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COMPARATIVE HPTLC FINGERPRINT PROFILING OF LEAF AND RHIZOME EXTRACTS OF *CYPERUS ROTUNDUS* L. PREPARED USING DIFFERENT SOLVENT SYSTEMS: IMPLICATIONS FOR HERBAL STANDARDIZATION AND QUALITY CONTROL

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ABSTRACT
Cyperus rotundus L. is a medicinal plant widely recognized for its diverse therapeutic properties, which are primarily attributed to its rich phytochemical composition. Reliable phytochemical fingerprinting is essential for the authentication, quality control, and standardization of herbal medicines. High-Performance Thin-Layer Chromatography (HPTLC) is a rapid, reproducible, and cost-effective analytical technique extensively employed for the qualitative characterization of medicinal plant extracts. The present study aims to develop comparative HPTLC fingerprint profiles of leaf and rhizome extracts of *Cyperus rotundus* prepared using ethanol, methanol, ethyl acetate, and chloroform, and to evaluate solvent-dependent variations in phytochemical composition using quercetin as a reference marker. During the work, distinct chromatographic fingerprints were obtained for all extracts, indicating solvent-dependent variation in phytochemical composition. Leaf extracts exhibited two characteristic peaks, with the ethyl acetate extract showing the highest major peak area (60%), followed by ethanol (58%), methanol (52%), and chloroform (46%). Rhizome extracts also exhibited two prominent peaks, with ethanol showing the highest peak area (65%), followed by methanol (62%), ethyl acetate (59%), and chloroform (54%). Similar major Rf values among different extracts suggested the presence of common flavonoid constituents, whereas differences in peak intensity reflected variations in extraction efficiency associated with solvent polarity. The developed HPTLC fingerprint profiles provide reproducible chromatographic characteristics for the qualitative evaluation of *Cyperus rotundus* leaf and rhizome extracts. The findings demonstrate that extraction solvent significantly influences phytochemical recovery, with ethanol, methanol, and ethyl acetate showing superior extraction efficiency compared with chloroform. The proposed HPTLC fingerprints may serve as valuable analytical reference standards for the authentication, quality assessment, and standardization of *Cyperus rotundus* and its herbal formulations.

KEYWORDS: *Cyperus rotundus* L., HPTLC fingerprinting, Phytochemical profiling, Herbal drug standardization, Chromatographic analysis, Phytoconstituents.

1. INTRODUCTION

Medicinal plants have served as an indispensable source of therapeutic agents since ancient times and continue to play a pivotal role in modern healthcare and pharmaceutical research. The increasing prevalence of antimicrobial resistance, adverse effects associated with synthetic drugs, and growing demand for natural products have renewed scientific interest in medicinal plants as promising reservoirs of bioactive compounds. According to the World Health Organization (WHO), traditional medicine remains an essential component of primary healthcare, particularly in developing countries, where approximately 80% of the population relies on herbal medicines for their primary health needs (World Health Organization, 2013). The pharmacological potential of medicinal plants is primarily attributed to their diverse secondary metabolites, including flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, glycosides, and saponins, which exhibit antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and antiparasitic activities (Wachtel-Galor & Benzie, 2011; Harborne, 1998).

Cyperus rotundus L. belonging to Cyperaceae family, commonly known as nut grass or purple nutsedge, is a perennial medicinal herb widely distributed throughout tropical and subtropical regions of the world. Although considered an invasive weed in agricultural ecosystems, it occupies a prominent position in Ayurveda, Traditional Chinese Medicine, Siddha, and Unani systems of medicine. Different parts of the plant, particularly the rhizomes and tubers, have traditionally been used for the treatment of gastrointestinal disorders, dysentery, fever, inflammation, menstrual irregularities, pain, skin diseases, and microbial infections. Modern pharmacological investigations have further demonstrated that *C. rotundus* possesses antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, anticancer, and

antiparasitic activities, thereby supporting many of its traditional medicinal applications (Kamala et al., 2018; Xue et al., 2023).

The broad therapeutic potential of *C. rotundus* is associated with its rich phytochemical composition. Previous phytochemical investigations have identified numerous bioactive constituents, including flavonoids, phenolic acids, sesquiterpenes, terpenoids, alkaloids, tannins, steroids, glycosides, and volatile oils. Among these, flavonoids have attracted considerable attention because of their potent antioxidant and antimicrobial activities and their contribution to the pharmacological efficacy of herbal medicines. Quercetin, a naturally occurring flavonol, is frequently employed as a chemical marker for the standardization of medicinal plants due to its well-established biological properties and chromatographic stability. Consequently, chromatographic fingerprinting using quercetin as a reference marker has become an effective strategy for evaluating the phytochemical quality of herbal extracts (Xue et al., 2023).

Standardization of herbal medicines has become an important prerequisite for ensuring their quality, safety, efficacy, and batch-to-batch consistency. Since the chemical composition of medicinal plants is influenced by geographical location, environmental conditions, harvesting season, plant part, and extraction solvent, reliable analytical techniques are required for accurate phytochemical characterization. High-Performance Thin-Layer Chromatography (HPTLC) has emerged as one of the most reliable and cost-effective chromatographic techniques for herbal drug standardization. Compared with conventional thin-layer chromatography, HPTLC offers superior resolution, reproducibility, automated sample application, densitometric quantification, and simultaneous analysis of multiple samples. These advantages make HPTLC an indispensable analytical tool for authentication, detection of adulteration, marker compound identification, quality control, and phytochemical fingerprinting of medicinal plants (Reich & Schibli, 2007; Wagner & Bladt, 2009).

Although numerous studies have reported the phytochemical composition and pharmacological activities of C. rotundus, comprehensive comparative HPTLC fingerprint profiling of leaf and rhizome extracts prepared using different extraction solvents remains limited. Since solvent polarity significantly influences the extraction of phytoconstituents, comparative chromatographic evaluation is essential for selecting suitable extraction solvents and establishing reproducible phytochemical fingerprints for quality assessment. Therefore, the present study was undertaken to develop comparative HPTLC fingerprint profiles of ethanol, methanol, ethyl acetate, and chloroform extracts prepared from the leaves and rhizomes of *Cyperus rotundus* L. using quercetin as a reference marker. The generated chromatographic fingerprints are expected to provide a valuable analytical reference for authentication, quality control, and future standardization of *C. rotundus* and its herbal formulations.

2. MATERIALS AND METHOD

2.1 Plant Material

Fresh leaves and rhizomes of *Cyperus rotundus* L. were collected from the Marathwada region, Maharashtra, India. The collected plant material was taxonomically authenticated by a qualified botanist, and a voucher specimen was deposited in the departmental herbarium for future reference. The samples were thoroughly washed with distilled water to remove adhering impurities, shade-dried at room temperature, and pulverized into a fine powder using a mechanical grinder. The powdered samples were stored in airtight containers until further analysis.

2.2 Preparation of Plant Extracts

Dried powdered leaf and rhizome samples were extracted separately using ethanol, methanol, ethyl acetate, and chloroform by Soxhlet extraction. Briefly, 10 g of powdered plant material was extracted with 250 mL of the respective solvent for approximately 8 h. The extracts were concentrated under reduced pressure using a rotary evaporator and further dried to remove residual solvent. The dried extracts were preserved at 4°C in airtight containers until HPTLC analysis.

2.3 Chemicals and Reagents

Analytical-grade methanol, ethanol, ethyl acetate, chloroform, toluene, and formic acid were used throughout the study. Quercetin (≥98% purity) was employed as the reference standard. Pre-coated silica gel 60 F_{254} aluminium-backed HPTLC plates (200 × 100 mm; Merck, Darmstadt, Germany) were used as the stationary phase.

2.4 Preparation of Sample Solutions

For HPTLC analysis, 5 mg of each dried extract was dissolved in 5 mL of distilled water and sonicated for 5 min to obtain a homogeneous solution. The volume was adjusted to 10 mL with methanol. Subsequently, 1 mL of this solution was diluted to 10 mL using methanol (1:1, v/v), yielding a final concentration of 50 ppm. The prepared sample solutions were filtered through a 0.45 µm membrane filter prior to chromatographic application.

2.5 HPTLC Fingerprint Analysis

HPTLC fingerprint profiling was carried out using a CAMAG HPTLC system (Muttenz, Switzerland) consisting of a Linomat 5 automatic sample applicator, twin-trough glass developing chamber, TLC Visualizer 2, TLC Scanner 3, and visionCATS software for instrument control and data acquisition.

Sample solutions were applied as 6.0 µL bands on silica gel 60 F_{254} HPTLC plates using the Linomat 5 applicator while maintaining uniform band width and spacing. Chromatographic development was performed by the linear ascending technique in a twin-trough chamber pre-saturated for 20–25 min with the mobile phase comprising toluene acetate acid (6:3:1, v/v/v). The chromatograms were developed up to a migration distance of 70 mm under ambient laboratory conditions.

Following development, the plates were air-dried and examined under ultraviolet light at 254 nm and 366 nm using the TLC Visualizer 2. Densitometric scanning was carried out at 329 nm in absorbance mode using the TLC Scanner 3. Chromatographic fingerprints were generated using visionCATS software, and phytochemical constituents were evaluated based on Rf values, peak area percentages, peak height, and peak distribution patterns. All leaf and rhizome extracts, along with the quercetin standard, were applied and developed simultaneously on the same HPTLC plate under identical

chromatographic conditions to ensure analytical consistency, reproducibility, and reliable comparison among different solvent extracts.

The HPTLC procedure was performed following established chromatographic fingerprinting protocols for medicinal plant analysis with appropriate modifications to suit the present investigation (Wagner & Bladt, 2009).

3. RESULT AND DISCUSSION

3.1. HPTLC Fingerprint Analysis of *Cyperus rotundus* L.

High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profiling was performed to evaluate the phytochemical composition of leaf and rhizome extracts of *Cyperus rotundus* prepared using different extraction solvents. HPTLC is widely recognized as an effective analytical technique for herbal drug authentication because it generates reproducible chromatographic fingerprints that facilitate the qualitative assessment of complex phytochemical mixtures. In the present study, all extracts were analysed under identical chromatographic conditions using quercetin as the reference marker, allowing direct comparison of chromatographic profiles among different solvent extracts.

3.1.1. HPTLC Profile of Quercetin Standard

The HPTLC chromatogram of the quercetin standard produced a single, sharp, and symmetrical peak with excellent chromatographic resolution, confirming the suitability of the selected mobile phase for marker separation. The standard migrated within the expected Rf region and exhibited consistent peak symmetry without evidence of tailing or peak distortion, indicating satisfactory chromatographic performance. The optimized chromatographic conditions therefore provided a reliable reference for comparison with the phytochemical constituents detected in the plant extracts. Similar application of quercetin as a marker compound has been reported for the chromatographic standardization of medicinal plants because of its stability, characteristic chromatographic behaviour, and widespread occurrence among flavonoid-rich species (Reich & Schibli, 2007; Wagner & Bladt, 2009).

3.1.2. HPTLC Fingerprint Profile of Leaf Extracts

Distinct chromatographic fingerprints were obtained for all leaf extracts, demonstrating that solvent polarity markedly influenced the extraction of phytochemical constituents. The ethanol, methanol, ethyl acetate, and chloroform extracts each produced two well-resolved chromatographic peaks within comparable Rf regions, indicating the occurrence of chemically related flavonoid constituents. However, considerable variation was observed in peak intensity and peak area among the different solvent extracts.

The ethyl acetate leaf extract exhibited the highest chromatographic response, with the major peak contributing approximately 60% of the total peak area, followed by ethanol (58%), methanol (52%), and chloroform (46%). The superior chromatographic response observed for the ethyl acetate extract suggests that this semi-polar solvent extracted flavonoid constituents more efficiently than the other solvents evaluated. Conversely, the comparatively lower peak area recorded for the chloroform extract indicates limited extraction of polar flavonoids owing to the lower polarity of the solvent.

The consistent occurrence of a dominant chromatographic band near the quercetin reference region across all solvent extracts suggests the presence of common flavonoid constituents in the leaves of C. rotundus. Variations in peak area are likely attributable to differences in solvent polarity, which strongly influences the solubility and extraction efficiency of phenolic compounds and flavonoids. Similar observations have been reported in previous phytochemical investigations of medicinal plants, where semi-polar solvents demonstrated greater efficiency for the extraction of flavonoid-rich fractions than non-polar solvents (Kamala et al., 2018; Xue et al., 2023).

Overall, the chromatographic fingerprint analysis demonstrated that ethyl acetate and ethanol were the most suitable solvents for recovering flavonoid-rich constituents from *C. rotundus* leaves, while methanol showed moderate extraction efficiency and chloroform exhibited comparatively lower phytochemical recovery. These findings indicate that solvent selection is a critical factor in developing reproducible chromatographic fingerprints for herbal standardization.

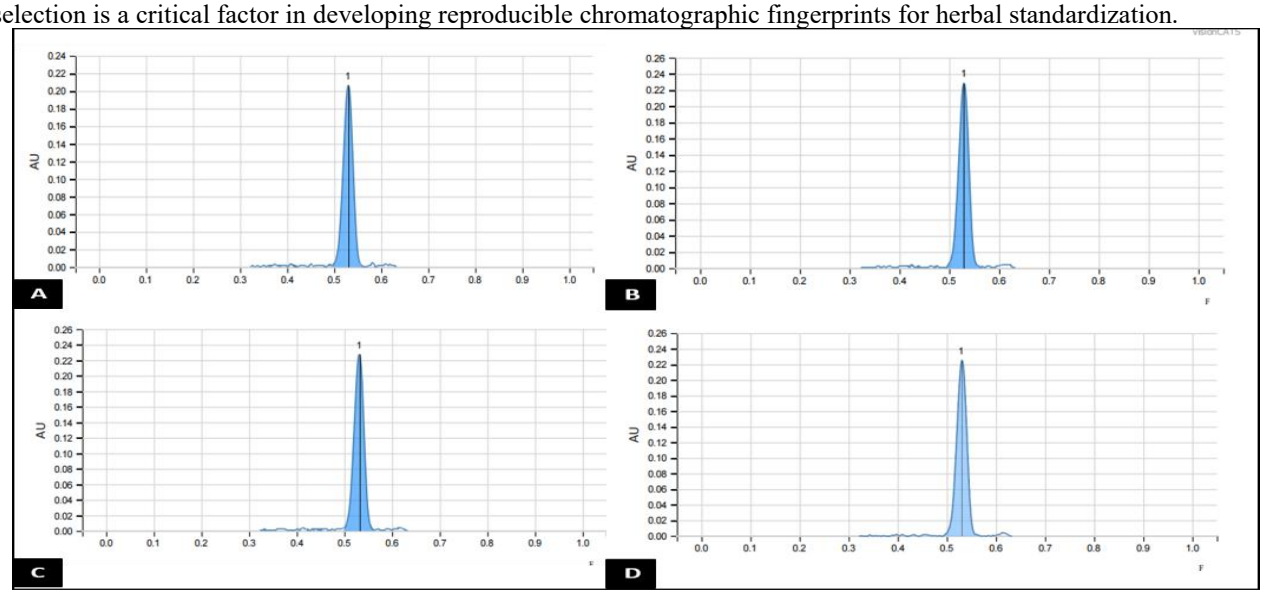

Figure 01. HPTLC densitograms of *Cyperus rotundus* leaf extracts prepared using different solvents. (A) Ethanol, (B) Methanol, (C) Ethyl acetate, and (D) Chloroform.

Table 01. HPTLC Fingerprint Profile of *Cyperus rotundus* Leaf Extracts Obtained Using Different Solvents.

Extracts	Plant Part	No. of Peaks	Major Rf	Major Peak Area (%)	Interpretation
Ethanol	Leaf	2	0.54	58	High flavonoid content
Methanol	Leaf	2	0.54	52	Moderate flavonoid extraction
Ethyl acetate	Leaf	2	0.54	60	Highest flavonoid recovery
Chloroform	Leaf	2	0.53	46	Lower extraction efficiency

3.1.3 HPTLC Fingerprint Profile of Rhizome Extracts.

The HPTLC fingerprint analysis of *Cyperus rotundus* rhizome extracts revealed distinct chromatographic profiles with noticeable variations among the extraction solvents. Similar to the leaf extracts, all rhizome extracts exhibited two well-resolved peaks, indicating the presence of major flavonoid constituents with comparable chromatographic behaviour. However, differences in peak intensity and peak area percentage suggested solvent-dependent variation in phytochemical extraction efficiency.

Among the investigated solvents, the ethanolic rhizome extract produced the highest chromatographic response, with the dominant peak contributing approximately 65% of the total peak area. The methanolic extract exhibited the second-highest peak area (62%), followed by the ethyl acetate extract (59%), whereas the chloroform extract showed the lowest peak area (54%). The higher chromatographic response observed in the ethanolic and methanolic extracts indicates their superior ability to extract flavonoid-rich phytoconstituents from the rhizome. Ethanol, owing to its intermediate polarity, facilitates efficient solubilization of both moderately polar and polar secondary metabolites, thereby enhancing the recovery of flavonoid compounds.

The major chromatographic peaks detected in all rhizome extracts migrated within a similar Rf region as the quercetin standard, suggesting the presence of structurally related flavonoid constituents. Although HPTLC fingerprinting alone does not permit unequivocal identification of individual compounds, similarity in chromatographic migration provides valuable preliminary evidence regarding the occurrence of comparable phytochemical classes. Such characteristic fingerprint profiles are widely employed for herbal authentication and routine quality control of medicinal plant materials.

Compared with the leaf extracts, rhizome extracts exhibited comparatively higher peak areas irrespective of the extraction solvent employed. This observation indicates that the rhizome contains a relatively greater abundance of flavonoid constituents than the leaves. The results are consistent with previous phytochemical investigations reporting that the underground parts of *C. rotundus* accumulate higher concentrations of secondary metabolites, particularly flavonoids, sesquiterpenoids, and phenolic compounds, which contribute significantly to the medicinal value of the species (Kamala et al., 2018; Xue et al., 2023).

Overall, the HPTLC fingerprint analysis demonstrated that ethanol was the most efficient solvent for extracting flavonoid-rich constituents from *C. rotundus* rhizomes, followed closely by methanol. The comparatively lower chromatographic response observed for chloroform suggests that non-polar solvents are less effective for the extraction of polar flavonoid compounds from rhizome tissues.

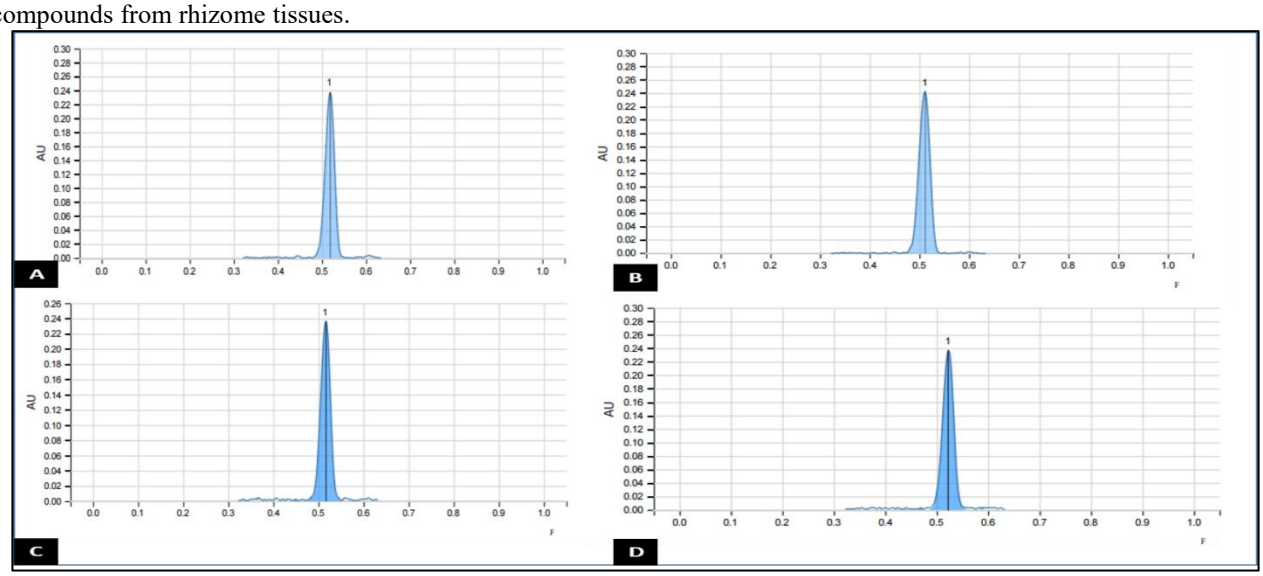

Figure 02. HPTLC densitograms of *Cyperus rotundus* rhizome extracts prepared using different solvents. (A) Ethanol, (B) Methanol, (C) Ethyl acetate, and (D) Chloroform

Table 02. HPTLC Fingerprint Profile of *Cyperus rotundus* Rhizome Extracts Obtained Using Different Solvents.

Extract	Plant Part	No. of Peaks	Major Rf	Major Peak Area (%)	Interpretation
Ethanol	Rhizome	2	0.49	65	Highest flavonoid content
Methanol	Rhizome	2	0.52	62	High flavonoid extraction
Ethyl acetate	Rhizome	2	0.45	59	Moderate flavonoid extraction
Chloroform	Rhizome	2	0.44	54	Lower extraction efficiency

3.1.4 Comparative Evaluation of Leaf and Rhizome Fingerprints

Comparative evaluation of HPTLC fingerprints revealed clear differences in the phytochemical composition of leaf and rhizome extracts. Although both plant parts exhibited similar chromatographic patterns with two characteristic peaks, considerable variation was observed in peak area percentages and chromatographic intensity. Rhizome extracts consistently produced larger peak areas than the corresponding leaf extracts, indicating a higher concentration of flavonoid-rich phytoconstituents.

Among the leaf extracts, ethyl acetate produced the highest chromatographic response, whereas ethanol demonstrated the greatest extraction efficiency for rhizome samples. These observations indicate that solvent polarity influences phytochemical extraction differently depending on the plant organ. Semi-polar solvents such as ethyl acetate appear more suitable for recovering flavonoids from leaf tissues, while ethanol proved more effective for extracting flavonoid-rich constituents from rhizomes.

The reproducible chromatographic profiles obtained under identical experimental conditions demonstrate the reliability of the developed HPTLC method for comparative phytochemical evaluation. The presence of common chromatographic bands across different solvent extracts further confirms the occurrence of similar classes of phytochemicals, whereas differences in peak intensity reflect variations in their relative abundance. Such reproducible HPTLC fingerprints provide valuable analytical markers for authentication, detection of adulteration, quality assessment, and standardization of *C. rotundus* raw materials and herbal formulations.

The present findings corroborate previous reports emphasizing the usefulness of HPTLC as a rapid and reliable analytical technique for medicinal plant standardization. However, unlike previous studies that primarily focused on single plant parts or individual solvent extracts, the present investigation provides a comprehensive comparative assessment of four solvent systems applied to both leaf and rhizome tissues under identical chromatographic conditions. This comparative approach offers valuable baseline information for selecting suitable extraction solvents and establishing reproducible chromatographic fingerprints for future phytochemical investigations and quality-control applications.

4. CONCLUSION

The present study successfully established comparative HPTLC fingerprint profiles of leaf and rhizome extracts of *Cyperus rotundus* L. prepared using ethanol, methanol, ethyl acetate, and chloroform. Distinct chromatographic fingerprints were obtained for all extracts, demonstrating that solvent polarity significantly influences the extraction of phytochemical constituents. Rhizome extracts exhibited comparatively higher peak area percentages than leaf extracts, indicating a greater abundance of flavonoid-rich phytoconstituents. Among the investigated solvents, ethanol proved to be the most effective for the extraction of rhizome constituents, whereas ethyl acetate showed the highest extraction efficiency for leaf extracts. The consistent chromatographic patterns and characteristic Rf values obtained using quercetin as the reference marker confirmed the reproducibility and reliability of the developed HPTLC method.

The established HPTLC fingerprints provide valuable baseline data for the authentication, quality assessment, and standardization of *Cyperus rotundus* raw materials and herbal formulations. Furthermore, the comparative evaluation of different plant parts and extraction solvents offers useful insights for selecting appropriate extraction systems in future phytochemical investigations. Although HPTLC provides reliable qualitative fingerprinting, complementary analytical techniques such as HPLC-MS/MS, LC-MS, or NMR may further facilitate the identification and characterization of individual bioactive constituents. Overall, the developed chromatographic fingerprints represent a useful analytical tool for quality control and support the future pharmaceutical and phytochemical utilization of *Cyperus rotundus* L.

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