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Comparative Analysis of Hydro-Distillation and Steam-Distillation Techniques on the Chemical Composition of Turmeric (*Curcuma longa* Linn) Rhizomes

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Abstract

Turmeric (*Curcuma longa* Linn) is a well-known medicinal and aromatic plant, traditionally used for centuries due to its bioactive rhizomes, which offer significant health benefits. To fully harness these benefits, a suitable method capable of extracting these bioactive compounds is required. This research, therefore, seeks to compare the effect of two different techniques (hydro-distillation: HD and steam-distillation SD) on the chemical composition of *Curcuma longa* Linn rhizomes, which were obtained from the local market in Ijebu-Ode, Ogun State. The extracted oils were analyzed using Gas Chromatography Mass Spectrometry (GC-MS). A total of 23 compounds were found in the HD oil and 25 in the SD oil. In the HD oils, Sesquiphellandrene (18.77%), Tumerone (18.83%), and Terpinolene (13.28%) were the most abundant compounds, followed by α-Zingiberine (7.61%), Germacrene (7.43%). Other compounds were present in negligible amounts. While in Steam distillation, the order of abundance was Tumerone (24.23%), Sesquiphellandrene (11.20%), and Aromadendrene (10.07%), besides other compounds. The HD method had a higher composition of Sesquiphellandrene (18.77%), Aromadendrene oxide (2) (5.30%), and Terpinolene (13.28%), while Tumerone (24.23%) and Aromadendrene (10.07%) were more abundant in the SD oil. Although both hydro-distillation and steam-distillation successfully extracted various bioactive compounds from *Curcuma longa* rhizomes, the methods yielded distinct chemical profiles, with only eight compounds in common. These differences suggest that the choice of extraction method significantly influences the chemical composition and potential applications of the extracted oils.

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Introduction

From a long time ago, medicinal and aromatic plant species have been used by mankind as a source of medicines, food flavourings, preservatives and ornaments, as well as beauty and personal delight products, becoming natural alternatives over synthetic products that offer reliability, safety and sustainability to the populations (Osinubi et al., 2024). Most people's first healthcare comes from traditional medicine. The use of medicinal plants has been the foundation for maintaining good health in the majority of developing countries (Tsai *et al.,* 2011).

Various human societies around the world have reportedly made use of more than 35,000 different plant species for medical needs, taking advantage of the various components in the different plant parts such as the leaves, stems, stem and root-back, and seeds, as well as flowers. These components are extracted either by making decoctions of the plants' parts to obtain their crude extracts (Osinubi *et al.,* 2015) or by using their oil extracts.

For thousands of years, plant oils and their crude extracts have served a wide variety of purposes, one of such is as a medication source since they include organic molecules with medicinal worth (Osibote *et al*., 2021). In addition to their therapeutic uses, the plants are utilised as herbs and spices because they are believed to be both safe and effective in treating specific health conditions. Between these two, natural products from essential oils have attracted the most attention over the years.

The oils are usually derived from aromatic plants. These oils are referred to as essential oils; they are volatile oils that are produced from aromatic plants, and they encapsulate the essence of the aromatic plant. Essential oils have a variety of health benefits due to their intriguing biological activities, which include insecticidal, antiviral, antioxidant, and antibacterial properties (Costa e*t al*., 2021). Among the widely used essential oils in the world such as lavender, turmeric, citrus, eucalyptus, lemongrass, tea tree, etc., turmeric (*Curcuma longa)* essential oil is particularly popular due to its many pharmacological properties, including antioxidant, anticancer, anti-inflammatory, neuro- and

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dermo protective, antiasthmatic, and flavouring properties, as well as the use of its rhizomes as a traditional treatment for diseases like malaria, jaundice, gastric ulcers, skin conditions, emotional disorders, and convulsions (Oyemitan *et al*., 2017; Nascimento *et al*., 2019)

Curcuma longa, the scientific name for turmeric, is a Southeast Asian native plant that is a member of the Zingiberaceae family. Turmeric's biological activities are among the qualities that make it unique, and it has been observed to be toxic to fungi involved in the deterioration of agricultural products, interfering with the development of mycelia (Ferreira *et al.,* 2013). For centuries, it has also been used as a flavouring, colouring, and preservative in the preparation of traditional Indian curries (Tsai *et al.,* 2011). India produces about 400,000 tons of fresh weight per year, which accounts for about 80% of the world's supply of commercial turmeric annually (Cousins *et al.,* 2007). Three to five per cent of curcuminoids are responsible for the turmeric rhizome's distinctive yellow colour. *Curcuma longa's* rhizomes are a rich source of two major products with remarkable attributes, including curcuminoids and essential oils (Li et al., 2011). The essential oil with its distinctive chemical composition provides the *C. longa* rhizome with a unique spicy and aromatic flavour (Meng *et al.,* 2018).

The essential oil of turmeric contains aromatic-turmerone (ar-turmerone), β-turmerone, α-turmerone, α-zingiberene, β-sesquiphellandrene, and β-bisabolene (Singh et al. 2010). Turmeric's rhizome essential oil has biological properties such as antibacterial, antifungal, anticancer, insect repellent, and anti-snake venom properties (Funk et al. 2010; Singh *et al*. 2010). According to Ibáñez *et al.,* (2021), its essential oil has numerous health benefits, including cardiovascular protection, antihyperlipidemic, antiglycemic, antioxidant, antiplatelet, anti-inflammatory, antioxidant, antiarthritic, and antibacterial properties.

Essential oils are becoming more and more in demand these days in a variety of industries, including food and beverage, pharmaceutical, perfume and cosmetics, and agriculture. Replacing synthetic products with more sustainable, environmental, and human-health-friendly ones while applying benign practices is vital for sustainable research. Numerous methods for obtaining active principles from plants' volatile oils have been studied. These methods however have drawbacks such as low yields, differences in the quality of oil as well as a non-holistic extraction of active principles from the plants, among others. The most popular methods that have been reported are steam distillation and hydro distillation due to their affordability, ease of use, and simplicity, all of which are further benefits for investigating plant resources (Povh *et al.,* 2001).

This study aims to compare the effects of hydro-distillation (HD) and steam-distillation (SD) techniques on the chemical composition of *Curcuma longa* Linn rhizomes, intending to identify the more effective method for extracting bioactive compounds. Comparing the effect of these two methods on the essential oils of C*urcuma longa* rhizome will not only provide insight into the method that results in higher oil yield but also provide guidance on which produces better oil quality in terms of bioactive composition, stability and potential for therapeutic and industrial application.

Materials and Methods

Collection of Plant Materials

The healthy rhizomes of *Curcuma Longa* (Turmeric) were procured locally from the Ita-Osu market (popularly called new market) in Ijebu-ode, Ogun State, Nigeria, and washed thoroughly before being used.

Method of Extraction of Essential Oil

Hydro-distillation

The apparatus used was washed with distilled water and liquid soap and was allowed to dry before extraction. 150 g of the dried *Curcuma Longa* seeds were loaded into a Clevenger-type hydrodistillation setup. Hydrodistillation was carried out continuously for 5 hours. The extract (essential oil, hexane, and water) was dried over anhydrous sodium sulphate to remove any traces of water and then concentrated. The dried oil was then collected in a sealed, air-tight vial. The concentrate was refrigerated at 4°C until further analysis (Njoku *et al.,* 2017).

Steam distillation

A known weight of rhizomes (150 g) was placed in the reaction vessel, which was attached to a steam generator, and a water-cooled condenser was loaded into the steam distillation setup, and the extraction was carried out for 5 hours. The extract (essential oil, hexane, and water) was then collected and dried over anhydrous sodium sulphate to remove any traces of water and was then concentrated and stored in a sealed air-tight vial. The concentrate was refrigerated at 4°C until further analysis by Gas Chromatography-Mass Spectrometry (GC-MS).

Each distillation method was carried out once.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Following the method of Pharm-Huy *et al.* (2008) with minor modifications, the gas chromatography/mass spectrometry (GC/MS) analyses was performed on a

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Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with an ion source temperature of 230 °C, quadrupole temperature of 150 and transfer line temperature of 280 °C, employing an Elite-5 column (5 % phenyl and 95 % dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (PerkinElmer, USA). Acquisition of ion was via Scan mode (scanning mass range of 40–500 amu at 2.0 s/scan rate). Helium (1 mL/min) was used as a carrier gas. The initial oven temperature was 60 °C (1 min), this was increased to 240 °C at the rate of 6 °C / min, remained at 240 °C for 6 min, and then continued to increase to 250 °C at the rate of 10 °C/min, with a final stage of 10 min at 250 °C. The sample (0.1 uL) was injected with a splitless mode.

Identification of Volatile Oil Constituents

Component identification was accomplished by comparison of the retention indices (RI) of the GC peaks with those obtained using saturated n-alkanes (C8–C30) (Aldrich, USA), those reported in the literature (Njoku et al, 2021, Koenig *et al.,* 1998; NIST Library, 2020) and by comparison of the mass spectra of the peaks with those reported in the literature (Jennings and Shibamoto, 1989) and stored in the NIST library.

Results and Discussion

The Chemical Composition of Turmeric Volatile Oils extracted using hydro distillation and steam distillation methods was analyzed using Gas Chromatography. The results (Table 1) revealed significant differences in the profiles of the volatile compounds.

Table 1: Chemical Composition of Turmeric Volatile Oils Extracted by Hydrodistillation and Steam Distillation

S/N	Component	RI_{cal}	RI_L	Steam Distillation	Hydrodistillation
1.	Santolina triene	902	904	-	0.24
2.	β-Thujene	902	904	0.33	-
3.	3-Carene	948	946	0.47	-
4.	α-Pinene	948	946	4.14	-
5.	Sabinene	967	970	9.44	-
6.	β-Myrcene	983	980	0.34	-
7.	Terpinolene	1052	1054	6.13	-
8	Terpinolene	1052	1055	-	13.28
9	cis-p-Menth-2-en-1-ol	1109	1111	0.96	-
10	(+)-2-Carene, 4-.alpha.-isopropenyl-	1175	1177	-	2.03
11.	Isopentyl hexanoate	1212	1214	-	0.83
12	β-Copaene	1216	1218	-	4.36
13	Copaene	1221	1219	1.06	
14	α-Cubebene	1344	1344	1.18	-
15	γ-Pyronene	1345	1346	-	4.59
16	α-cubebene	1351	1352	1.18	-
17	δ-elemene	1377	1379	0.78	
18	Ledane	1380	1381	-	0.25
19	Aromadendrene	1386	1384	10.07	6.14
20	γ-Caryophyllene	1424	1427	-	3.15
21	γ-elemene	1431	1428	0.78	
22	Cis-Muurola-4(15),5-diene	1435	1433	0.36	
23	Cis-β-farnesene	1440	1444	-	4.77
24.	Farnesene	1458	1456	3.34	-
25	Aromadendrene oxide	1462	1466	3.43	5.30
26	Cadina-1(10),4-diene	1469	1464	1.28	-

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27	δ-Cadinene	1469	1474	6.92	1.74
28	Pinane	1480	1478	-	1.96
29	α-Zingiberine	1492	1490	5.84	7.61
30	α-Himachalene	1494	1496	-	1.79
31	γ-Cadinene	1507	1505	0.44	-
32	γ- cadinene	1507	1509	0.44	-
33	Germacrene D	1515	1513	3.99	7.43
34	Epiglobulol	1530	1528	-	1.19
35	β-Sesquiphellandrene	1537	1534	11.20	18.77
36	α –Calacorene	1547	1544	-	1.78
37	Nerolidol	1564	1562	-	0.82
38	Globulol	1575	1573	-	1.57
39	Humulene	1579	1581	2.12	-
40	Epicubenol	1580	1584	0.65	-
41	Tumerone	1650	1653	24.23	18.83
42	Humulane-1,6-diene-3-ol	1757	1755	-	0.96

RI_{cal}: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column. R_l: literature retention indices;

The result of GC analysis of the hydro-distilled and steam-distilled turmeric essential oil is presented in Table 1. In the essential oil obtained from the hydrodistilled rhizomes, a total of 23 compounds were identified. This oil was composed mainly of β-Sesquiphellandrene (18.77%), Tumerone (18.83%), and Terpinolene (13.28 %) as the most abundant compounds. Other compounds included β-Copaene (4.36%), Cis-β-farnesene (4.77%), γ-Caryophyllene (3.15%), Germacrene (7.43%), Aromadendrene oxide (5.30 %), and Aromadendrene(1) (4.80 %) and the rest were only available in trace amounts.

In the Steam-distilled turmeric essential oil, a diverse array of compounds were also identified, totalling 25 with Tumerone (24.23%) being the most abundant. β-Sesquiphellandrene (11.20%) and Aromadendrene (10.07%), Sabinene (9.44%), (+)-δ-Cadinene (6.92 %), Terpinolene (6.13 %), Zingiberene (5.84%), Germacrene D (3.99%), Farnesene (3.34 %) and Aromadendrene Oxide (3.43 %) were also present in significant amounts. The other compounds identified were only present in traces.

Interestingly, the two extraction methods had eight compounds in common (Table 2), however at different percentage compositions. These compounds include β-Sesquiphellandrene (HD: 18.77 %, SD: 11.20 %), Terpinolene (HD: 13.28 %, SD: 6.13 %), Aromadendrene (HD: 6.14 %, SD: 10.07 %), Aromadendrene oxide (HD: 5.30 %, SD: 3.43 %), Tumerone (HD: 18.83 %, SD: 24.23 %), δ-Cadinene (HD: 1.74 % SD: 6.92), Germacrene D (HD: 7.43 % SD: 3.99 %), α-Zingiberine (HD: 7.61 % SD: 5.84 %).

Table 2: Compounds common to both extraction methods.

S / N	Compound	Hydro-distillation (HD)	Steam-distillation (SD)
1	β-Sesquiphellandrene	18.77 %	11.20 %
2	Terpinolene	13.28 %	6.13 %
3	Aromadendrene	6.14 %	10.07 %
4	Aromadendrene oxide	5.30 %	3.43 %
5	Tumerone	18.83 %	24.23 %
6	δ-Cadinene	1.74 %	6.92 %
7	Germacrene D	7.43 %	3.99 %
8	α-Zingiberine	7.61 %	5.84 %

β-Sesquiphellandrene, Terpinolene, α-Zingiberine, Germacrene D and Aromadendrene oxide were more abundant in HD oil than in the SD oil while Tumerone and Aromadendrene andδ-Cadinene were more abundant in the SD oil than in the HD oil. Other compounds were specific to the method used, showing a marked difference in their extraction potential.

While certain chemicals were present in both steam-distilled and hydro-distilled oils, the findings of the two extraction techniques showed that their relative abundances differed. Specifically, compounds like

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terpinolene, sesquiphellandrene, and tumerone varied significantly between the two oils. For example, the amount of tumerone in the steam-distilled oil was higher (24.23%) than in the hydro-distilled oil (18.83%), suggesting that steam distillation may be a better method for extracting tumerone from turmeric. However, compared to the steam-distilled oil, chemicals such as sesquiphellandrene and terpinolene were more prevalent in the hydro-distilled oil (18.77% and 13.28%, respectively), indicating that hydro-distillation is a more effective method of extracting these specific compounds. This fluctuation in compound concentrations highlights how the extraction process affects the solubility and volatility of different components of the oil (Figueiredo *et al.,* 2010; Da Porto *et al.,* 2012).

This disparity in the percentage composition of the different compounds could be associated to different factors which include but are not limited to the fact that hydro-distillation is more selective in general when it comes to removing particular volatile chemicals, especially those that have lower boiling temperatures or are more soluble in water (Baser et al., 2015). Given that components in essential oils are more likely to be dissolved in water during the distillation process due to their hydrophilic nature (Masyita et al., 2022), this could account for the greater concentration of β-Sesquiphellandrene, Terpinolene, α-Zingiberine, Germacrene D and Aromadendrene oxide in the hydro-distilled oil.

The presence of chemicals like Zingiberene, Sabinene, Germacrene D, and β-Myrcene in steam-distilled oil that were either absent or present in minimal amounts in the hydro-distilled oil indicates that a wider range of compounds were recovered using the steam distillation process. These compounds are well-known for their antibacterial, anti-inflammatory, and antioxidant qualities, which may enhance the essential oil's medicinal potential, according to Gupta *et al.* (2013) and Wang *et al.* (2016). One important component of *Curcuma longa* essential oil is zingiberene, which has been associated with anti-inflammatory and antinociceptive effects (Saxena *et al.,* 2018). These extra bioactive compounds found in the steam-distilled oil re-affirms the possibility that steam distillation is a more thorough extraction technique for obtaining turmeric's entire medicinal components compared to Hydro-distillation.

Notable compounds like Tumerone (24.23%) Zingiberene (5.84%), Sabinene (9.44%), Germacrene D (3.99%), α-Pinene (4.14%), β-Myrcene (0.34%), in the steam-distilled oil which are known for their antimicrobial and anti-inflammatory properties adds to the oil's therapeutic potential (Ariani *et al.,* 2023; Orellana-Paucar, 2024). The oil also contains compounds like Aromadendrene Oxide (3.43%), δ-Elemene (0.41%), and Humulene (2.12%), which are commonly found in essential oils with flavour and fragrance applications (Sarkic and Stappen, 2018; van Beek and Joulain, 2018). The Steam distillation method appears to be effective in extracting a wide range of compounds from turmeric, resulting in an oil with a unique chemical profile. Furthermore, steam distillation is more effective in extracting a wider variety of chemicals, including those with higher boiling temperatures or those less soluble in water, despite being less selective (Lopez *et al.,* 2014). A more complicated chemical profile is produced by extracting both hydrophilic and lipophilic molecules due to the dynamic environment created by the steam distillation process. This idea is supported by the steam-distilled oil's inclusion of chemicals like Zingiberine and germacrene D, which are frequently found in oils with a variety of medicinal uses (Wang *et al.,* 2016).

Conclusion

The essential oil's chemical compositions and potential for therapeutic use are largely determined by the extraction process as observed from the result because a wider range of chemicals, some of which may have additional therapeutic effects, are captured by steam distillation, whereas the hydro-distillation process seems to favour the extraction of certain volatile compounds. The significance of choosing the right extraction technique according to the essential oil's intended application is highlighted by these findings. To improve the quantity and quality of essential oils, future research should concentrate on adjusting extraction parameters and assessing the pharmacological characteristics of these oils in clinical settings.

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