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Phytochemical Profiling and Spectroscopic Elucidation of a Terpenoid from *Nigella sativa* Seeds

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Abstract
The present study focuses on the extraction, isolation, and structural characterization of bioactive constituents from the ethyl acetate extract of *Nigella sativa* (black seed). Using Soxhlet extraction and silica gel column chromatography, ten fractions (A–J) were obtained, with fraction F showing significant similarity to the standard terpenoid (carvacrol) based on thin-layer chromatography (TLC) with an Rf value of 0.64. UV-Visible spectroscopy of fraction F revealed a λmax at 292 nm, indicating aromatic characteristics. FTIR analysis confirmed the presence of phenolic terpenoids. 1H NMR spectra indicated the presence of methyl, isopropyl, and aromatic protons, while mass spectrometry confirmed the molecular ion peak at m/z 150.2000, corresponding to the compound 2-methyl-5-(propan-2-yl)phenol ($C_{10}H_{14}O$). This study validates the presence of pharmacologically relevant terpenoids in N. sativa and supports its traditional medicinal applications. The identified compound can serve as a potential lead for future drug development due to its reported antioxidant and antimicrobial properties.

Keywords: *Nigella sativa*, Phytochemical screening, Terpenoid, TLC, FTIR, NMR, Mass spectrometry, 2-methyl-5-(propan-2-yl)phenol

1. Introduction

Medicinal plants have played a crucial role in drug discovery and therapeutic development for centuries. It is estimated that approximately 20% of all known plant species have been investigated for their potential pharmaceutical applications, leading to significant contributions in the treatment of life-threatening conditions such as cancer and other chronic diseases **(Naczk and Shahidi, 2006).** The therapeutic potential of plants lies in their ability to produce a large number of bioactive secondary metabolites. These phytochemicals, particularly when present in high concentrations in fruits and vegetables, are known for their antioxidant properties and protective effects against oxidative stress and cellular damage **(Suffredini *et al*., 2004; Boots *et al*., 2008).** Natural antioxidants derived from plants are increasingly valued for their role in enhancing human health and preventing degenerative diseases.

Among these medicinal plants, *Nigella sativa* L. (family: Ranunculaceae), commonly known as black cumin or black seed, stands out due to its diverse pharmacological profile and historical importance in traditional medicine systems. Widely recognized for both its culinary and therapeutic uses, *N. sativa* has been traditionally used in the treatment of various ailments including asthma, bronchitis, rheumatism, headaches, anorexia, amenorrhea, back pain, eczema, and hypertension **(Chaudhry *et al.*, 2020).** Its seeds and oil preparations have been attributed with a broad spectrum of biological activities such as antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, anticancer, neuroprotective, cardioprotective, antidiabetic, gastroprotective, nephroprotective, and hepatoprotective effects **(Yimer *et al.*, 2019).**
Despite its rich history and wide usage, phytochemical investigations of *N. sativa* remain limited. The current study focuses on the isolation and structural elucidation of novel terpenoid compounds from *N. sativa*, with the aim of uncovering potential therapeutic agents and contributing to the scientific understanding of its medicinal value.

2. Materials and Methods

2.1 Collection and authentication of plant material

The *Nigella sativa* was collected from Local market of Bhopal, M.P.

2.2 Extraction:

Nigella sativa powder was defatted with petroleum ether and successively extracted further with ethyl acetate in a Soxhlet extractor.

2.3 Preliminary phytochemical investigations

Preliminary phytochemical investigations of the *Nigella sativa* extracts **(Kokate, 1991)** *Nigella sativa* extract were subjected to qualitative analysis for various phytochemical constituents.

2.4 Thin layer chromatography: -

Pre-coated silica gel G TLC plates were cut into strips, and the origin was marked with a straight line approximately 1 cm from the bottom edge. The ethyl acetate extract of *Nigella sativa* was applied at the origin and placed in a pre-saturated TLC chamber containing the solvent system. Preliminary TLC analysis of the ethyl acetate extract revealed that the mobile phase consisting of toluene: ethyl acetate (4.8:0.2) provided the best resolution, producing the maximum number of visible spots in comparison to other solvent systems. Carvacrol , a standard terpenoid , was also run alongside for comparison. The solvent front was allowed to rise by capillary action until it reached the upper limit, marked with another straight line. After development, the plates were removed, air-dried, exposed to iodine vapors, and examined under UV light **(Kumar *et al.*, 2009).** The retention factor (Rf) values for each spot were calculated using the following formula:

$$\mathbf{R_f\ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

2.5 Column chromatography

The extracts were subjected to silica gel column chromatography for the isolation of terpenoids from the ethyl acetate extract of *Nigella sativa*. A vertical glass column (30 mm diameter) made of borosilicate material was used for the chromatography. The column was rinsed with acetone and completely dried before packing. It was packed using the wet packing technique with silica gel (60–120 mesh) as the

adsorbent. Slurry was prepared using toluene and poured into the column. One gram of extract was loaded onto the top of the column. Gradient elution was performed using a solvent system of toluene:ethyl acetate (4.8:0.2), and multiple fractions were collected. The collected fractions were concentrated, and thin-layer chromatography (TLC) was performed to identify the presence of compounds **(Srivastava *et al*., 2021).**

2.6 Spectroscopic characterization: -

2.6.1 UV-visible Spectroscopy

The isolated fraction (F) of *Nigella sativa* ethyl acetate extract was scanned in the wavelength range of 200 to 800 nm using a UV-Visible spectrophotometer (Shimadzu UV-1700), and the characteristic absorption peaks were detected and recorded **(Patel *et al.*, 2022)**.

2.6.2 FT-IR

To establish the presence of functional groups in the isolated fraction (F) of *Nigella sativa* ethyl acetate extract, FT-IR spectroscopy was performed using a Perkin Spectrum 95763 spectrophotometer. The sample was dried and finely ground with potassium bromide (KBr) to form pellets and analyzed using a Thermo Nicolet Model 6700 spectrometer. A disk was prepared by mixing 2% of the finely dried sample with 100 mg of KBr. The infrared spectra were recorded in the range of 400–4000 cm^{-1} **(Moraes *et al.*, 2008).**

2.6.3 NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy was performed to identify the structure of the compound present in the isolated fraction (F) of *Nigella sativa* ethyl acetate extract. The analysis was carried out using a JNM EC-500 NMR spectrometer **(Zia *et al.*, 2019).**

2.6.4 Mass Spectroscopy

Mass spectrometry was employed to determine the molecular weight of the isolated fraction (F) of *Nigella sativa* ethyl acetate extract. The analysis was carried out using a micrOTOF-Q mass spectrometer (Instrument ID: 228888.10348), which ionizes molecules and separates the resulting ions based on their mass-to-charge (m/z) ratios **(Wiley *et al.*, 1995).**

3. Results

3.1 Percentage yield

Table 1 Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Nigella sativa*	Petroleum ether	Dark yellow to brown	200.00 gm	1.361	0.680
2.	*Nigella sativa*	Ethyl acetate	Brown	198.60gm	2.465	1.241

3.2 Qualitative Phytochemical Analysis of different extracts

Table 2 *Phytochemical* analysis of *Nigella sativa* extracts

S. No.	Experiment	Result	
		Petroleum ether	Ethyl acetate
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	+	+
3.	Benedict's Test	+	+
4.	Barfoed's test	-	-
Test for Alkaloids			
1.	Mayer's Test	+	+
2.	Hager's Test	-	+
3.	Wagner's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	+	+
Test for Glycosides			
1.	Legal's Test	-	-
2.	Keller Killani Test	+	+
3.	Borntrager's Test	-	+

3.3 Quantitative Phytochemical analysis of extracts of *Nigella sativa* -

3.3.1 Total phenolic and flavonoid content Estimation

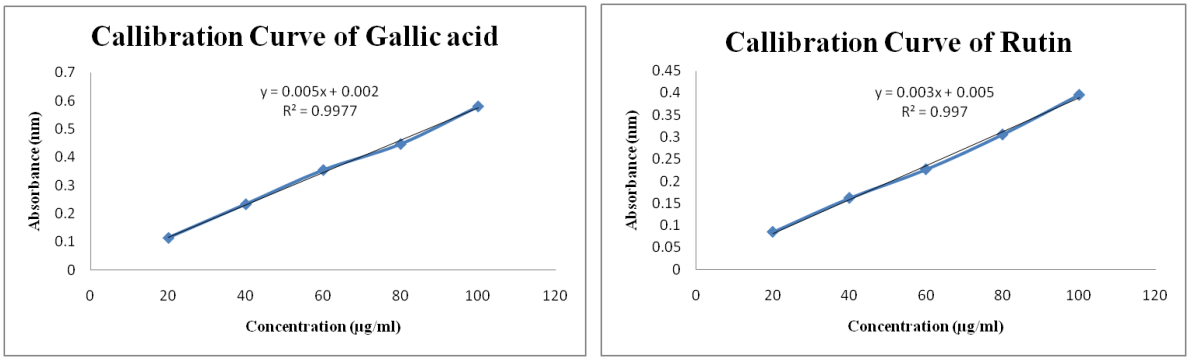

Figure 1 Graph represent standard curve of Gallic acid and Rutin

Table 3 Total phenolic and flavonoid content in Ethyl acetate extracts of *Nigella sativa*-

Total Phenolic and flavonoid content (mg/gm equivalent to Gallic acid and Rutin)	
TPC	85.06
TFC	355.93

3.4 Preliminary TLC preparation for the estimation of active constitutes –

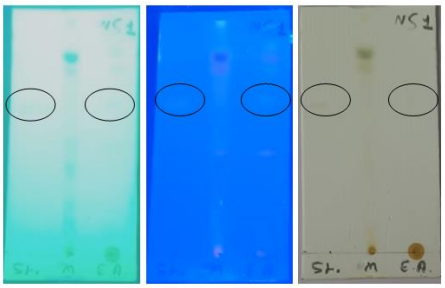

Figure 2 TLC estimation of *Nigella sativa* ethyl acetate extract by UV lamp for NS with Std. terpenoid (Carvacrol) (a) Shoet UV- 254nm, (b)Long UV-365nm, ad (c)Visible light) (Where, Std.= Standard, NS= *Nigella sativa,* M=Methanol, EA=Ethyl acetate)

Thin-layer chromatography (TLC) performed using a solvent system of toluene:ethyl acetate (4.8:0.2) for the *Nigella sativa* ethyl acetate extract showed clearly visible bands corresponding to the standard terpenoid and fatty acid. The Rf values of the N. sativa ethyl acetate extract and the standard terpenoid were both found to be 0.64, indicating the presence of terpenoids in the extract

3.5 Column Chromatography

The fractions (elutes) obtained from silica gel column chromatography of the ethyl acetate extract of *Nigella sativa* were tested for the presence of various phytochemicals using thin-layer chromatography (TLC). The collected fractions were properly handled, and UV spectroscopic analysis was subsequently performed to further characterize the compounds present.

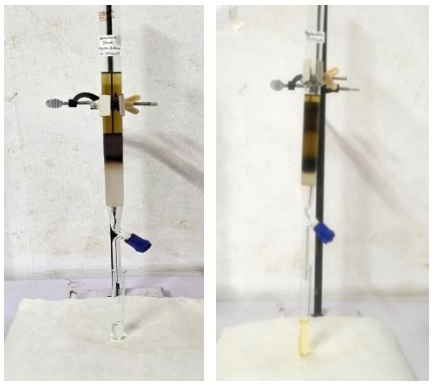

Figure 3 Isolation of components by column chromatography

3.5.1 TLC of all collected fractions-

TLC of all collected fractions of NS (Ethyl acetate) Extract-

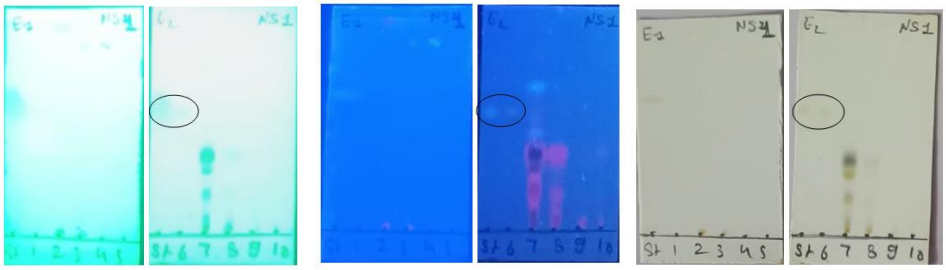

Figure 4 TLC estimation by UV lamp for NS fractions after column chromatography with Std. terpenoid (Carvacrol) a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light (Std. = Standard, NS= *Nigella sativa*)

Ten elutes (A, B, C, D, E, F, G, H, I, and J) were collected from column chromatography. TLC was performed using a mobile phase of toluene: ethyl acetate (4.8:0.2), and the Rf values were determined to confirm the presence of the active constituent in the isolated fraction. Fraction F was finalized based on the comparison of its Rf value with that of the standard terpenoid.

3.6 Spectroscopic characterization: -

3.6.1 Active constitutes estimation by UV-Spectroscopy-

The UV spectrum of the isolated fraction (F) of *Nigella sativa* ethyl acetate extract was recorded using a Shimadzu 1700 double-beam UV-Visible spectrophotometer over a scanning range of 200–800 nm. The maximum absorption wavelength (λ max) of the isolated compound was found to be at 292 nm.

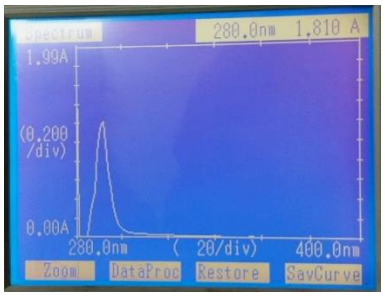

Figure 5 Active constitutes estimation by UV- Spectra of fraction (F) of NS (Ethyl acetate) extract after column chromatography

3.6.2 *FTIR* spectra of the isolated fraction (F) of NS (Ethyl acetate) extract

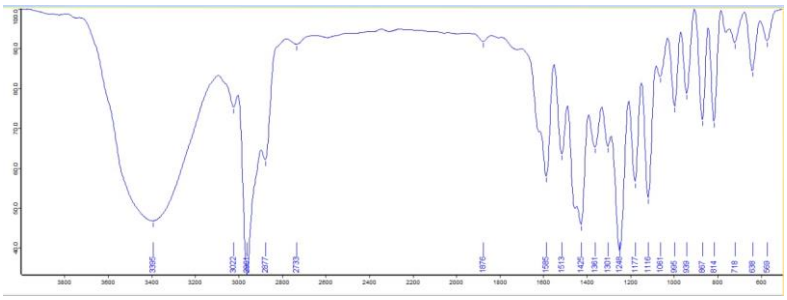

Figure 6 FTIR spectra of the isolated fraction (F) of NS (Ethyl acetate) extract

The FTIR Spectra of isolated fraction (F) of NS (Ethyl acetate) extract showed that -OH group strong, Broad peaks appeared at 3395 cm-1, OH stretching of Alcohol weak, Broad peaks appeared at 3022 cm-1, C-H stretching peak of alkane at 2991 & 2877 cm-1, C-H bending peak of Aromatic compound at 1876 cm-1, C=C stretching peak of Cyclic alkene at 1585 & 1513 cm-1, C-H bending peak of Alkane at 1425 cm-1, O-H bending peak of Alcohol & Phenol at 1361 & 1301 cm-1, C-O stretching peaks of Ether at 1248 cm-1, C-O stretching medium peak of Alcohol at 1177, 1116 & 1061 cm-1, C=C bending strong peaks of Alkene at 995 & 939 cm-1 and C=C bending strong peaks of Substituted at 867, 814, 718 & 638 cm-1.

3.6.3 ^{1}H NMR - Spectroscopy-

^{1}H NMR spectra of fraction (F) from the *Nigella sativa* ethyl acetate extract were recorded using an NMR spectrometer. Tetramethylsilane (TMS) was used as the internal standard. The proton signals were denoted by the symbols s, d, t, and m, representing singlet, doublet, triplet, and multiplet, respectively.

- ***1H NMR spectra of the isolated fraction (F) of NS (Ethyl acetate)*** –

In ^{1}H-NMR spectra isolated fraction (F) of NS (Ethyl acetate) showed that H-6 protons appeared at 1.104-1.121 (d) ppm, H-3 protons appeared at 2.047 (s) ppm, H-1 proton appeared at 2.661-2.730 (sept) ppm, H-1 proton appeared at 3.460 (s) ppm, H-1 proton appeared at 6.502-6.521 (dd) ppm, H-1 proton appeared at 6.626-6.629 (dd) ppm, H-1 proton appeared at 6.881-6.899 (1H, dd) ppm.

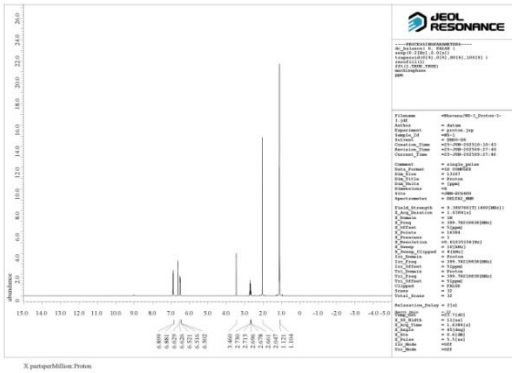

Figure 7 ^{1}H-NMR spectra of the **isolated fraction (F) of NS (Ethyl acetate)**

3.6.4 Mass – Spectroscopy-

The mass spectrum of the isolated fraction (F) of *Nigella sativa* ethyl acetate extract was recorded using a Bruker micrOTOF-Q mass spectrometer.

- ***Mass spectra of the isolated fraction (F) of NS (Ethyl acetate)*** -

Mass spectra of isolated fraction (F) of NS (Ethyl acetate) showed molecular ion [M^+] peaks at mlz 150.2000 which obtained 2-methyl-5-(propan-2-yl)phenol in which presence of carbons (C_{10}), Hydrogen's (H_{14}) and Oxygen (O). Finally the molecular formula of isolated Fraction (F) of NS (Ethyl acetate) extract was found to be $C_{10}H_{14}O$ according to their fragments (91, 132, 107, 70, 88, and 127 m/z).

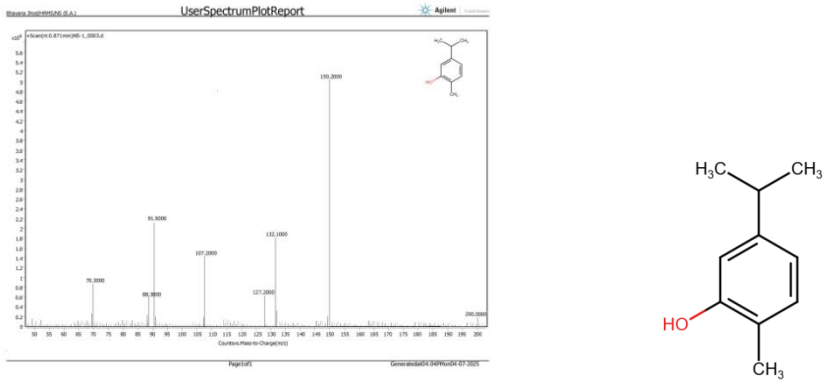

Figure 8: Mass spectra of the isolated fraction of NS (Ethyl acetate) **2-methyl-5-(propan-2-yl)phenol**

4. Discussion

The present study involved the extraction, isolation, and characterization of bioactive constituents from the ethyl acetate extract of *Nigella sativa*. The percentage yield of the ethyl acetate extract was found to be 1.241%, which is higher than that obtained using petroleum ether, suggesting better solubility and extractability of phytochemicals in ethyl acetate.

Phytochemical screening revealed the presence of multiple classes of compounds, including carbohydrates, alkaloids, terpenoids, flavonoids, tannins, saponins, proteins, and glycosides. Notably, both the Salkowski and Liebermann–Burchard tests confirmed the presence of terpenoids in the ethyl acetate extract.

Preliminary TLC analysis using a mobile phase of toluene:ethyl acetate (4.8:0.2) demonstrated the presence of bands corresponding to standard terpenoids (carvacrol), with an Rf value of 0.64 in both the extract and standard. This suggests the presence of similar compounds in the extract. Further purification using silica gel column chromatography yielded ten fractions (A–J), of which fraction F was finalized based on TLC similarity with the standard terpenoid.

UV-visible spectroscopy of fraction F showed a characteristic absorption peak at 292 nm, which is consistent with aromatic and phenolic compounds. This was further supported by FTIR analysis**,** where absorption peaks indicated the presence of -OH, C-H, C=C, and C-O functional groups—typical features of phenolic and terpenoid structures.

Further confirmation came from ^{1}H NMR analysis, which showed distinct chemical shifts corresponding to proton environments of a substituted phenolic compound. Signals in the aliphatic and aromatic regions confirmed the presence of methyl, isopropyl, and phenolic protons, consistent with 2-methyl-5-(propan-2-yl)phenol**.**

Finally, mass spectrometry of the isolated fraction revealed a molecular ion peak at *m/z* 150.2000, indicating a molecular formula of $C_{10}H_{14}O$. Fragmentation patterns further confirmed the identity of the compound as 2-methyl-5-(propan-2-yl)phenol, a known phenolic compound with reported biological activity, including antioxidant and antimicrobial properties.

5. Conclusion

The study successfully extracted, isolated, and identified 2-methyl-5-(propan-2-yl)phenol**,** a bioactive phenolic terpenoid compound, from the ethyl acetate extract of *Nigella sativa* seeds. The process involved TLC-guided fractionation, followed by characterization using UV-Visible, FTIR, NMR, and mass spectrometric techniques. The isolated compound shares structural similarity with carvacrol, a known terpenoid with therapeutic potential.

These findings substantiate the traditional medicinal value of *Nigella sativa* and highlight its potential as a source of pharmacologically active terpenoid compounds. The identified compound can serve as a lead molecule for further pharmacological studies and potential drug development.

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