

ISOLATION AND COMPARATIVE CHEMICAL CHARACTERIZATION OF ESSENTIAL OIL FROM FRESH AND DRIED STEMS OF *ARTEMISIA SCOPARIA* **(VIRGATE WORMWOOD)**

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Received: April 16, 2024 Accepted: June 28, 2024

Introduction

Nature stands as the foremost chemist, its aromatic essences, present throughout the known flora, proving beyond doubt its unparalleled capability. Despite millennia of collective human endeavor in chemistry, the synthesis of these essences remains beyond reach (Butnariu & Sarac, 2018). Aromatic plants, in general, boast volatile oils in various proportions and concentrations across their different parts, ranging from leaves and petals to stems, seeds, and even roots (Hariri *et al*., 2018). Essential oils, nestled within select plants' specialized cells or glands, serve multifaceted roles. They act as defenses against predators and pests while attracting pollinators, forming a part of the plant's immune system. Paracelsus, the renowned Swiss alchemist, famously dubbed distilled oils from herbs as "quinta essentia," denoting them as the quintessence of the plant, hence the term essential oils (Jahan *et al*., 2015).

These oils, highly concentrated and volatile, are extracted from various plant parts and exhibit distinct therapeutic and energetic effects. Despite being termed oils, they lack fatty substances and are instead derived from the essence rich in natural flavors and active ingredients secreted by the plant's cells. Techniques such as distillation or pressing of secretory organs yield these precious liquids. For instance, citrus peel undergoes cold pressing, while other plant parts are subject to distillation (Ghaderinia & Shapouri, 2017; Faramarz Hariri Moghadam *et al*., 2018). Essential oils find relatively widespread occurrence in the plant kingdom, especially among superior plants from about 50 families, including various angiosperm orders. While terpenic compounds dominate, certain biosynthesized monoterpenes have been observed in soil bacteria, insects, and even in some sesquiterpenes and diterpenes of animal origin (Kumar & Senapati, 2015; Georgieva & Kosev, 2018; Vasileva, 2015).

Synthesis and accumulation of essential oils take place both within and outside plant structures. Glandular brushes and papillae external to the plant and secretory cells within, along with intercellular spaces and secretory bags, serve as reservoirs. These oils are distributed across various plant organs, albeit in varying quantities, from roots and leaves to flowers, fruits, stems, and bark. While some species boast substantial oil content, others possess therapeutic substances derived from essential oils despite less pronounced aromatic qualities (Marinova *et al*., 2018; Ouis & Hariri, 2017; Olufeagba *et al*., 2016). Biosynthesis of these aromatic substances occurs primarily in leaves, persisting until flowering, after which migration to flowers ensues. Postfertilization, oils accumulate in fruits and seeds or migrate to other plant parts such as leaves, bark, and roots (Bakari & Yusuf, 2018; Jasim, 2016).

As plants mature, essential oil composition undergoes transformation, with young plants exhibiting terpenic hydrocarbons and simpler molecules, while reproductive organs harbor etheric oils richer in oxygenated compounds. With over 3,000 characterized oils, approximately 150 are industrially manufactured due to their physical and chemical distinctiveness (Nikolova & Georgieva, 2018; Eed & Burgoyne, 2015; Rahimian et al., 2018).

The chemical composition of essential oils, ranging from mono- to sesquiterpene constituents, encompasses aromatic compounds, phenylpropane derivatives, and occasionally, diterpenes. Obtaining these oils necessitates meticulous harvesting to avoid contamination, with flowers and leaves being primary reservoirs (Hassan & Soleimani, 2016; Saidi *et al*., 2017).

Additionally, the technological process significantly influences oil composition and quality, particularly in hydrodistillation. Crushing, cutting, and grinding prepare vegetable products for extraction, with steam or solvent-based methods commonly employed. Distilled or demineralized water serves as the solvent, with varying plant-to-solvent ratios dictated by oil

content and solubility (Belkhodja *et al*., 2017; Mahmoodi *et al*., 2018; Menkovska et al., 2017). The separation of excess undiluted volatile oil from the saturated aqueous solution marks the culmination of the extraction process. Techniques such as pressing, distillation, solvent extraction, and extraction from concentrated solutions contribute to industrial-scale essential oil production (Satimehin, Tiamiyu, & Okayi, 201). Artemisia scoparia is a specie in the genus Artemisia in the sunflower family (Asteraceae), commonly known as redstem wormwood in English, 'dongbei yin chen hao' or 'binhao' in Chinese and 'baelteran' in Saudi Arabia (Haibo *et al*., 2016). The leaves, stem, bark and seeds of Artemisia scoparia have been extensively used as a natural therapeutic agent (Anwar et al., 1994) in traditional medicine as it has proved effacious in the treatment of various diseases such as human periodontal infection (Liu *et al*., 2013). Studies undertaken on the plant shows that it possess several biochemical and biological activities which affirms and justifies its folkloric uses; Antibacterial (Altunkaya *et al*., 2014), Antifungal (Hosagoudar, 2012), Antimalarial (Mojarrab *et al*., 2016), Anti-inflammatory (Taherkhani, 2014), Anticancer (Shafi *et al*., 2012), Antidiabetic (Taherkhani, 2014), Antioxidants (Shafi et al., 2012; Hailu *et al*., 2013). This study aims to isolate and compare chemical characterization of essential oils from fresh and dried stems of *Artemisia scoparia.*

Materials and Methods

Plant Materials

Stems of *Artemisia scoparia* was harvested in the morning and afternoon at a flower garden located at Air Force road, Ilorin West Local Government Area of Kwara state. The samples was identified and authenticated by a plant taxonomist at the Herbarium of the Plant Biology Department, University of Ilorin, with a voucher number of UILH/ 018/0037. After identification, the stems from each harvest was separately pulverised prior to extraction.

Isolation of Essential oil

A 500 g each of fresh and dried pulverized stems of *Artemisia scoparia* was hydrodistilled for 3 hours in a Clevenger-type apparatus. The extracted oil was collected, the yield was measured and preserved in a labelled sealed sample tube and stored at 4 °C for GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis sample of *Artemisia scoparia* volatile oils was carried out on a GCMS spectrometer (QP 2010SE, SHIMADZU, JAPAN) at B.O.B laboratory located in Abuja, Nigeria, using the following operating conditions. Column (optima 5MS by Macherey-Nagel; length 30m, 0.25mm I.D, 0.25m thickness) with helium used as carrier gas and flows at 2ml per minute, oven temperature placed at 60 $^{\circ}$ C – 180 $^{\circ}$ C with rate of 10 °C minute. Initial temperature at 60 °C, injector mode as split injection, where with split ratio at 50, injection at 1µL, injection temperature at 250 °C, ionization source at 200°C, ionization mode (EI) at 70ev 38 and interface temperature at 250 °C. The MS data was processed by on-line desktop computer equipped with disk memory. The percentage compositions of the constituents of the oil was computed in each case from GC peak areas. The identification of the components was based on the comparism of their relative retention time and Mass Spectra with authentic samples and data from literature and computerised MS-data bank. (Jennings and Shibamito, 1980; Adams, 1995; Joulain and Koenig, 1998).

Results and Discussions

Oil yields from fresh stem and the dried stem of *Artemisia scoparia* harvested in the morning (7am) and afternoon (1pm) are presented in Table 1 below

KEY: Fresh: Fresh stem, DD1: Stem dried for one day, DD2: Stem dried for two days, DD3: Stem dried for three days and DD4: Stem dried for four days

The oils from the stem harvested in the morning afforded oil in the yield of $0.10 - 0.30$ % (w/w). The yield increased steadily from 0.10 % in fresh stem and subsequently increased to 0.30 % in the stem that were dried for four days. Similarly, the oils from the stem that were harvested in the afternoon afforded oil in the yield of 0.11 - 0.31% (w/w). The fresh stem yielded 0.11% of the oil which increased steadily to 0.31% in the stem that were dried for four days. The yield increases with increase in period of drying. This increase may be attributed to the

reduction in the moisture content of the stems as the days of drying increased. However, the dry stems afforded more oil than fresh stem. Afternoon harvest also yielded more essential oil than morning harvest.

Figure 1: Yield of stem essential oil of *Artemisia scoparia* KEY: DSM: Dry season morning harvests DSA: Dry season afternoon harvest

Table 2: Chemical Composition (%) of essential oils from fresh stem and the dried stem of Artemisia scoparia harvested in the morning.

FUW Trends in Science & Technology Journal, www.ftstjournal.com e-ISSN: 24085162; p-ISSN: 20485170; August, 2024: Vol. 9 No. 2 pp. 300 – 308 303

Table 2 presents a comprehensive analysis of the chemical composition of essential oils from the fresh and dried stems of *Artemisia scoparia,* harvested in the morning. The table highlights the presence and concentration of various compounds, demonstrating significant variability influenced by the drying process. The total composition percentages range from 91.1 % to 99.6 %, indicating that drying impacts the presence of specific compounds.

One notable compound is 2-tert-butyl-1,4-dimethoxybenzene, which is present at 24.6% in the fresh sample but disappears completely in some dried samples (DD2 and DD3). This compound's significant reduction or absence in dried samples suggests that it is highly volatile and sensitive to the drying process (Rao et al., 2023). Such volatility underscores the importance of careful handling and processing of fresh stems to retain certain beneficial compounds.

Thymol methyl ether is another major component, present in the fresh sample at 16.5% and showing consistent presence across dried samples, albeit at slightly lower percentages (ranging from 8.9% to 11.7%). This stability indicates that thymol methyl ether is less affected by the drying process, retaining its presence in dried essential oils. Thymol methyl ether is known for its antimicrobial properties, making it valuable in both fresh and dried forms of the essential oil (Singh et al., 2023).

The presence of monoterpenoids such as β-pinene and $α$ pinene also highlights interesting trends. β-Pinene increases from 2.0% in the fresh sample to up to 4.2% in DD1, showing that drying can enhance its concentration. Similarly, α-pinene appears only in dried samples, with a peak at 1.6% in DD1. These findings suggest that the drying process can potentially improve the yield of certain monoterpenoids, which are valued for their anti-inflammatory and bronchodilator properties (Kaur & Arora, 2023).

The sesquiterpenoid composition, including compounds such as β-caryophyllene and γ-humulene, also exhibits notable changes. β-Caryophyllene, present at 4.7% in the fresh sample, shows variability in dried samples, peaking at 8.6% in DD3. This indicates that drying can either concentrate or dilute certain sesquiterpenoids depending on the conditions. β-Caryophyllene is recognized for its anti-inflammatory and analgesic effects, highlighting the potential therapeutic benefits of dried Artemisia scoparia essential oils (Silva et al., 2023).

γ-Humulene, absent in the fresh sample, appears in dried samples (1.6% in DD2 and 2.2% in DD3), suggesting that it may be a degradation product or form through transformation during the drying process. This compound is noted for its antiinflammatory properties, adding to the medicinal value of the dried essential oils (Mehdi et al., 2022).

Certain compounds, such as 4-tert-butylcatechol, dimethyl ether, and 1,4-diacetyl benzene, appear exclusively in some dried samples. For instance, 4-tert-butylcatechol, dimethyl ether is present at 10.3% in DD2 and fluctuates in other dried samples. This suggests that drying can lead to the formation of new compounds, potentially altering the essential oil's profile and therapeutic properties (Moghtader, 2022).

Table 3: Chemical Composition (%) of essential oils from fresh stem and the dried stem of *Artemisia scoparia* harvested in the **afternoon.**

Table 3. presents the percentage composition of various compounds in the essential oils extracted from fresh and dried stems of Artemisia scoparia, harvested in the afternoon. The table lists compounds identified by their retention indices (RI) and details their presence across different drying methods (DD1 to DD4), along with mass spectral data for each compound.

α-Thujene (RI 873) is present in fresh stems (0.4%) and increases slightly in DD1 (0.7%) but is absent in DD2 and DD4, and minimally present in DD3 (0.3%). Sabinene (RI 897) appears only in DD2 (1.2%), indicating that drying can either create or stabilize certain volatile compounds not initially present in the fresh stems (Khan et al., 2023).

β-Pinene (RI 943) shows significant variability, with its presence increasing from 2.1% in fresh stems to 4.7% in DD1 and peaking at 5.5% in DD4. It is absent in DD2 and reduced in DD3 (1.5%) . Similarly, α -Pinene (RI 948) appears in DD1 (1.6%) and DD4 (2.1%), but not in fresh stems, indicating that certain drying methods enhance the concentration of these monoterpenes, which are valued for their aromatic properties (Singh & Kaur, 2022).

β-Myrcene (RI 958) is detected in DD1 (1.4%), DD3 (0.5%), and DD4 (2.8%), but not in fresh stems. α -Phellandrene (RI 969) is present in fresh (4.1%) and increases slightly in DD4 (7.5%), though it decreases significantly in DD1, DD2, and DD3. These findings suggest that drying processes can both generate and diminish certain compounds, depending on the method used (Verma et al., 2023).

β-Ocimene (RI 976) is found in all dried samples except DD2, with the highest concentration in DD4 (2.1%) . Benzaldehyde (RI 982), present in fresh (0.8%) and all dried samples, peaks in DD3 (5.6%), indicating that drying can significantly impact the preservation and concentration of aromatic aldehydes (Patel et al., 2022). α-Limonene (RI 1018) is only present in fresh stems (0.8%), suggesting it is highly volatile and not retained through drying. Artemisia ketone (RI 1042) appears consistently but varies, being highest in DD1 (1.4%) and lowest in fresh stems (0.6%), highlighting the stability of this compound in dried forms (Ahmad et al., 2022). p-Cymene (RI 1042) shows a marked increase from 1.9% in fresh stems to 5.5% in DD1 and DD4. Terpinolene (RI 1052) is only detected in DD4 (0.5%), indicating selective stability and formation during specific drying processes (Mohammadi et al., 2023).

Thymol methylether (RI 1231) is a major component, especially in DD2 (32.7%), compared to fresh stems (17.9%). Thymol acetate (RI 1421) shows consistent presence across all samples, with the highest in DD2 (11.5%). These compounds are key constituents, significantly influenced by drying methods (Rao et al., 2023).

Bornyl acetate (RI 1277) and neryl acetate (RI 1352) show selective presence, with bornyl acetate decreasing from 2.1% in fresh to lower levels in dried forms, while neryl acetate is only found in DD3 (1.7%), suggesting differential stability under drying conditions (Banik et al., 2021).

Cedrene (RI 1398) is present in fresh stems (9.4%) but significantly drops or is absent in dried forms. Nauurolane (RI 1419) appears mainly in DD3 (5.5%) and minimally in other forms, indicating selective retention during drying (Chaudhary et al., 2023).

Other sesquiterpenoids, like α-bergamotene (RI 1430) and γ-muurolene (RI 1435), show varied presence, with significant peaks in specific drying methods. For example, γ-muurolene is highest in DD3 (9.6%) and $α$ bergamotene shows consistent but variable presence across all samples. These compounds' concentrations are highly dependent on the drying process (Sharma et al., 2023).

The total percentage of chemical compounds varies, with fresh stems containing 99.8%, DD1 97.5%, DD2 99.5%, DD3 97.8%, and DD4 95.61%. These variations highlight the impact of drying period on the chemical composition of essential oils, which can significantly alter their therapeutic and aromatic properties (Rao et al., 2023).

Conclusion

The study successfully isolated and characterized the essential oils from the fresh and dried stems of *Artemisia scoparia*, highlighting the impact of drying on oil yield and chemical composition. Drying the stems increased the oil yield and significantly influenced the concentration of various bioactive compounds. Afternoon-harvested stems yielded more oil than morning-harvested ones. Key findings include the substantial presence of compounds such as thymol methyl ether, β-pinene, and β-caryophyllene, which are known for their therapeutic properties. The drying process enhanced the concentration of certain monoterpenoids and sesquiterpenoids, potentially improving the oils' medicinal value. However, some compounds, like 2-tert-butyl-1,4-dimethoxybenzene, were reduced or absent in dried samples, indicating a loss of certain volatile components during drying. These results underscore the need for precise drying techniques to retain and enhance the desired chemical profiles of essential oils. Overall, the study provides valuable insights into the optimal processing of Artemisia scoparia for maximizing the benefits of its essential oils.

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