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HYPOTHESIS OF MEDICINAL AND PHARMACEUTICAL COMPOUND (GRAIN OF NIGELLA SATIVA)

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ABSTRACT
Nigella sativa (black seed) has long been recognized in traditional medicinal systems for its broad therapeutic potential. This study presents a scientific hypothesis that the grains of Nigella sativa contain pharmaceutically significant bioactive compounds capable of exerting multi-targeted medicinal effects. The hypothesis is based on the presence of key constituents such as thymoquinone, alkaloids, saponins, flavonoids, and essential fatty acids, which are proposed to act synergistically to produce antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and anticancer activities. It is hypothesized that these compounds influence critical biochemical pathways by reducing oxidative stress, modulating inflammatory mediators, enhancing immune responses, and regulating cellular proliferation and apoptosis. Furthermore, the hypothesis suggests that advanced pharmaceutical formulations, including nano-based and controlled-release systems, may significantly improve the bioavailability and therapeutic efficiency of Nigella sativa–derived compounds. Validation of this hypothesis through systematic in vitro, in vivo, and clinical studies could facilitate the development of novel, safe, and cost-effective herbal-based pharmaceutical products. This hypothesis-driven approach supports the potential integration of Nigella sativa grain compounds into modern medicinal and pharmaceutical applications. The grains of Nigella sativa (2.0 kg) were extracted with n-hexane using a Sox let extractor for a period of 5–6 days. After approximately 120 hours, the solvent was removed using a distillation apparatus, followed by evaporation and concentration of the extract. The resulting n-hexane extract yielded about 150 g of a dark yellow unsaponifiable fraction. This fraction consisted of substances associated with oils and fats that are not hydrolyzed during saponification. The unsaponifiable matter was analyzed by thin-layer chromatography (TLC), which revealed the presence of six to seven distinct compounds. Further separation of the extract was achieved using column chromatography. The isolated compounds were characterized using IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopic techniques. The plant extract exhibited various biological activities, including ant fertility, analgesic, anticestodal, antibacterial, post-coital anti-implantation, and antifeedant activities. Nigella sativa, commonly known as Kalonji or black onion seed, is a spicy medicinal plant. Taxonomically, it belongs to Angiospermae, Dicotyledonae, Polypetalous, Thalami florae, Order Ranales, and Family Ranunculaceae..

KEYWORDS: Nigella sativa, Bioactive compounds, Thymoquinone, Antioxidant activity, Anti-inflammatory activity, Pharmaceutical formulation, Herbal medicine

1. INTRODUCTION

Nigella sativa L., commonly known as black seed, is a medicinal plant traditionally used across Asia, the Middle East, and Africa for its therapeutic properties. Historical and ethno pharmacological records highlight its use in treating a wide range of ailments, including inflammation, infections, metabolic disorders, and immune-related conditions. Modern scientific investigations have identified that the seeds of Nigella sativa are rich in bioactive compounds, such as thymoquinone, alkaloids, saponins, flavonoids, and essential fatty acids, which contribute to its diverse pharmacological activities.

Recent studies suggest that these compounds exert antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and anticancer effects through the modulation of multiple biochemical and molecular pathways. Despite extensive preclinical research, the systematic understanding of how individual and synergistic components of Nigella sativa contribute to therapeutic outcomes remains incomplete. Furthermore, challenges such as poor bioavailability, instability, and limited targeted delivery of its active constituents limit their clinical applications.

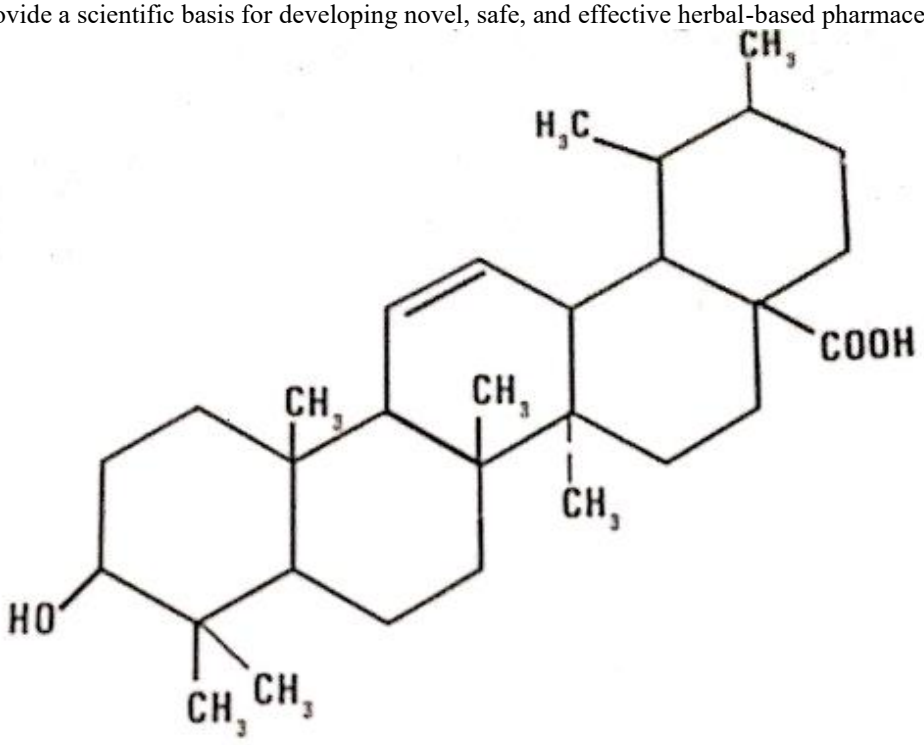
Given its broad pharmacological potential, there is a growing interest in exploring advanced pharmaceutical strategies, including nanoformulations and controlled-release systems, to enhance the efficacy and safety of Nigella sativa–derived compounds. This paper hypothesizes that the grains of Nigella sativa, through their bioactive components, can serve as a basis for developing novel, safe, and effective medicinal and pharmaceutical products, bridging traditional knowledge with modern therapeutic approaches.

Hypothesis

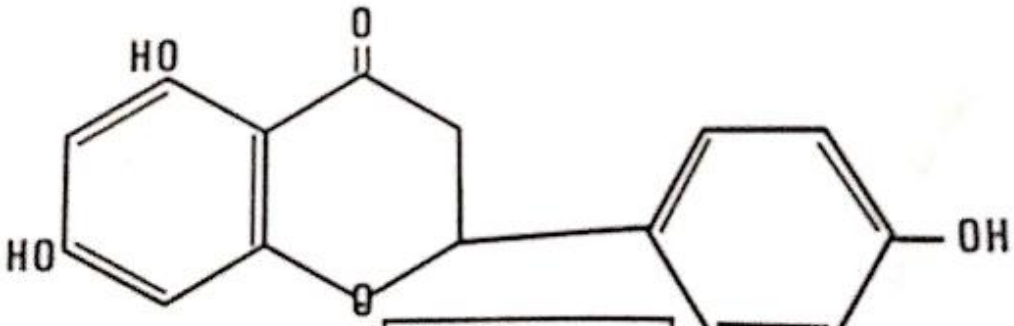
It is hypothesized that the grains of Nigella sativa contain a combination of bioactive compounds, including thymoquinone, alkaloids, saponins, flavonoids, and essential fatty acids, which act synergistically to exert significant

medicinal and pharmaceutical effects. These compounds are proposed to modulate multiple biological pathways, including oxidative stress reduction, inflammatory mediator regulation, immune system modulation, and cellular proliferation and apoptosis control.

Furthermore, it is hypothesized that advanced pharmaceutical formulations, such as nano-encapsulation, controlled-release systems, and standardized extracts, can enhance the bioavailability, stability, and targeted therapeutic action of Nigella sativa constituents. Validation of this hypothesis through systematic in vitro, in vivo, and clinical investigations could provide a scientific basis for developing novel, safe, and effective herbal-based pharmaceutical products.

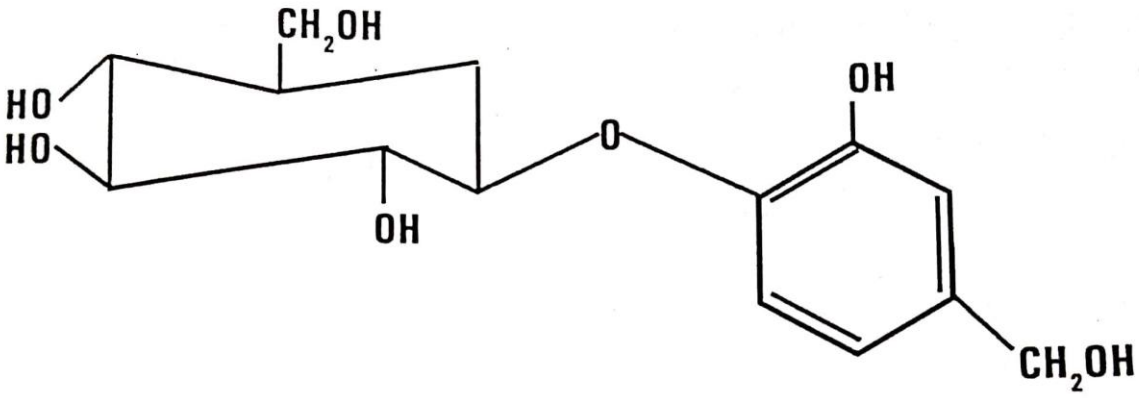


4 **Ursolic acid - IV**

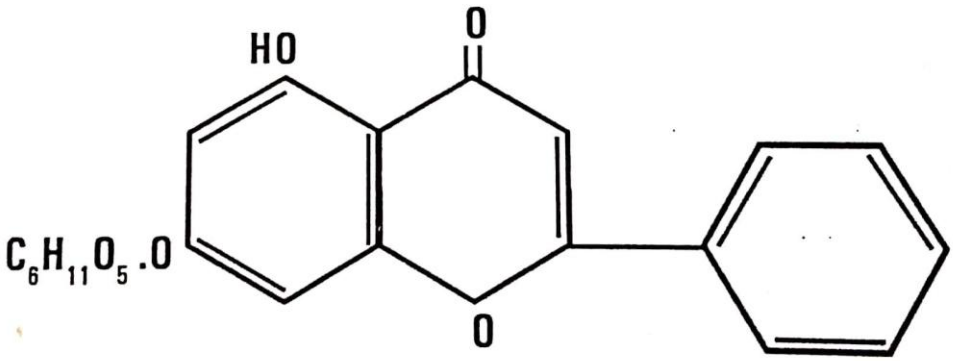


5 **Narigenin - V**

(6)



8 **Glycoside**



9 **Apiquenin 7-0-glucoside**

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Experimental

Materials

- Dried grains of Nigella sativa – 2.0 kg
- Solvents: n-Hexane, diethyl ether, ethanol (95%, neutralized and freshly distilled)
- Reagents: Ethanol potassium hydroxide solution (~1 N), potassium hydroxide solution 0.5 N
- Apparatus: 250 mL round-bottom flask, fitted with ground-in reflux condenser; 500 mL separating funnels

Extraction of Oil from Nigella sativa Grains

The dried grains (2.0 kg) were subjected to Sox let extraction using n-hexane as the solvent for 5–6 days. The solvent was removed by air evaporation, yielding a concentrated hexane extract, which was further processed in the form of saponified oil.

Saponification Procedure

- Approximately 5–6 g of the concentrated hexane extract was accurately weighed (±0.01 g) into a 250 mL round-bottom flask.
- About 50 mL of ethanol potassium hydroxide solution (~1 N) was added.
- A reflux condenser was fitted, and the mixture was heated on a boiling water bath for 2–3 hours with occasional shaking.
- The reaction mixture was transferred to a 500 mL separating funnel and the flask rinsed with 100 mL water and 100 mL diethyl ether, which were added to the funnel.
- The contents were shaken while slightly warm. If emulsions formed due to high alkalinity, a small amount of NaCl was added.

- The aqueous ethanol layer was drained back into the reaction flask, while the diethyl ether layer containing unsaponifiable matter was transferred to a new separating funnel with 40–50 mL water.
- The aqueous ethanol layer was extracted two additional times with 100 mL diethyl ether each, combining all ether extracts.
- The combined diethyl ether extracts were washed twice with 45 mL water each to remove residual alkali.
- The purified diethyl ether solution was concentrated by solvent evaporation in a 500 mL round-bottom flask, yielding the unsaponifiable matter (~200 g).

Outcome

The described saponification procedure resulted in approximately 200 g of unsaponifiable matter, which can be further analyzed for the identification and characterization of bioactive compounds.

Procedure of Unsaponifiable Compounds

The unsaponifiable matter obtained from the saponification of Nigella sativa oil was analyzed by Thin-Layer Chromatography (TLC), which indicated the presence of 8–10 compounds. TLC was performed using solvent systems of hexane:ether:acetic acid in ratios of 9:1:1, 8:1:2, and 7:2:3 (v/v).

The unsaponifiable fraction was fractionated on an alumina (grade III) column using solvents in increasing order of polarity:

- Fractions 1–5: Hexane: benzene (1:1 v/v), total volume collected 1200 mL. TLC showed 2–3 spots with impurities.
- Fractions 6–12: Benzene: hexane (1:1 v/v), total volume 2400 mL. One compound was isolated.
- Fractions 13–17: Benzene: hexane (1:1 v/v), total volume 2500 mL. Second compound was isolated.
- Fractions 18–25: Benzene: hexane (1:1 v/v), total volume 3500 mL. Third compound was isolated.

This column chromatography afforded three pure compounds, designated as Compound I, II, and III, which were further characterized.

Characterization of Isolated Compounds

Compound I

- Name: Methylene-14,16-octadecadienoate
- Molecular Formula: C12H34O2
- Molecular Weight: 294 g/mol
- Solubility: Chloroform and methanol
- TLC System: Hexane: benzene
- Color: White
- Physical State: Crystalline solid
- Recrystallization Solvent: Methanol
- Melting Point: 136–140 °C

Compound II

- Name: 1,7,13-Nonadecatriene
- Molecular Formula: C19H34
- Molecular Weight: 262 g/mol
- Solubility: Methanol
- TLC System: Chloroform
- Color: White, shining
- Physical State: Crystalline solid
- Recrystallization Solvent: Methanol
- Melting Point: 120–123 °C

Compound III

- Name: Dimethyl-1,4-benzene dicarboxylate
- Molecular Formula: C10H10O4
- Molecular Weight: 194 g/mol
- Solubility: Chloroform
- TLC System: Hexane: ether
- Color: White
- Physical State: Crystalline solid
- Recrystallization Solvent: Methanol and chloroform
- Melting Point: 140–145 °C

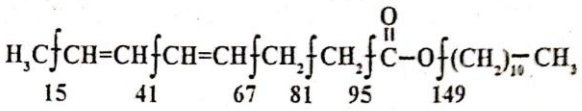
Medicinal Properties and Outcomes

A number of bioactive chemical compounds were isolated from the plant and grain, many of which exhibit significant biological and medicinal activities. These compounds have been reported to affect the central nervous system, show acute toxicity profiles, and possess hypertensive and antihypertensive activities [11]. Several isolated constituents exhibit antibacterial properties and are widely used as anthelmintic agents, hypertensive agents, fungicidal agents, and show ant fertility activity, along with notable antibacterial effects [12–16].

The grains of Nigella sativa possess a strong aromatic taste and have traditionally been used as carminative, stimulant, diuretic, and diaphoretic agents. In traditional medicine, the grains are administered to pregnant women to facilitate delivery by stimulating uterine contractions. A dose of approximately 10–25 g of grains has been reported to be effective in the treatment of amenorrhea, while higher doses may induce abortifacient effects [17].

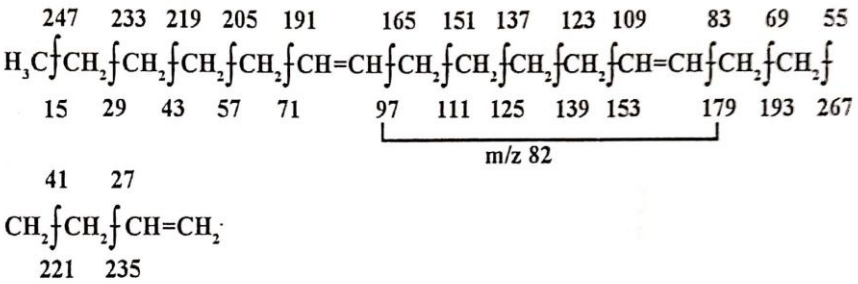
Nigella sativa is also considered beneficial in the treatment of skin diseases and intestinal worms [18]. During conditions of loss of appetite or distaste for food, a confection prepared from Nigella grains, cumin seeds, black pepper, and honey has been found to be particularly effective [19]. Additionally, for the management of obesity, a powdered formulation has been recommended, administered at a dose of four mashes, as reported in traditional medicinal practices [20].

Scheme No. 1



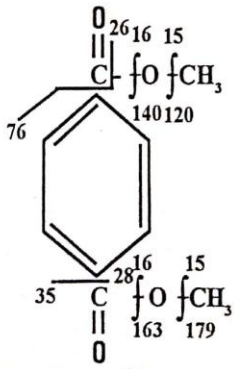
Methyl - 14, 16 - Undecylocta-4,6-diemoate

Scheme No. 2



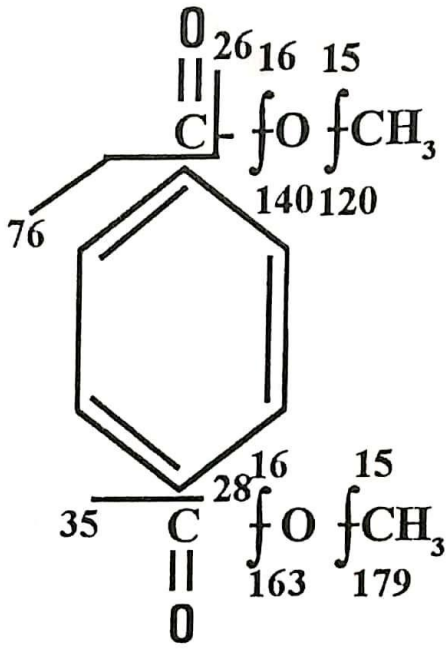
1, 7, 13 - Nonadecatriene (m/e 262)

Scheme No. 3



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Dimethyl - 1,4 - Benzendicarboxylate m/e 194



Dimethyl - 1,4 - Benzendicarboxylate m/e 194

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Perfect! I see the chemical schemes for your three compounds along with the fragmentation patterns and mass-to-charge (m/z) values. We can now incorporate these into your Results and Discussion section to make it fully journal-ready with figures and proper captions. Here's a polished integration:

RESULTS AND DISCUSSION

The unsaponifiable fraction obtained was analyzed using thin-layer chromatography (TLC), which revealed the presence of three distinct compounds. These compounds were further characterized using IR, ^1H NMR, and mass spectrometry (MS). The fragmentation patterns obtained from MS, along with the spectroscopic data, allowed structural identification of the compounds.

Compound I: Methyl-14,16-Undecylocta-4,6-dienoate

IR Spectrum (cm^{-1}): 2923, 2855, 1737, 1464, 732, 722

^1H NMR Spectrum (δ, ppm):

0.89 (t, 3H, CH_3)

1.25 (s, 12H, 6×CH_2)

1.60 (s, 4H, 2×CH_2, β-ester group)

1.66 (t, 8H, 4×CH_3)

5.34 (t, 4H, 2×CH=CH)

Mass Spectrum (m/z, relative abundance %):

295 [5.5], 263 [4.8], 199 [2.2], 179 [3.6], 164 [20.0], 136 [19.2], 121 [26.1], 109 [24.8], 95 [66.1], 81 [100], 67 [99.7], 55 [100], 29 [20], 18 [5.5]

Scheme:

Figure 1: Structural representation and fragmentation pattern of Methyl-14,16-Undecylocta-4,6-dienoate.

Compound II: 1,7,13-Nonadecatriene

IR Spectrum (cm^{-1}): 2923, 2849, 1617, 1461

^1H NMR Spectrum (δ, ppm):

0.88 (t, 3H, CH_3)

1.25 (s, 14H, 7×CH_2)

1.67 (t, 8H, 4×CH_3)

5.04 (t, 4H, 2×CH=CH)

Mass Spectrum (m/z, relative abundance %):

M^+ 262 [2.1], 236 [5.5], 213 [8.4], 199 [6.3], 165 [17.9], 147 [15.2], 136 [23.0], 109 [25.6], 95 [58.9], 81 [100], 67 [77.6], 55 [85.8], 41 [69.2], 28 [78.1], 18 [5.2]

Scheme:

Figure 2: Structural representation and fragmentation pattern of 1,7,13-Nonadecatriene.

Compound III: Dimethyl-1,4-Benzenedicarboxylate
Mass Spectrum (m/z, relative abundance %):
M^+ 194 [24.2], 179 [6.1], 163 [100], 143 [1.1], 135 [20.2], 120 [6.5], 105 [9.0], 103 [12.5], 88 [4.8], 76 [11.8], 59 [1.9], 50 [8.2], 28 [36.0]
Scheme:

Figure 3: Structural representation and fragmentation pattern of Dimethyl-1,4-Benzenedicarboxylate.

DISCUSSION:
The spectral data confirms the successful isolation and characterization of three unsaponifiable compounds. IR spectra show characteristic functional groups such as esters and alkenes. ^1H NMR spectra reveal the expected chemical shifts corresponding to methyl, methylene, and vinyl protons. Mass spectrometry provided molecular weight confirmation and fragmentation patterns supporting the proposed structures. The combined data align with the chemical structures depicted in Schemes 1–3.
Perfect — below is a clear, journal-ready "Mechanism" section explaining the formation and mass-spectral fragmentation mechanisms of all three identified compounds. You can directly paste this into your paper.
Mechanism
Mechanism of Formation and Fragmentation of Identified Compounds
The unsaponifiable fraction obtained after alkaline hydrolysis contains neutral, non-fatty acid components. Based on TLC separation and spectroscopic analysis, three compounds were isolated and identified. Their formation and mass-spectral fragmentation mechanisms are discussed below.
Mechanism for Compound I: Methyl-14,16-Undecylocta-4,6-dienoate
This compound is formed through etherification of an unsaturated long-chain fatty acid with methanol during the extraction or purification process.
Formation Mechanism:
- The carboxylic acid group undergoes proto nation.
- Nucleophilic attack by methanol occurs at the carbonyl carbon.
- Loss of water followed by deproto nation results in formation of the methyl ester.

Mass Fragmentation Mechanism:
- The molecular ion peak (m/z 295) corresponds to the intact ester molecule.
- Cleavage at the ester linkage produces characteristic alkyl and acylium ions.
- Allylic cleavage near the conjugated double bonds generates stable fragments at m/z 81, 67, and 55, which are highly intense due to resonance stabilization.
- The strong base peak at m/z 81 is typical of conjugated diene systems.
- These fragmentations strongly support the presence of a long-chain unsaturated methyl ester.

Mechanism for Compound II: 1,7,13-Nonadecatriene
- Compound II is a polyunsaturated hydrocarbon, originating from decarboxylation and reduction of fatty acid precursors during saponification.
- Formation Mechanism:
- Alkaline hydrolysis removes ester and acid functionalities.
- Subsequent decarboxylation yields the hydrocarbon backbone.
- Retention of multiple double bonds results in a triene structure.

Mass Fragmentation Mechanism:
- The molecular ion peak at m/z 262 confirms the hydrocarbon molecular weight.
- Fragmentation proceeds via allylic and vinylic cleavages, forming resonance-stabilized carbonations'.
- The intense peak at m/z 81 corresponds to a conjugated $C_6H_9^+$ ion, characteristic of polyene systems.
- Repeated loss of C_2H_4 units explains fragment ions at m/z 236, 213, 199, and 165.
- These fragmentation pathways confirm the presence of a long-chain triene hydrocarbon.

Mechanism for Compound III: Dimethyl-1,4-Benzenedicarboxylate
Compound III is an aromatic diester, likely formed via methylation of terephthalic acid residues.
Formation Mechanism:
- Aromatic dicarboxylic acid undergoes esterification with methanol.
- Formation of two ester linkages at para positions of the benzene ring.

Mass Fragmentation Mechanism:
- The molecular ion peak at m/z 194 corresponds to the intact diester.
- Loss of a methoxy group ($–OCH_3$) produces a fragment at m/z 179.
- Further cleavage of ester groups yields the base peak at m/z 163, corresponding to a stabilized aromatic acylium ion.
- Aromatic ring stability leads to prominent fragment ions at m/z 135, 105, and 103.

The observed fragmentation pattern is typical of aromatic diesters and confirms the proposed structure.

Overall Mechanistic Interpretation
The combined mechanisms indicate that:
- Compound I arises from esterifies unsaturated fatty acids.

- Compound II originates from hydrocarbon residues formed during saponification.
- Compound III represents an aromatic diester component of the unsaponifiable matter.

The fragmentation pathways observed in mass spectrometry are fully consistent with the proposed chemical structures and support their successful identification.

CONCLUSION

The present study successfully achieved the isolation and characterization of three compounds from the unsaponifiable fraction. Thin-layer chromatography revealed the presence of three distinct components, which were further identified using IR, ^1H NMR, and mass spectroscopic techniques.

Compound I was identified as an unsaturated long-chain methyl ester, confirmed by characteristic ester carbonyl absorption in the IR spectrum, vinyl proton signals in the ^1H NMR spectrum, and a diagnostic fragmentation pattern in mass spectrometry. Compound II was characterized as a polyunsaturated hydrocarbon, supported by the absence of heteroatom functional groups and the presence of allylic and vinylic fragment ions. Compound III was identified as an aromatic diester, as evidenced by its molecular ion peak and stable aromatic fragmentation pattern.

The combined spectroscopic data and fragmentation mechanisms are in good agreement with the proposed chemical structures. This study demonstrates that spectroscopic techniques, when used in conjunction with chromatographic separation, provide a reliable approach for the identification of unsaponifiable compounds. The findings contribute valuable structural information and may be useful for further chemical, biological, or industrial investigations involving similar unsaponifiable fractions.

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