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(REVIEW ARTICLE)

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An article reviews of extraction, isolation and separation techniques of Zingiberene from *Zingiber officinale* by using Soxhlet distillation

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Abstract

One of the most sought-after natural items on the domestic or global market is essential oils. Numerous plant components can be used to extract essential oils. Collectively, essential oils lack any particular chemical or medicinal properties. Rather, what defines them is what they communicate distinctive scents. Consequently, the widespread propensity to discuss crucial oils as a group, as though that suggested something specific regarding their health, medicinal or culinary qualities are quite erratic and frequently genuinely hazardous. Until recently, ginger, a very beneficial herb plant, was widely used in both traditional and contemporary natural therapy. Research on this product should be conducted to determine how to meet the nation's need for ginger oil and the most effective method and the least expensive way to increase the product's yield. One of the traditional techniques for extracting essential oils is Soxhlet Distillation. The purpose of Soxhlet distillation is to producing effective ginger oil in a large amount This is because some components cannot be extracted using a particular approach, yet they could be effectively removed with the Soxhlet Extractor.

Keywords: Zingiberene; Ginger (*Zingiber officinale*); Soxhlet Extraction; Essential Oils

1. Introduction

For thousands of years, people have used herbs and spices to improve the color, flavor, and perfume of food. Herbs and spices are known for their anti-oxidant, antibacterial, preservative, and other therapeutic properties in addition to their ability to enhance flavor.4. However, there is a lot of interest in creating high-value, natural goods that are functional and free of solvent or additive residues and chemical alteration. Since the pharmaceutical and nutritional industries are very interested in essential oils, which are naturally occurring volatile extracts of plant components, they have a significant potential for export. For several centuries, plant extracts and essences that are now known as essential oils were widely used in Egypt, Greece, Rome, and other parts of the Middle and Far East.

1.1. Chemical Structure and Properties of Zingiberene

The main source of zingiberene, a sesquiterpene hydrocarbon, is ginger essential oil (Zingiber officinale). It offers a variety of biological actions and adds to ginger's distinctive flavor and scent.

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1.1.1. Chemical Structure

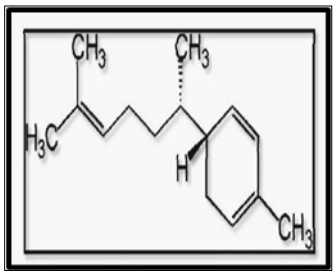

Figure 1 Molecular Structure of Zingeberene

- **IUPAC Name:** 1,4,7,10,10-pentamethyl-3,6-dodecadiene
- **Molecular Formula:** C15H24
- **Molecular Weight:** 204.36 g/mol

The structure of zingiberene consists of a 15-carbon backbone (dodecadiene) with several methyl groups (–CH3) attached at different positions on the molecule. Specifically, it contains:

A diene (C=C) functional group, which gives it its unsaturated nature.

A series of methyl groups (–CH3) attached to the carbon atoms along the chain.

1.2. Properties

- Volatility: Zingiberene is a volatile compound, which is why it is commonly found in the essential oils of ginger.
- Hydrophobicity: As a non-polar hydrocarbon, zingiberene is hydrophobic and is insoluble in water but soluble in organic solvents like ethanol, ether, and chloroform.
- Aroma and Flavor: Zingiberene imparts a spicy, slightly sweet, and peppery aroma to ginger and contributes to its characteristic flavor.
- Biological Activity:
- Antioxidant Properties: Zingiberene has been shown to exhibit antioxidant activity, which can help protect cells from oxidative damage.
- Antimicrobial Activity: Some studies have suggested that zingiberene may possess antibacterial and antifungal properties.
- Anti-inflammatory Effects: Like other compounds found in ginger, zingiberene may contribute to ginger's known anti-inflammatory effects.

1.3. Occurrence

Zingiberene is primarily found in ginger essential oil, but it is also present in smaller amounts in other plants such as cardamom, turmeric, and some species of the Zingiberaceae family.

In summary, zingiberene is a key compound in the essential oil of ginger, contributing both to its sensory characteristics (taste and smell) and its potential therapeutic effects.

2. General significance of ginger (Zingiber officinale) in food health and industry

Ginger (Zingiber officinale) is valuable in food, health, and industry because of its many uses and adaptable qualities. An outline of its significance in these domains is as follows:

2.1. In Food

- Flavoring Agent: Ginger is a common spice that gives savory and sweet foods a distinct, zesty flavor.
- Preservative Property: -Natural antibacterial qualities found in ginger contribute to food preservation and prolong its shelf life.
- Digestive Aid: As a food ingredient, ginger is believed to aid digestion and help alleviate symptoms of nausea and indigestion, often used in teas or as a remedy for motion sickness.

2.2. In Health

- Anti-inflammatory and Antioxidant Properties: Zingeberene, one of the bioactive substances found in ginger, has analgesic, anti-inflammatory, and antioxidant properties. Arthritis and other inflammatory diseases are treated with it.
- Immune System Support: Vitamin C and other antioxidants found in ginger can boost immunity and aid in the battle against illnesses.

2.3. In Industry

- Pharmaceutical Industry: Because of its medicinal qualities, ginger is a common component of over-the-counter medications, natural treatments, and supplements for conditions like motion sickness, nausea, and joint pain.
- Essential Oils: The rhizome of ginger is used to extract essential oil, which is utilized in massage and aromatherapy for its alleged calming and energizing benefits on circulation.

2.4. Objectives of the review

- To Summarize the Methods of Zingiberene Extraction: Review the various techniques used for extracting zingiberene from ginger, with a focus on Soxhlet extraction.
- To Examine the Soxhlet Extraction Process: Provide a detailed explanation of the Soxhlet extraction process, including the principles behind it (e.g., continuous solvent reflux) and the types of solvents typically used
- To Highlight the Applications of Extracted Zingiberene: Investigate the potential industrial applications of zingiberene extracted from ginger, particularly in the pharmaceutical, food, cosmetic, and fragrance industries.
- To Address Environmental and Economic Considerations: Discuss the environmental impact of the Soxhlet extraction process, such as the use of solvents and energy consumption.
- To Identify Gaps and Future Research Directions: Identify knowledge gaps in the extraction and application of zingiberene, and propose areas where further research is needed, such as the development of greener solvents or more efficient extraction methods.

3. Ginger composition: Zingeberene vs Other bioactive chemical

An overview of the main elements in ginger's chemical profile is provided below. Ginger (Zingiber officinale) has a complex chemical profile that includes a wide range of bioactive compounds that contribute to its flavor, aroma, and therapeutic properties. These include essential oils, phenolic compounds, carbohydrates, proteins, vitamins, and minerals.

Ginger's characteristic aroma and flavor come primarily from its essential oil, which contains a variety of terpenoid compounds. The most significant constituents include:

- Essential Oils (Volatile Compounds): Zingiberene, gingerol, shogaol, beta-bisabolene, cineole.
- Phenolic Compounds: Gingerol, shogaol, paradol, and gingerdiol.
- Carbohydrates: Starch and natural sugars (glucose, fructose).
- Proteins and Amino Acids.
- Minerals: Potassium, magnesium, calcium, phosphorus.
- Vitamins: Vitamin C, Vitamin B6, and trace amounts of Vitamin E.
- Fatty Acids: Linoleic acid.
- Dietary Fiber: Contributes to gastrointestinal health.

3.1. Concentration of zingiberene in ginger

- **Essential Oil Content:** Zingiberene is one of the main ingredients of ginger essential oil, which is a combination of chemicals derived from the rhizomes. While the overall amount of essential oil extracted from ginger might vary, it usually ranges between 1 and 3% of the fresh ginger rhizome's weight.
- **Essential Oil Concentration:** Typically, between 25% and 35% of ginger's essential oil is composed of gingeberene. According to certain research, it is approximately 35%.
- **Variation by Extraction Method:** The extraction technique employed may also have an impact on the zingiberene concentration. Different essential oil yields can be obtained using techniques including steam distillation and Soxhlet extraction.

3.2. Variation in concentration across different ginger variety and growing condition

The factors influence the chemical composition of ginger, including the concentration of essential oils and specific compounds like zingiberene. Here's a closer look at how these factors can affect the zingiberene content:

- **Variety of Ginger:** The amount of zingiberene in different types of ginger can vary; mature ginger usually has more of it than younger ginger. In comparison to Chinese or other kinds, Indian varieties could possibly have higher levels of zingiberene.
- **Growing Conditions:** The biosynthesis of zingiberene is influenced by soil type, agricultural techniques, and climate (temperature, humidity). For example, ginger cultivated under ideal circumstances (balanced irrigation, nutrient-rich soil) is more likely to contain zingiberene and other essential oils.
- **Geographical Differences:** Due to variations in growth circumstances, altitude, and cultivation methods, ginger from different regions (such as China versus India) may show variances in zingiberene concentration.
- **Processing and Storage:** During processing (such drying) and prolonged storage in unfavorable conditions, the amount of zingiberene in ginger may drop.

4. Factor influencing Zingeberene yield

Numerous elements, including the genetic traits of the ginger plant, the growing environment, and the extraction techniques, affect the amount of zingiberene that is produced in ginger. The following are the main elements influencing zingiberene yield:

4.1. Ginger Type

4.1.1. Genetic Variations:

The output of zingiberene is directly impacted by the unique chemical profiles of various ginger varieties, such as Chinese, Indian, young, and mature ginger. Certain cultivars may naturally contain higher levels of zingiberene and other essential oils, while others may have lower levels.

4.1.2. Age of Ginger Rhizomes

Compared to young ginger, mature ginger rhizomes usually have higher levels of zingiberene and more essential oils. As the ginger plant ages, its levels of gingiberene rise, particularly in the latter stages of growth cycle.

4.1.3. Conditions for Growth

Climate

Two important environmental elements are temperature and humidity. Ginger grows best in tropical regions with high humidity and moderate temperatures (around 25 to 30 °C), which can promote the development of essential oils like zingiberene.

The plant may become stressed by extreme heat or cold and be less able to produce essential oils, which could diminish the amount of zingiberene produced.

Rainfall and Watering:

For ginger plants to yield superior rhizomes, they need enough water. Lower essential oil yields can result from stressing the plant with too much or too little water. Higher oil concentrations are usually produced under regular, moderate watering circumstances.

4.1.4. Fertility and Soil Type

Nutrient-rich soils

Ginger grows best in nutrient-rich soils that drain well, which also encourages the development of essential oils. Healthy growth and higher oil yields, particularly zingiberene, are supported by soils high in organic matter.

Soil pH

Another factor is the pH of the soil; ginger usually grows best in slightly acidic soils (pH 5.5–6.5). Unbalanced nutrient levels or poor soil quality can inhibit the synthesis of zingiberene.

Altitude and Geographical Location

Because of differences in temperature, soil composition, and growing conditions, ginger cultivated at higher elevations or in particular geographic regions may yield variable amounts of essential oil. For instance, ginger from mountainous areas, such as India's Western Ghats, typically contains higher levels of zingiberene and other essential oils.

4.1.5. Environmental Stress and Plant Health

- Diseases & Pests: When illnesses or pests are present, ginger plants may become stressed, which lowers their capacity to generate zingiberene and other essential oils. The amount of bioactive chemicals produced by a healthy plant is often higher.
- Environmental Stress: Because the plant puts survival before oil production, stressors including drought, heavy rains, or unfavorable soil conditions can cause a reduction in the production of zingiberene and essential oils.
- By carefully managing these variables, zingiberene yield and quality can be increased for usage in a variety of industries, such as food, medicine, and cosmetics.

4.2. Uses of Zingeber Officinale

- Both traditional and modern natural medicine make extensive use of ginger, commonly known as Zingiber officinale.
- Ginger is used in Chinese and Unani-Tibb medical systems to treat Gingivitis, toothache, rheumatism, mental disorders, uncomfortable menstruation, migraine, diabetes, constipation, asthma, and stroke. It is utilized in Asian medicine as a carminative.
- Ginger has a wide range of medicinal applications, including comprise the management of colds, illness, diarrhea, colic, dyspepsia, and poor appetite.
- Additionally, ginger is suggested as an anti-inflammatory treatment for rheumatoid arthritis and muscle diseases and to lengthen life. Ginger's use is supported by clinical trials.
- Measures to avoid pregnancy-related motion nausea and vomiting, while for musculoskeletal problems, the data is less clear (Lee et al.,2007; Zancan, K. C. et al., 2002).
- Products made from ginger, like oleoresin and essential oils, are sold for use in the manufacturing of food and medications.

4.3. Essential Oil

Plants and other natural sources are the source of essential oils, which are liquid, volatile scent molecules. It also contains fragrant plant essences that are highly concentrated (Khairu Aizam, 2006). Despite not being technically oils, essential oils often have a limited solubility in water. According to Jenny Jobling, the plant extracts are thought to be more tasty and definitely less harmful than the manufactured drug. Essential oils typically consist of a complex mixture of chemical compounds, primarily composed of several linear and cyclic molecules with relatively modest molecular masses that are either saturated or moderately unsaturated. Hydrocarbons and oxygenated molecules are present.(Ozel and Kaymaz, 2004).

5. Method of extraction

5.1. Extraction of Ginger Oil by Soxhlet Distillation

Soxhlet extraction is one of the laboratory instruments designed especially for extracting lipids from solid materials; it is extensively employed (Ayuso et al., 1998) and has received official approval in many countries. Though any solution can be used with this method if desired, the Soxhlet Extractor is not limited to merely extracting lipids per se.The substance has a certain solubility in the solvent, whereas the impurities are insoluble. A Soxhlet extraction can be used when a material with limited solubility needs to be extracted from a solid mixture. The technique places a specific glassware between a flask and a condenser. By repeatedly washing the solid, the refluxing solvent removes the required component into the flask.

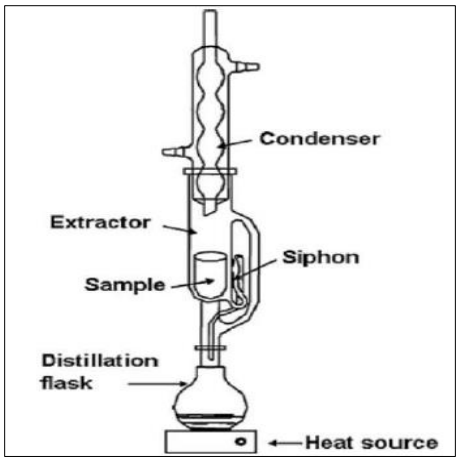

Figure 2 Soxhlet Extractor

6. Methodology

6.1. Materials

The extraction was performed using a sample of Zingiber officinale. Acetone, one of the chemical reagents utilized as the solvents in this extraction study, Benzene, methanol, and dichloromethane have a boiling point of 56.53-degree C, 39.6 degree C, 64.7 degree C, 80.1 degree C respectively. In this study, hexane was also utilized to dilute the oil samples prior to GC-MS analysis

6.2. Apparatus

The primary tools utilized in the Zingiber extraction process.The Soxhlet extractor is officinale. To separate the materials, a rotary evaporator was utilized. solvent as well as the oil that the Soxhlet extractor produces. The GC-MS was then employed for identification and detection of the oil samples' constituent parts.

6.3. Experimental Procedure

There were 4 main steps in this experiment which is sample preparation, extraction of ginger oil (Zingiber officinale), separation of the solvent from the oil and the last one was sample analysis.

6.3.1. Sample Preparation

Zingiber officinale's rhizome was cut into tiny pieces, which were thereafter dried in an oven for a full day or two. The rhizome was ground into extremely fine particles (powders) using a blender once it had completely dried.

Figure 3 Grinding of dried ginger

6.4. Extraction of ginger (Zingiber officinale) oil by using Soxhlet Distillation

The thimble was filled with 30 grams of ginger powder before being put into the Soxhlet chamber. The volumetric flask at the bottom of the Soxhlet extractor was filled with the solvent. Once the heating process had started to bring the

solvent's temperature up to a steady level, the extraction process began. Finally, the product was gathered and placed in the flask.

Figure 4 Soxhlet Extractor with the thimble inside.

6.5. Separation of the mixture

In this phase, the rotary evaporator was used to separate the solvent from the extracted oil.After the combination was added to the boiling flask, the temperature rose over the solvent's boiling point. The oil was left in the boiling flask while the solvent was gathered in the condenser flask. The yield % was then calculated by obtaining the mass of oil.

Figure 5 Rotary Evaporator

6.6. Sample Analysis

The Zingiber officinale oil was analyzed by GC-MS Agilent 6890 gas chromatography instrument

Figure 6 Gas Chromatography- Mass Spectrometer

7. Conclusion

For several decades, conventional Soxhlet extraction has been the most popular extraction method in the world. It outperforms other extraction options and serves as a benchmark for efficiency when comparing its traditional and novel competitors. This study demonstrates that Zingiber officinale essential oil may be extracted using the Soxhlet extraction method. Using methanol as the solvent extraction, it was discovered that the most effective conditions for extracting Zingiber officinale oil using the Soxhlet extraction method were 8 hours of extraction time.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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