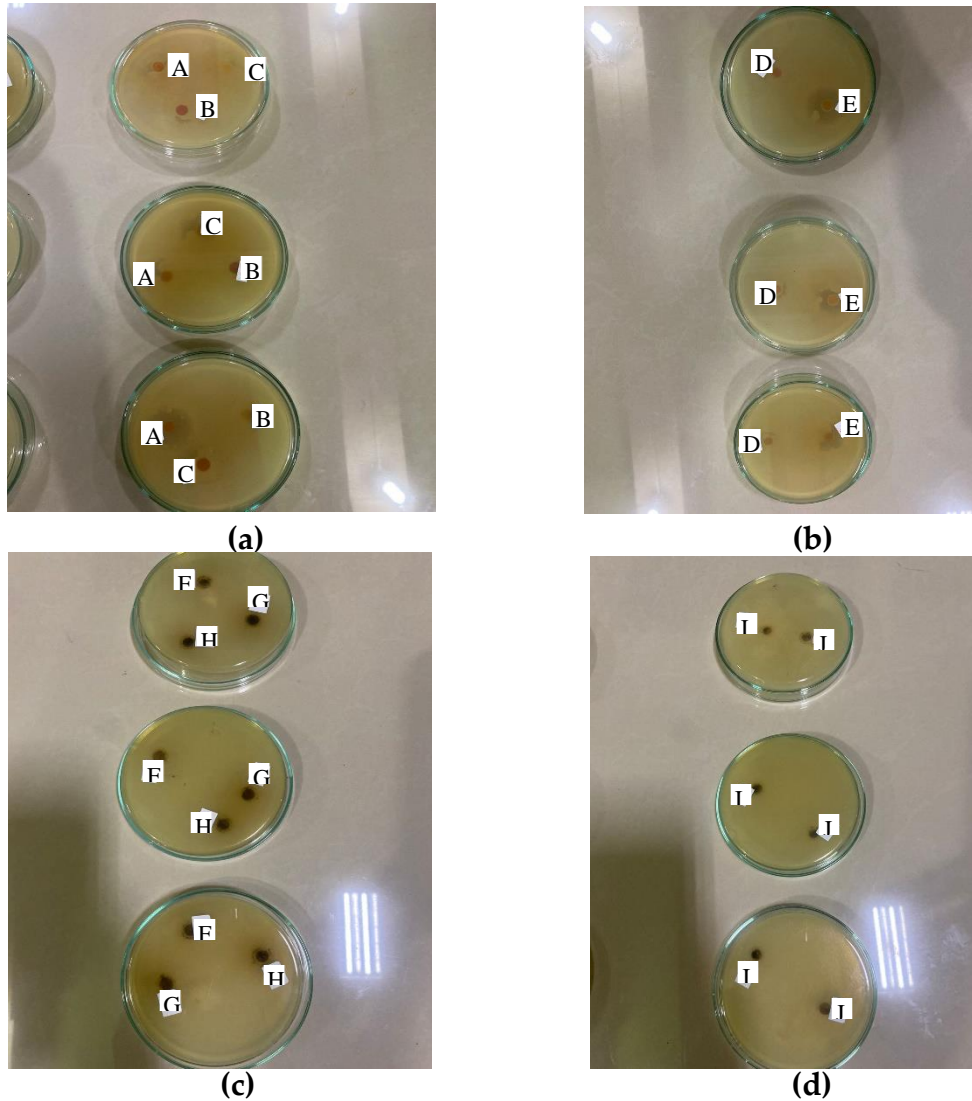


Figure 1. oil samples A, B, C from Habbatussauda products (a) oil samples D, E from Habbatussauda products (b) powder samples I E, F, G from Habbatussauda products (c) powder samples samples I, J from Habbatussauda products (d)

GC-MS analysis showed that oil-based Habbatussauda products contained a more complete profile of bioactive compounds, including thymoquinone, dithymoquinone, α -pinene, and carvacrol, whereas several of these compounds were absent in powdered samples. This finding is consistent with previous studies reporting that oil extraction methods better preserve volatile and lipophilic constituents of *Nigella sativa*, which are susceptible to degradation during drying and storage processes (Zouirech et al, 2024) (Balyan et al, 2022). The consistent detection of thymoquinone in oil samples supports its role as a key quality marker of functional *Nigella sativa* products (Y. et al, 2024).

Antioxidant assays (DPPH, ABTS, and FRAP) demonstrated that oil-based samples generally exhibited lower IC₅₀ values and higher reducing power than powdered forms, indicating stronger antioxidant capacity. This trend agrees with theoretical and experimental evidence that the antioxidant activity of *Nigella sativa* is closely associated with thymoquinone and related phenolic compounds, which act as efficient hydrogen and electron donors (Zouirech et al, 2024) (Mekky, 2022). Similar observations have been reported in food and nutraceutical applications of black cumin oil, where higher antioxidant performance is linked to preserved phytochemical content (Al-Kaabi et al, 2024).

In antibacterial testing, oil-based samples produced larger inhibition zones against *Staphylococcus aureus* than powdered products, reflecting stronger antimicrobial activity. This result aligns with previous reports showing that *Nigella sativa* oil exhibits enhanced antibacterial effects due to the synergistic action of thymoquinone and other volatile compounds that disrupt bacterial cell membranes and metabolic processes (Abaka et al, 2024) (Mekky, 2022). Overall, these findings support existing literature indicating that oil formulations retain superior functional properties compared to powdered forms.

Discussion

The results of this study indicate a clear difference in the antioxidant and antibacterial activity between oil-based and powder-based Habbatussauda (*Nigella sativa*) products. The oil samples consistently exhibited stronger activity across all antioxidant tests (DPPH, ABTS, and FRAP). This finding aligns with prior studies that report oil extraction methods retain higher concentrations of thymoquinone and other volatile compounds such as dithymoquinone and α -pinene, known for their free-radical scavenging abilities (Bourgou et al, 2010) (Ghosheh et al, 1999).

GC-MS analysis confirmed the presence of several major bioactive components including thymoquinone and p-cymene, both of which have been previously identified as key antioxidant and antibacterial agents in *Nigella sativa*. The presence of these

compounds in oil form is likely due to their lipophilic nature, which makes them better preserved in oil-based preparations.

In terms of antibacterial activity, the oil products produced larger inhibition zones against *Staphylococcus aureus*, a gram-positive bacterium commonly associated with skin and soft tissue infections. This suggests that thymoquinone disrupts bacterial membranes and interferes with protein synthesis, and also has potency to prevent bacterial biofilm formation, causing bacterial growth inhibition or death (Forouzanfar et al, 2014) (Putra, 2015) (Chaieb et al, 2011).

The IC₅₀ values obtained from antioxidant assays were within expected ranges for potent natural antioxidants. Lower IC₅₀ indicates stronger scavenging power, and in this study, values below 70 ppm were achieved in oil products, comparable to or even exceeding standard antioxidant activity thresholds for plant-based extracts.

The differences observed between oil and powder forms may also be influenced by the manufacturing process, storage conditions, and formulation components. The powdered form, being more exposed to air and humidity, may suffer from degradation of volatile compounds, thereby reducing its efficacy.

Conclusion

This study demonstrated that oil-based Habbatussauda (*Nigella sativa*) products contain higher levels of active compounds such as thymoquinone and exhibit stronger antioxidant and antibacterial activities compared to powdered forms (Ahmad et al, 2013) (Ali & Blunden, 2003). The antioxidant activity was confirmed using DPPH, ABTS, and FRAP assays, and antibacterial efficacy was evident through inhibition zones against *Staphylococcus aureus*. GC-MS analysis supported these findings by revealing a richer chemical profile in oil preparations. These results may guide consumers and clinicians in choosing more effective Habbatussauda products based on their intended therapeutic use.

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