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RESEARCH ARTICLE

Phytochemical Extraction and Chromatographic Characterization of *Chrysopogon zizanioides* (Vetiver) Root

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Abstract: The extraction and analysis of bioactive constituents from aromatic medicinal plants remain a cornerstone of pharmaceutical and cosmetic research. *Chrysopogon zizanioides* (L.) Roberty, commonly known as Vetiver, is a perennial grass renowned for its complex essential oil sequestered within its fibrous root system. This study focuses on the extraction efficiency and phytochemical profiling of Vetiver roots utilizing solvent extraction methodologies. The primary objective was to isolate the crude extract and characterize its constituents through Thin Layer Chromatography (TLC). Root samples were subjected to rigorous processing, including shade drying and pulverization, followed by continuous hot percolation using a Soxhlet apparatus. This method facilitated the exhaustion of phytochemicals, yielding a viscous, aromatic extract rich in sesquiterpenoids. Subsequent chromatographic analysis employed a silica gel stationary phase and a specific solvent system of n-hexane and ethyl acetate to resolve the complex mixture. The analysis revealed three distinct phytochemical fractions with Retardation factor (Rf) values of 0.42, 0.60, and 0.76, indicating the presence of compounds with varying polarities, likely corresponding to the main sesquiterpene alcohols such as Khusimol and its derivatives. These results validate the efficacy of the extraction protocol and highlight the pharmaceutical viability of Vetiver root beyond its traditional olfactory applications. The study suggests that refined fractionation could further isolate specific therapeutic agents applicable in anti-inflammatory and antiseptic formulations.

Keywords: *Chrysopogon zizanioides*; Soxhlet Extraction; Thin Layer Chromatography; Sesquiterpenes; Phytochemical Analysis.

1. Introduction

Medicinal and aromatic plants constitute a significant segment of the global pharmaceutical and horticultural economy, providing essential raw materials for drug discovery, perfumery, and cosmetics. Among these, *Chrysopogon zizanioides* (L.) Roberty (syn. *Vetiveria zizanioides* Nash), belonging to the Poaceae family, occupies a prominent position due to the unique olfactory and therapeutic properties of its root system [1]. Indigenous to the Indian subcontinent, this perennial grass, locally known as "Khus," has been utilized since antiquity in traditional systems of medicine, particularly Ayurveda, for its cooling properties and ability to alleviate inflammatory disorders [2]. Taxonomically, while previously classified under the genus *Vetiveria*, molecular and morphological data have supported its reclassification under *Chrysopogon*, a change now widely accepted in the scientific community [3].

The economic value of *C. zizanioides* is primarily derived from vetiver oil, a complex essential oil extracted from the roots. This oil is characterized by a high tenacity and a woody, earthy aroma, making it an indispensable fixative in the fragrance industry. Beyond perfumery, the plant exhibits remarkable physiological resilience, forming dense clumps with a massive, spongy root system that is instrumental in soil conservation and environmental rehabilitation, particularly in the remediation of heavy metal-contaminated soils [4], [5].

While the agricultural benefits of Vetiver are well-documented, its chemical diversity presents a vast scope for pharmaceutical exploration. The root oil is reported to contain over 150 sesquiterpenoid compounds, including α-vetivone, β-vetivone, and khusimol, which contribute to its biological activities ranging from anti-inflammatory to sedative effects [6]. Recent pharmacological screenings have also highlighted the plant's antioxidant potential, attributed to the presence of phenolic compounds and specific sesquiterpenes that scavenge free radicals [7], [8]. The investigation into the plant's cytotoxicity have revealed promising activity against specific cancer cell lines, suggesting a potential role in oncology [9].

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Despite its extensive traditional usage, the precise characterization of its extracts requires robust analytical methodologies. The extraction process serves as the critical first step in phytochemical analysis, determining the yield and integrity of the bioactive moieties. Traditional methods like steam distillation often struggle with the complete recovery of high-molecular-weight compounds; however, continuous hot extraction techniques, such as those involving the Soxhlet apparatus, have proven superior in isolating both volatile and non-volatile constituents from recalcitrant plant matrices [10], [11].

Figure 1. Whole Plant of *Chrysopogon zizanioides* (L.)

This study aims to optimize the extraction of *C. zizanioides* roots and employ Thin Layer Chromatography (TLC) to establish a preliminary phytochemical fingerprint. By correlating the extraction efficiency with chromatographic profiles, this research seeks to validate the therapeutic potential of the root extracts and establish quality parameters for future pharmaceutical applications.

2. Materials and Methods

2.1. Plant Material Collection and Preparation

Fresh root samples of *Chrysopogon zizanioides* were collected from cultivating fields. The roots were thoroughly washed with distilled water to remove adhering soil and debris. To prevent the thermal degradation of thermolabile volatile constituents, the cleaned roots were subjected to shade drying at ambient temperature (25 $\pm$2^{o}C) for 14 days. The dried roots were subsequently pulverized into a coarse powder using a mechanical grinder and stored in airtight, amber-colored containers to protect against photo-oxidation prior to extraction.

2.2. Extraction Methodology

The extraction was executed using a standard Soxhlet apparatus to ensure exhaustive extraction of phytochemicals, a method preferred for its ability to cycle fresh solvent repeatedly through the biomass [12].

1. Approximately 50 g of the coarse root powder was packed into a porous cellulose thimble and placed in the extraction chamber.
2. A round-bottom flask was charged with 300 ml of analytical grade solvent (Ethanol/Methanol), chosen for its ability to solubilize a broad spectrum of polar and non-polar compounds.
3. The solvent was heated to its boiling point. The solvent vapor traveled up the side arm, condensed, and dripped onto the thimble, immersing the powder. Once the liquid level reached the siphon top, the extract-laden solvent siphoned back into the flask.
4. This cycle was maintained continuously for 6–8 hours until the solvent in the siphon tube appeared colorless, indicating complete extraction.
5. The resulting miscella was concentrated using a rotary evaporator under reduced pressure to yield a viscous crude extract. The percentage yield was calculated, and the extract was stored at 4°C.

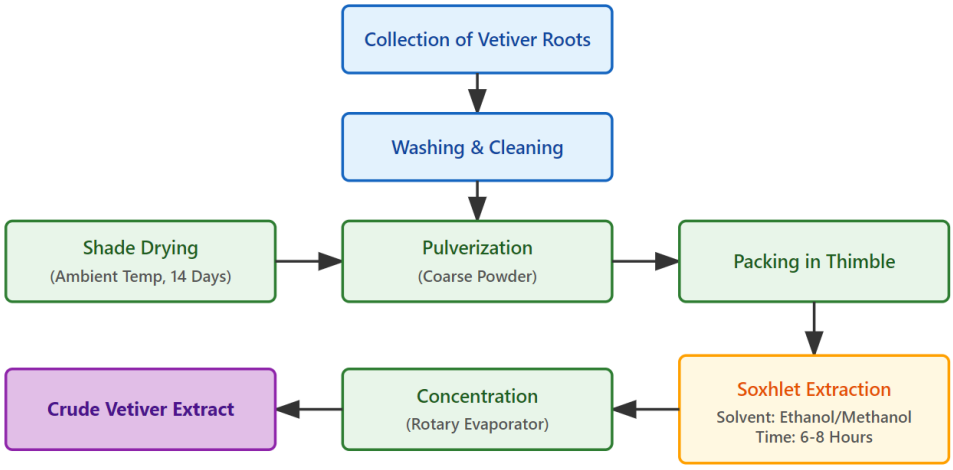

Figure 2. Soxhlet Extraction

2.3. Thin Layer Chromatography (TLC) Analysis

Qualitative profiling of the extract was performed using Thin Layer Chromatography to assess the chemical complexity of the extract [13].

- Stationary Phase: Silica gel G pre-coated aluminum plates (Merck) served as the stationary phase.
- Mobile Phase: A binary solvent system comprising n-hexane and ethyl acetate was optimized to achieve separation. The non-polar nature of hexane combined with the polarity of ethyl acetate facilitates the differential migration of sesquiterpenoids.
- Sample Application: The crude extract was dissolved in a minimal amount of solvent and spotted onto the TLC plate using a capillary tube, 1 cm from the bottom edge (origin).
- Development: The plate was developed in a saturation chamber until the solvent front reached approximately three-quarters of the plate height.
- Visualization: The developed plates were air-dried and visualized under UV light (254 nm and 366 nm). For distinct band identification, a derivatizing agent (Vanillin-Sulfuric acid reagent) was sprayed, followed by heating at 105°C to visualize separated compounds.

The Retardation factor (Rf) was calculated for each resolved spot using the formula:

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front}}$$

3. Results and Discussion

3.1. Physical Characterization

The solvent extraction process yielded a dark brownish-yellow viscous residue with a distinct, tenacious, earthy aroma characteristic of vetiver oil. The yield varied between 0.5% and 5% depending on the specific solvent efficiency, consistent with literature values for root essential oil content [14]. The high viscosity of the extract suggests a significant concentration of sesquiterpenoids, which are known for their heavy molecular weight and fixative properties. Comparatively, solvent extraction often yields a higher mass percentage than hydro-distillation due to the co-extraction of heavier non-volatile waxes and pigments [11], [15].

3.2. Chromatographic Profiling

The TLC analysis revealed a distinct separation profile, indicating the presence of multiple phytochemical constituents. The analysis yielded three prominent spots with the following retention factor shown in Table 1.

Table 1. Results of TLC and Rf values

Compound Identifier	Distance Traveled by Spot (cm)	Distance Traveled by Solvent Front (cm)	Rf Value
Spot 1	2.1	5.0	0.42
Spot 2	3.5	5.0	0.60
Spot 3	3.8	5.0	0.76

Interpretation: The separation of compounds into three distinct zones suggests the presence of constituents with varying degrees of polarity.

- **Spot 1 (**Rf **0.42):** The lower Rf value indicates a compound with higher polarity, likely interacting more strongly with the silica stationary phase. This fraction may correspond to oxygenated sesquiterpenes or alcohols such as Khusimol, which possess hydroxyl groups capable of hydrogen bonding with the silica [16].
- **Spot 2 (**Rf **0.60) & Spot 3 (**Rf **0.76):** Higher Rf values denote less polar compounds that have a higher affinity for the non-polar mobile phase components (n-hexane). These spots likely represent sesquiterpene hydrocarbons or specific ketone derivatives like α-vetivone, which are less polar than their alcohol counterparts [17].

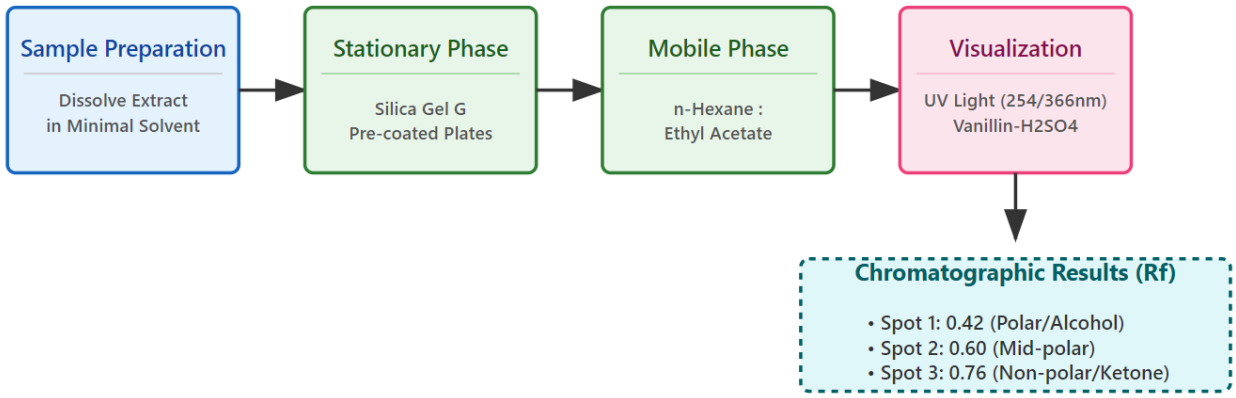

Figure 3. Phytochemical Profiling (TLC)

The distinct separation achieved confirms that the root extract is a complex mixture of phytochemicals, validating the use of the n-hexane/ethyl acetate solvent system for preliminary identification. The presence of these multiple fractions supports the traditional use of Vetiver, as the therapeutic effects are likely synergistic interactions between these various sesquiterpenoids. Similar chromatographic patterns have been observed in studies focusing on the anti-acne potential of Vetiver extracts, where mid-polar fractions demonstrated significant antimicrobial activity [13].

4. Conclusion

The present study successfully demonstrated the extraction and preliminary phytochemical characterization of *Chrysopogon zizanioides* root. The Soxhlet extraction method proved effective in isolating the bioactive principles, yielding a concentrate rich in aromatic constituents. Chromatographic analysis via TLC effectively resolved the crude extract into three major components with Rf values of 0.42, 0.60, and 0.76. These findings underscore the chemical diversity of Vetiver root and support its application in pharmaceutical formulations. The identified fractions serve as a foundation for further isolation and structural elucidation studies, potentially leading to the development of novel therapeutic agents for anti-inflammatory and anxiolytic applications. Future research should focus on High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) to structurally identify the specific compounds corresponding to these Rf values

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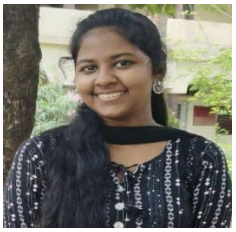

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