



Extraction of essential oil from plant material (orange peels)

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Abstract

In this study, the peels of *Citrus sinensis* (sweet orange) were used for the extraction and characterization of essential oils. Since these oils are volatile and aromatic, they can be widely used in pharmaceuticals, cosmetics, food preservatives, and aromatherapy. The oils were obtained through steam distillation with the help of highly modified Clevenger Apparatus and analyzed by phytochemical screening and thin layer chromatography (TLC). The Liebermann-Burchard test confirmed the presence of terpenes through the appearance of a wine-red color. TLC analysis gave an R_f value of 0.87, consistent with the reported value of limonene which is the major constituent of citrus essential oil. This work demonstrates that discarded orange peel waste can serve as a sustainable source of valuable natural products with potential applications in pharmaceutical, cosmetic, and food industries.

Keywords: Extraction of essential oil; Citrus sinensis; Steam distillation; Phytochemical screening; TLC analysis; R_f value

1. Introduction

Essential oils are natural, highly concentrated aromatic compounds, that are extracted from different parts of plants such as leaves, flowers, roots, seeds, and peels [1]. Unlike fatty oils, they are volatile, non-greasy, and non-saponifiable [2]. These bioactive oils have numerous applications in pharmaceuticals as antiseptic and anti-inflammatory agents, in cosmetics for skincare and therapeutic purposes, in the food and beverage industry as preservatives and flavoring agents, and across several other industries [3,4]. Essential oils can be obtained through mechanical pressing or, more commonly, by steam distillation due to their volatility [5]. Among citrus fruits, *Citrus sinensis* (sweet orange) peel is particularly rich in limonene, a cyclic monoterpene hydrocarbon responsible for its characteristic citrus aroma [6]. In this study, discarded orange peel waste was utilized for the extraction and characterization of essential oil, demonstrating both its chemical properties and potential industrial applications.

1.1. Previous Studies:

Previous studies have demonstrated that volatile oils can be recovered from citrus wastes more efficiently through steam distillation and hydro-distillation [7,8]. Reportedly, these oils contain a high number of monoterpenes, which possess antimicrobial, antioxidant, and aromatic properties [3,4]. The presence of limonene and related compounds can be confirmed using Thin Layer Chromatography (TLC) and phytochemical screening [9]. Moreover, citrus peel waste can be utilized to provide a value-added product, which will further help in minimizing the waste from food processing industry [10].

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1.2. Research Gap

Although research is available on citrus essential oils, very few studies have demonstrated the use of phytochemical screening and TLC analysis for characterizing the extracted oil from locally available *citrus sinensis* peel [11]. Furthermore, the retention factor (Rf) values of limonene obtained from experimental extraction need to be documented and compared with standard literature value under specific conditions [12].

Objectives of the Study

The main objective of this study was to confirm the presence of terpenes in essential oils, extracted through steam distillation of *citrus sinensis* peels by the help of phytochemicals screening and to determine the Rf value of extracted oils using TLC and compare it with reported values.

This study therefore focuses on extracting essential oil from locally available *Citrus sinensis* peels, characterizing it through phytochemical tests and thin-layer chromatography, and documenting the Rf value of limonene to compare with reported standards. By using discarded orange peel, this study also highlights the potential for sustainable, value-added products in pharmaceuticals, food preservation, cosmetics, and aromatherapy, while contributing to waste minimization in the food processing industry.

2. Materials and Methods

2.1. Plant material

Fresh oranges (which contain a high concentration of aromatic compounds)

2.2. Apparatus Used

- Clevenger's apparatus
- Round-bottom flask (500 mL)
- Beakers and test tubes
- Condenser for condensation
- Steam generator
- TLC plate (silica gel coated)
- Glass rods
- Capillary tube
- Cotton
- Asbestos cloth

2.3. Chemicals Used

- Acetone
- Distilled water
- Chloroform
- Silica gel
- conc.H₂SO₄
- Acetic Anhydride

2.4. Theory

Essential oils from plants can be released and separated by the process called steam distillation which is a widely used classical method of separation [13]. This technique remains highly relevant even today due to its efficiency in extracting heat-sensitive compounds without decomposition [14]. A distillation flask is used to hold the plant material and water. The steam generated in the flask comes out through an outlet which is connected with a long glass tube. The connecting tube is wrapped with a piece of asbestos cloth to prevent the condensation of steam in it so that vapor and oil won't condense prematurely, thereby minimizing oil loss. When the distillation flask was gently heated, the vapors containing essential oils were formed which was then condensed and collected as distillate for further use.

In this experiment a specially modified Clevenger Apparatus was used to extract the essential oil from peels of orange [15]. The distillate obtained was subjected to qualitative phytochemical screening and TLC analysis for detection and

identification of natural products or bioactive substance particularly focusing on limonene, the major terpene present in citrus oils.

2.5. Experimental Method

2.5.1. Extraction of essential oil

Preparation of plant material

Clean orange peel was taken and chopped into small pieces, from which 50 grams was taken for the experiment.

Setup and procedure

Small pieces of orange peel were placed into a 500 mL flask, in which 50 ml of distilled water was added. In this technique a distillation flask was used which was connected with the outlet of steam generator and also with the collector [16]. Steam Distillation was used to avoid condensation, for which the outlet tube was wrapped with asbestos cloth and the flask, was heated gently while the steam was passed through it [17]. As the distillation process completed, distillate was taken into the beaker and proceeded for further examination.

2.6. Analysis

2.6.1. Phytochemical screening test (Liebermann Burchard test)

The Liebermann–Burchard test is a colorimetric chemical test used for the detection of terpenes in the extracted essential oil [18].

Table 1 Liebermann–Burchard Test for Detection of Terpenes in Extracted Orange Peel Essential Oil

Experiment	Observation	Inferences
2mL of chloroform was added to the extracted oil, which is dissolved in 3mL of acetic anhydride and 2mL of conc. H ₂ SO ₄	Wine red color solution was observed	Presence of terpenes confirmed.

2.6.2. Thin layer Chromatography Analysis

TLC is a method used to identify and separate the components of mixture using a thin stationary phase by observing their movement [19]. In this experiment this technique is employed for the separation and identification of major constituents in the extracted oil.

2.6.3. Solvent system/ mobile phase

Chemicals used as a solvent were - Acetone (7 mL), chloroform (3 mL) and distilled water (2 mL) [20].

2.6.4. Procedure

First, solvent (acetone, chloroform, water) was prepared in a clean beaker. The prepared TLC plate was spotted with the extracted essential oil extraction by using capillary tube then placed in the developing chamber containing the mobile phase. The position of spot of solvent was marked with sample spot. The TLC plate was visualized by spraying acetic anhydride dissolved in conc. H₂SO₄ and a red spot was observed, indicating the presence of Terpene compound [21].

2.6.5. Calculation of R_f

- The R_f value is calculated by using this formula,
- $RF = \text{Distance travelled by sample} / \text{Distance travelled by solvent} = 4.7 / 5.4 = 0.87$
- The R_f value was calculated as 0.87 for this extracted oil.

3. Results

In this experiment, valuable essential oil (bio-based product) was extracted from plant material particularly from orange peels or discarded waste material by using an apparatus named Clevenger's apparatus. To confirm the presence

of oil in the composition, phytochemical screening was done by using Liebermann-Burchard test and also through chromatographic analysis.

3.1. Phytochemical screening test (Liebermann-Burchard test)

2mL of chloroform extract was reacted with conc. Sulfuric acid and acetic anhydride which produced wine red color solution indicating the presence of Terpenes in an essential oil.

3.2. Chromatographic Analysis

The extracted essential oil was analyzed using thin layer chromatography. Acetone, chloroform and distilled water were used as a solvent in the ratio of 7:3:2. When acetic acid and conc. Sulfuric acid was sprayed, red colored spot was observed which indicates the presence of essential oil.

The retention factor (RF) was calculated and found to be 0.87 which is within the reported range for limonene and other terpene hydrocarbons in citrus essential oils analyzed by TLC using silica gel and hydrocarbon solvents [22].

4. Discussions

Steam distillation is a separation technique used for extraction of essential oils from orange peel or (discarded fruit waste) and volatile organic compounds, which are confirmed by TLC analysis and phytochemical test [23,24]. Terpenes are aromatic compounds found in various plants such as- orange, lemon, limes, and grapefruits and its presence is confirmed by using Liebermann-Burchard reaction [25,26]. The main terpene present in citrus essential oils is d-limonene, which contributes to their characteristic aroma and properties. During the process steam distillation was carried out immediately after crushing the peels to prevent oil from evaporation. Clevenger's apparatus was effective for condensation, which increased the oil yield. Similarly, TLC method was used for component confirmation which helped to identify the individual compounds.

5. Conclusion

This experiment shows the use of steam distillation, phytochemical screening as well as TLC analysis methods to extract an essential oil from *C. sinensis*. Confirmation of terpenes was done through chemical test, and the calculated value of retention factor was found to be 0.87. The observed Rf value (0.87) suggested the possible presence of limonene in the extracted essential oil. This study demonstrates the value of bio-degradable product (orange peel oil) which has potential applications in several fields like food industries, pharmaceuticals as anti-inflammatory, antiseptic cosmetics, preservatives and aromatic applications.

Compliance with ethical standards

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Disclosure of Conflict of Interest

The authors declare no conflict of interest.

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