Chapter 7

Phytochemical Diversity in *Cyperus rotundus* L.

Abstract

Though considered as a noxious weed, *Cyperus rotundus* is the store house of several interesting phytochemicals. The phytochemicals reported from *Cyperus rotundus* can be broadly classified into volatile compounds and non-volatile compounds, and the chapter enlists a total of 684 compounds (294 non-volatile compounds and 390 volatile compounds) reported from *Cyperus rotundus.*The volatile chemical profiles of *Cyperus rotundus* reported from 24 countries have been compared with a biogeographic perspective. Also, the chapter presents the essential oil and head space volatiles of *Cyperus rotundus* rhizomes and leaves collected from Kerala, south India. Sesquiterpenoids, especially guaiane and patchoulane type sesquiterpenoids are the major volatile compounds reported, while phenolics are the predominant non-volatile compounds in the plant. Correlation of the vast data on chemical diversity with phenology, genetics and ecology is yet to be explored.

Introduction

Among the various species coming under the Cyperaceae family, the most extensively investigated species is *Cyperus rotundus* L. The plant is a perennial sedge exhibiting erect stem with open umbel inflorescence and fibrous roots with underground, composite network of tubers, basal bulbs and rhizomes which ensure its spreading even in unfavourable conditions (**Figure 1**).

Figure 1. *Cyperus rotundus* (inset: tubers)

The plant had been used from prehistoric time onwards in food, medicinal and perfumery sectors. The wide use of the plant in traditional medicines and the potential bioactivities of the species are attributed to the characteristic chemicals present in the plant. A number of reports are available on the phytochemistry of *Cyperus rotundus* and several researchers; Singh *et al*., (2012), Srivastava *et al.,* (2013), Imam *et al.,* (2014), Samraj *et al.,* (2014), Hemanth Kumar *et al*., (2014), Gamal *et al*., (2015); Pirzada *et al.,* (2015), Al-Snafi (2016), Priyanka *et al.,* (2017), Saragih *et al.,* (2019), Taheri *et al.,* (2021), Wang *et al.,* (2022), Ross (2003) and Lu *et al.,* (2022), have reviewed the literature on *Cyperus rotundus* including phytochemistry, traditional uses and pharmacological activities. Recently Babiaka *et al.* (2021) reviewed 192 compounds reported from *C. rotundus*, that includes volatile compounds as well.

It is quite interesting to note that conventional phytochemical techniques such as derivatisation, adduct formation, years long structural interpretation through decomposition and semi-synthesis along with the most modern hyphenated analytical techniques were employed in the phytochemical investigation of *Cyperus rotundus* (**Figure 2**).

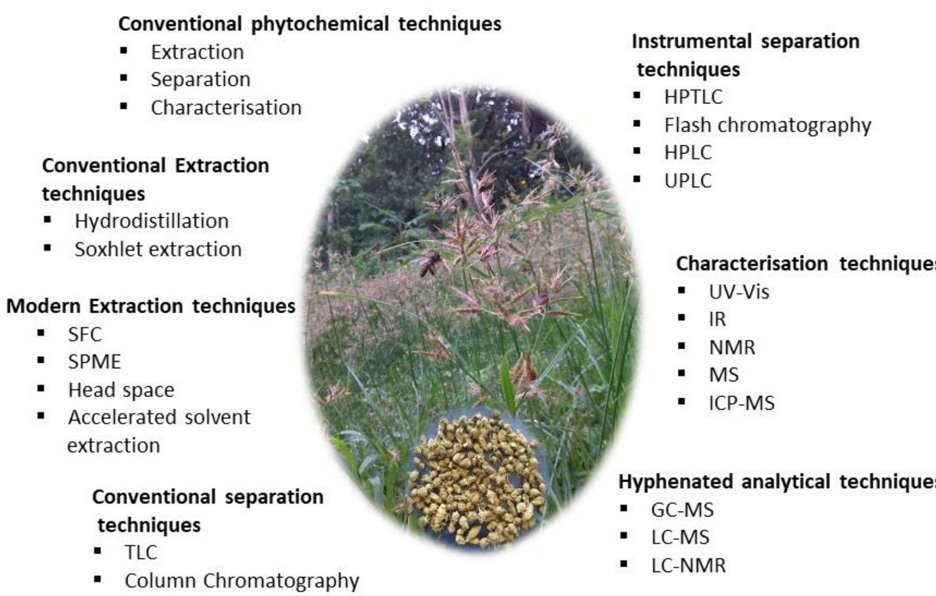

Figure 2. Major phytochemical techniques as applied in *Cyperus rotundus*

The present chapter elaborates a total of 684 compounds, with 294 non-volatile compounds reported from various solvent extracts of *C. rotundus*, and 390 volatile compounds in essential oils, head space and solvent extracts of *C. rotundus* (**Figure 3**).

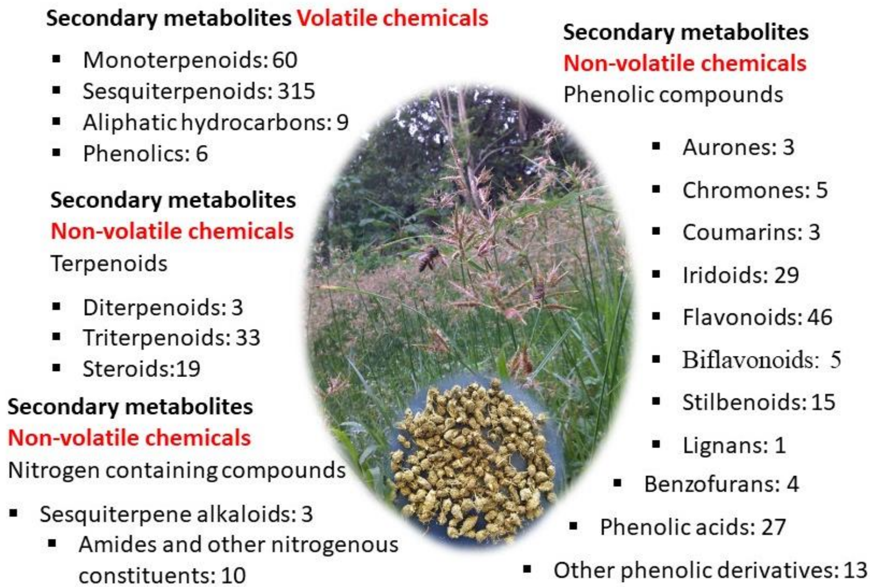

Figure 3. Phytochemical diversity in *Cyperus rotundus*

Cyperus rotundus in prehistoric times- A phytochemical exploration

Exploring the plant remaining in prehistoric skeletons using modern analytical tools reveal remarkable evidence about the herbal usage in prehistoric times (**Figure 4)**. The information hidden in the dental calculus of the skeletal remaining unearthed from the burial grounds of Motya's Phoenician community, located in Al Khidayparts of central Sudan, and lived around 7000 years before, during the pre-Mesolithic to Meroitic period, discloses the dietary ecology and phytomedicinal practices, especially the usage of *Cyperus rotundus* during that time. Samples of dental calculus taken from the burial grounds were analyzed by gas chromatography-mass spectrometry (GC-MS) and microscopy. It is interesting to note that the characteristic compounds such as rotundene, norrotundene, calamine, calamenene, cadalene and calarene present in *C. rotundus* were detected from the dental plaque of skeletal remaining. Further the starch granules extracted from dental calculus was superficially similar to that of *C. rotundus*. The finding revealed that the plant had been used either as medicinal or food material from ancient time (Buckley *et al.*, 2014). Also, the usage of *C. rotundus* explains the unexpectedly low frequency of caries among the Meroitic populations of Al Khiday, as *C. rotundus* has the ability to inhibit *Streptococcus mutans* that causes dental caries.

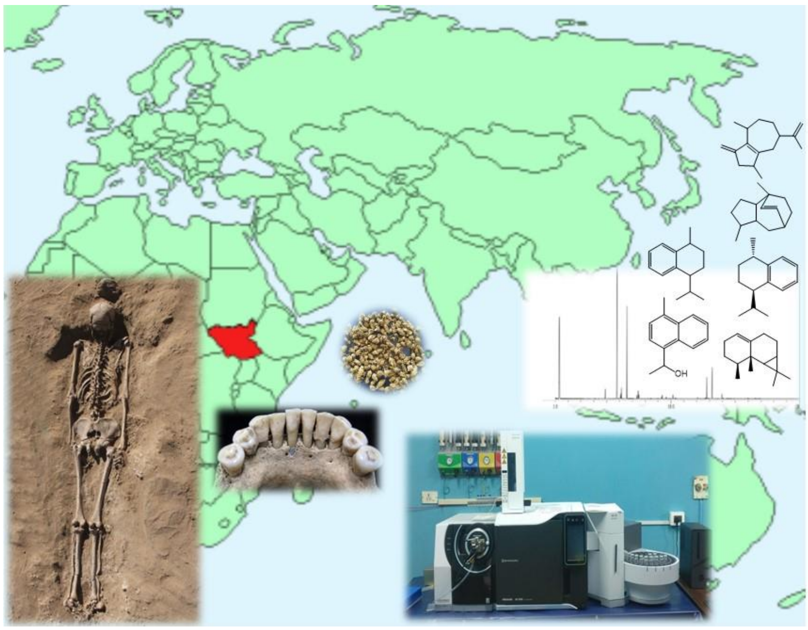

Figure 4. Schematic diagram of the discovery of *Cyperus rotundus* used in prehistoric time based on phytochemical tracing

The species has been a source of interesting structural skeletons in the history of phytochemistry. Doyens in the field of phytochemistry in India and abroad such as Sukh Dev *et al*., Nigam *et al*. andHarborne *et al*. had contributed significantly in understanding the complex structural features of phytochemicals in *C. rotundus* (Kapadia *et al.*, 1963; Kapadia *et al.*, 1967; Harborne *et al.*, 1982). Several interesting structures have been reported for the first time from the species, and several phytochemicals were named after the plant.

The plant still remains to be source of novel phytochemicals and biological activities, and 3,9-peroxy sesquiterpene-15-O-glucoside, a new sesquiterpene with fungicidal, bactericidal and cytotoxic properties has been reported from *C. rotundus* recently (Sabir *et al.*, 2020). In the COVID-19 pandemic period, the phytochemicals from the plant have been found promising. Kumar *et al*. (2021) reported sugetriol-3,9-diacetate from *C. rotundus* as one among the active phytochemicals, screened for binding affinity to PL^{pro} protein of SARS-CoV-2. Recently Majeed *et al.* (2022) has reported the stilbene derivatives piceatannol, scirpusin A and scirpusin B as the pharmacologically active molecules from the ethyl acetate extract of *C. rotundus* rhizomes. The present chapter elaborates the phytochemicals reported from the species.

Physicochemical profile and proximate composition of *Cyperus rotundus*

The phytochemicals reported from *Cyperus rotundus* can be broadly classified into proximate components, volatile compounds and non-volatile compounds from solvent extracts. Proximate analysis is used to estimate the relative amounts of protein, lipid, water, ash, carbohydrate *etc* in any sample, and are the first and foremost step to determine the identity and to assess the quality of plant material. The proximate analysis of *C. rotundus* gain much attention since the rhizomes are edible as a staple carbohydrate and is a famine food in some agrarian cultures (Farre, 2003; Prakash *et al.*, 2019; Ibrahim and Abdullahi, 2013; Umerie and Ezeuzo, 2000).

Physicochemical properties and extractive values of *Cyperus rotundus* rhizome such as total ash, acid-insoluble ash, water-soluble ash, loss on drying, sulfated ash, water-soluble extractive, alcohol-soluble extractive were determined along with successive extractive values with different solvent systems including petroleum ether, *n*-hexane, benzene, acetone, chloroform, ethyl acetate, alcohol, methanol and water (Sri Ranjani, 2017; Surendra Kumar and Ajay Pal, 2011;Emelugo *et al.*, 2011; WHO, 1998).

Various physicochemical parameters were determined for Cyperus *rotundus* and the tuber has been found to be good source of carbohydrates, minerals and fibre (Nalini *et al.*, 2014). Emelugo *et al.* (2011) reported 9.0% moisture, 1.75% crude protein, 9.50% oil, 7.87% ash, 17.48% crude fibre and 63.60% carbohydrate in *Cyperus rotundus* tubers. The high content of crude fibre in the rhizome helps to increase the bioavailability of nutrients in human diet (Sivapalan, 2013). The carbohydrate content in *Cyperus rotundus* is comparable with that of peanut, suggesting the rhizomes as a healthy component in human diet. The starch extracted from tuberous part of the plant has various applications in food and confectionary industries. The starch yield was 24.1% (on a dry weight basis) by wet-milling process

(Umerie and Ezeuzo, 2000).The large sized starch granules were comparable to the potato starch and with high amylose content (26.7%).

Mineral composition of *Cyperus rotundus*

The mineral content of plants has an important role in human conception since these are necessary vital elements for metabolic processes. Both the micro and macro elements in the *Cyperus rotundus* tubers were critically analysed, since the tubers of the plant were included in diet since ancient times (**Table 1)** (Oladunni *et al.*, 2011).

Table 1. Mineral composition of *Cyperus rotundus* (Ref. Oladunni *et al.*, 2011)

Sl. No.	Mineral	Content
1.	Phosphorus (mg/100ml)	0.52
2.	Zinc (ppm)	0.79
3.	Copper (ppm)	0.065
4.	Cadmium (ppm)	0.002
5.	Cobalt (ppm)	< 0.03
6.	Sodium (mg/g)	119.29
7.	Potassium (mg/g)	110.11
8.	Magnesium (mg/g)	50.76
9.	Calcium (mg/g)	16.40

The mineral analysis revealed the presence of sufficient amounts of macro (magnesium, phosphorus, potassium, calcium) as well as micro (sodium, copper, zinc) elements in *C. rotundus* rhizomes. The mineral concentration showed that sodium was found to be most abundant followed by potassium and also significant quantity of magnesiumand calcium while manganese and iron were not detected. The determination of heavy metals has drawn a significant attention due to the toxic and nutritional effects of these elements.

Phytoremediation potential of *Cyperus rotundus*

Phytoremediation refers to the use of plants to reduce the concentrations of contaminants in the environment. Heavy metal contamination is a major threat to the environment and human health, and phytoremediation is widely accepted as a cost-effective environmental restoration technology and considered as an alternative to industrial process for removing heavy metals from the surroundings (Onakpa *et al*. 2018; Das and Maiti 2007; Pinto *et al*.; 2014). Phytoremediation use a variety of mechanisms such asphytoextraction, phytostabilization, phytodegradation, phytovolatilization and phytofilteration (Dary *et al.*, 2010; Kumar and Maiti, 2015; Saran *et al*., 2020).

Cyperus rotundus is a hyper tolerant plant with high ability to take up heavy metals from soil, accumulate it in its underground tissues. Jahan-Nejati *et al*. (2021) reported that *Cyperus rotundus* is a safe forge or phytostabilizer species in copper contaminated soils. The plant is suggested as safe for grazing and forage production in copper contaminated environments as the translocation factor of copper is very low. Sultana *et al*. (2018) studied the interaction of *Cyperus rotundus* in nickel contaminated water, and found the Ni phytoremediation potential of *Cyperus rotundus* at 14mg/L. The plant showed remarkable metal uptake which remain accumulated near the root tips in contact with the medium. The plant is considered as a good accumulator of cadmium and chromium as well, and was recommended for remediation of cadmium and chromium contaminated soils (Subhashini and Swamy, 2014). Phytoremediation potential of *Cyperus rotundus* against Pb contamination revealed that the Pb concentrations were maximum in roots in comparison to shoots and the plant reduced more than 90% of lead in 30 days (Sunil Kumar *et al*., 2020). Hence, *Cyperus rotundus* could be used in natural treatment of polluted soil and in the rehabilitation programmes as a safe tool and treatment of polluted soil on road side (Elsayed Nafea and Šera, 2020).

Secondary metabolite diversity in *Cyperus rotundus*

The structural features of secondary metabolites in *Cyperus rotundus* is astonishingly diverse, and in addition to the proximate composition, the general secondary metabolites reported from *Cyperus rotundus* can be broadly classified into volatile compounds and non-volatile compounds.

Volatile chemical composition of *Cyperus rotundus*

Cyperus rotundus was used in ancient India, Egypt, Greece and elsewhere as an aromatic and perfume agent. Ancient Egypt and Greece literature reveals the use of *Cyperus rotundus* for aromatic purposes, and the plant had reputation as a source of perfume during the time of Greek physicians Hippocrates (5th century BC), Theophrastus, Pliny and Dioscorides (1st century AD). Dioscorides highlights the use of *Cyperus rotundus* tubers as an ingredient of ancient Egypt's best-known perfume, kuphi or kyphi, an incense that also had medicinal properties. The perfume as described by Dioscorides, is similar to the one in the *Ebers Papyri*, demonstrating its continuity over 1600 years (Negbi,1992). The plant is known in Ayurveda as *sugandhamusthaka* (aromatic *Cyperus*) and also being suggested as substitute for *karpura* (*Cinnamomum camphora*) based on the concept of drug substitution (*AbhavaPratinidhi Dravya*) (Venkatasubramanian *et al.,* 2010).

The aromatic nature of *Cyperus rotundus* is due to the presence of volatile compounds. Volatile compounds are typically small molecules with low boiling points and high vapour pressure at ambient temperature. Plant volatiles are generally made up of terpenoids, phenylpropanoids, benzenoid compounds, amino acid derivatives and fatty acid derivatives. Unconjugated volatiles can cross the cell membranes freely to be released from flowers, fruits and vegetative tissues into the atmosphere and from roots into the soil. The array of volatile compounds, released into the atmosphere by plants are responsible for attracting pollinators and other beneficial insects, providing a means of inter-plant communication, and directly repelling or intoxicating attacking herbivores. The plant volatiles have been investigated intensively with respect to integrated pest management (Ahuja *et al.,* 2010), defence against herbivores (Mithöfer and Boland, 2012; Das *et al.,* 2013), below-ground emissions (Ali *et al.*, 2012; Ghimire *et al.*, 2013), detection of disease infestation (Sankaran *et al.,* 2010; Cevallos-Cevallos *et al.*, 2011), food quality (Oms-Oliu *et al.*, 2013), chemotaxonomy (Sajewicz *et al.*, 2009; Liu *et al.,* 2013), biological control mechanisms (Smith and Beck, 2013; Wheeler and Schaffner, 2013) and metabolomics (Roze *et al.*, 2010; Cevallos-Cevallos *et al.*, 2011). The essential oils and volatile organic compounds are also found to be responsible for a variety of biological and pharmacological activities of *C. rotundus* (Sonwa and Konig, 2001; Jirovetz *et al.*, 2004; Kilani *et al.,* 2005).

The volatile aroma chemicals in plants are generally investigated through essential oils (EO) and recently head space (HS) analysis has received much attention as a rapid tool for volatile aroma chemical analysis.

Essential oil volatiles: Essential oils are steam volatile components of plants responsible for the aroma of the plant and mainly constitute terpenes, some phenolics, and aliphatic derivatives. Essential oils, as the name implies, bears the essence of the plant. Essential oils are not directly involved in growth and reproduction, but rather in fitness of plant life. Essential oils act as a safe guard against pathogens and insects, attractant for pollinator and fruit dispersers, and as deterrents to the growth of competing plants, and thus have a major role in chemical ecology.

Essential oils have important role in various industrial sectors such as perfumery, aromatherapy, insect control, flavor, medicines and preservatives**.** The distribution of the volatile chemicals can be utilized forchemotaxonomic purpose, to subgroup the species and also for phylogenic evaluation.The distribution of some of the essential oil constituents can be used as chemotaxonomical markers that aid in the identification of taxonomically closely related plants. Further, standardization with respect to the volatile chemicals will help authenticate marketsamples of the economically important species.

Essential oils are seen in specialized plant cells, glands or vessels and are isolated by different methods, of which distillation, especially hydro distillation is the most widely used technique. Gas Liquid Chromatography (GLC) coupled with Mass Spectrometry (MS) is used for the qualitative and quantitative analysis of mixtures of volatile compounds. Gas chromatography is the ideal separator, whereas mass spectrometry is excellent for identification. The GC-MS with computerized library search facility can be regarded as the best single tool for plant volatile chemical analysis.

Head space volatiles: Headspace refers to the gas phase above a solid or liquid sample. Headspace analysis is a simple, non-destructive and solvent-free technique used to analyse the volatile compounds from both liquid and solid samples. The technique has been widely used in food, cosmetic, flavour, perfume and forensic sectors. Headspace is an equilibrium state, depending on the partition coefficient of the analytes, and not all of the volatile analytes of the solid or liquid sample will evolve into the headspace gas volume.

The sample is placed into a sealed headspace vial of 10 to 20 ml capacity, and generally the sample is heated to a predetermined temperature for an incubation time. During this time, volatile compounds travel between the matrix and the headspace, and reach an equilibrium where the rate of migration from the matrix into the headspace equals the rate of migration from the headspace back to the matrix. The equilibrium condition does not mean that the concentration of the analyte is equal between the headspace and the matrix. The concentration is determined by partition coefficient (K) of the analyte.

Two types of sampling, static and dynamic, are widely used for headspace volatile investigation. Static headspace encloses the sample for a set period of time, and volatiles collected at the end of the period are analysed. Generally, in static HS analysis, the volatile analytes present above the solid or liquid sample are evolved into the headspace by heating the sample at a fixed temperature and for a fixed length of time in a vial of known volume. The volatiles are then adsorbed on solid-phase microextraction (SPME) unit. Dynamic headspace essentially moves the volatile chemicals continuously from the sample directly to the collecting matrix. In dynamic HS, the sample is heated and agitated in a sealed vial and the headspace above the sample is purged onto a solid sorbent tube. This is also known as purge and trap technique, where volatile organic compounds are purged out of the sample matrix by an inert gas and carried onto a sorbent trap, where they are concentrated and later introduced into an analytical instrument such as GC-MS.

The head space volatiles of *Cyperus rotundus* have seldom been analysed. α-Copaene, cyperene, valerenal, caryophyllene oxide, trans-pinocarveol and valencene have been identified as the head space volatiles through SPME-headspace analysis by Jirovetz *et al.* (2004), while Ilham *et al*. (2018) reported cyperene, α-copaene and α-ylangene as the major volatiles through headspace SPME analysis of *Cyperus rotundus* rhizomes. The study has reported variation in percentage composition of the identified components depending on the temperature of SPME exposure.

Essential oil, generally isolated through steam/hydro distillation process, represent a comprehensive volatile chemical profile. However, during the distillation process of essential oil isolation, the exact nature of the oil may be destructed as the compounds may change by oxidation, hydrolysis, decompose by heat, may polymerize or resinify or some of the delicate constituents may escape the process. While head space profile depicts the fine aroma of the plant material without any extraction process. However, HS has only the most volatile chemicals, while EO has a wide representation of the whole volatile chemicals, both low volatile and high volatile compounds.

The hyphenated analytical technique GC-MS is perhaps the most widely used analytical technique for *Cyperus rotundus* phytochemical evaluation, especially the volatile chemical profile. However, as several complex sesquiterpenoid structures such as endoperoxides, norsequiterpenoids, secosesquiterpenoids, hydroazulene, eudesmane, elemene, aristolane, eremophilane and aromadendrene are present in *Cyperus rotundus*, it is difficult to identify the constituents by GC-MS alone. Researchers have used both electron impact (EI) and chemical ionization (CI) detection modes on nonpolar and polar stationary phases in GC-MS. In addition to essential oils, volatile compounds were isolated from different solvent extracts, including supercritical fluid extracts (Wang *et al.,* 2012). Several investigators have tried separation of the volatile components through ordinary column chromatography (CC), CC at low temperature, CC over silver nitrate precoated silica, and preparative TLC and other techniques such as high-speed counter-current chromatography. Further characterization of the isolated compounds was done by various spectroscopic techniques such as high-resolution electrospray ionization mass spectrometry, and 1D and 2D nuclear magnetic resonance spectroscopy to establish the structures of the compounds from *C. rotundus* essential oils (Thebtaranonth *et al.,* 1995; Ohira *et al.,* 1998; Sonwa and Konig, 2001; Shi *et al.,* 2009; Tsoyi *et al.,* 2011; Zhou and Yin, 2012; Sultana *et al.,* 2019; Wang *et al.,* 2021; Xu *et al.,* 2009).

Cyperus rotundus contains essential oil in its roots, rhizomes, tubers and leaves, of which the rhizomes are rich in essential oils and the oil content varied in rhizomes from 0.5 to 1.0 % v/w, depending on the geographical origin (Ohira *et al.,* 1998). Among the various components present in the essential oil of *Cyperus rotundus* rhizomes, sesquiterpenoids, especially oxygenated sesquiterpenoids, are the most important category. Wang *et al*. (2022) has reviewed around 100 volatile chemicals from *Cyperus rotundus*, and α-cyperone and cyperenonewere the main components of volatile oil of *Cyperus rotundus.*

Table 2 enlists a total of 390 volatile chemicals reported from *Cyperus rotundus* essential oils, head space as well as in solvent extracts, belonging to sesquiterpene- oxygenated (208 numbers), sesquiterpene- hydrocarbons (107 numbers), monoterpene- oxygenated (46 numbers), monoterpene- hydrocarbons (14 numbers), aliphatic hydrocarbons (9 numbers) and phenolic derivatives (6 numbers).

Table 2: Volatile phytochemicals reported from *Cyperus rotundus* tubers

Sl. No.	Class of compounds	Phytochemicals	Reference
1.	Monoterpene hydrocarbons	1. Camphene 2. Limonene 3. Myrcene 4. o-Cymene 5. p-Cymene 6. Sabinene 7. Terpinolene 8. Verbenene 9. α-Pinene 10. β-Phellandrene 11. β-Pinene 12. β-Thujene 13. γ-Terpinene 14. α-Phellandrene	Bisht *et al.*,2011 Chang *et al.*, 2012 El-Gohary, 2004 Essaidi *et al.*, 2014 Ghannadi *et al.*, 2012 He *et al.*, 2018 Hu *et al.*, 2017 İlham *et al.*, 2018 Janaki *et al.*, 2018 Jin *et al.*, 2011 Kapadia *et al.*, 1967 Richa and Suneet, 2014 Xu *et al*. 2010 Yagi *et al.*, 2016 Zoghbi *et al.*, 2008 Fenanir *et al.*, 2021 Samra *et al.*, 2020
2.	Monoterpene oxygenated	1. (-)-Dihydrocarveol 2. (+)-Dihydrocarvone 3. 1,8-Cineole 4. 6-Camphenol 5. Borneol 6. Bornyl acetate 7. Camphene hydrate 8. Camphor 9. Carvacrol 10. Carvenone 11. Carvone 12. cis-Carveol 13. cis-Dihydrocarvone 14. cis-Verbenol 15. Citronellal 16. Cuminaldehyde 17. Dihydrocarvone 18. Dihydro carvylacetate 19. Geraniol 20. iso-Pinocamphone 21. Linalool	Bisht *et al.*,2011 El-Gohary, 2004 İlham *et al.*, 2018 Jin *et al.*, 2011 Chang *et al.*, 2012 Yagi *et al.*, 2016 Janaki *et al.*, 2018 Al-Snafi 2016 Liu *et al.*, 2016 Hu *et al.*, 2017 Fenanir *et al.*, 2021 Eltayeib and Ismaeel, 2014 Ghannadi *et al.*, 2012 Eröz Poyraz *et al.*, 2018 Yagi *et al.*, 2016 Hu *et al.*, 2017 Janaki *et al.*, 2018

		22. Myrtenal 23. Myrtenol 24. Myrtenyl acetate 25. Nerol 26. Nopinone 27. p-Cymen-8-ol 28. p-Cymol 29. Perilla alcohol 30. Pinocamphone 31. Pinocarvone 32. Piperitone 33. p-Menth-2-en-1-ol 34. p-Mentha-1,5-diene-8-ol 35. Terpinen-4-ol 36. Thymol 37. trans-Carveol 38. trans-Pinocarveol 39. trans-Verbenol 40. Verbenone 41. α-Campholenal 42. α-Fenchol 43. α-Ionone 44. α-Terpineol 45. α-Terpineol 46. β-Citronellol	He *et al*., 2018 Zhang *et al*., 2017 Kilani *et al*., 2008 Lawal and Oyedeji 2009 Ohira *et al*., 1998 Fang *et al*., 2004 Chang *et al*., 2012 Zoghbi *et al*., 2008 Richa and Suneet 2014
3.	Sesquiterpene hydrocarbons	1. (-) Cypera-2,4-diene 2. (-)-Cypera-2,4(15)-diene 3. (-)-Eudesma-2,4(15),11-triene 4. (-)-iso-Rotundene 5. (-)-iso-Sativene 6. (-)-Norrotundene 7. (+)-Calarene 8. (+)-Ylanga-2,4(15)-diene 9. δ-Cadinene 10. (E,E)-α-Farnesene 11. 1-Isopropyl 2,7 dimethyl naphthalene 12. 1-Isopropyl-2,7 dimethylnaphthalene 13. 4,5-Secoeudesmane 14. 5,10-Cycloaromadendrane 15. 8,8-Dimethyl-9-methylene-1,5-cycloundecadiene 16. 8,9-Dehydrocycloisolongifolene 17. 8,9-Dehydro neoisolongifolene 18. 9,10-Dehydro isolongifolene	Bisht *et al*., 2011 Chen *et al*., 2011 El-Gohary, 2004; He *et al*., 2018 Jirovetz *et al*., 2004 Eröz Poyraz *et al*., 2018 Essaidi *et al*., 2014 Fenanir *et al*., 2021 Ghannadi *et al*., 2012 Ilham *et al*., 2018 Janaki *et al*., 2018 Qu *et al*., 2021; Fenanir *et al*., 2021 Jirovetz *et al*., 2004

		19. allo-Aromadendrene 20. Aromadendrene 21. Cadalene 22. Cadina-1,4-diene 23. Calarene 24. cis-Calamenene 25. cis-α-Bisabolene 26. cis-γ-Bisabolene 27. Copadiene 28. Cyclosativene 29. Cypera-2,4-diene 30. Cyperene 31. Cyprotene 32. Dehydrocostuslactone 33. Dihydro aromadendrene 34. E-Caryophyllene 35. epi-α-Selinene 36. Eudesma-1,4(15),11-triene 37. Eudesma-2,4(15)-11-triene 38. Eudesma-2,4,11-triene 39. Germacrene B 40. Germacrene D 41. Gurjunene 42. iso-Aromadendrene 43. iso-Germacrene D 44. iso-Ledene 45. iso-Longifolene 46. iso-Patchoula-3,5-diene 47. iso-Rotundene 48. Longifolene 49. Longipinene 50. Nootkatene 51. Norrotundene 52. Patchoula-2-4-diene 53. Rotundene 54. Selina-4,11-diene 55. Selinatriene 56. trans-Calamenene 57. trans-β-Bergamotene 58. trans-γ-Bisabolene 59. Valencene 60. Ylanga-2,4-diene 61. α-Amorphene 62. α-Aromadendrene 63. α-Bergamotene 64. α-Bulnesene	Kandikattu *et al.*, 2015 Kilani *et al.*, 2008 Lawal and Oyedeji 2009 Li, 2013 Liu *et al.*, 2016 Narasimhan and Senich, 1956; Trivedi *et al.*, 1964 Ohira *et al.*, 1998; Lu *et al.*, 2022 Ohira *et al.*, 1998 Richa and Suneet, 2014 Sonwa and König, 2001 Wang *et al.*, 2021 Xu *et al.*, 2015 Yagi *et al.*, 2016; Hu *et al.*, 2017 Yang and Shi, 2012 Zhou and Yin, 2012 Zoghbi *et al.*, 2008

		65. α-Cadinene 66. α-Calacorene 67. α-Caryophyllene 68. α-Copaene 69. α-Cubebene 70. α-Elemene 71. α-Farnesene 72. α-Guaiene 73. α-Gurjunene 74. α-Himachalene 75. α-Humulene 76. α-Longipinane 77. α-Maaliene 78. α-Muurolene 79. α-Selinene 80. α-Ylangene 81. β-Acoradiene 82. β-Bourbonene 83. β-Calacorene 84. β-Caryophyllene 85. β-Cedrene 86. β-Copaene 87. β-Cubebene 88. β-Elemene 89. β-Farnesene 90. β-Guaiene 91. β-Gurjunene 92. β-Humulene 93. β-Selinene 94. β-Vatirenene 95. γ-Cadinene 96. γ-Calacorene 97. γ-Elemene 98. γ-Gurjunene 99. γ-Muurolene 100. γ-Selinene 101. γ-Vetivenene 102. δ-Cadinene 103. β-Calacorene 104. β-Elemene 105. β-Gurjunene 106. δ-Copadiene	
4.	Sesquiterpene oxygenated	1. (-)-10-epi-α-Cyperone 2. (-)-Clovane-2,9-diol 3. (-)-Eudesma-3,11-diene-5-ol 4. (-)-Isobicyclogermacrenal	Ahmed *et al.*, 1998 Ahn *et al.*, 2015 Al-Massarani *et*

		5. (+) Oxo-α-ylangene 6. (+) α-Cyperone 7. (+)-Alismoxide 8. (+)-Cyperadione 9. (4S, 5E, 10R)-7-Oxo-trinoreudesm-5-en-4β-ol 10. (4αS, 7S), -7-Hydroxy-1,4a-dimethyl-7-(prop-1-en-2-yl)-4,4α,5,6,7,8-hexahydronaphthalen-2 (3H)-one 11. (4αS,7S, 8R)-8-Hydroxy-1,4a-dimethyl-7-(prop-1-en-2-yl)-4,4α,5,6,7,8-hexahydronaphthalen-2(3H),-one 12. (6S)-Patchoulan-4-ene-6-ol 13. (E, E)-Farnesol 14. 1,4-Epoxy-4-hydroxy-4,5-seco-guain-11-en-5-one 15. 10,12-Peroxycalamenene 16. 10-Epieudesm-11-ene-3β, 5α-diol 17. 10-epi-α-Cyperone 18. 10-Hydroxy amorph-4-en-3-one 19. 11(13)-Eudesmene-3,4,12-triol 20. 11,12 Dihydroxy eudesm-4en,3-one 21. 12-Hydroxy nootkatone 22. 12-Methyl cyprot-3-en-2-one-13-oic acid 23. 14-Acetoxy cyperotundone 24. 14-Hydroxy cyperotundone 25. 14-Hydroxy-α-cyperone 26. 1β,4α-Dihydroxyeudesm -11-ene 27. 1β-Hydroxy-α-cyperone 28. 2-(4α,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol 29. 2-Hydroxy-14-calamenenone 30. 2-Methyl cyprot-3-en-2-one-13-oic acid 31. 2-Oxo-α-cyperone 32. 2α-(5-Oxypentyl)-2β-methyl-5β-isopropenyl cyclohexanone 33. 2β-Hydroxy-α-cyperone 34. 3,5,6,7,8,8α-Hexahydro-4,8α-dimethyl-6-(1-methyl	*al.*, 2016 Bisht *et al.*, 2011 Carvalho *et al.*, 2003 Chang and Lee, 2016 Chiu *et al.*, 2001 Dhillon *et al.*, 1993 El-Gohary, 2004; Fang *et al.*, 2004 El-Gohary, 2004 Eltayeib and Ismaeel, 2014 Eneh *et al.*, 2016 Fenanir *et al.*, 2021 Fraga *et al.*, 1995 Fu *et al.*, 2010 Ghannadi *et al.*, 2012 Gliszczyńska *et al.*, 2011 He *et al.*, 2018 Hikino *et al.*, 1971 Hikino *et al.*, 1975 Hu *et al.*, 2017 Huffman *et al.*, 1980 Ibrahim *et al.*, 2007 İlham *et al.*, 2018 Jiang *et al.*, 2011 Jirovetz*et al.*, 2004 Kandikattu *et al.*, 2015 Kapadia *et al.*, 1967 Khan *et al.*, 2011 Tsoyi *et al.*, 2011 Priya Rani and Padmakumari 2012 Kim *et al.*, 2013 Lawal and

		ethenyl)2(1H) naphthalenone 35. 3β, 4α-Dihydroxy-7-epieudesm-11 (13)-ene 36. 3β, 4α-Dihydroxy-7-epi-eudesm-11(13)-ene 37. 3β-Hydroxy cyperenoic acid 38. 3β-Hydroxy ilicic alcohol [11(13)-eudesmene-3,4,12-triol] 39. 4,5-Secoeudesmane epimer/2β-(5-oxopentyl)-2β-methyl- 5β-isopropenylcyclohexanone 40. 4,5-Secoeudesmane/2α-(5-oxopentyl)-2β-methyl-5β-isopropenylcyclohexanone 41. 4,5-seco-Eudesmanolide 42. 4,5-seco-Guaia-1(10) ,11- diene-4,5-dioxo 43. 4-Oxo-α-ylangene 44. 4α,5α-Oxido eudesm-11-en-3-one 45. 4α,5α-Oxido eudesm-11-en-3α-ol 46. 5-Hydroxy lucinone 47. 6,9-Diacetoxy cyperene 48. 6-Acetoxy cyperene 49. 6-Acetoxypatchoul-4-en-3-one 50. 6-Acetyl sugebiol 51. 7-epi-Teucrenone 52. 7α (H), 10β-Eudesm-4-en-3-one-11,12-diol 53. 8-Oxo-9H-cycloisolongifolene 54. 9-Methoxycalamenene 55. Agarospirol 56. Alismoxide 57. Argutosine D 58. Aristol-9-en-3-one 59. Aristolene epoxide 60. Aristolone 61. Aromadendrene epoxide 62. Britanlin E 63. Carophylla-6-one 64. Carotol 65. Caryophylla-2(12), 6(13) dien-5-one 66. Caryophylladienol 67. Caryophyllane-2-6-β-oxide	Oyedeji, 2009 Luo *et al.*, 2014 Morikawa *et al.*, 2002 Morimoto *et al.*, 2009 Nyasse *et al.*, 1988 Ohira *et al.*, 1998 Qin *et al.*, 2006 Rani *et al.*, 2012 Rukachaisirikul *et al.*, 2005 Ryu *et al.*, 2015 Wang *et al.*, 2021b Sabrin *et al.*, 2018 Sanz and Marco, 1990 Sun *et al.*, 2000 Wang *et al.*, 2021 Wu *et al.*, 2007 Xu *et al.*, 2004 Xu *et al.*, 2008 Xu *et al.*, 2009 Xu *et al.*, 2013 Xu *et al.*, 2015 Xu *et al.*, 2016 Yagi *et al*. 2016 Yang and Shi, 2012 Yang, 2012 Zhang *et al.*, 2007 Zhou and Yin, 2012 Zoghbi *et al.*, 2008

		68. Caryophyllene acetate 69. Caryophyllene alcohol 70. Caryophyllene ketone 71. Caryophyllene oxide 72. Caryophyllenol-I 73. Caryophyllenol-II 74. Cedrol 75. cis-12-Caryophyll-5-en-2-one 76. cis-Nerolidol 77. cis-Valerenol 78. cis-Valerenyl acetate 79. cis-α-Bisabolene epoxide 80. Clovane-2,9-diol 81. Cubebol 82. Cyclic acetal 83. Cyper-11-ene-3,4-dione 84. Cyperadione 85. Cyperenal 86. Cyperene epoxide 87. Cyperene-3,6-diol 6-acetate 88. Cyperene-3,8-dione 89. Cyperene-3,8-dione, 14-hydroxy 90. Cyperenoic acid 91. Cyperenol 92. Cyperenone 93. Cyperensol A 94. Cyperol 95. Cyperolone 96. Cyperotundic acid 97. Cyperotundol 98. Cyperotundone 99. Cyperusol 100. Cyperusol A1 101. Cyperusol A2 102. Cyperusol A3 103. Cyperusol C 104. Cyperusol D 105. Cyprotene 106. Dehydrocostuslactone 107. diepi-α-Cedrenepoxide 108. Elema-1,3,11 (13)-trien-12-ol 109. Elemol 110. epi-Cubebol 111. epi-Cubenol 112. epi-Guaidiol A 113. epi-α-Cadinol	

		114. epi-α-Muurolol 115. Epoxy caryophyllane-5α,15-diol 116. Epoxyguaiene 117. Eudesm-7(11)-en-4-ol 118. Eudesma-4(14),11(13)-diene-7α,8α,12-triol 119. Eudesma-4(14),11-dien-3β-ol 120. Eudesmene-3, 4, 12-triol 121. Globulol 122. Guaidiol 123. Guaidiol A 124. Guaiol 125. Humulene epoxide II 126. Humulene oxide 127. iso-Aromadendrene epoxide 128. iso-Corymbolone 129. iso-Curcumenol 130. iso-Cyperol 131. iso-Cyperotundone 132. Isokobusone 133. iso-Longifolen-5-one 134. iso-Longifolenone 135. iso-Mustakone 136. iso-Patchoul-4-en-3-on-8α-ol 137. iso-Patchoulenone 138. iso-Rotundenol 139. iso-Spathulenol 140. Khusinol 141. Kobusone 142. Ledene alcohol 143. Ledene oxide 144. Ledol 145. Ligucyperonol 146. Longifolinaldehyde 147. Longipinocarvone 148. Longiverbenone 149. Mandassidione 150. Methoxy cyperotundol 151. Mustakone 152. Nardol 153. Nootkatone 154. Norcyperone 155. Oplopanone 156. Oxyphyllenone C 157. Oxyphyllenones B 158. Oxyphyllol C	

		159. Palustrol 160. Patchoulenone 161. Patchoulenyl acetate 162. Perilla alcohol 163. Rhombitriol 164. Rotundene 165. Rotundenol 166. Rotundone 167. Rotundusolide A 168. Rotundusolide B 169. Santalol 170. Scariodione 171. Solavetivone 172. Spathulenol 173. Sugebiol 174. Sugebiol 6-acetate 175. Sugeonol 176. Sugeonyl acetate 177. Sugetriol 178. Sugetriol triacetate 179. Sugetriol-3,9-diacetate 180. Sugetriol-6,9-diacetate 181. Torreyol 182. Valeranone 183. Valerenal 184. Valerianol 185. Vellerdiol 186. Viridiflorol 187. Vulgarol A 188. Vulgarol B 189. Widdrol 190. Zerumbone 191. Zierone 192. α-Cadinol 193. α-Calacorene 194. α-Cedrene epoxide 195. α-Corymbolol 196. α-Cyperol 197. α-Cyperolone 198. α-Cyperone 199. α-Muurolol 200. α-Rotunol 201. β-Bisabolol 202. β-Cyperone 203. β-Eudesmol 204. β-Hydroxycyperone	

		205.β-Rotunol 206.γ-Eudesmol 207.γ-Gurjunene epoxide 208.δ-Epoxyguaiene	
5.	Phenolic derivatives	1. Carvacrol 2. Cinnamaldehyde 3. Cuminaldehyde 4. Eugenol 5. Thymol 6. trans-Anethole	Chang *et al.*, 2012 Eltayeib and Ismaeel, 2014 Ghannadi *et al.*, 2012 Hu *et al.*, 2017 Janaki *et al.*, 2018 Yagi *et al.*, 2016 Zhang *et al.*, 2017
6.	Aliphatic hydrocarbons	1. 3-Methyl heneicosane 2. Eicosane 3. Hentriacontane 4. Hexadecane 5. Nonacosane 6. Octacosane 7. Octadecane 8. Pentatriacontane 9. Triacontane	Nidugala *et al.*, 2015

Sesquiterpenoids reported from *Cyperus rotundus*

Terpenoids are the major class of compounds reported from *Cyperus rotundus* essential oils (**Table 2**). A characteristic of naturally occurring terpenes is the bewildering array of structures, varying from acyclic chains to cyclic ring systems with ring sizes ranging from three to eleven carbons, with an obvious prevalence for five and six membered rings. The sesquiterpenoids form a major group of natural products with extraordinary structural variety, and different polycyclic carbon skeletons were elaborated for sesquiterpenoids that are derived biosynthetically from farnesyl pyrophosphate.

Sesquiterpenoids are the major subclass of natural products reported from *Cyperus rotundus,* and sesquiterpenoids possessing diverse skeletons such as eudesmane, patchoulane, cadinane, rotundane, guaiane, caryophyllane, clovane, copaene anderemophilanehave been reported from *Cyperus rotundus* (Yang and Shi, 2012).In addition, sesquiterpene endoperoxides, norsequiterpenoids and secosesquiterpenoids are also reported from *Cyperus rotundus*.It is interesting to note that several sesquiterpenoids were isolated for the first time from *Cyperus* species, and the compounds names are thus associated with *Cyperus* (Nigam, 1965; Hikino *et al.*, 1967; Hikino *et al.*, 1968; Neville *et al.*, 1968; Hikino *et al.*, 1971). Even recently, new sesquiterpenoids were reported from *C. rotundus* (Wang *et al.*, 2021). **Table 2** enlists 315 sesquiterpenoids reported from *C. rotundus,* of which 107 are sesquiterpene hydrocarbons, while 208 are oxygenated sesquiterpenoids.

Guaiane and patchoulane type sesquiterpenes

Among the various sesquiterpenoids reported from *Cyperus rotundus,* guaiane and patchoulane sesquiterpenes, with a characteristic fused structure of five and seven membered rings, are peculiar to the plant group (Sonwa and Konig, 2001; Kim *et al.,* 2012; Yang and Shi, 2012). Rotundone, (6S)-6-acetoxy cyperene, (6S)-cyperene-6-ol, sugetriol triacetate, (6S,9S)-6,9-diacetoxy cyperene, 14-acetoxy cyperotundone, 14-hydroxy cyperotundone, 3β-hydroxy cyperenoic acid, 6,12 cyperotunone, cyperene-3,8-dione, cyperenoic acid and sugetriol-3, 9-diacetate are few examples of guaiane/ patchoulane type sesquiterpenoids reported from *Cyperus rotundus* (Xu *et al.*, 2015). Guaiane/patchoulanetype sesquiterpenoids were found to act as allelochemicals and growth inhibitors on the surrounding plants (Morimoto and Komai, 2005; Yang and Shi, 2012).

Sugetriol triacetate

The patchoulane type sesquiterpenoid sugetriol triacetate and analogues sugetriol-3,9-diacetate and sugetriol-6,9-diacetate areimportant secondary metabolites reported from *Cyperus rotundus* (**Figure 5**) (Kim *et al*., 2012; Kim *et al*., 2013; Wang *et al*., 2021). Sugetriol triacetate has been attributed with tumour necrosis factor-alpha (TNF-α)-induced NF-κB activation inhibition with IC_{50} value of 72.6 ± 3.0 μM (Wang *et al*., 2021).Sugetriol-3,9-diacetate exhibited remarkable binding affinity to PL^{pro} of SARS CoV-2(Wu *et al*., 2020; Birendra Kumar *et al*., 2021).

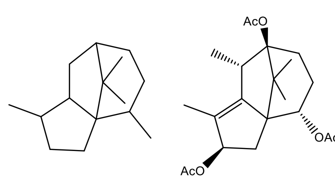

Figure 5. Patchoulane skeleton and sugetriol triacetate

Rotundone: The guaiane sesquiterpene rotundone is so called because it was originally isolated and characterised from the rhizomes of *Cyperus rotundus* (**Figure 6**) (Kapadia *et al.,* 1967). Rotundone is also present in several aromatic plants, including black pepper, marjoram, oregano, rosemary, basil, thyme, boswelia and geranium (Johannes *et al.*, 2016). However, till the discovery of the odour impact of the compound by Siebert *et al.*(2008), the compound was relatively unknown. Rotundone is an important component of agarwood scent and patchouli scent, and recently cypriol oil (*Cyperus scariosus*) with rotundol is emerging as an alternative to the costly agarwood oil.

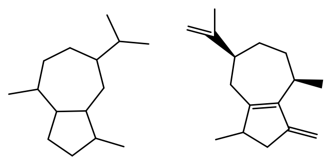

Figure 6. Guaiane skeleton and rotundone

Rotundone, the stealthy structure behind the peppery note for Australian Shiraz wines: The popular Australian Shiraz wines have the characteristic spicy black pepper aroma, and rotundone was identified as the major contributor to peppery characters in Shiraz grapes and wine, with an odor threshold of 8 ng/L in water and 16 ng/L in red wine (**Figure 7)** (Siebert *et al.*, 2008; Wood *et al.*, 2008). In addition to the peppery note, rotundone is responsible for spicy and woody odors of several aromatic plants. Rotundone was found to have the highest odor activity value among the measured compounds, and together with the other ketones, contributes to the woody amber character of cypriol oil (Clery *et al.*, 2016). An investigation of the aromas of grapefruit, orange, apple and mango revealed the presence of rotundone as the odor-active compound that gave off a strong woody odor (Akira *et al.*, 2017).

Figure 7. Rotundone, the peppery odour compound in Australian Shiraz wines

Cyperenone (Cyperotundone, isopatchoulenone): The sesquiterpene was isolated for the first time from the essential oil of *Cyperus scariosus* by Nigam (1965), and the structure was characterised by hydrogenation and deoxidation **(Figure 8)**. The compound was synthesized from cyperene by chromic acid oxidation, and named cyperenone in view of its relationship to cyperene.

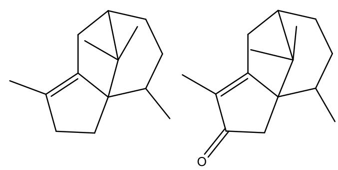

Figure 8. Cyperene and cyperenone

Mustakone: The tricyliccadinane sesquiterpenoid mustakone has its name derived from the common name in *Sanskrit* for *Cyperus rotundus* 'mustuka'. The compound was first isolated from *Cyperus rotundus* by Kapadia *et al.* (1963) (**Figure 9**). Mustakone is an important odour component of *Cyperus rotundus.*

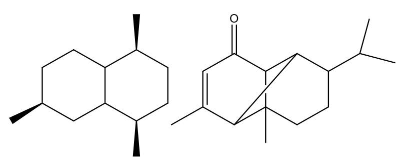

Figure 9. Cadinane skeleton and the structure of mustakone

Eudesmane type sesquiterpenoids

Eudesmane type sesquiterpenes are another important class of volatile compounds reported from *C. rotundus* essential oil. α-Cyperone, isocyperol, cyperol, 1β-hydroxy-α-cyperone, 10-epieudesm-11-ene-3β, 5α-diol, 3β-hydroxyilicic alcohol, eudesmene-3, 4, 12-triol, cyperusol C, α-corymbolol, 3β, 4α-dihydroxy-7-epieudesm-11 (13)-ene, 7α (H), 10β-eudesm-4-en-3-one-11,12-diol and rhombitriolare few examples of eudesmane type sesquiterpenoids reported from *C. rotundus* (Kim *et al.*, 2012).

α-Cyperone: The sesquiterpene ketone α-cyperone is the most important eudesmane sesquiterpenoid reported from *Cyperus rotundus* **(Figure 10)**. The compound exhibits insecticidal properties against diamondback moth larvae (Dadang *et al.*, 1996).

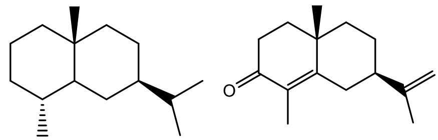

Figure 10. Eudesmane skeleton and the structure of α-cyperone

Rotundusolide A is a rare, rearranged secoeudesmane sesquiterpenoid skeleton reported from *Cyperus rotundus* (**Figure 11)** (Yang and Shi, 2012). Eudesmane type sesquiterpenes and their glycosides show broad bioactivities, including anti-inflammatory, anticancer, anti-angiogenic, antifungal, and anti-hepatitis B virus activities (Yang *et al.*, 2018).

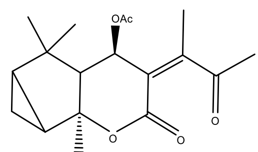

Figure 11. Rotundusolide A

Norsesquiterpenes

Norsesquiterpenes are compounds formally derived from a sesquiterpene by the removal of a methylene or methyl group. Several norsesquiterpenes have been reported from *Cyperus* species, and it seems to be a peculiarity of *Cyperus* species to produce norsesquiterpenes (Sonwa and Konig, 2001). A novel norsesquiterpene, named norcyperone was reported from *Cyperus rotundus* by Xu *et al.* in 2008 (**Figure 12)**.

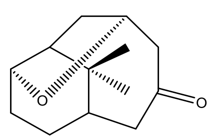

Figure 12. Structure of norcyperone

Volatile aroma profiles of *Cyperus rotundus* tubers and leaves collected from Kerala, south India

The volatile chemicals of *Cyperus rotundus* tubers and leaves collected from Kerala, south India were analysed through essential oil and head space GC-MS.

Headspace volatile chemical analysis of *Cyperus rotundus*

The head space volatile chemical profiles of *Cyperus rotundus* rhizomes and leaves, collected from south India were analysed through Shimadzu Gas Chromatograph- Mass

Spectrometer, model QP2020C NX attached to Shimadzu Head Space Sampler (HS 20), by static method.

Plant sample preparation: The plant samples, 1.0 g of the fresh leaves and rhizomes, cut into fine pieces of 2 x 2 mm, were taken in 20 ml headspace vials and introduced into the Shimadzu Head Space Auto-Sampler (HS 20 with 90 vials capacity).

Head space volatile extraction and transfer to GC: The sealed vial containing the plant sample were kept at 60° C for 10 minutes equilibrating time at shaking level 3 and after equilibration, a sampling needle is inserted into the vial through the vial septum to pressurize the sample vapor using He gas.The head space volatiles were introduced to GC inlet through valve loop injection, where the pressurized vapor is allowed to escape to a sampling loop of 1mL capacity and held at sample line temperature of 150℃, instead of being directly diverted into the GC column. From the sampling loop, the sample vapor is mixed with carrier gas and moved to a transfer line connected to the GC, at transfer line temperature of 150℃.

GC analysis: The column used was SH-Rxi-5ms capillary column (30m, 0.25mm ID, 0.25um). GC oven temperature was from 50ºC (2 min. hold) to 200ºC (3 min. hold), at the rate of 10° C/ min., and the analysis time was 20 minutes. The carrier gas (Helium) flow rate was 1.5 mL/min, at split ratio 20.0.

MS analysis: The ionization mode was electron impact ionization (EI); 70 eV with source temperature 200°C and interface temperature 220 °C. The mass analysis was done with Shimadzu single quadrupole 8030 series mass selective detector. The MS was done at full scan mode, at the range 45.0 m/z to 300.0 m/z.

Data processing: The data was processed using the software GC-MS solution Ver. 4.

Compound identification: The constituents were identified by MS library search (NIIST 17, Wiley 275), comparison of the relative retention indices (RRI) calculated with respect to homologous of n-alkanes, mass fragmentation analysis, and compared with literature data and published Mass spectra (Adams, 2017). Relative retention indices (RRI) of essential oil constituents were calculated on the same column using C_8-C_{30} straight chain alkanes as standards (Aldrich Chemical Company, USA).

$$RRI=100[(RT-H_n)/(H_{n+1}-H_n)]+100n$$

Where RT is the retention time of the compound (oil constituents), H_n and H_{n+1} are retention times of reference hydrocarbons with n and n+1 carbon respectively.

Essential oil analysis of Cyperaceae members

The plant material (fresh leaves 300 g; fresh rhizomes 200 g) were hydrodistilled using a Clevenger type apparatus for 3 hr. The oil obtained was dried over anhydrous sodium sulphate and stored in 4° C prior to further analysis. The essential oils were analyzed by injecting 1 µL of the diluted essential oil in diethyl ether (1:10 dilution) to Shimadzu Gas Chromatograph Mass Spectrometer (QP2020C NX), fitted with a cross bond 1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane Rxi-5 Sil MS capillary column (30 m x 0.32 mm, film thickness 0.25 µm) coupled with Shimadzu single quadrupole 8030 series mass selective detector. The injector temperature was 240°C, and the oven temperature was 60-250°C at the rate 3°C/minute. The ionization mode was electron impact ionization (EI); 70 eV. The ion source temperature of the mass detector was 240°C, and the interphase temperature was 260°C.

The tuber essential oils of *Cyperus rotundus* were yellow in colour and the yield was 0.6% v/w. Patchoulane, copaene and eudesmane type skeletons were predominated in the essential oil samples of the tuber and leaf essential oils of *Cyperus rotundus* collected from Kerala, south India.

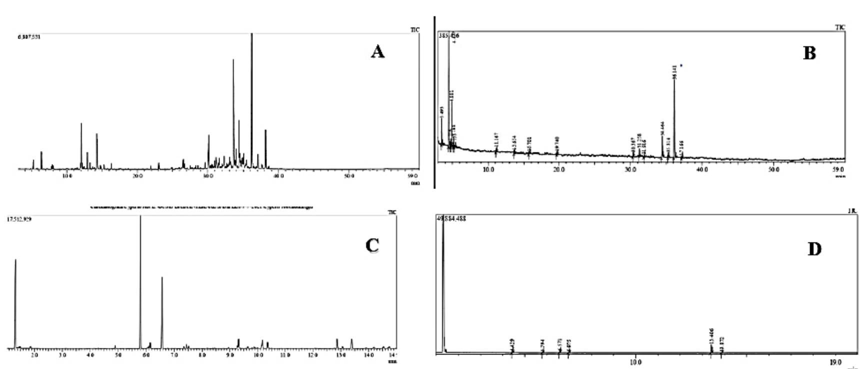

Figure 13. Essential oil and head space GC-MS TICs of *Cyperus rotundus.* **A.** Rhizome essential oil, **B.** Leaves essential oil, **C.** Rhizome head space and **D.** Leaves head space

The major compounds in rhizome essential oil were identified as α-cyperone (18.0%), mustakone (14.0%) and caryophyllene oxide (5.7%), while the leaf oil had γ-himachalene (13.1%), α-humulene (12.3%), cyperene (8.8%), β-caryophyllene (7.8%), δ-amorphene (7.6%), humulene epoxide II (6.6%) and longipinanol (6.2%) as the major compounds (**Table 3**, **Figure 14**).

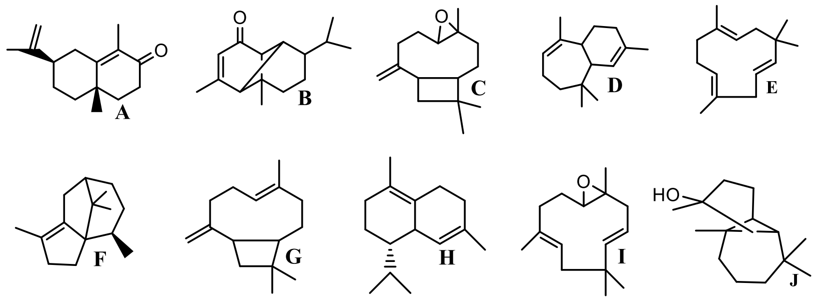

Figure 14. Major essential oil volatile chemicals of *Cyperus rotundus.*Rhizome, **A-** α-Cyperone, **B-** Mustakone, **C-** Caryophyllene oxide. Leaves, **D-** γ-Himachalene, **E-** α-Humulene, **F-** Cyperene, **G-** β-Caryophyllene, **H-** δ-Amorphene, **I-** Humulene epoxide II, **J-** Longipinanol

The major head space volatiles of the rhizome were α-pinene (43.1%) and β-pinene (25.7%) while the leaves HS showed β-caryophyllene (70.7%) and β-pinene (11.3%) as the major constituents (**Figure 15)**.

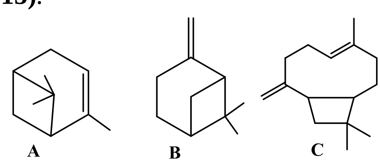

Figure 15. Major head space volatile chemicals of *Cyperus rotundus.*
A- α-Pinene, **B-** β-Pinene and **C-** β-Caryophyllene

Table 3. Essential oil and head space composition of *Cyperus rotundus*

Sl. No.	RRI $_{cal}$	RRI $_{lit}$	Compound	Essential oil Area %		Head space Area %	
				Rhizome	Leaf	Rhizome	Leaf
1	842	844	3-Hexenol	-	-	-	4.4
2	932	932	α-Pinene	1.2	-	43.1	3.3
3	950	946	Camphene	-	-	0.4	-
4	955	952	α-Fenchene	-	-	3.0	-
5	957	953	Thuja-2,4(10)-diene	-	-	1.2	-
6	974	977	β-Pinene	2.2	-	25.7	11.3
7	988	988	Myrcene	-	1.4	-	-

8	1021	1022	o-Cymene	-	-	0.9	-
9	1023	1026	1,8-Cineole	-	-	1.1	-
10	1024	1028	Limonene	0.6	4.1	2.0	-
11	1043	1032	β-Ocimene	-	0.6	-	-
12	1135	1139	trans-Pinocarveol	3.4	-	-	-
13	1143	1135	Nopinone	-	-	0.4	-
14	1160	1159	Pinocarvone	1.5	-	0.5	-
15	1193	1192	neo-Dihydro carveol	3.1	-	-	-
16	1194	1193	Myrtenol	1.8	-	-	-
17	1199	1195	Myrtenal	-	-	1.3	-
18	1204	1203	Verbenone	0.3	-	0.4	-
19	1215	1216	trans-Carveol	0.3	-	-	-
20	1239	1240	Carvone	0.4	-	-	-
21	1371	1369	Cyclosativene	-	1.3	0.2	-
22	1389	1389	β-Elemene	-	1.8	0.2	-
23	1398	1398	Cyperene	0.7	8.8	8.8	-
24	1414	1408	β-Caryophyllene	-	7.8	-	70.7
25	1443	1437	α-Guaiene	-	2.0	-	-
26	1454	1452	α-Humulene	-	12.3	-	3.7
27	1455	1457	Rotundene	-	1.3	-	-
28	1469	1458	Aromadendrene	-	2.0	-	-
29	1472	1480	Germacrene D	-	1.4	-	-
30	1475	1475	δ-Gurjunene	-	1.0	-	-
31	1478	1478	γ-Muurolene	-	9.6	-	-
32	1487	1481	γ-Himachalene	1.5	13.1	-	-
33	1489	1493	β-Selinene	0.9	-	-	-
34	1492	1471	4,5-di-epi-Aristolochene	-	-	1.3	-
35	1501	1496	Viridiflorene	-	3.0	-	-
36	1513	1511	δ-Amorphene	-	7.6	-	-
37	1517	1509	Nootkatene	0.3	-	-	-
38	1582	1575	Caryophyllene oxide	5.7	-	-	-
39	1590	1584	β-Copaen-4-α-ol	1.1	-	-	-
40	1574	1562	Longipinanol	-	6.2	-	-
41	1607	1597	β-Oplopenone	1.2	-	-	-
42	1608	1602	Humulene epoxide II	2.2	-	-	-
43	1609	1608	Humulene epoxide II	-	6.6	-	-
44	1616	1613	Patchoulenone	1.1	-	-	-
45	1655	1651	Pogostol	-	1.3	-	-

46	1676	1667	Mustakone	14.0	-	-	-
47	1695	1687	Cyperotundone	4.3	-	-	-
48	1770	1767	13-Hydroxyvalencene	-	3.0	-	-
49	1727	1735	α-Cyperone	18.0	2.0	-	-
50	1755	1759	Cyclocolorenone	4.2	-	-	-
51	1806	1792	Nootkatone	4.2	-	-	-

Notes: RRI[a]: Relative retention index from literature (Adams, 2017). RRI[b]: Relative retention index calculated on SH-Rxi-5Sil column with respect to homologous of n-alkanes (C7-C30, Aldrich Chem. Co. Inc.).

Isolation and characterisation of components from the essential oil of *C. rotundus*

The major compounds from the tuber essential oil were isolated by column chromatography, using hexane: ethyl acetate gradient elution and characterized by EI-MS analysis. The major compounds identified were; trans-pinocarveol (100% hexane), caryophyllene oxide (100% hexane), pathchoulenone (100% hexane), α-cyperone (1% ethyl acetate) and mustakone (1% ethyl acetate) (**Figure 16)**.

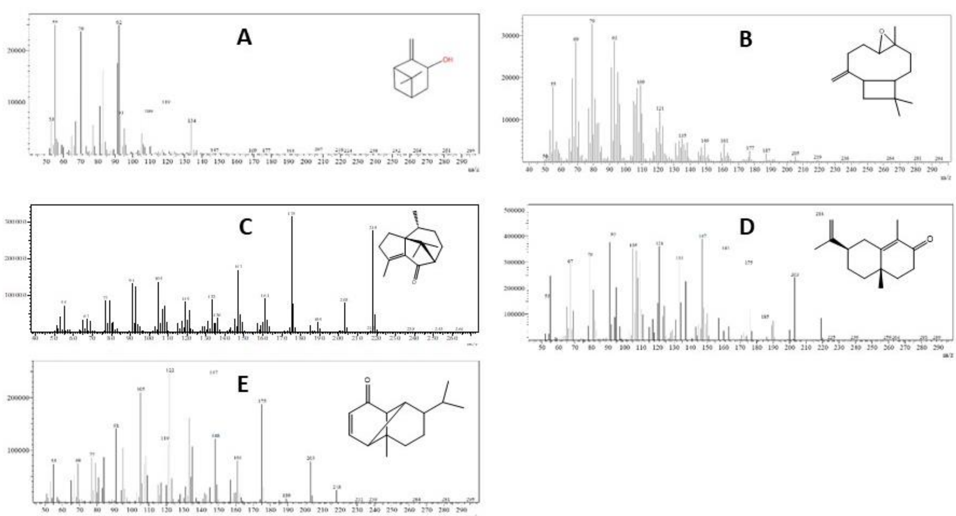

Figure 16. Electron Impact Mass Spectrum (EI-MS) and structure of major compounds isolated from *Cyperus rotundus* rhizome essential oil by column chromatography. **A-** trans-Pinocarveol, **B-** Caryophyllene oxide, **C-** Pathchoulenone, **D-** α-Cyperone and **E-** Mustakone

Chemical profiling of solvent extract of *Cyperus rotundus*

Solvent extraction: About 50g of dried rhizomes of *Cyperus rotundus* were extracted using hexane by Soxhlet apparatus. The hexane extract was submitted to column chromatography on silica gel (60-120 mesh size) by gradient elution of hexane: ethyl acetate. Fractions eluted at 5% ethyl acetate yielded the pure compounds. The compounds were characterized through ^{13}C NMR and GC-MS analysis as α-cyperone and cyperotundone. Quantification of the isolated pure compounds was done through HPTLC.

α-Cyperone: ^{13}C NMR showed that the compound has 15 carbon signals with chemical shifts at δ (ppm); 198.9, 162.0, 147.7, 130.3, 110.6, 43.5, 39.0, 37.4, 36, 33.9, 31.4, 26.6, 22.5, 21.5 and 10.3 (**Figure 17)**.

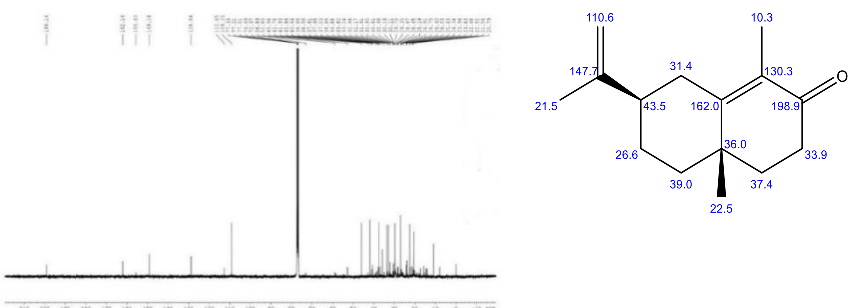

Figure 17. ^{13}CNMR of α-cyperone

Cyperotundone: ^{13}C NMR showed that the compound has 15 carbon signals with chemical shifts at δ (ppm); 207.6, 166.4, 137.9, 57.2, 49.0, 41.2, 40.5, 33.0, 37.8, 28.2, 28.0, 10.0, 20.5, 20.5 and 17.0 (**Figure 18)**.

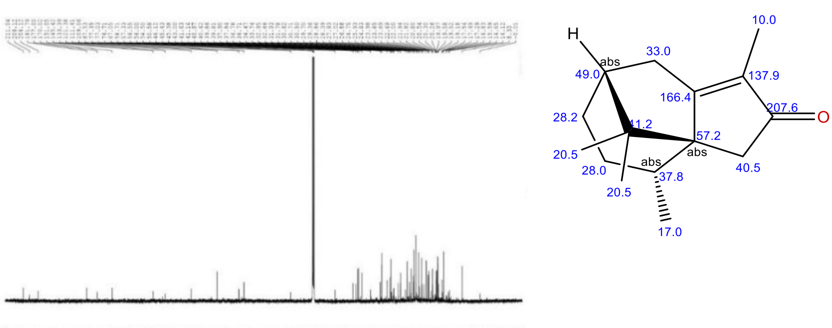

Figure 18. ^{13}CNMR of cyperotundone

HPTLC profiling

The isolated compounds α-cyperone and cyperotundone were profiled in the hexane extracts of different assessions of *Cyperus rotundus* rhizomes using HPTLC (**Figure 19)**. The HPTLC chromatogram gave a better resolution in the solvent system, chloroform: methanol (9.5:0.5 v/v) with R_f value 0.58 and 0.49 respectively, and was recorded at 366 nm. Linear regression analysis was performed with peak area and concentration to calculate the calibration equation and correlation coefficients. α-Cyperone in the range of 0.4 to 3 µg per band gave linear response with regression equation$y = 5825x + 0.694$. The correlation coefficient 0.995, indicated good linear relationship of peak area with concentration of the standard. Cyperotundonein the range of 0.6 to 5 µg per band gave linear response with regression equation$y = 5965x + 0.694$ with the correlation coefficient 0.984, indicating good linear relationship of peak area with concentration of the standard.

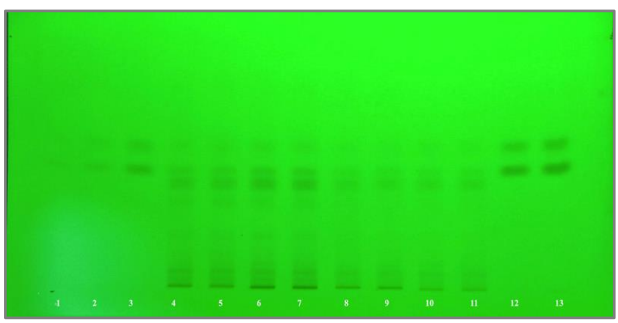

Figure 19. HPTLC profile (366 nm) of standard α-cyperone and cyperotundone (1,3 and 5 µg of std 1 and 2 in tracks 1-3), hexane extracts of different accessions of *Cyperus rotundus* rhizomes (15µg in track 4 and 5, 3µg in track 6 and 7, 8µg in track 8 and 9, 3µg in track 10 and 11 respectively of *Cyperus rotundus*Vaikom, Kannur, Thiruvananthapuram and Karunagappally accessions) 5,7µg of standard 1 and 2 in track 12-13.

Biogeographic variation and chemotaxonomy

The constitution of *Cyperus rotundus* essential oils from different parts of the world has been studied, and literature review revealed the report of essential oils from 24 different countries across the world (**Table 4, Figure 20**).

Several attempts have been made to utilize the diversity of volatile chemicals in *Cyperus rotundus* for chemosystematic purpose, and various chemotypes of *Cyperus rotundus* were expected, and variations were observed in the phytochemical composition. Based on the volatile chemicals, four chemotypes (H, K, M and O) were reported for *C. rotundus* from different parts of Asia (Komai and Tang, 1989; Komai *et al.*, 1994).

H-type (Japan): α-Cyperone, β-selinene, cyperol and caryophyllene.

M-type (China, Hong Kong, Japan, Taiwan and Vietnam): α-Cyperone, cyperotundone, β-selinene, cyperene and cyperol.

O-type (Japan, Taiwan, Thailand, Hawaii and the Philippines): Cyperene, cyperotundone and β-elemene.

K-type (Hawaii): Cyperene, cyperotundone, patchoulenyl acetate and sugeonyl acetate.

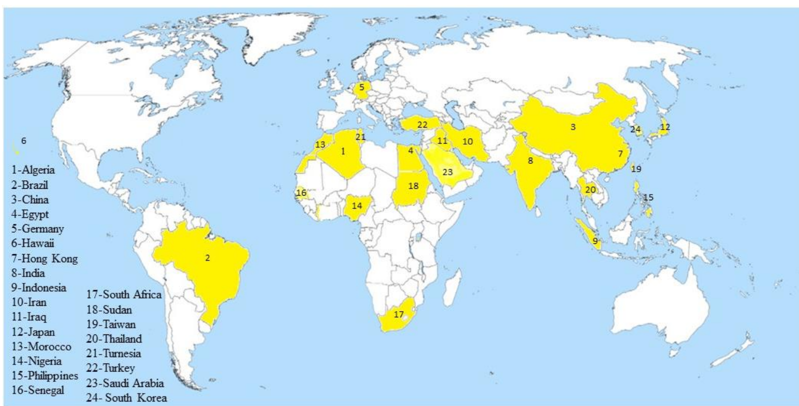

Figure 20. *Cyperus rotundus* essential oil composition reported from the world

Table 4. Country wise distribution of volatile phytochemicals in *Cyperus rotundus*

Sl. No.	*Cyperus rotundus* origin	Major compounds	References
1.	Algeria	Humulene oxide-II, caryophyllene oxide, khusinol, agarospirol, spathulinol, trans-pinocarveol	Fenanir *et al.*, 2022
2.	Brazil	α-Cyperone, cyperotundone	Zoghbi *et al.*, 2008
3.	China	α-Cyperone, cyperol, α-copaene, cyperene	Komai *et al.* 1994
		Cyperene, b-caryophyllene oxide, a-selinene, a-copaene,	Chen *et al.*, 2011
		α-Cyperone, cyperene, caryophyllene oxide, β-selinene	Xin *et al.*, 2016
		α-Cyperone, cyperene, α-selinene	Hu *et al.*, 2017

		α-Cyperone, cyperene, caryophyllene oxide, β-selinene, trans-pinocarveol, aristolone, α-copaene	Liu *et al.*, 2016
		Cyperone, cyperene, α-selinene	Zhang *et al.*, 2017
		Cyperenone, α-cyperone, cyperene	Qu *et al.*,2021
		α-cyperone, aristolone	Wu, 2007
		Cyperene, α-cyperone, β-selinene, aristolone	Feng *et al.*, 2006
		Caryophyllene oxide, cyperene, α-cyperone, β-selinene, aristolone, α-copaene, longiverbenone, isolongifolen-5-one	Li, 2013
		Cyperene, α-cyperone,	Jin *et al.*, 2006
		Cyperene, α-cyperone, β-selinene	Lin *et al.*, 2006
		α-Cyperone, aristolone	Xu *et al.*, 2006
		Isolongifolen -5-one	He *et al.*, 2015
4.	Egypt	(+) Oxo-α-ylangene, α-cyperone, trans-pinocarviol, cyperene	Gohary *et al*., 2004
		Humulene epoxide, caryophyllene oxide	Samra *et al.*, 2020
5.	Germany	Cyprotene, α-copaene, cyperene, α-selinene, rotundene	Sonwa and Konig, 2001
6.	Hawaii	Cyperotundone, cyperene	Komai *et al*., 1989
		Cyperene, α-cyperone	Komai *et al*., 1994
		Cyperone, patchoulenyl acetate, sugeonyl acetate, β-elemene	Komai *et al*., 1994
		Caryophyllene	Komai *et al*., 2005
7.	Hong Kong	Cyperotundone	Komai *et al*., 1994
8.	India	α-Copaene, cyperene, valerenal, caryophyllene oxide	Jirovetz *et al*., 2004
		Cyperene, humulene, α-selinene	Tiwari *et al*., 2014
		Caryophyllene alcohol	Dhillon *et al.*, 1993
		β-Selinene, α-cyperone, anethole	Singh *et al.*, 2018
		Cyperene	Richa and Suneet, 2014
		Caryophyllene oxide	Gupta *et al.*, 2016
9.	Indonesia	Longiverbenone, β-silinene, 3,4-isopropylidene, caryophyllene oxide	Busman *et al.*, 2018

10.	Iran	Cyperene, caryophyllene oxide, α-longipinane	Ghannadi *et al.*, 2012
		Cyperene, cyperotundone	Ali *et al.*, 2013
		Elemenone, αcyperone, and caryophyllene oxide	Janaki *et al.*, 2018
		α-Cyperone	Mojab *et al.*,2009
11.	Iraq	Cyperol, caryophyllene, cyperene	Nima *et al.*, 2008
12.	Japan	α-Cyperone, β-selinene, cyperene	Komai *et al.*, 1991
		Cyperol	Komai *et al.*, 1994
13.	Morocca	Longiverbenone, cyperotundone, (-) eudesma-1,4(15),11-triene, β-copaen-4α-ol, humulene epoxide-2	Karima *et al.*, 2022
14.	Nigeria	Cyperene, α-cyperone	Kilani *et al.*, 2008
		Cyperene, α-cyperone	Ekundayo *et al.*, 1991
15.	Philippines	Cyperotundone, β-selinene	Komai *et al.*, 1991
16.	Saudi Arabia	α-Cyperone, 4-oxo-α-ylangene	Al-Massarani *et al.*, 2016
17.	Senegal	Caryophyllene oxide, humulene oxide II, longiverbenone	Thiam *et al.*,2022
18.	South Africa	α-Cyperone, myrtenol, caryophyllene oxide, β-pinene	Lawal and Oyedeji, 2009
		β-Pinene, α-pinene, α-cyperone, myrtenol, α-selinene	Lawal *et al.*, 2009
19.	South Korea	α-Cyperone, β-selinene, cyperene, aristolone, caryophyllene oxide	Chang *et al.*, 2012
20.	Sudan	Humulene epoxide 2, allo-aromadendrene, cyperene, α-calacorene, 3,7-guaiadiene, humulene epoxide-II	Eltayeb *et al.*, 2016
		Longiverbenone	Yagi *et al.*, 2016
		Longiverbenone	Eltayeib and Ismaeel, 2014
		Isolongifolen -5-one	Eltayeib and Ismaeel, 2014
		Isolongifolen -5-one	Eltayeib and Ismaeel, 2014
21.	Taiwan	Cyperene	Kilani *et al.*, 2008
22.	Thailand	Cyperene, cyperotundone	Ohira *et al.*, 1998
23.	Tunesia	Cyperene, α-cyperone	Kilani *et al.*, 2005
		Cyperene, α-cyperone, isolongifolen-5-one, rotundene, cyperorotundene	Kilani *et al.*, 2008
		Cyperotundone, cyperene	Essaidi *et al.*, 2014
24.	Turkey	Cyperene, α-copaene, α-ylangene	Ilham *et al.*, 2018

A systematic investigation of the *Cyperus rotundus* volatile chemicals reported from different regions of the world reveals that the essential oil composition varied considerably. However, few characteristic compounds such as α-cyperone, cyperene and cyperotundone can be considered as the major compounds in most of the accessions (**Figure 21)** (Komai *et al.,* 1994; Lawal *et al.,* 2009).

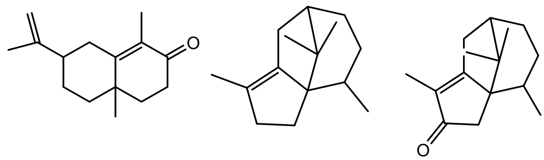

Figure 21. Major volatile compounds reported from the essential oils of *Cyperus rotundus*- Cyperone, Cyperene and Cyperotundone

Several factors such as time of the day, temperature, diurnal/nocturnal nature, soil, nutrient levels, water availability, fungal or endophyte presence, systemic pathogens, and mechanical or herbivore damage need to be considered for getting consistent volatile chemical profiles (Holopainen and Gershn-hexaneenzon, 2010; Kusari *et al.*, 2013). It is always advisable to mention the exact plant parts, phytogeographical location of plant collection, season of collection, extraction technique and analytical technique.

The constituents of volatile compounds of *C. rotundus* obtained by hydrodistillation, supercritical fluid extraction, pressurized liquid extraction and headspace techniques vary considerably (Jirovetz *et al.,* 2004). Tam *et al.* (2007) tried three methods; hydrodistillation (HD), pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) for extraction of volatile compounds from *C. rotundus* and found that the contents varysignificantly with HD, PLE and SFE. PLE had the highest extraction efficiency for α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone, while SFE had the best selectivity for extraction of β-cyperone and α-cyperone.

Non-volatile chemical composition of *Cyperus rotundus*

In addition to the wide array of volatile constituents, the relevance of the plant also depends on different classes of non-volatile compounds as well. Phenolic acids, flavonoids, iridoids, furochromons, stilbenoids, triterpenoids, steroids, alkaloids and fatty acids are the major class of non-volatile compounds reported from *C. rotundus* (Babiaka *et al.,* 2021).

The non-volatile phytochemicals from various solvent extracts are generally analysed through conventional techniques such as extraction, separation and characterisation, while phytochemical screening through LC-MS is another approach. The reports of phytochemicals through the conventional techniques are much less, while the hyphenated techniques such as LC-MS/MS predominates.

In the beginning, conventional phytochemical techniques such as solvent extraction and column chromatographic separation were used for isolating the phytochemicals from *C. rotundus*, and spectroscopic and synthetic methods were adopted for structure elucidation. Currently several novel techniques such as supercritical fluid extraction, solid phase micro extraction, high-speed counter current chromatography, reverse phase chromatography, supercritical fluid chromatography (SFC), ultraperformance convergence chromatography (UPCC), high resolution mass spectrometry (HRMS), inductively coupled plasma mass spectrometry (ICPMS), 1D and 2D nuclear magnetic resonance spectroscopy, and various hyphenated analytical techniques such as GC-MS, GC-MS/MS, LC-MS, LC-MS/MS and LC-NMR are used for the phytochemical investigation of *Cyperus rotundus*.

Liquid Chromatography- Mass Spectrometry (LC-MS) analysis: While GC-MS is the apt technique for analysing volatile components, LC-MS is the preferred analytical technique for non-volatile components such as flavonoids, triterpenoids, iridoid glycosides and alkaloids (Kilani *et al.,* 2005; Chen *et al.,* 2011; Madhulika and Varsha, 2015). Recent technological developments and methodological advances of both liquid chromatography (LC) and mass spectrometry (MS) have allowed LC-MS based plant metabolomics to become a common tool for qualitative and quantitative investigation of plant metabolites. Tandem mass spectrometry (LC-MS/MS), especially triple quadrupole mass spectrometers and quadrupole time of flight (QToF) are the most commonly used hyphenated analytical technique for solvent extracts. Different solvent extracts of *C. rotundus* rhizomes were analysed by various LC-MS/MS techniques, yielding elaborate chemical profiles (Singh and Singh, 1980; Jeong *et al.,* 2000; Kilani *et al.,* 2005; Sayed *et al.,* 2008; Kilani *et al.,* 2009; Chen *et al.,* 2011; Zhou and Yin, 2012; Zhang *et al.,* 2014; Madhulika and Varsha, 2015; Kakarla *et al.,* 2015; Hemanth Kumar *et al.,* 2015; Singh and Sharma, 2015; Gamal *et al.*, 2015; Zhou *et al.*, 2016; Kakarla *et al.*, 2016; Sultana *et al.*, 2017; Sabrin *et al.*, 2018; Kamala *et al.*, 2018; Majeed *et al.*, 2022; Xu *et al.*, 2015).

The non-volatile phytochemicals reported from *Cyperus rotundus* are tabulated in **Table 5**. A total of 294compounds comprising aurones (3), chromones (5), coumarins (3), quinanoids (2), iridoids (29), flavonoids (46), biflavonoids(5), stilbenoids (15), lignans (1), benzofurans (4), phenolic acids and derivatives(27), phenolic derivatives (13), sesquiterpene alkaloids (3), diterpenoids (3), triterpenoids (33), steroids (19), organic acids (8), aliphatic acids and derivatives(28), amides and other nitrogenous constituents(10) and miscellaneous compounds (37) were reported from *Cyperus rotundus* (**Table 5)**.

Table 5. Non-volatile phytochemicals reported from *Cyperus rotundus*

Sl. No.	Class of compounds	Phytochemicals	Reference
1.	Aurones	1. Aureusidin 2. 4,6,3,4-Tetramethoxy aurone 3. 6,3,4-Trihydroxy-4-methoxy-5-methylaurone	Harborne *et al.*, 1982
2.	Chromones	1. Ammiol 2. Isorhamnetin 3. Khellin 4. Khellol-β-D-glucopyranoside 5. Visnagin	Sayed *et al.*, 2007
3.	Coumarins	1. 6,7-Dimethoxy coumarin 2. 6-O-p-Coumaroyl genipingentiobioside 3. Coumarin	Sayed *et al.*, 2008
4.	Quinanoids	1. Catenarin 2. Physcion	Wu *et al*., 2008
5.	Iridoids	1. 10-Hydroxyoleuropein 2. 10-O-p-Hydroxybenzoyl theviridoside 3. 10-O-Vanilloyl theviridoside 4. 6″-O-(trans-p-Coumaroyl)-procumbide 5. 6-Hydroxy ipolamiide 6. 6'-O-p-Coumaroylgenipin gentiobioside 7. 6-O-p-Hydroxybenzoyl-6-epi-monomelittoside 8. 6-O-p-Hydroxybenzoyl-6-epi-aucubin	Sayed *et al*. 2008 Zhou and Yin, 2012 Zhou *et al*., 2013 Zhou and Zhang, 2013 Zhang *et al*., 2014 Gamal, 2015 Jeong *et al.*, 2017 Zhang *et al*.; 2016 Cheng *et al.*, 2014 Lin *et al*., 2015

		9. 7-O-p-Hydroxybenzoyl-8-epi-loganic acid 10. Ipolamiide 11. Isooleuropein 12. Loganic acid 13. Negundoside 14. Neonuezhenide 15. Nishindaside 16. Oleuropeinic acid 17. Oleuroside 18. Rotunduside A 19. Rotunduside B 20. Rotunduside C 21. Rotunduside D 22. Rotunduside E 23. Rotunduside F 24. Rotunduside G 25. Rotunduside H 26. Senburiside I 27. Syringopicroside B 28. Syringopicroside C 29. Verproside	
6.	Flavonoids	1. (2RS,3SR)-3,4′,5,6,7,8-Hexahydroxyflavane 2. 5,7,4'-Trihydroxy-2'-methoxy-3'-prenylisoflavone 3. 5,7-dihydroxy-4'-methoxy-8-C-[2''-(2''-methylbutyryl)]-β-D-glucopyranosyl flavone 4. 5-Hydroxy-4'methoxy-7-[(3-methyl-2-buthenyl) oxy]-isoflavone 5. 7,8-Dihydroxy-5,6-methylenedioxyflavone 6. 7-Methoxy-isoflavone 7. Afzelechin 8. Apigenin 9. Biochanin 10. Biochanin A 11. Catechin 12. Chrysoeriol 13. Cinaroside 14. Cyperaflavoside 15. Epiorientin 16. Isorhamnetin 17. Isovitexin	Harborne *et al.*, 1982 Kilani-Jaziri *et al.*, 2009 Zhou *et al.*, 2012 Sayed *et al.*, 2008 Sayed *et al.*, 2008 Ibrahim *et al.*, 2007 Sayed *et al.*, 2001 Sayed *et al.*, 2007 Sayed *et al.*, 2008 Krishna and Renu, 2013 El-Habashy *et al.*, 1989 Singh and Singh, 1986 Kasala *et al.*, 2016 Cheng *et al.*, 2014 Gamal *et al.*, 2015 Xu *et al.*, 2016

		18. Kaempferol 19. Leucocyanidin 20. Licoricone 21. Luteolin 22. Luteolin 3`-methyl ether 23. Luteolin 4′-glucoside 24. Luteolin 5,3'-dimethyl ether 25. Luteolin 5'-methyl ether 26. Luteolin 5-methyl ether 27. Luteolin 7,3′-dimethyl ether 28. Luteolin 7-diglucoside 29. Luteolin 7-O-glucoside 30. Luteolin 7-O-β-D-glucuronopyranoside-6"-methyl ester 31. Myricetin 32. Myricetin 3-O-β-D-galactopyranoside 33. Myricetin 3-O-β-D-glucopyranoside 34. Orientin 35. Pinoquercetin 36. Pongamone A 37. Pongamone A/4'-Methoxyl-8-methoxyl-7-γ, γ-dimethylallyloxy isoflavone 38. Quercetin 39. Quercetin 3-O-β-D-glucopyranoside 40. Quercitrin 41. Rhamnetin 3-O-rhamnosyl (1→4) rhamno-pyranoside 42. Rutin 43. Scaberin 44. Tricin 45. Tricin 5-glucoside 46. Vitexin	
7.	Biflavonoids	1. Amentoflavone 2. Bilobetin 3. Ginkgetin 4. Isoginkgetin 5. Sciadopitysin	
8.	Stilbenoids	1. (–)-(Z)-Cyperusphenol A 2. (+)-(Z)-Cyperusphenol A 3. (E)-Cyperusphenol C 4. (E)-Mesocyperusphenol A	Ito *et al*., 2012 Tran *et al*., 2014 Majeed *et al*., 2022

		5. (Z)-Mesocyperusphenol A 6. Cassigarol E 7. Cyperusphenol A 8. Cyperusphenol B 9. Cyperusphenol C 10. Cyperusphenol D 11. Mesocyperusphenol 12. Piceatannol 13. Piceid 14. Scirpusin A 15. Scirpusin B	
16.	Lignans	1. Liriodendrin	Xu *et al*., 2016
17.	Benzofurans	1. 1-[2,3-Dihydro-6- hydroxy-4,7-dimethoxy2S-(prop-1-en-2-yl)benzofuran-5-yl]ethenone 2. 2S-Isopropenyl-4,8- dimethoxy-5-hydroxy-6- methyl-2,3-dihydrobenzo[1,2-b;5,4-b`]difuran 3. 2S-Isopropenyl-4,8- dimethoxy-5-methyl-2,3- dihydrobenzo-[1,2-b;5,4- b`]difuran 4. Sulfuretin	Amesty *et al.*, 2011
18.	Phenolic acids and derivatives	1. (-)-(E)-Caffeoylmalic acid 2. 1-[2,3-Dihydro-6-hydroxy-4,7-dimethoxy-2S-(prop-1-en-2yl) benzofuran-5-yl] ethanone 3. 3-Hydroxy,4-methoxybenzoicacid 4. 4',6'-Diacetyl-3,6-diferuloylsucrose 5. 4-Hydroxy benzoic acid 6. 4-Hydroxy butyl cinnamate 7. 4-Hydroxy cinnamic acid 8. 6'-Acetyl-3,6-diferuloylsucrose 9. Benzoic acid 10. Caffeic acid 11. Caffeoylmalic acid 12. Chlorogenic acid 13. Cinnamic acid 14. Ellagic acid 15. Ferulic acid 16. Gallic acid 17. Galloyl quinic acid 18. Hydroxybenzoic acid 19. Methyl 3,4-dihydroxy benzoate 20. Methyl ferulate	Sayed *et al*., 2008 Jahan *et al*., 2013, Samariya and Sarin, 2013 Zhou *et al*., 2013, Li, 2014 Amestry *et al*., 2011 Zhou and Yin, 2012 Zhou and Zhang, 2013 Zhang *et al*., 2014, Gamal, 2015 Komai and Kunikazu, 1981 Kowthar *et al.*, 2010 Chen *et al.*, 2011 Zhou and Zhang, 2013 Komai and Kunikazu, 1981

		21. p-Coumaric acid 22. p-Hydroxybenzoic acid 23. Propyl gallate 24. Protocatechuic acid 25. Salicylic acid 26. Vanillic acid 27. Vanillin lactoside	
19.	Other phenolic derivatives	1. 1-(3, 4-Methylenedioxyphenyl)-1E-tetradecene 2. 1-(3,4-Methylenedioxyphenyl)-1E-tetradecene 3. 1α,3β-Dihydroxy-4α-(3′,4′-dihydroxyphenyl) -1,2,3,4-tetrahydronaphthalene 4. 1α-Methoxy-3βhydroxy-4α-(3′,4′- dihydroxyphenyl)-1, 2,3,4-tetrahydro naphthalin 5. 3-(4-Hydroxy-3-methoxy phenyl)-methyl ester 6. 3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-[4-(3-hydroxy-1-(E)-propenyl)-2,6-dimethoxy phenoxy] propyl-β-D-glucopyranoside 7. Catechol 8. Chionoside A 9. Cyperine 10. Helioside C 11. Isoaragoside 12. Pungenin 13. Salidroside	Zhou and Yin, 2012 Zhou and Zhang, 2013
20.	Sesquiterpene alkaloids	1. Rotundine A 2. Rotundine B 3. Rotundine C	Jeong *et al.*, 2017 Jeong *et al.*, 2000
21.	Diterpenoids	1. Dolabella-3,7,18-triene 2. Phytol 3. Rosenonolactone	Xu *et al*., 2008
22.	Triterpenoids	1. 18-epi-α-Amyrin glucuronoside 2. 3,4-seco-Mansumbinoic acid 3. 3β-Hydroxyolean-12-en-28-oic acid α-D-arabinofuranoside 4. 5α,8α-epidioxy-(20S,22E,24R)-Ergosta-6,22-dien-3β-ol/ergosterol peroxide 5. 9,10-seco-Cycloartane 6. 9,10-seco-Cycloartane α-D-	Singh *et al.*, 1980 Alam *et al.*, 2012 Sultana *et al.*, 2017 Singh and Sharma, 2015 Yang *et al.*, 2010 Zhou *et al.*, 2012 Lin *et al.*, 2018 Zhou *et al.*, 2016

		arabinofuranoside 7. Cyperalin A 8. Cyprotuside A 9. Cyprotuside B 10. Cyprotuside C 11. Cyprotuside D 12. Dammaradienyl acetate 13. Dammaradienyl acetate 14. Daucosterol 15. epi-α-Amyrin glucuronoside 16. Lup-12, 20 (29)-dien-3β-ol-3-α-L-arabinopyranosyl-2'-oleate 17. Lupenyl 3β-O-arabinpyranosyl 2'-Oleate /Lupenylarabinopyranosyl oleate 18. Lupenylarabinosyl oleate 19. Lupeol 20. Oleanolic acid 21. Oleanolic acid 3-O-(2-rhamnosylglucosyl) 22. Oleanolic acid arabinoside 23. Oleanolic acid-3-O-neohesperidoside 24. Oleanolic acid-3-O-neohesperidoside/3-O-(2-rhamnosylglucosyl)-oleanolic acid 25. Rotundusolide C 26. Secomacrogenin A 27. Secomacrogenin B 28. Taraxerone 29. Zeorin 30. α-Amyrin glucopyranoside 31. β-Amyrin 32. β-Amyrin acetate 33. β-Amyrin glucopyranoside	Sabrin *et al.*, 2018 Yang and Shi, 2012
23.	Steroids	1. 4,4-Dimethylandrost-5-en-3-one 2. 5,16-Pregnadiene 3. 5α,8α-Epidioxy-(20S,22E,24R)-ergosta-6,22-dien-3β-ol 4. Daucosterol 5. Sitosteryl (6`-hentriacontanoyl)-β-D-galactopyranoside 6. Stigmast-5,22-dien-3β-olyldodecanoate 7. Stigmast-5,22-dien-3β-olyltetradecanoate	Singh *et al.*, 1980 Sultana *et al.*, 2017 Gamal, 2015 Samra *et al.*, 2021 Xu *et al.*, 2008 Singh *et al.*, 2017 Luo*et al.*, 2014 Abo-Altemen *et al.*, 2019

		8. Stigmast-5-ene 9. Stigmasterol 10. Stigmasterol laurate 11. Stigmasterol laurate 12. Stigmasterol myristate 13. Stigmasterol myristate 14. Stigmasterol-n-dodecanoate 15. Stigmasterol-n-tetradecanoate 16. Taraxerone 17. β-Sitosterol 18. β-Sitosterol-3β-O-glucoside 19. β-Stigmasterolglucoside	
24.	Organic acids	1. (-)-(E)-Caffeoylmalic acid 2. 2-Hydroxy-2-methylmalonic acid 3. 2-Hydroxypropanoic acid 4. 2-Propenoic acid 5. 3,4-O-Isopropylidene shikimic acid 6. Lactic acid 7. Methyl tartronic acid 8. Propanoic acid	Sayed *et al.*, 2008 Zhou and Yin, 2012
25.	Aliphatic acids and derivatives	1. (9Z,12Z,15Z)-Octadecatrienoic acid methyl ester 2. 12-Dienoate n-pentadecanyl linoleate 3. 22-Dien-3β-olyl n-dodecanoate 4. 5-Hydroxy-4-oxo-10-pentadecenoic acid lactone 5. 9,12,15-Octadeca trienoic acid 6. Behenic acid 7. Behenic acid monoglyceride 8. Fulgidic acid 9. Linoleic acid 10. Linolenic acid 11. Methyl (Z)-5,11,14,17-eicosatetraenoate 12. Methyl linoleate 13. Myristic acid 14. n-Hexadecanoic acid 15. n-Hexadecanyl linoleate 16. n-Hexadecanyl oleate 17. n-Pentacos-13′-enyl octadec-9-enoate 18. n-Pentacos-13'-enyl oleate 19. n-Pentadecanyl linoleate 20. n-Pentadecanyl octadec-9, 12-	Jin *et al.*, 2015 Sultana *et al.*, 2017 Singh and Sharma, 2015 Samra *et al.*, 2021 Sim *et al.*, 2016 Shin *et al.*, 2015

		dienoate 21. n-Pentadecanyl-9-octadecenoate 22. n-Tetradecanyl-n-octadec-9, 12-dienoate 23. Oleanolic acid 24. Oleic acid 25. Palmitic acid 26. Pinellic acid 27. Stearic acid 28. Tetradecanyl linoleate	
26.	Amides and other nitrogenous constituents	1. 6,7-Dihydro-2,3-dimethyl-5-cyclopentapyrazine 2. Adenosine 3. Aristolochic acid 4. Caprolactam 5. Cerebroside 6. Guineensine 7. Octopamine 8. Pellitorine 9. Sarmentine 10. Uridine	Xu *et al*., 2016 Smith, 1977 Cantalejo, 1997 Wu, 2009 Chen *et al*., 2017
27.	Miscellaneous compounds	1. 1(2)-Acetyl-3(5)-styryl-5(3)-methylthiopyrazole 2. 1-(3,4-Methylenedioxyphenyl)-1E-tetradecene 3. 1,4,4-Trimethyl bicyclo [3.2.0] hept-6-en-2-ol 4. 1-[2,3-Dihydro-6-hydroxy 4,7-dimethoxy-2S-(prop-1-en-2yl)benzofuran-5-yl] ethenone 5. 1-Isopropyl-2,7 dimethylnaphthalene 6. 2,4-Decadienal 7. 2-Furfural 8. 2S-Isopropenyl-4,8-dimethoxy-5-hydroxy-6-methyl-2,3-dihydrobenzo[1,2-b;5,4b’] difuran 9. 2S-Isopropenyl-4,8-dimethoxy-5-methyl-2,3-dihydrobenzo-[1,2-b;5,4b'] difuran 10. 3`, 4`-Nonadecanetriol 11. 3-Furfural 12. 4',6' Diacetyl-3,6-diferuloylsucrose 13. 4,7-Dimethyl-1-tetralone	Sonwa *et al.*, 2001 Ohira *et al.*, 1998 Gamal, 2015 Syed *et al.*, 2008 Thebtaranonth *et al.*, 1995 Smith,1977 Chen *et al.*, 2011 Sim *et al.*, 2016 Kamala *et al.*,2018 Luo *et al*., 2014

		14. 4-Hydroxy-4,7-dimethyl-1-tetralone 15. 5-Hydroxymethyl furfural 16. 6,7-Dihydro-2,3-dimethyl,5-cyclopentapyrazine 17. 6'-Acetyl-3,6-diferuloylsucrose 18. 7-Hydroxy-1,4α-dimethyl-7-(prop-1-en-2-yl),-4,4α,5,6,7,8-hexahydronaphthalen-2 (3H),-one 19. 8-Hydroxy-1, 4α-dimethyl- 7-(prop-1-en-2-yl),-4,4a,5,6,7,8-hexahydronaphthalen-2 (3H)-one 20. Ascorbic acid 21. Cyclohexane,1,1,2-trimethyl,3,5 bis-(1-methyl ethyl) 22. Cyclopentene-3-ethylidene-1-methyl 23. Ethyl acetate 24. Ethyl ethanoate 25. Ethyl-α-D-glucopyranoside 26. Glycerol 27. N-(1-Deoxy-D-fructos-1-yl)-L-tryptophan 28. Naphthalene 29. n-Butyl, β-D-fructopyranoside 30. n-Dodecanol 31. n-Dotriacontan-15-one 32. n-Dotriacontan-16-one 33. n-Tetracontan-7-one 34. n-Tricont-1-ol-21-one 35. n-Tritriacontan-16-one 36. o-Methylacetophenone 37. Tryptophan α-D fructofuranoside	

Phenolic derivatives in *C. rotundus*

The major class of non-volatilecompounds are phenolic derivatives (total 140 compounds), belonging to aurones (3), chromones (5), coumarins (3), quinanoids(2), iridoids (29), flavonoids (46), biflavonoids(5), stilbenoids (15), lignans (1), benzofurans (4), phenolic acids and derivatives (27) and phenolic derivatives (13). Phenolic compounds such as flavonoids are the key ingredients in natural products, purported to have several health benefits. However, most of the phenolic compounds are detectedthrough various LC-MS analyses, rather than conventional phytochemical analytical techniques. Out of the 40 flavonoid derivatives reported in *C. rotundus*, luteolin and derivatives, quercetin and derivatives, and myricetin glycosides are the major flavonoids (**Table 5, Figure 22**).

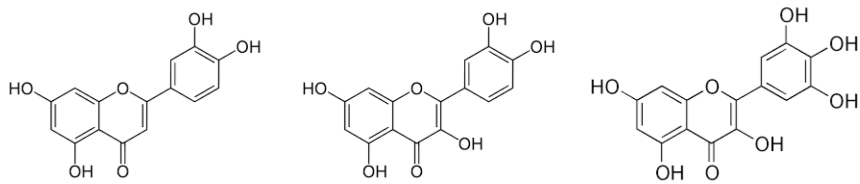

Figure 22. The common flavonoid skeletons in *Cyperus rotundus*- Luteolin, quercetin and myricetin

Biflavonoids

Biflavonoidsare characteristic class of phenolics with diverse biological activities and has significance in chemotaxonomy as well. Amentoflavone, bilobetin, Ginkgetin, isoginkgetinand sciadopitysin are the bioflavonoids reported from *Cyperus rotundus.* Amentoflavone, isolated from *C. rotundus* showed a significant inhibitory effect on uterine tumours in rats (**Figure 23**) (Ying and Bing, 2016). The mechanism of action has been suggested as elevating Bax protein expression, down-regulating Bcl-2 expression, forming homodimers Bax/Bax, and reducing plasmaestradiol and progesterone to promote apoptosis of uterine fibroid cells. Amentoflavone has previously been reported as bioactive constituent from several medicinal plants including *Ginkgo biloba*, *Biophytum sensitivum, Selaginella tamariscina* and *Hypericum perforatum.*

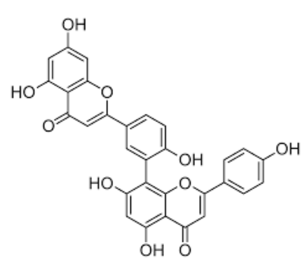

Figure 23. Amentoflavone, the bioactive biflavonoid in *Cyperus rotundus*

Stilbenes

Cyperaceae family is known for the presence of bioactive stilbenes, that possess cardioprotective, anticancer, anti-inflammatory, anti-obesity, chemopreventive, antioxidative and antimicrobial activities (Arraki *et al.*, 2017; Majeed *et al.*, 2022). Fifteen stilbenoidswere reported from *Cyperus rotundus* (**Table 5, Figure 24**). Ito *et al.* (2012) isolated enantiomeric and meso-stilbene trimers (+)- and (−)-(E)-cyperusphenol A, (E)-mesocyperusphenol A, a trimer bearing a novel hexacyclic ring system, cyperusphenol B, together with the known stilbenoids, cyperusphenol C, cyperusphenol D, trans-scirpusin A and scirpusin B from the rhizomes of *C. rotundus*. Tran *et al.* (2014) isolated the stilbene dimers cassigarol E, scirpusin A and B from *C. rotundus* rhizomes. Majeed *et al.* (2022) investigated the components in ethyl acetate extract of *Cyperus rotundus* rhizomes and reported piceatannol, scirpusin A and scirpusin B as the pharmacologically active molecules responsible for the antiobesity properties.

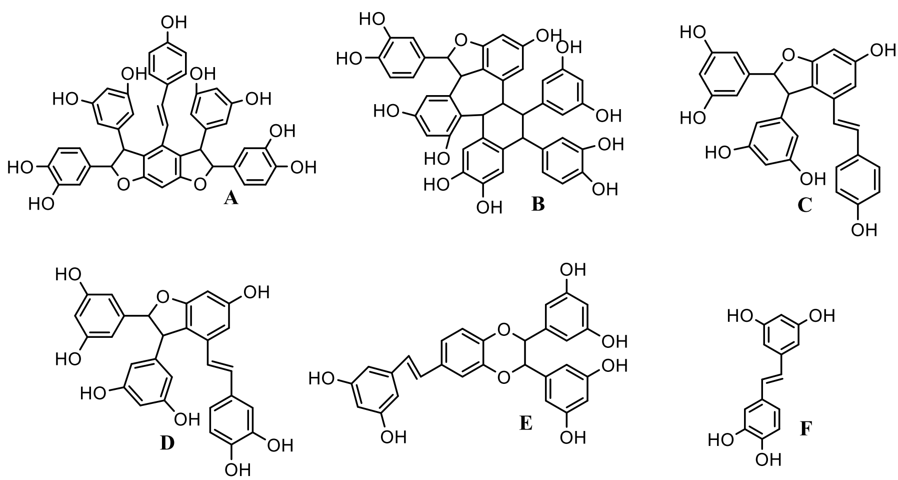

Figure 24. Major stilbenes reported from *Cyperus rotundus*; **A-** (E)-Cyperusphenol, **B-** Cyperusphenol B, **C-** Scirpusin A, **D-** Scirpusin B, **E-** Cassigarol E and **F-** Piceatannol

Iridoids

Iridoids are derivatives of monoterpenes and occur usually as glycosides, and provide a biogenetic and chemotaxonomic link between terpenes and alkaloids. Twentynine iridoids were reported from *Cyperus rotundus* (**Table 5, Figure 25**). The cleavage of the cyclopentane ring of iridoids produces secoiridoids. Iridoids have bitter taste and have antifeedant and growth inhibitory activities against insects, and have been regarded as defense chemicals against herbivores and pathogens. Iridoid glycosides exert inhibitory effects in numerous cancers. *C. rotundus* rhizome is reported to possess several iridoid glycosides (Zhou and Zhang, 2013; Gamal, 2015).

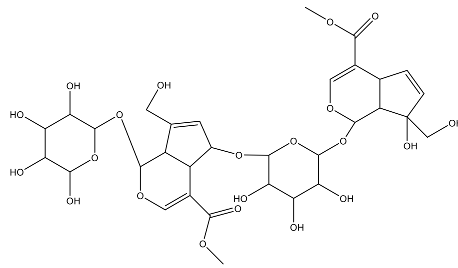

Figure 25. The iridoid glycoside rotunduside A reported from *Cyperus rotundus*

Triterpenoids and steroids

Triterpenoids and steroids are common secondary metabolites among plants, and around 33 terpenoids and 19 steroids were reported from *C. rotundus* (**Table 5, Figure 26**). The major triterpenoids are oleanolic acid and glycosides, amyrin and glycosides. Cycloartane terpenoid glycosides such as cyprotusides are characteristiccompounds reported from *C. rotundus* (Zhou *et al.*, 2016). Rotundusolide C is a triterpenoid with rare 9,10-seco-cycloartane skeleton, reported from *C. rotundus* (Yang and Shi, 2012; Lin *et al.*, 2018). β-Sitosterol and stigmasterol glycosides and esters are the major steroids reported from *C. rotundus* (Singh *et al.*, 2017).

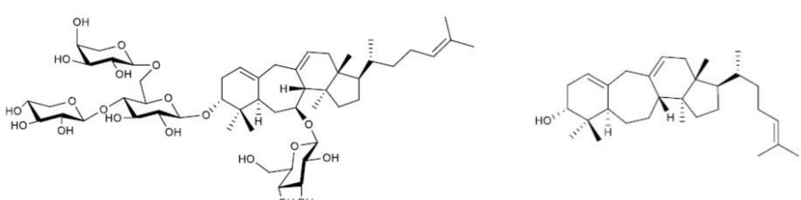

Figure 26. The cycloartane terpenoid glycoside cyprotuside A and the 9,10-seco-cycloartane triterpenoid rotundusolide C

Sesquiterpene alkaloids

Sesquiterpenes with nitrogen atom within the basic carbon skeleton of the sesquiterpenoid structure are reported from *Cyperus rotundus*. Three novel sesquiterpene alkaloids rotundines A, B, and C, with an unprecedented carbon skeleton of cyclopentane ring attached to the pyridine ring, were isolated from the methanol extract of *C. rotundus* (**Figure 27)** (Jeong *et al*., 2000).

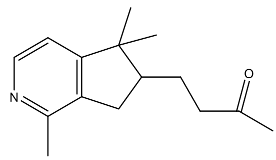

Figure 27. The sesquiterpene alkaloid rotundine A

Fatty acids

Table 5 shows 28 long chain aliphatic acids and derivatives reported from *Cyperus rotundus*. The fatty acids are generally identified through GC-MS method, after making the volatile Fatty Acid Methyl Esters (FAME). In addition to the common fatty acids, the species has been reported as source of characteristic fatty acids as well. Ceramides are a family of lipid molecules, composed of sphingosine and a fatty acid, and are found in the cell membrane (**Figure 28)**. The new ceramide, 2`-[2-hydroxypentacosanoylamino]-1`,3`,4`-nonadecanetriol reported from *Cyperus rotundus* has displayed inhibitory activity against HepG2 with IC_{50} value 6.81 to 8.075 µM, and PC3 with IC_{50} of 11.92 to 14.48 µM (Samra *et al*., 2021).

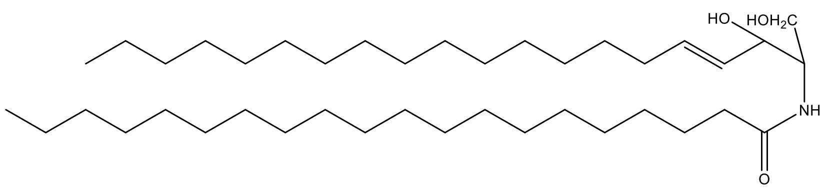

Figure 28. Ceramide derivative, a new cytotoxic lipid from *Cyperus rotundus*

Two unsaturated trihydroxy C_{18} fatty acids fulgidic acid and pinellic acid were isolated from *Cyperus rotundus* rhizomes, of which fulgidic acid possess anti-inflammatory activity (**Figure 29)** (Shin *et al*., 2015).

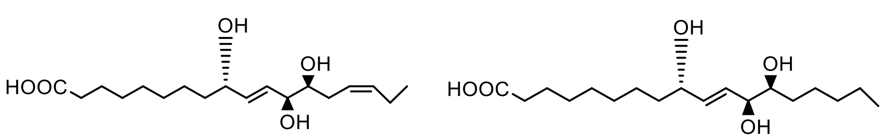

Figure 29. Fulgidic acid and pinellic acid reported from *Cyperus rotundus*

It is interesting to note that in Ayurveda, the rhizomes of *Cyperus rotundus* has been recommended as substitute for tubers of *Aconitum heterophyllum* (Family: Ranunculaceae), and *karpura* (*Cinnamomum camphora*), based on the concept of drug substitution (*Abhava Pratinidhi Dravya*). A comparative HPLC profile of *Aconitum heterophyllum* and *Cyperus rotundus* revealed the same pattern for HPLC peaks, however, there is scope for detailed phytochemical investigation using modern hyphenated analytical techniques such as LC-MS/MS and LC-MS/NMR (Venkatasubramanian *et al.*, 2010; Nagarajan *et al*., 2015).

Conclusion

Though the plant *Cyperus rotundus* is considered as a problematic weed all over the world, a review of the phytochemistry of the plant suggested the species as a store house of exiting structural features, with 294 non-volatile organic compounds, and 390 volatile organic compounds reported so far in the species. Though the phytochemistry of the species has been explored extensively, systematic studies are yet to be done to correlate the chemical diversity with phenology, genetics and ecology. Also, the seasonal, geographical and climatic effects need to be studied in detail in correlation with chemical diversity. More effective statistical approaches such as chemometry can also be employed in *Cyperus rotundus* metabolomics. Recently, ethno-botanical and traditional uses of natural compounds, especially of plant origin, received much attention as they are well tested for their efficacy and generally safe for human use, and *Cyperus rotundus* isone of the oldest herbs being used by mankind. Being a noxious weed widely distributed globally, *C. rotundus* rhizomes can be collected in huge quantity, and a systematic approach employing the recent developments in science and technology tools can yield value added herbal products from the weed.

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