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Original Article

Development of HPTLC Fingerprints for *Cymbopogon citratus, Ocimum sanctum* and *Asparagus racemosus* for Identification and Product Safety

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ABSTRACT

Background and Objectives: The present study aimed to develop HPTLC fingerprint profiles and perform densiometric quantification of specific phytochemical markers in *Cymbopogon Citratus* (Lemongrass), *Ocimum Sanctum* (Tulasi) and *Asparagus Racemosus* (Shatavari) for herbal quality control and authentication. **Materials and Methods:** Plant materials were systematically collected and authenticated. Extraction was done with aqueous, hydroalcoholic and petroleum ether to obtain diverse phytoconstituents. HPTLC analysis was established on silica gel plates using optimized mobile phases for effective separation of phytochemical markers: Citral from *Cymbopogon Citratus*, Ursolic acid from *Ocimum Sanctum* and Shatavarin-IV from *Asparagus Racemosus*. Following derivatization with anisaldehyde sulfuric acid reagent, densiometric scanning and fingerprint profiling were performed under UV light at 254 nm and 366 nm. Quantification of marker compounds were carried out using calibration curve analysis. **Results:** Distinct chromatographic fingerprints were obtained for all the three medicinal plants. Citral (R_f 0.78-0.79), Ursolic acid (R_f 0.70), and Shatavarin IV (R_f 0.45) were successfully identified and quantified from the respective extracts. Higher phytochemical distribution was shown by hydroalcoholic and aqueous extracts due to better extraction of polar and moderately polar constituents. The developed fingerprints and densiometric profiles confirmed the presence of characteristic phytochemical markers useful for herbal standardization. **Conclusion:** Our study proves that HPTLC fingerprinting is a trustworthy method for ensuring the safety, potency, and quality control of herbal medications. The developed fingerprints can be used as reference standards for routine analysis, increasing the legitimacy and acceptance of herbal compositions around the world.

Keywords: *Asparagus Racemosus* (Shatavari), *Cymbopogon Citratus* (Lemongrass), HPTLC fingerprinting, *Ocimum Sanctum* (Tulasi).

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INTRODUCTION

High-Performance Thin Layer Chromatography (HPTLC) is a rapid, sensitive, and economical analytical method that can analyse multiple samples quickly with least amount of solvent. Major and minor constituents can be detected by means of UV imaging and derivatization. The WHO and ICH also recommended HPTLC for quality control of herbal drugs, active pharmaceutical ingredients and their formulations. HPTLC can be employed for a wide range of applications such as identification, quantitative analysis, impurity profiling, method development, and cleaning validation. HPTLC offers outstanding separation for pharmaceuticals, nutraceuticals, traditional medicines and radio-labelled compounds.

The main advantages include the ability to analyze multi-component crude samples, use multiple standards on a single plate, process samples and standards identically, have a wide range of solvents, have a variety of detection techniques, *in situ* spectra can be recorded, and have less exposure to hazardous solvents.[1-4]

Medicinal plants

The leaves of the plant *Cymbopogon Citratus*, also known as lemongrass are the sources of essential oil. It is a tall perennial grass belonging to the Poaceae family and widely cultivated in tropical regions. Citral (a mixture of neral and geranial) accounts for about 65-85% of the plant oil. The other constituents vary with the source and chemotype and are myrcene, limonene and geraniol. The plant is known for the utilization of antibacterial, anti-inflammatory, antipyretic and carminative properties. The bioactivity that has been reported very recently of lemongrass

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oil and its major constituent citral comprising antioxidant, gastroprotective, and hypolipidemic activities, which could be a common profile. India is one of the major countries that grows lemongrass and it is grown commercially in several parts of the country namely Kerala, Karnataka, Assam and Uttar Pradesh. The oil is valuable in food, pharmaceutical and perfume industries globally. The standardized HPTLC fingerprints have helped in enhancing its acceptance and ensuring quality control of the plant oil.[5-7]

Ocimum sanctum, also known as Tulasi belongs to Lamiaceae family. It is a fragrant perennial shrub that is extensively grown in different agroclimatic zones in India. The two main varieties of Tulasi are green (Rama) and purple (Krishna), and they are highly priced for their cultural and therapeutic significance. Significant phytochemical groups present in *Ocimum Sanctum* are Phenylpropanoids (eugenol, methyl eugenol) with analgesic and antimicrobial activity, triterpenoids (ursolic acid) with

anti-inflammatory and hepatoprotective properties, and phenolic acids and flavonoids (rosmarinic acid, apigenin) with antioxidant and cardioprotective effects. Volatile compounds with respiratory and anxiolytic effects include linalool and 1,8-cineole, the composition varies according to the variety and growing techniques. Tulasi has diverse therapeutic properties, which include antibacterial, immunomodulatory, respiratory-protective, antioxidant and hepatoprotective effects. Therefore, it can be used in the management of systemic inflammation, immunological support, respiratory conditions and stress. India is the primary producer of Tulasi, and the plant has considerable commercial value in nutraceuticals, ayurvedic medicines, herbal teas, and personal care items. Due to increasing worldwide demand, standardized extracts with confirmed chemical markers, such as ursolic acid and eugenol, are important for product quality, efficacy, and compliance to regulations.[8-10]

Asparagus Racemosus (Shatavari) which is a climbing perennial plant in the Asparagaceae family is found in many tropical and sub-tropical areas of India. In terms of chemistry, it includes steroidal saponins for example shatavarins I-IV, aglycones like sarsasapogenin also we see other phytochemicals in the form of flavonoids and alkaloids. What we also note is that the level of Shatavarin IV does play its variable role by geography and plant type which in turn puts into play the issue of standardization. Traditionally this plant is known for use as a galactagogue, adaptogen and a rejuvenating agent. There are also scientific researches offering evidence that the plant indeed has large scale immunomodulatory, antioxidant, anti-inflammatory and gastroprotective properties, especially in women's health. India is the main player in production and export of this plant which at the same time plays a large role in commercial and therapeutic fields of adaptogenic formulations, nutraceuticals and herbal supplements. HPTLC fingerprinting especially for the quantification of Shatavarin IV is of great importance in terms of quality control and which in turn facilitates its acceptance in international markets.[8,9]

Therefore, the present study aims to develop and compare HPTLC fingerprints of *Cymbopogon Citratus, Ocimum sanctum*, and *Asparagus Racemosus* for their key phytochemical markers.

Establishing reliable chromatographic profiles that may be utilized for authentication, quality control, and standardization of these medicinal plants used in herbal formulations.

TLC of Medicinal plants

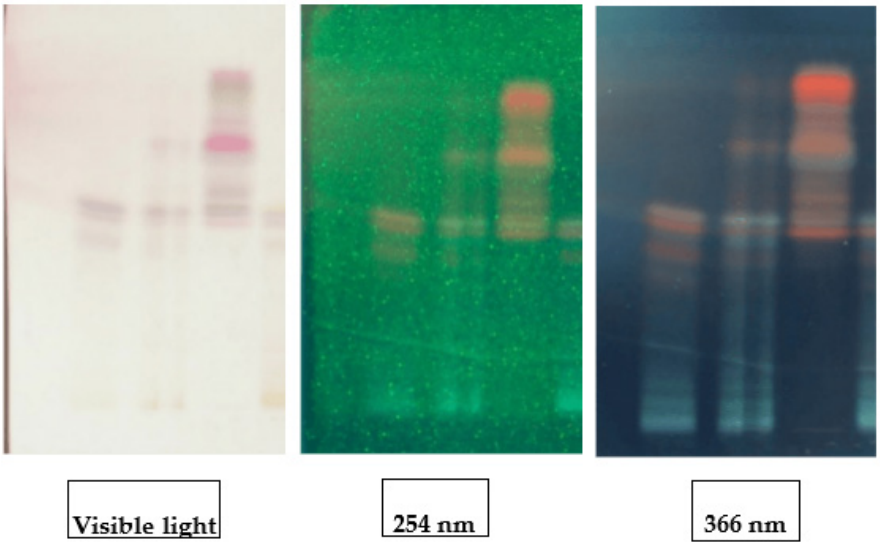

Figure 1: TLC of *Cymbopogon Citratus* extracts.

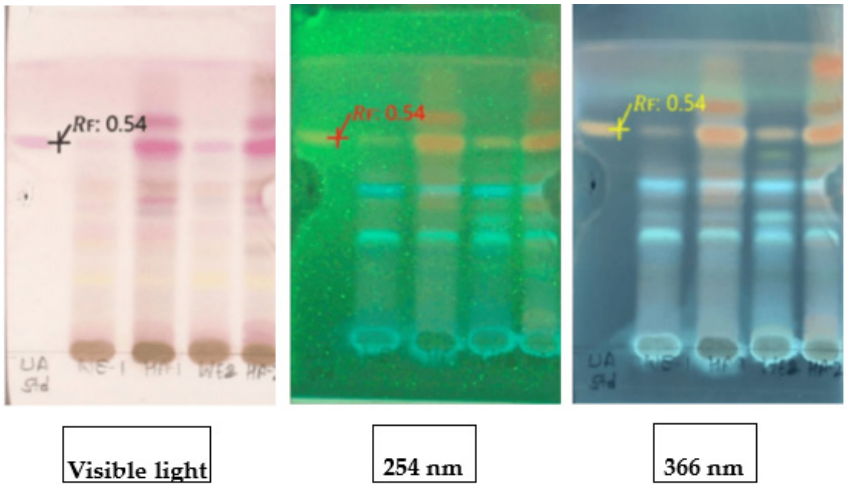

Figure 2: TLC of *Ocimum Sanctum* extracts.

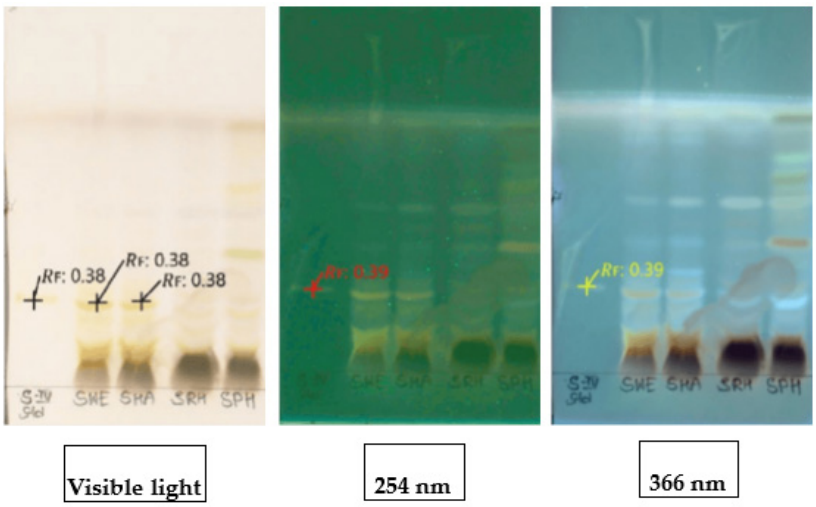

Figure 3: TLC of *Asparagus Racemosus* extracts.

HPTLC FINGERPRINTING

HPTLC fingerprints of *Cymbopogon Citratus* extracts

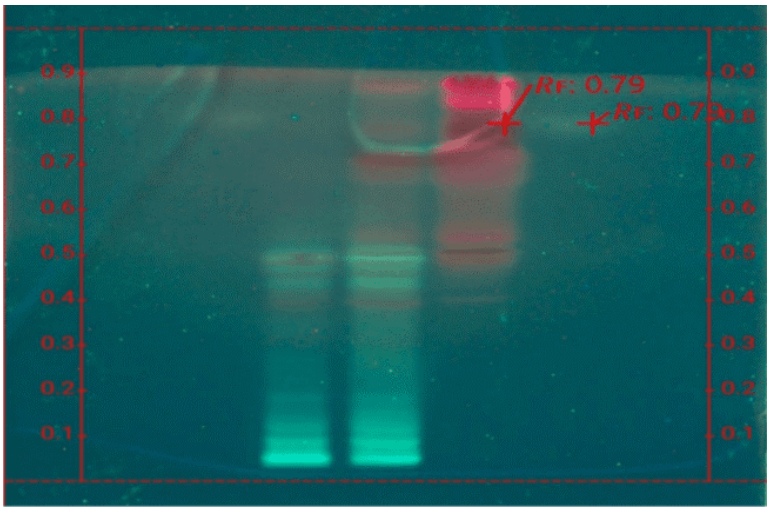

Figure 4: Visualization at 366nm of *Cymbopogon Citratus* extracts.

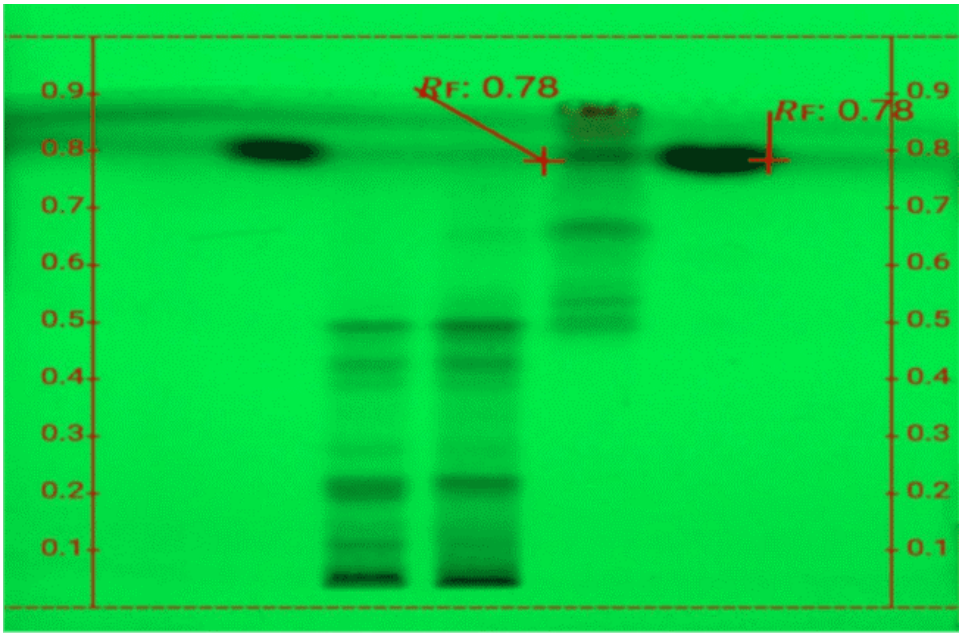

Figure 5: Visualization at 254nm of *Cymbopogon Citratus* extracts.

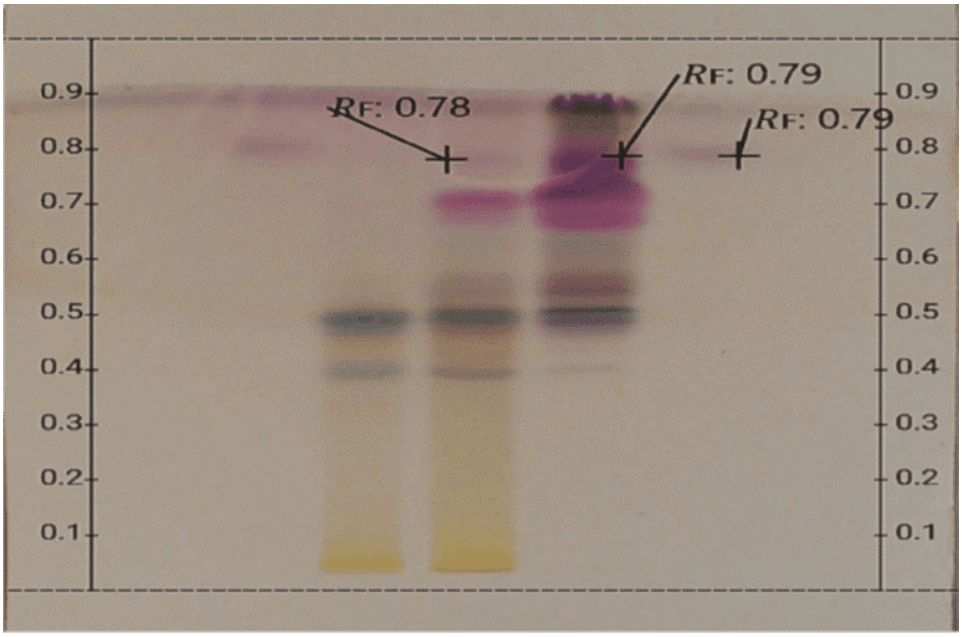

Figure 6: Visualization at visible light of *Ocimum Sanctum* extracts Figure 6: Visualization at 366nm, 254nm and visible light of *Asparagus Racemosus* extracts.

MATERIALS AND METHODS

Dried leaves of *Cymbopogon Citratus* and *Ocimum Sanctum* and dried roots of *Asparagus Racemosus* were used in the investigation. Commercial-grade solvents (ethanol, ethyl acetate), demineralized water, and LR-grade chemicals (petroleum ether, chloroform) were among the necessary supplies. An extractor, rotary evaporator, vacuum tray drier, beakers, precoated silica gel 60F254 TLC and HPTLC plates, and a CAMAG scanner were among the tools utilized. A taxonomist at Natural Remedies Pvt. Ltd., in Bengaluru verified the authenticity of the raw plant components that were gathered from Tamil Nadu's Krishnagiri district. The labels for the verified batches were Shatavari-RHD-961, Tulasi-HARDOT-194, and Lemongrass- 2526SBP002.

Four techniques were used to extract *Cymbopogon Citratus*: hydro-alcoholic extraction, water extraction, petroleum ether extraction, and hydro-distillation. 150 g of raw material were put in an extraction flask and treated with the appropriate solvent (50% ethanol, water, or 100% petroleum ether) for each solvent-based extraction. Filtration came after 3. 45-min washes with constant monitoring at each stage of extraction. After concentrating the filtrates via solvent evaporation and drying in

a Vacuum Tray Dryer (VTD) which upon completion the dried extracts were removed and their yields noted. Using a Clevenger device, 250 g of plant material was hydro-distilled with water for 3 hr. Later, the essential oil was separated, collected, yielded, and refrigerated.

There were four ways to extract the *Ocimum Sanctum* (Tulasi) leaves: 50% hydro-alcoholic extraction, water extraction, 100% hydro-alcoholic extraction and 100% water extraction. 250g of raw material was added to the extraction flask and treated with either water or 50% alcohol. Three 45-min washes were taken and a water bath/heating mantle was monitored throughout. The mixture was filtered. The bioactive compound-containing filtrate was dried in a Vacuum Tray Dryer (VTD) and concentrated using solvent evaporation. Percentage yields after scraping of dried extract, gathering and packaging were noted.

The four different methods adopted for extracting *Asparagus Racemosus* (Shatavari): Hydroalcoholic extraction of whole root, Water extraction of whole root, Hydroalcoholic extraction

HPTLC fingerprints of *Ocimum Sanctum* extracts

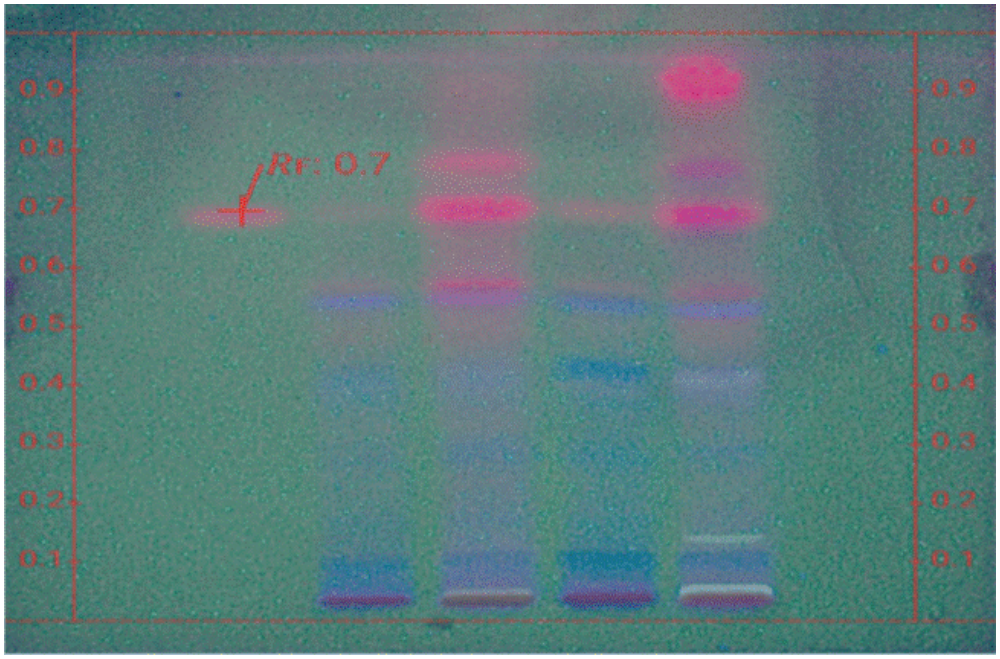

Figure 7: Visualization at 366nm of *Ocimum Sanctum* extracts.

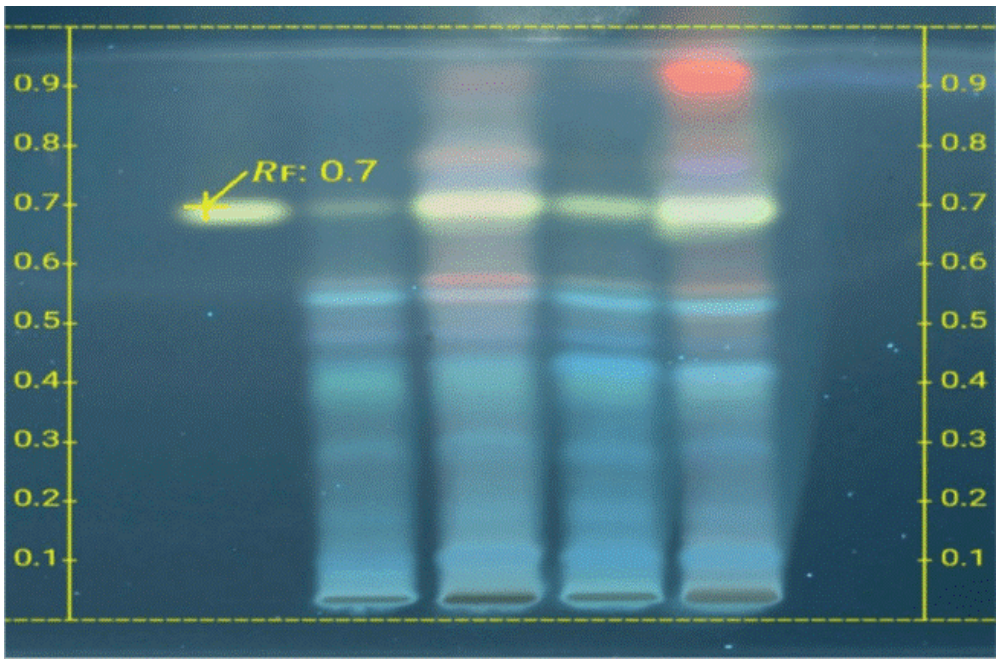

Figure 8: Visualization at 254nm of *Ocimum Sanctum* extracts.

of root peel, Hydroalcoholic extraction of peeled root. Method consists of taking 250 g of raw material in each extraction flask and extracted by water / 50% Ethanol solution. Water bath / Heating mantle was applied on extraction by 45 min, 3 washes under continuous supervision. All samples then filtered and the bioactive containing filtrate was dried in Vacuum Tray Dryer (VTD) and concentrated by solvent evaporation and the % yield was noted after the dried extracts were scrapped out, collected and packed.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was optimized by testing different solvent systems and selecting the best for separation of specified phytocompound as shown in Figures 1-3. 10 mg of each extract were dissolved in 1 mL of methanol to create sample solutions. Anisaldehyde sulfuric acid reagent was sprayed after TLC was carried out on silica gel 60 F254 plates (0.25 µm) and observed under UV at 254 nm and 366 nm. Anisaldehyde, glacial acetic acid, methanol, and concentrated sulfuric acid were combined in an ice bath to create the reagent, which was then kept in an amber bottle. For optimal viewing, plates were heated to 105 °C. For *Cymbopogon Citratus*, the ideal mobile phases were toluene: ethyl acetate (9.3:0.7), for *Ocimum sanctum*, toluene: ethyl: acetate: acetic acid (4.5:5.5:0.5), and for *Asparagus Racemosus*, ethyl acetate: methanol: water (7.5:1.5:1).

HPTLC fingerprinting

A CAMAG-HPTLC machine with VisionCATS software was used to perform HPTLC fingerprinting of all plant extracts. 10 mg of extract was dissolved in 1 mL of methanol for each sample, which was then heated, sonicated, and filtered through a 0.45 µm syringe filter. 100 mg of extract per milliliter was used in a similar manner to prepare standards. A Linomat 5 applicator with an 8 mm band width and a 4-6 µL application volume was used to apply the samples onto Supelco HPTLC silica gel 60 F254 plates. Depending on the plant extract, the optimized mobile phases were toluene: ethyl acetate (93:7), toluene: ethyl acetate: acetic acid (4.5:5.5:1), and ethyl acetate: methanol: water (7.5:1.5:1).

Using a 10 mL mobile phase, a 25 mL saturation volume, and a 70 mm migration distance, chromatogram development was carried out in an Automated Chamber (ADC2) in 4 min and 50 sec. In order to capture R_f values and fingerprint profiles, plates were dried, derivatized with anisaldehyde sulfuric acid, heated for visualization, and examined using CAMAG TLC Visualizer 3 and fingerprints were recorded as shown in the Figures 4-6.

RESULTS AND DISCUSSION

The polarity and solubility of the plant components are reflected in the extraction yields. Because they can effectively extract polar chemicals such phenolics, flavonoids, saponins, and glycosides, water and hydroalcoholic extracts demonstrated higher yields. The low output of essential oil from steam distillation is similar with the low yield of petroleum ether in *Cymbopogon Citratus*, which suggests fewer non-polar ingredients. The higher yields on mature leaves and hydroalcoholic solvents of *Ocimum Sanctum* suggest an increase in amounts of moderately polar metabolites, whereas higher yield in water extracts of *Asparagus Racemosus* shows the predominance of very polar saponins and glycosides. In general, it shows the importance of solvent polarity

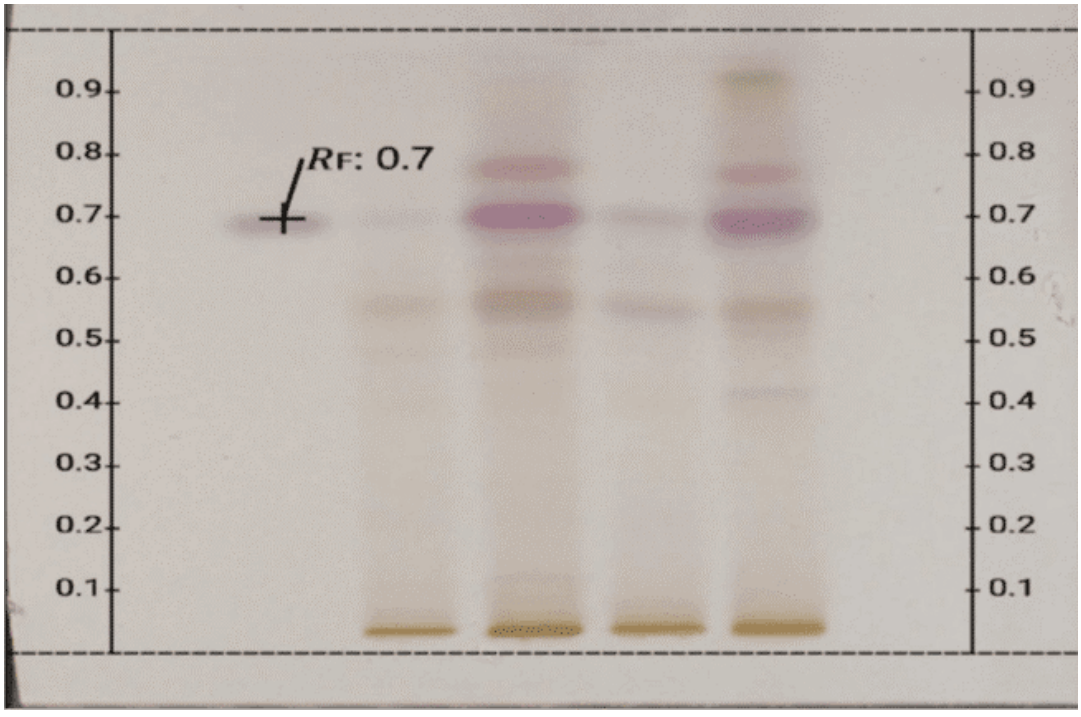

Figure 9: Visualization at visible light of *Ocimum Sanctum* extracts.

HPTLC fingerprints of *Asparagus Racemosus* extracts

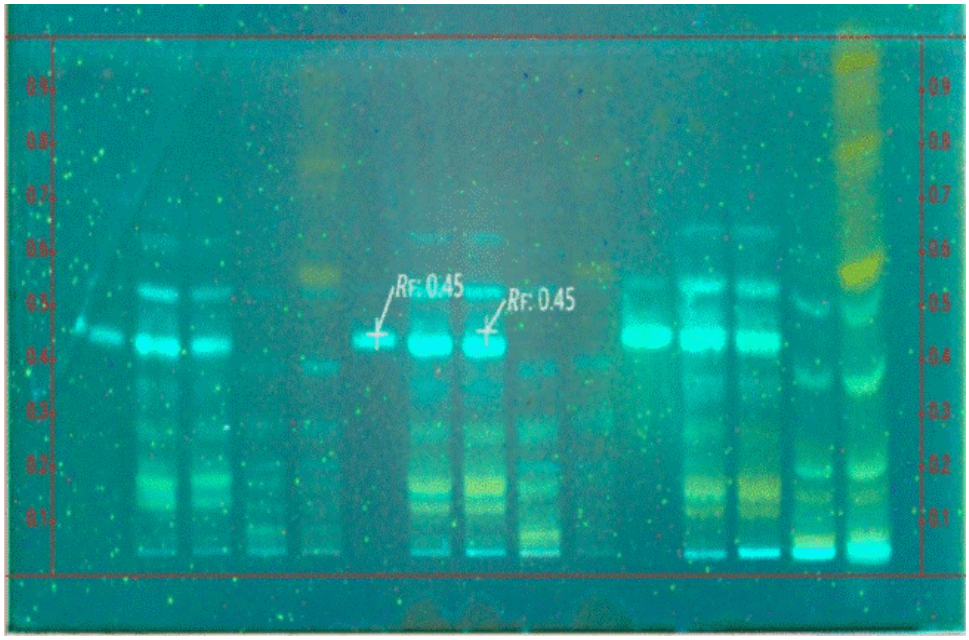

Figure 10: Visualization at 366nm of *Asparagus Racemosus* extracts.

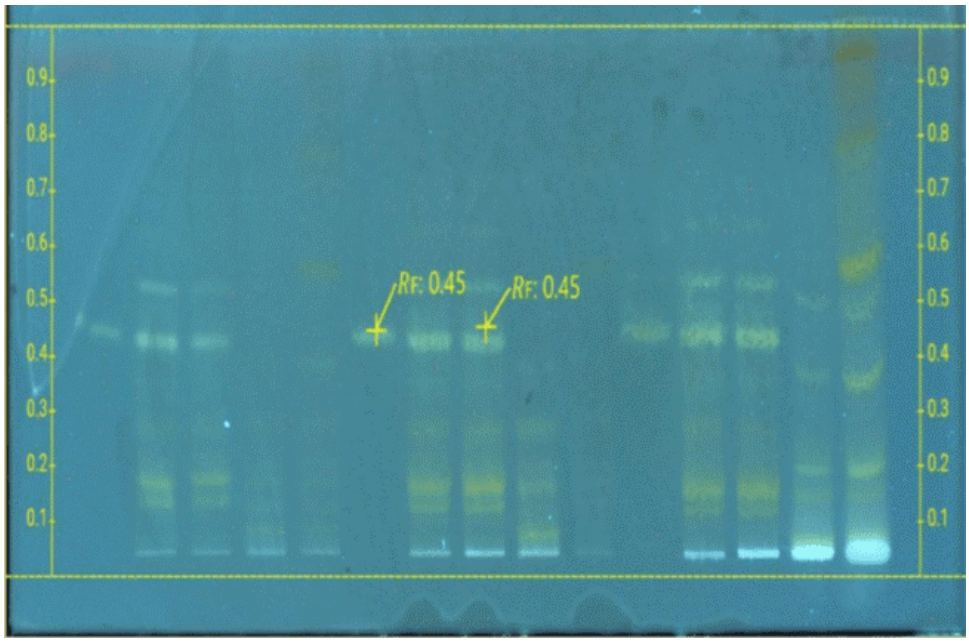

Figure 11: Visualization at 254nm of *Asparagus Racemosus* extracts.

in extracting specific components and also provides direction for the preparation of herbal drug formulae and phytochemical profiling. These observations are consistent with previously reported studies on *Cymbopogon Citratus, Ocimum Sanctum* and *Asparagus Racemosus,* where aqueous and hydroalcoholic solvents showed superior extraction efficiency due to their ability to dissolve a wide range of phytoconstituents. The results further demonstrate that solvent polarity plays a critical role in determining the distribution of phytochemicals among different extract fractions.

Via different band each matching with R_f of our standards, HPTLC Analysis of *Cymbopogon Citratus, Ocimum Sanctum* and *Asparagus Racemosus* confirmed presence of their marker chemicals, Citral, Ursolic acid and Shatavarin IV respectively. Lemongrass revealed important Citral band R_f 0.78.

0.79 at all wavelengths, showing maximum intensity in non-polar extract. Tulasi demonstrated the Ursolic Acid Band R_f 0.70 under 254nm, 366nm and visible light. Enhanced expression in hydroalcoholic extract was observed due to better solubility. The

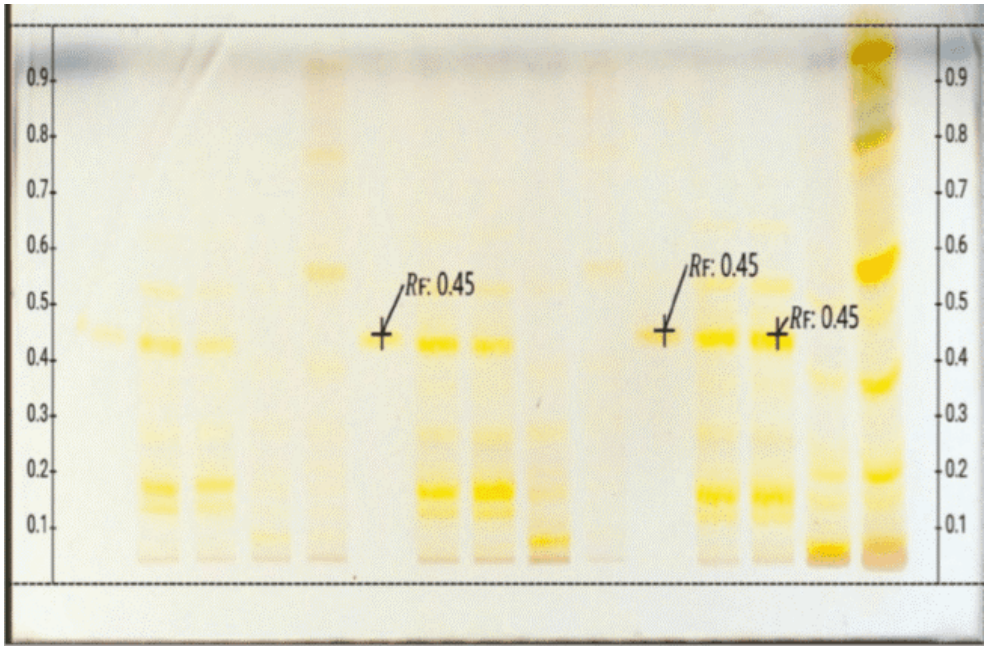

Figure 12: Visualization at visible light of *Asparagus Racemosus* extracts.

HPTLC fingerprints of Ocimum sanctum recorded under 366nm, 254nm and visible light are shown in Fig 7, 8 and 9, respectively. Shatavari revealed the unique Shatavarin IV Band at Rf 0.45 in all detection methods, which was strongest in hydroalcoholic fraction. The HPTLC fingerprints of Asparagus racemosus recorded under 366nm, 245nm and visible light are shown in Figures 10, 11 and 12, respectively. All three plants confirmed other phytoconstituents like glycosides, terpenoids, flavonoids, phenolic acid and saponin, which formed basis of diverse herbal formulations.

The developed HPTLC fingerprints showed good agreement with previously reported chromatographic studies in which citral, ursolic acid, and shatavarin IV were employed ass characteristic marker compounds for authentication of these medicinal plants. The variation in band intensity among different solvent extracts reflects the selective extraction of phytochemicals according to their polarity and solubility characteristics, Hydroalcoholic extracts generally produced richer fingerprint profiling, indicating the presence of both polar and moderately polar constituents. Through this technique, we successfully assigned these compounds to be trustworthy markers and complete fingerprint profiles were achieved, which can be utilized for purpose of authentication, standardization and quality control of the herbals.

In addition, the generated fingerprint profiles may serve as reference chromatograms for routine herbal quality control, batch-to-batch consistency evaluation, and detection of adulteration in raw materials and finished herbal formulations. The comparative assessment of extraction efficiency and HPTLC fingerprinting also highlights the practical relevance of the present study for herbal standardization and future phytopharmaceutical development.

CONCLUSION

In order to highlight the chemical constituents and solvent wise extraction behavior of *Cymbopogon Citratus* (Lemongrass), *Ocimum Sanctum* (Tulasi) and *Asparagus Racemosus* (Shatavari) the present study evaluated extraction yield and HPTLC finger printing. Polar metabolites such as phenolics, flavonoids, glycosides, saponins and polysaccharides were more abundant in water and hydro alcoholic solvents than petrol ether. Low production of petroleum ether in Lemongrass is equivalent to less abundance of non-polar molecules and needed oil. Better yield in older leaf in Tulasi showed high abundance of metabolites whereas in Shatavari water turned out to be the best due to high polarity of saponin and glycosides present in root. Citral (R_f 0.785-0.785) of lemongrass, ursolic acid (R_f 0.70) of Tulasi and Shatavarin IV (R_f 0.45) of shatavari were satisfactorily detected using HTPLC. Solvent-solute property relationship of the above marker bands was performed by noting the changes in clarity and intensity of band using polar and non-polar solvents. Chemical diversity of the plant extracts revealed from various bands at different R_f represents different types of phytoconstituents. So, the suitability of choice of solvent for better yield and exactness of the obtained chromatogram is identified. This study validates HPILC is a reliable process in raw material and formulation verification, quality and standards of herbs.

ACKNOWLEDGEMENT

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ABBREVIATIONS

%: Percentage; **TLC:** Thin Layer Chromatography; **HPTLC:** High-Performance Thin-Layer Chromatography; **R_f:** Retention Factor; **LR:** Laboratory Reagent; **mL:** Milliliter; **µL:** Microliter; **mg:** Milligram; **g:** Gram; **mm:** Millimeter; **nm:** Nanometer; **UV:** Ultraviolet; **PE:** Petroleum Ether; **EA:** Ethyl Acetate.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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