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Optimize of Extraction Method on Yield of Ocimum Leaf Oil in Two Species: (*Ocimum Santum & Ocimum gratissimum*)

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Abstract: ***Among the plants known for medicinal value, the plants of genus Ocimum belonging to plant family Lamiaceae are very important for their therapeutic potentials. The therapeutic potential of it has been established on the basis of several pharmacological studies carried out of various extraction methods, various solvent, various species and different parts of the plant. But there is no published comparative report in the literature about the chemical compositions of various Ocimum species (O.basilicum, O. Sanctum, O. gratissimum) essential oil from various oil extraction techniques. So this studied has been made to investigate the effect on quality and quantity of essential oil of various oil extraction techniques. This research could help optimized extraction methods to maximize the yield of its bioactive compounds. In this research result found that O. gratissimum consistently yields higher essential oil than O. sanctum across all extraction methods. Supercritical CO_2 extraction provides the highest oil yield for both species. The choice of extraction method depends on the desired oil yield and quality. Supercritical CO_2 extraction provides the highest yield and preserves volatile compounds effectively but is costly. Soxhlet and solvent extraction also offer high yields but may involve solvent residues. Steam and hydro distillation are traditional methods with moderate yield but produce high-quality essential oils suitable for therapeutic applications. In this study found that supercritical CO_2 extraction was an advanced, eco-friendly, and highly efficient method for extracting Ocimum essential oil with high purity and no solvent residues. Although it has higher initial costs, its superior yield, selectivity, and sustainability make it the preferred choice for pharmaceutical, food, and cosmetic applications. In this finding, it is important to note that the efficiency of each extraction method can vary depending on the specific plant species and its unique characteristics. Therefore, selecting the most appropriate extraction technique should consider both the desired oil yield and the quality requirements for the intended application. A) If higher yield is the priority, O. gratissimum is the better choice, especially when using supercritical CO_2 or Soxhlet extraction. B) If medicinal properties and aromatic purity are required, O. sanctum oil from steam distillation is preferable.***

Keywords: Ocimum Gratissimum, Ocimum sanctum, supercritical CO_2, Soxhlet and Solvent extraction

1. Introduction

Among the plants known for medicinal value, the plants of genus Ocimum belonging to plant family *Lamiaceae* are very important for their therapeutic potentials (Yang et al, 2001). Tulsi is an important symbol of the Hindu religious tradition. Although the word, Tulsi‟ gives the implication of the incomparable one, its other name, Vishnupriya means the one that pleases Lord Vishnu. Found in most of the Indian homes and worshipped, its legend has permeated Indian ethos down the ages. Ocimum sanctum L. (Tulsi), Ocimum gratissium (Ram Tulsi), Ocimum canum (Dulal Tulsi), Ocimum basilicum (Ban Tulsi), Ocimum kilimandscharicum, Ocimum ammericanum, Ocimum camphora, Ocimum minimum L., Ocimum tenuiflorum L. and Ocimum micranthum are examples of known important species of genus Ocimum which grow in different parts of the world and are known to have medicinal properties. Ocimum sanctum L., known as ‘Tulsi’ in Hindi and ‘Holy Basil’ in English, is an erect softy hairy aromatic herb or under shrub found throughout India. Tulsi plant is a shrub reaching a height of 0.5 to 1.5 m. The leaves are 2-4 cm in length (Mundy et al,2004). In Ayurveda Ocimum sanctum L.(Tulsi) has been well documented for its therapeutic potentials and described as antiasthmatic (Dashemani Shwasaharni) and cough suppressant drugs (Kaphaghna) Khanna and Bhatia (2003).

The essential oil extracted from Tulsi leaves contains various volatile and non-volatile compounds responsible for its medicinal properties. The primary constituents include phenylpropanoids, terpenoids, and flavonoids. Pandey and Madhuri,2010. In last few decades several studies have been carried out by scientists and researchers to suggest the role of essential oils & eugenol in therapeutic potentials of Tulsi Rajeshwari, (1992).

The essential oil of *Ocimum sanctum* (Tulsi) is rich in bioactive compounds, with eugenol Prakash and Gupta (2005) as the primary component. In traditional systems of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chromic fever, cold cough, malaria, dysentery, convulsions, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite etc and in the treatment of gastric, hepatic, cardiovascular and immunological disorders (Singh et al, 2005). The holy basil is an herbal remedy for a lot of common ailments such as healing power, fever, common cold coughs, sore throat, respiratory disorder, kidney stone, heart and vascular protection, children's ailments, stress, mouth infections, insect bites, skin disorders, teeth disorder, headaches, eye disorders, liver support, lung and bronchial support, radiation protection, immunity tune-up, anti-inflammatory Action, antibiotic protection, nutrition, high safety margin, food additive and perfume etc. (Panda, 2005).

The therapeutic potential of it has been established on the basis of several pharmacological studies carried out with eugenol and steam distilled, petroleum ether and benzene extracts of different parts of this plant Pandey (2009). But there is no published comparative report in the literature about the chemical compositions of various Ocimum species (*O.basilicum, O. Sanctum, O. gratissimum*) essential oil from

various oil extraction techniques. So this studied has been made to investigate the effect on quality and quantity of essential oil of various oil extraction techniques. Both *Ocimum sanctum* (Holy Basil) and *Ocimum gratissimum* (Clove Basil) are well known for their medicinal and aromatic properties due to their rich essential oil content. In this research, studies different oil extraction techniques to find out the Maximize Yield and Quality of Essential Oils, determine the optimum extraction conditions for maximizing oil yield and recommend the Most Suitable Extraction Technique for High-Quality *Ocimum* leaves Oil. Identify the most effective and sustainable method that balances yield, quality, and economic feasibility.

2. Material and Methods

The plant material (leaves) of both species *Ocimum sanctum and Ocimum gratissimum* were collected from Botanical Garden of MJB Govt. college, Indore, MP. area and washed with water to remove mud and dust particles. The leaves were first dried at sunlight and then in an oven at 40 0 C. The dried leaves were grinded to powder by a cyclotec grinder (200 meshes) and the powder was stored in an air tight bottle and this was used throughout the investigations.

The essential oils from leaves of both Ocimum species were extracted by using various methods. In this research, studies different oil extraction techniques to find out the Maximize Yield and Quality of Essential Oils, determine the optimum extraction conditions. In this research were used five various extraction methods; i.e. Steam distillation, Hydro distillation Method, Solvent extraction method, Soxhlet apparatus method and Supercritical CO_2 extraction.

In steam distillation method, leaves powder apporx 250gm of each Ocimum species (*O. gratissimum* and *O. Sanctum)* were steam distilled separately using copper still fitted with spiral glass condensers for 3 h. Water distillate was extracted with hexane. While Ocimum species were subjected to hydro distillation method for 3 h using a Clevenger type apparatus. Sodium chloride (1 g) and 20 mL of diethyl ether was added with the distillate in a separatory funnel; shaking were continued for 40 min and allowed to stand for 15 min.

In solvent extraction method, leaf of ocimum species (*O. gratissimum* and *O. Sanctum)* and solvents (n- hexane) 40/1 ratio (mL solvent/gm leaf powder) were placed in the extraction reaction flask (Khalid et al, 2018) for 4-6 hours until complete extraction is achieved at 60 °C and mixing speed 300 rpm for solvent. In this method, solvent was removed by using a rotary evaporator to under reduced pressure, leaving behind the extracted oil.

The Soxhlet apparatus method is a widely used technique for extracting essential oils. In this method 20 g leaf powder were subjected in Soxhlet chamber with 500 ml Polar solvent methanol at 50-70°C using heating mantle. The process was continuing for 4-6 hours, the solvent was removed from the extract using a rotary evaporator under reduced pressure and the remaining Ocimum essential oil was separated from the solvent.

Supercritical CO_2 extraction is an advanced and efficient method used to extract essential oils and bioactive compounds from Ocimum (basil) leaves. This technique utilizes carbon dioxide (CO_2) in its supercritical state, allowing it to act as both a gas and a liquid, enhancing the extraction process while preserving heat-sensitive compounds. Supercritical CO_2 State: CO_2 was used above its critical temperature **(**31.1°C**)** and pressure **(**73.9 bar), making it behave like both a gas and a liquid. Supercritical CO_2 was penetrates plant material and dissolves oils. After the completion of process, the CO_2 was reused for further extractions, making the process cost-effective and environmentally friendly

3. Result & Discussion

The efficiency and suitability of different extraction methods play a crucial role in oil yield.%. In this research observed that the oil yield from Ocimum (basil) leaves varies significantly among different species, with factors like the variety, growth stage, and environmental conditions influencing the essential oil content and composition. Kholiya et al 2022 also reported this type finding on his research. Oil content of leaf of Holy Basil (*Ocimum sanctum species*) was found range from 1.30% to 7.05% (Presented in table 1) supported by Ashok Kumar et al 2010. While the essential oil yield was found higher side, ranging from 2.84% to 9.90% (w/w) in the leaf of *Ocimum gratissimum* species as compared than *Ocimum sanctum*. Kaliyaperumal et al (2020) supported this result.

Table 1: Comparative Oil Yield (%) Based on Different Extraction Methods

Extraction Method	Oil yield {(%) (w/w)} from dry leaf of Ocimum Species	
	Ocimum Sanctum	*Ocimum gratissimum*
Steam distillation	1.51± 0.5	3.05± 0.5
Hydro-distillation	1.30± 0.5	2.84± 0.5
Solvent Extraction	5.05± 0.5	6.08± 0.5
Soxhlet extraction	5.99± 0.5	7.20± 0.5
Supercritical CO_2 Extraction	7.05± 0.5	9.90± 0.5

In this study also observed that different extraction methods were significantly impact the quantity and quality of leaf oil extracted from Ocimum species like Ocimum sanctum (holy basil) and Ocimum gratissimum (African basil), with factors like type of solvent, temperature, and extraction time influencing the yield of the extracted oil.

In this research found that, methods like Soxhlet extraction provide higher yields but can also lead to potential degradation of sensitive compounds, while Super critical CO2 extraction methods was preserve more beneficial components along with high yield of Oil content. Data presented in table 1 that the oil yields of leaf of both Ocimum species were found 5.05% and 6.08 % respectively in solvent extraction method. This method was more efficient than steam or hydrodistillation. Non-polar solvents (hexane) were used for high oil yield, but may extract fewer polar bioactive compounds like antioxidants and also extract unwanted compounds. Yields were found almost similar and slightly higher to solvent extraction method, Oil was found 5.99% and 7.20% respective Ocimum species. Polar solvents

(ethanol, methanol) **(**Chatterjee et al 2011) was extract a wider range of phytochemicals including antioxidants and phenolic compounds. Supercritical CO_2 Extraction method was extracted more essential oil compared to conventional methods. Employing supercritical carbon dioxide as the solvent, this modern method produced yields ranging from 7.05% to 9.90% to *O. Sanctum and O. gratissimum* species respectively. It's environmentally friendly and produces high-quality oils. Supercritical CO2 considered a **"green"** method, allowing for selective extraction based on pressure and temperature, potentially preserving sensitive compounds. It is environmentally friendly method, because CO_2 is non-toxic, non-flammable**,** and reusable. In this method extraction was fast so more efficient than steam distillation or Soxhlet methods**.**

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