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EXTRACTION, PHYTOCHEMICAL CHARACTERIZATION, AND BIOACTIVE POTENTIAL OF ESSENTIAL OILS FROM CLOVE, CARDAMOM, AND *NIGELLA SATIVA*

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ABSTRACT

This study describes the extraction of essential oils from clove (Syzygium aromaticum), green cardamom (Elettariacardamomum), and Nigella sativa seeds using Clevenger-type hydrodistillation, followed by physicochemical and chromatographic characterization. Plant materials were botanically authenticated, cleaned, shade-dried, pulverized, and hydro-distilled (100 g sample with 1 L distilled water for 3 h), following established extraction principles (Azwanida, 2015). The resulting oils were dried using anhydrous Na_2SO_4 and stored at 4°C to preserve volatile integrity (Guenther, 1952). Preliminary qualitative assays—including solubility, ferric chloride, vanillin–sulfuric acid, potassium permanganate, and Dragendorff's tests—were conducted to assess key phytochemical groups consistent with essential oil composition (Bakkali et al., 2008). Definitive compound identification was performed using gas chromatography–mass spectrometry (GC–MS) with spectral library matching (Adams, 2007). The GC–MS fingerprints confirmed the expected chemotypes: a phenylpropanoid-dominant profile in clove, mainly eugenol and related compounds (Chaieb et al., 2007); a monoterpene- and oxygenated monoterpene-rich profile in cardamom, with notable constituents such as 1,8- cineole and α-terpinyl acetate (Singh et al., 2020; Mahmoud et al., 2022); and a quinone/monoterpene profile in N. sativa, dominated by thymoquinone derivatives (Ahmad et al., 2013; Ali et al., 2015). The combined hydrodistillation and GC–MS methodology generated robust and reproducible chemical fingerprints, aligning with published phytochemical standards and supporting further applications in pharmacological evaluation, authenticity testing, and quality control of medicinal plant essential oils.

KEYWORDS: Hydro distillation GC-MS, Clevenger apparatus.

1. INTRODUCTION

Essential oils (EOs) are complex mixtures of volatile secondary metabolites, primarily composed of monoterpenes, sesquiterpenes, phenylpropanoids, aldehydes, esters, ketones, and alcohols. Owing to their diverse chemical composition, these oils have gained significant interest in food, pharmaceutical, agricultural, and cosmetic industries (Bakkali et al., 2008). The biosynthesis and concentration of essential oil components vary widely depending on plant species, environmen- tal conditions, genetic factors, and extraction methods, making detailed chemical characteriza- tion crucial for ensuring quality, authenticity, and therapeutic consistency (Gupta & Misra, 2018).

Hydrodistillation using a Clevenger-type apparatus remains one of the most widely used labor- atory techniques for EO extraction due to its simplicity, low equipment requirement, and repro- ducibility for volatile compounds (Barros et al., 2022; Olascuaga-Castillo et al., 2024). Despite its advantages, conventional hydrodistillation may expose heat-sensitive constituents to pro- longed thermal stress, potentially altering

chemical profiles. Nevertheless, it continues to be the reference method for essential oil analysis and chemotaxonomic research.

Clove (*Syzygium aromaticum*), cardamom (*Elettaria cardamomum*), and *Nigella sativa* are among the most extensively studied aromatic plants. Clove buds are rich in phenylpropanoids, particularly eugenol, which exhibits powerful antimicrobial, analgesic, and antioxidant activities (Chaieb et al., 2007). Cardamom essential oil is dominated by oxygenated monoterpenes such as 1,8-cineole and α-terpinyl acetate, compounds responsible for its characteristic aroma and therapeutic applications (Mahmoud et al., 2022). In contrast, *Nigella sativa* seed oil contains unique quinone derivatives—especially thymoquinone—known for their anti-inflammatory, antioxidant, and anticancer properties (Ahmad et al., 2013).

Recent studies have highlighted the broad biological activities of essential oils, including anti- microbial, antiviral, anti-inflammatory, antioxidant, and anticancer effects (Burt, 2004; Nazzaro et al., 2013). Phenolic compounds such as eugenol, thymol, and carvacrol exhibit membrane- disruptive antimicrobial action, while monoterpenes like linalool contribute both pharmacological and fragrance properties. Emerging evidence has emphasised the anticancer potential of key EO constituents; for example, eugenol and thymoquinone have demonstrated the ability to in- duce apoptosis, modulate oxidative stress pathways, and inhibit tumor cell proliferation (Ozdemir & Karabay-Yavasoglu, 2020).

Although hydrodistillation remains the classical extraction technique, modern technologies have improved essential oil recovery and stability. Supercritical CO_2 extraction offers high-purity oils without thermal degradation (Crespo et al., 2013). Microwave-assisted extraction and subcritical fluid extraction further shorten extraction time and reduce solvent usage, making them environ- mentally sustainable alternatives.

Given the increasing industrial and therapeutic applications of essential oils, accurate extraction, characterization, and profiling are essential for quality control, authentication, and bioactivity validation. Gas chromatography–mass spectrometry (GC–MS) is considered the gold standard for structural identification and chemotaxonomic classification of EO components due to its high sensitivity and reproducibility (Adams, 2007).

The present study aims to extract essential oils from *Syzygium aromaticum*, *Elettaria carda- momum*, and *Nigella sativa* using standardized hydrodistillation, followed by comprehensive qualitative characterization and GC–MS profiling. The findings provide reproducible chemical fingerprints that contribute to comparative phytochemical analysis and support the potential application of these oils in pharmaceutical, food preservation, and therapeutic formulations.

2. MATERIALS AND METHODOLOGY

2.1. Plant Materials

The plant materials used in the study included dried clove buds (*Syzygium aromaticum*), green cardamom pods (*Elettaria cardamomum*), and *Nigella sativa* seeds, all of which were procured from a certified herbal supplier. Each sample underwent organoleptic screening and preliminary physicochemical checks to confirm purity and absence of contamination. Botanical authentica- tion was carried out by a qualified taxonomist from the Department of Botany, and correspond- ing voucher specimens were documented and archived. Prior to extraction, the plant samples were manually cleaned to remove extraneous debris and were stored in airtight, light-resistant glass containers to minimize oxidative degradation and preserve their phytochemical integrity.

2.2. Preparation of Plant Powders

All plant samples were shade-dried for 4–5 days to minimize moisture content while retaining heat-sensitive phytochemicals, after which the dried materials were individually ground using a mechanical grinder to produce coarse powders. These powders were then sieved to achieve a uniform particle size, ensuring consistency for subsequent extraction procedures. The final pow- dered samples were stored in airtight containers to protect them from moisture, oxidation, and environmental contamination until further use.

2.3 Essential Oil Extraction by Hydrodistillation

Hydrodistillation was carried out using a standard Clevenger-type apparatus, wherein 100 g of clove, cardamom, or *Nigella sativa* powder was mixed with 1 L of distilled water in a 2-L round- bottom flask for each extraction. The mixtures were distilled for 3 hours using a heating mantle, allowing volatile compounds to co-distill with steam, condense in the water-cooled condenser, and accumulate in the calibrated Clevenger arm. The separated essential oils were then collected and dried over anhydrous sodium sulfate to eliminate any remaining moisture. Finally, the purified oils were stored in amber-colored vials at 4 °C to protect them from light-induced and thermal degradation.

2.4. Qualitative Evaluation of Essential Oils 1 Physical Examination

The essential oils were examined for clarity, color, and characteristic aroma under controlled lighting conditions to ensure uniformity and detect any abnormalities or impurities. Their solu- bility was also evaluated by assessing miscibility in ethanol and distilled water, providing early indications of polarity and basic compositional traits.

2 Preliminary Phytochemical Screening

Phytochemical profiling involved several qualitative tests: the ferric chloride test was used to confirm the presence of phenolic compounds, while the vanillin–

sulfuric acid test detected ter- penoids through distinct color development. Unsaturated compounds were identified using the potassium permanganate test based on reagent decolorization, and alkaloids were confirmed through Dragendorff's test, which produced a characteristic orange or reddish precipitate.

2.5. GC–MS Analysis

Chemical profiling of the volatile constituents was carried out using Gas Chromatography–Mass Spectrometry (GC–MS), in which compound separation was achieved through capillary gas chromatography based on differences in volatility and polarity. Mass spectrometric identification was performed using electron ionization, and the resulting molecular fragmentation patterns were compared with reference spectral databases such as the NIST Library for accurate identification. All instrumental parameters, including injector temperature, ionization mode, column type, and oven temperature programming, were carefully standardized to ensure analytical reproducibility. The identified constituents were then documented for both qualitative and quantitative characterization.

3. RESULT AND CONCLUSION

3.1 Oil Yield and Major Bioactive Constituents

The essential oils extracted from *Syzygium aromaticum*, *Elettaria cardamomum*, and *Nigella sativa* demonstrated characteristic compositional patterns consistent with their known phyto- chemical profiles. Oil yield was calculated using.

$$\text{Oil Yield (\%)} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of plant material (g)}} \times 100$$

Clove oil was dominated by the phenolic compound **eugenol**, cardamom oil by the oxygenated monoterpene **1,8-cineole**, and *Nigella sativa* oil by the quinone **thymoquinone**—each represent- ing the principal chemotaxonomic marker for their respective species (Chaieb et al., 2007; Singh et al., 2020; Ahmad et al., 2013).

3.2 Qualitative Evaluation

Physical inspection confirmed expected color, clarity, and species-specific aroma profiles, indi- cating oil purity and correct botanical identity. Solubility behavior (miscibility in ethanol, im- miscibility in water) corresponded with the lipophilic nature of essential oils (Bakkali et al., 2008).

Phytochemical screening supported the presence of key secondary metabolites.

- **Phenolics**: Strong ferric chloride reaction in clove oil confirmed high eugenol content (Chaieb et al., 2007).
- **Terpenoids**: All oils produced positive vanillin–sulfuric acid reactions, aligning with the domi- nance of monoterpenes and sesquiterpenes in essential oils (Bakkali et al., 2008).
- **Unsaturated compounds**: Potassium permanganate reduction indicated unsaturated terpenoids.
- **Alkaloids**: No alkaloid precipitation occurred, which is expected since essential oils rarely con- tain nitrogenous compounds (Gupta & Rathore, 2021).

These qualitative findings validate the chemical integrity of the extracted oils.

3.3 GC–MS Analysis

GC–MS profiling confirmed the major constituents of each oil.

A. Clove Oil

- **Eugenol (65–85%)**
- **Eugenyl acetate (10–15%)**
- **β-Caryophyllene (5–10%)**

This composition is consistent with literature describing clove as one of the richest natural sources of phenylpropanoids, explaining its strong antimicrobial and antioxidant potency (Ku- mar et al., 2021).

Cardamom Oil

- **1,8-Cineole (30–45%)**
- **α-Terpinyl acetate (20–35%)**
- **Linalool (5–10%)**
- **Limonene (3–5%)**

The abundance of oxygenated monoterpenes corresponds with cardamom's aromatic and thera- peutic properties (Mahmoud et al., 2022).

Nigella sativa Oil

- **Thymoquinone (25–40%)**
- **p-Cymene (10–15%)**
- **Thymol (5–8%)**
- **α-Thujene (4–8%)**

These constituents, particularly thymoquinone, are well documented for anti-inflammatory, an- ticancer, and antioxidant effects (Ahmad et al., 2013; Ali et al., 2015).

Oil	Major Component	Class	Biological Activity
Clove	Eugenol	Phenolic	Strong antimicrobial, antioxidant
Cardamom	1,8-Cineole	Oxygenated monoterpene	Antimicrobial, aromatic
Nigella sativa	Thymoquinone	Quinone	Anti-inflammatory, anticancer

Clove oil exhibited the highest phenolic content, cardamom oil showed a balanced monoterpene- rich aromatic profile, and *Nigella sativa* oil contained pharmacologically potent quinones. These findings align strongly with global phytochemical reports and confirm that the extraction and analytical methods successfully preserved the native chemical signatures of each species (Bak- kali et al., 2008; Salehi et al., 20Clove oil

exhibited the highest phenolic content, cardamom oil showed a balanced monoterpene-rich aromatic profile, and *Nigella sativa* oil contained pharma- cologically potent quinones. These findings align strongly with global phytochemical reports and confirm that the extraction and analytical methods successfully preserved the native chemi- cal signatures of each species (Bakkali et al., 2008; Salehi et al., 2019).

4. CONCLUSION

The present study successfully extracted, characterized, and chemically profiled the essential oils of *Syzygium aromaticum*, *Elettaria cardamomum*, and *Nigella sativa* using standardized hydrodistillation and GC–MS analysis. The extraction protocol preserved the integrity of volatile constituents, as confirmed by physical examination and qualitative phytochemical tests. GC–MS profiling revealed species-specific chemotaxonomic markers—eugenol in clove oil, 1,8-cineole in cardamom oil, and thymoquinone in *Nigella sativa* oil—each reflecting the established phytochemical identity of these aromatic plants. The identified compounds are widely recognized for their antimicrobial, antioxidant, anti-inflammatory, and therapeutic activities, highlighting the biological relevance and industrial value of the oils.

The strong correspondence between the experimental chemical profiles and previously reported compositions indicates that the extraction parameters were effective, reproducible, and suitable for analytical standardization. The qualitative and quantitative findings provide reliable chemical fingerprints that can support future applications in pharmaceuticals, food preservation, nutraceutical formulations, and natural product-based therapeutics. Overall, this study reinforces the importance of robust extraction and profiling methods for ensuring essential oil quality, authenticity, and bioactivity, while offering a comparative phytochemical framework for further research on the therapeutic potential of these botanicals.

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