

In vitro Antioxidant Potentials of *Cyperus rotundus* L. Rhizome Extracts and Their Phytochemical Analysis

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ABSTRACT

Background: *Cyperus rotundus* L. (family Cyperaceae), native to India, is a multivalent medicinal plant widely used in conventional medicine. The research reports on bioactive components from *C. rotundus* L. are scanty. **Objective:** The objective of the study was to optimize the best solvent system and bioprospect the possible phytochemicals in *C. rotundus* L. rhizome (CRR). **Materials and Methods:** The phytochemicals were extracted from the rhizomes of *C. rotundus* L. by successive Soxhlet technique with solvents of increasing polarity. The resultant extracts were analyzed for their total flavonoid content (TFC), total phenolic content (TPC), total proanthocyanidin content (TPAC), *in vitro* antioxidant potential, and inhibition of lipid peroxidation. The 70% acetone extract of CRR was analyzed using gas chromatography–mass spectrometry (GC-MS) for probable phytochemicals. **Results and Discussion:** The TPC, TFC, and TPAC estimates ranged from 0.036 ± 0.002 to 118.924 ± 5.946 $\mu\text{g}/\text{mg}$ extract, 7.196 ± 0.359 to 200.654 ± 10.032 $\mu\text{g}/\text{mg}$ extract, and 13.115 ± 0.656 to 45.901 ± 2.295 $\mu\text{g}/\text{mg}$ extract, respectively. The quantities of TPC, TFC, and TPAC were found to be the highest in 70% acetone extract. The 70% acetone and 70% methanol extracts revealed best radical scavenging effect. GC-MS analysis of CRR extract revealed the presence of a novel compound 1 (2)-acetyl-3 (5)-styryl-5 (3)-methylthiopyrazole. **Conclusion:** The study indicated that 70% acetone and 70% methanol extracts of CRRs can be a potential source of antioxidants.

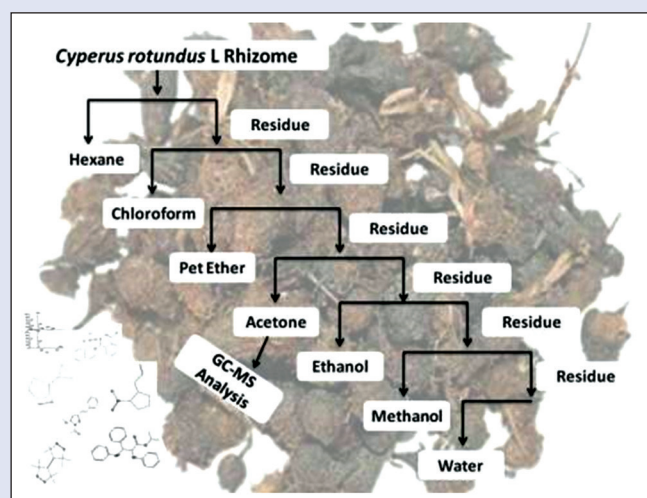
Key words: Alkaloids, antioxidants, *Cyperus rotundus* L. Rhizome, gas chromatography-mass spectrometry analysis, successive solvent extraction

SUMMARY

- The studies suggest 70% methanol and acetone as the suitable solvents for the extraction of phytochemicals
- Novel compound 1 (2)-Acetyl-3(5)-styryl-5(3)-methylthiopyrazole was detected in 70% acetone extract.

Abbreviations used: ACRE: Acetone *C. rotundus* L. rhizome extract; AlCl_3 : Aluminum chloride; AQRE: Aqueous *C. rotundus* L. rhizome extract; CE: Catechin Equivalent; CHRE: Chloroform *C. rotundus* L. rhizome extract; CRR: *C. rotundus* L. rhizome; DPPH: 2,2 diphenyl-1-picrylhydrazyl; ETRE: Ethanolic *C. rotundus* L. rhizome extract; EARE: Ethyl acetate *C. rotundus* L. rhizome

extract; FRP: Ferric reducing power; GAE: Gallic acid equivalent; GC-MS: Gas chromatography-mass spectrometry; HERE: Hexane *C. rotundus* L. rhizome extract; MERE: Methanolic *C. rotundus* L. rhizome extract; PERE: Petroleum ether *C. rotundus* L. rhizome extract; QE: Quercetin equivalent; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; TFC: Total flavonoid content; TPC: Total phenolic content; TPAC: Total proanthocyanidin content.



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INTRODUCTION

Natural products engross enormous chemical diversity. These phytochemicals find tremendous applications in agriculture, cosmetics, food, and medicine.^[1-3] The minimal side effects related to their use make them more popular than synthetic drugs.^[4] The Indian subcontinent, “The land of Ayurveda,” is known for its biological abundance of medicinal plants.^[5,6]

Cyperus rotundus L. (family: Cyperaceae) is popularly known as “Nut grass” in English, “*musta moola churna*” in ancient Ayurveda Charaka Samhita,^[7] and “Xiangfu or Xiangfuzi” in Chinese Traditional Medicine.^[8] It is a multivalent herb reported for its pharmacological properties such as an analgesic, antibacterial, antidiabetic, antidiarrheal,

anti-inflammatory, antioxidant, antipruritic, antisaturative, appetizer, diaphoretic, digestant, lactodepurant, thirst relieving, and tranquilizing effect.^[9-13] The phytochemical studies of *C. rotundus* L. led to the

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isolation of sesquiterpenes,^[11,14] flavonoids,^[15-17] phenylpropanoids,^[18,19] phenolic acids,^[18,20] alkaloids,^[21] and saponins.^[22] Investigations on *C. rotundus* L. rhizome (CRR) extract reported the presence of methyl 3,4-dihydroxybenzoate, ipolamiide, 6- β -hydroxyipolamiide, and rutin and also stated the uses of ethyl acetate fraction coupled with methanolic fraction in treatment of CCl₄-induced hepatic injury in rats.^[13] Lydia J and Sudarsanam (2014) identified 15-Hydroxy-4-oxo-10-pentadecynoic acid lactone from *C. rotundus* L.^[12] Recently, rotunduside, a phenolic compound from methanol extract of CRR, was isolated and its potential antidepressant activity with murine models was demonstrated.^[23]

However, there is limited information in the selection of the best solvent system to extract active molecules from CRR. Therefore, the current study aims to optimize the best solvent system and bioprospect the possible phytochemicals in CRR.

MATERIALS AND METHODS

Chemicals and reagents

Aluminum chloride, ascorbic acid, chloroform, ethanol, ethyl acetate, ferric chloride, ferrous sulfate, gallic acid, glacial acetic acid, hexane, hydrogen peroxide, α -naphthol, petroleum ether, potassium dihydrogen phosphate, potassium hydrogen phosphate, potassium chloride, potassium ferricyanide, sodium bicarbonate, trichloroacetic acid (TCA), and vanillin were procured from SD Fine Chemicals Limited, Mumbai, India. Acetone, hydrochloric acid, methanol, sodium hydroxide, and sulfuric acid were acquired from Spectrum Chemical Private Limited, Cochin. Acetic anhydride, Dragendorff reagent, Folin–Ciocalteu reagent, lead acetate, Mayer's reagent Sodium nitrate, and Wagner's reagent were purchased from MERCK Specialties Private Limited, Mumbai. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was procured from SISCO Research Laboratories, Maharashtra, India. Tris base was from NR Chemicals Private Limited, Mumbai, India. Thiobarbituric acid (TBA) was bought from Loba Chemie Private Limited, Mumbai, India. All chemicals and reagents were of analytical grade.

Collection and identification of plant material

The CRRs were obtained from the regional market, Bengaluru and authenticated by National Ayurveda Dietetics Research Institute, Bengaluru. A specimen copy was deposited in the herbarium of the Regional Research Centre (RRCBI-AP. 77). The cleaned and shade dried rhizomes were finely powdered using a mechanical blender (Kenstar, India) and were subjected to successive soxhlation using various solvents.

Preparation of *C. rotundus* L. rhizome extract

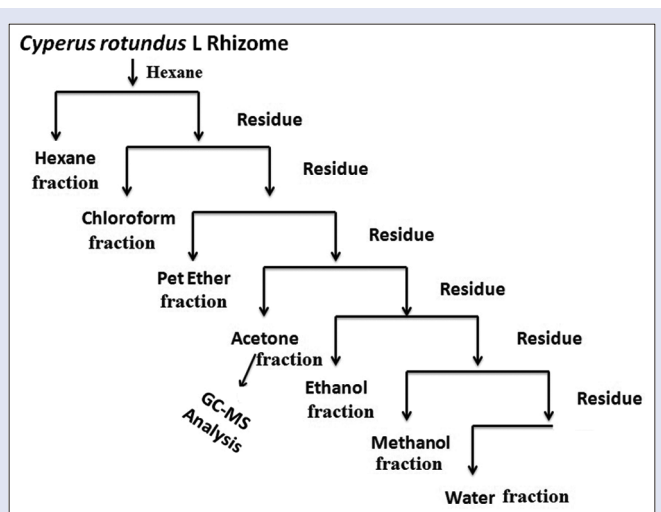
The powdered CRR was directed to successive Soxhlet extraction using eight different solvents, i.e., hexane, petroleum ether, ethyl acetate, chloroform, 70% acetone, 70% ethanol, 70% methanol, and water in their increasing polarity (1:12 w/v ratio) for 24 h at their respective boiling temperatures [Schema 1]. The lyophilized (LyoQuest, Telstar, Spain) samples were stored at 4°C for further use.

Quantification of *C. rotundus* L. rhizome extract yield

The obtained percentage yield of CRR extracts using various solvents was calculated using the following formula: % Yield = (W1/W2) \times 100; where W1 = the weight of the extract after solvent evaporation and W2 = the weight of the dry plant material.

Preliminary screening of phytochemicals in *C. rotundus* L. rhizome

Qualitative phytochemical analysis for the detection of alkaloids, carbohydrates, glycosides, flavonoids, resins, saponins, steroids, tannins,



Schema 1: Flowchart of sequential partitioning of active crude extract of rhizomes of *Cyperus rotundus* to obtain fractions

and phenols was carried for all the solvent extracts using standard methods.^[24] Each solvent extract was redissolved using Millipore water to obtain various concentrations and filtered. The filtrates were employed for further uses. All the tests were performed thrice.

Quantitative phytochemical assay

Determination of total phenolic content

The total phenolic content (TPC) was quantified by Folin–Ciocalteu method^[25] using gallic acid as standard for hexane *C. rotundus* L. rhizome extract (HERE), petroleum ether *C. rotundus* L. rhizome extract (PERE), ethyl acetate *C. rotundus* L. rhizome extract (EARE), chloroform *C. rotundus* L. rhizome extract (CHRE), 70% acetone *C. rotundus* L. rhizome extract (ACRE), 70% ethanolic *C. rotundus* L. rhizome extract (ETRE), 70% methanolic *C. rotundus* L. rhizome extract (MERE), and aqueous *C. rotundus* L. rhizome extract (AQRE). The absorbance of standard and test samples was read at 765 nm. TPC of the resulting successive extract was determined using standard curve and expressed as μ g gallic acid equivalent (GAE)/mg extract using $T = [C \times V]/M$ formula. Where T is the TPCs in μ g/mg of the extracts as GAE, C is the concentration of gallic acid in μ g/mL, V is the volume of the extracts in mL, and M is the weight in mg of the extract.

Determination of total flavonoid content

The estimation of total flavonoid content (TFC) was carried out for HERE, PERE, EARE, CHRE, ACRE, ETRE, MERE, and AQRE using AlCl₃ method^[26] with standard quercetin. The absorbance was read at 510 nm. The total amount of flavonoid was expressed as μ g of quercetin equivalents (QEs)/mg of extract.

Determination of total proanthocyanidin content

The total proanthocyanidin content (TPAC) of HERE, PERE, EARE, CHRE, ACRE, ETRE, MERE, and AQRE was quantified using vanillin–hydrochloride method as detailed by Usha *et al.*^[27] The absorbance was measured at 500 nm using vanillin–hydrochloride as blank. The standard curve was constructed by preparing catechin solutions at concentrations of 5–25 μ g/mL in methanol. TPAC contents were expressed as μ g catechin equivalents/mg of extract.

EARE, ACRE, ETRE, and MERE extracts were continued for further studies since they showed the better results.

In vitro assays

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The free radical scavenging activity of EARE, ACRE, ETRE, and MERE was evaluated according to the modified method described by Goyal *et al.*^[24] using DPPH. The free radical scavenging potentials are indicated by the degree of discoloration of DPPH caused due to the hydrogen-donating ability of the extract which was measured at 517 nm using methanol as blank. Quercetin was used as a reference standard. For control, only DPPH prepared in 95% methanol was taken without any extract.

The percentage of free radical scavenging capacity of the extracts was calculated using the following equation:

$$\text{DPPH radical scavenging effect (\%)} = (A_0 - A_1)/A_0 \times 100$$

where, A_0 : Absorbance of the control,

A_1 : Absorbance of the sample

The effectual inhibitory concentration of the sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotted between %inhibition (y-axis) and concentrations (x-axis).^[28]

Determination of ferric reducing power

The reducing power of CRR extracts (EARE, ACRE, ETRE, and MERE) was estimated by a reported method of Goyal *et al.*^[24] Absorbance was read at 700 nm using 0.2 M phosphate buffer (pH 6.6) as blank. Ascorbic acid was used as reference standard. The increase in absorbance of the reaction mixture indicated the increased reducing power of the samples.

Lipid peroxidation inhibition assay

The lipid peroxidation potential of extract was determined by TCA and TBA following the method of Okhawa *et al.*^[29] 200 mg of goat liver was homogenized with 10 mL of Tris-HCl buffer (40 mM, pH-7) and the homogenate was centrifuged for 10 min at 3000 rpm. Supernatant was collected. A volume of 0.5 and 1.0 mL aliquots of extract (1 mg/mL) was pipetted out into various tubes, and the volume was made up to 1 mL with Millipore water. 0.5 mL of supernatant was added to each tube, followed by addition of 200 μ L KCl (0.15 M), 1 mL $FeSO_4$ (15 mM), and 1 mL ascorbic acid (6 mM). The control was prepared without using the supernatant. The test tubes were incubated at 37°C for 1 h. 1 mL of TCA (10%) was added and the tubes were centrifuged at 3000 rpm for 20 min at 4°C. The supernatant in each tube was collected and treated with 1 mL TBA (0.8%). The test tubes were then heated at 90°C in water bath for 20 min and cooled. The absorbance of the TBA-malondialdehyde (MDA) complex was read at 532 nm after adding 2 mL of butanol. The percentage of lipid peroxidation potential by EARE, ACRE, ETRE, and MERE extracts was calculated as follows:

$$\% \text{ lipid peroxidation potential (MDA)} = [A_0 - A_1/A_0] \times 100$$

where, A_0 : Absorbance of the control

A_1 : Absorbance of the extract

Gas chromatography-mass spectrometry analysis of *C. rotundus* L. rhizome extracts

Gas chromatography-mass spectrometry (GC-MS) for ACRE extract was recorded with Thermo GC-Trace Ultra 5.0, Thermo MS DSQ II (Thermo Fisher Scientific, USA). TR 5-MS capillary standard nonpolar column with 30 m dimension, Id: 0.25 mm, 0.25 mm film was used. Helium gas was used as a carrier gas with flow rate of 1 mL/min.

Statistical analysis

All the experiments were carried out in triplicates and the results were expressed as mean \pm standard error of the mean. The data were statistically analyzed using Microsoft Office Excel 2007.

RESULTS

Yield of *C. rotundus* L. rhizome extracts

The yield of CRR extracts obtained after extraction with each solvent are given in Table 1. The highest yield of the extract was found to be in ETRE (22.72%) followed by MERE (3.22%). The lowest yield was obtained in PERE (0.133%).

Preliminary phytochemical screening of CRR extracts

HERE, PERE, and EARE extracts revealed the presence of alkaloids, carbohydrates, glycosides, and steroids. Flavonoids were not traced in CHRE extract. Saponins were present in ETRE, MERE, and AQRE whereas resins were present only in PERE and EARE extracts. CHRE showed the presence of tannins only. ACRE, ETRE, and MERE showed the presence of carbohydrates, glycosides, flavonoids, saponins, steroids, tannins, and phenols. Except HERE, PERE, and CHRE phenols were found in all the extracts [Table 2].

Quantitative phytochemical assay of *C. rotundus* L. rhizome extracts

Different extracts of CRR were quantitatively analyzed for TPC, TFC, and TPAC [Table 3]. According to the results, TPC of the CRR extract ranged from 0.0358 ± 0.002 to $118.924 \pm 5.946 \mu\text{g GAE/mg}$, TFC ranged from 7.196 ± 0.359 to $200.654 \pm 10.032 \mu\text{g QE/mg}$, and TPAC ranged from 13.115 ± 0.656 to $45.901 \pm 2.295 \mu\text{g CE/mg}$. ACRE showed the highest value of TPC, TFC, and TPAC. The lowest TFC and TPAC were observed in hexane extract whereas the lowest TPC was detected in PERE extract.

In vitro assays

2,2 diphenyl-1-picrylhydrazyl radical scavenging assay

Figure 1a indicated an increasing trend in DPPH radical scavenging activity with an increase in concentration of extract. It was found that ACRE showed highest scavenging activity compared to other extracts. Lower the IC_{50} value better is the extract. IC_{50} of the ACRE was found to be the lowest (0.901) and more effective than other extracts [Figure 1b]. The descending order of IC_{50} values was found to be EARE > MERE > ETRE > ACRE.

Reducing power of *C. rotundus* L. rhizome extracts

Ferric reducing power (FRP) assay determines the reducing power of the extracts. It was found that ACRE extract showed a greater reducing property compared to other solvent extracts [Figure 2].

Lipid peroxidation inhibition assay

Ascending order of lipid peroxidation inhibition was found as followed: ACRE > EARE > ETRE > MERE [Figure 3]. ACRE (23.65%) followed by

Table 1: Yield of *Cyperus rotundus* L. rhizome extracts after successive soxhlation

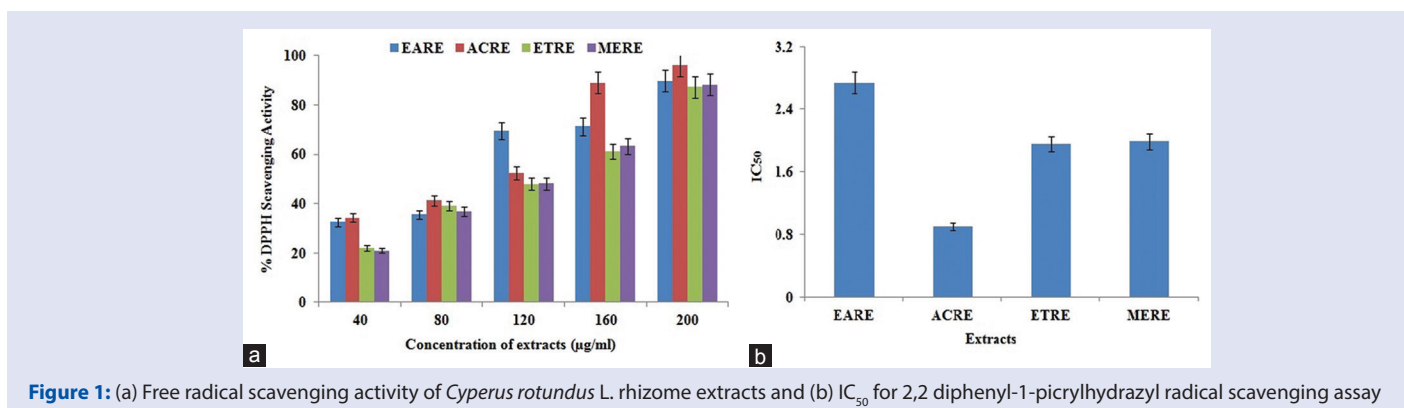
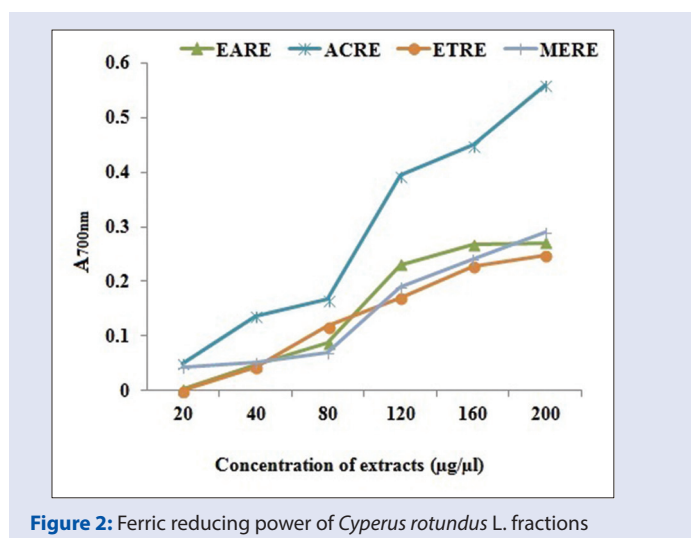
Solvent	Sample (g)	Extract (g)	Yield (%)
Hexane	25.00	0.4668(HERE)	1.87
Petroleum ether	24.61	0.0328(PERE)	0.133
Ethyl acetate	24.12	0.3248(EARE)	1.34
Chloroform	23.67	0.1857(CHRE)	0.78
70% acetone	23.43	0.4062(ACRE)	1.73
70% ethanol	23.03	5.2328(ETRE)	22.72
70% methanol	22.30	0.7170(MERE)	3.22
Water	22.02	0.3933(AQRE)	1.78

HERE: Hexane *C. rotundus* L. rhizome extract; PERE: Petroleum ether *C. rotundus* L. rhizome extract; EARE: Ethyl acetate *C. rotundus* L. rhizome extract; CHRE: Chloroform *C. rotundus* L. rhizome extract; ACRE: 70% Acetone *C. rotundus* L. rhizome extract; ETRE: 70% Ethanolic *C. rotundus* L. rhizome extract; MERE: 70% Methanolic *C. rotundus* L. rhizome extract; AQRE: Aqueous *C. rotundus* L. rhizome extract

Table 2: Preliminary phytochemical screening of *Cyperus rotundus* L rhizome

Plant constituents	HERE	PERE	EARE	CHRE	ACRE	ETRE	MERE	AQRE
Alkaloids	+	+	+	-	-	-	-	-
Carbohydrates	+	+	+	-	+	+	+	+
Glycosides	+	+	+	-	+	+	+	-
Flavonoids	+	+	+	-	+	+	+	+
Resins	-	+	+	-	-	-	-	-
Saponins	-	-	-	-	-	+	+	+
Steroids	+	+	+	-	+	+	+	-
Tannins	-	-	+	+	+	+	+	-
Phenols	-	-	+	-	+	+	+	+

+: Presence; -: Absence. *C. rotundus*: *Cyperus rotundus*; HERE: Hexane *C. rotundus* L. rhizome extract; PERE: Petroleum ether *C. rotundus* L. rhizome extract; EARE: Ethyl acetate *C. rotundus* L. rhizome extract; CHRE: Chloroform *C. rotundus* L. rhizome extract; ACRE: 70% Acetone *C. rotundus* L. rhizome extract; ETRE: 70% Ethanolic *C. rotundus* L. rhizome extract; MERE: 70% Methanolic *C. rotundus* L. rhizome extract; AQRE: Aqueous *C. rotundus* L. rhizome extract

**Figure 1:** (a) Free radical scavenging activity of *Cyperus rotundus* L. rhizome extracts and (b) IC₅₀ for 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay**Figure 2:** Ferric reducing power of *Cyperus rotundus* L. fractions

EARE (11.18%) extracts showed the highest inhibition as compared to other extracts.

Gas chromatography–mass spectrometry analysis of *C. rotundus* L. rhizome extracts

The detected compounds were 3,3,5,5,3',3',5',5'-Octamethyl-di-(delta-pyrazolinylidene), dimethyl-2- [o-(Ethoxycarbonyl) benzoyl]-1, 2-di hydro-1-iso quinolyphosphonate, 2-amino-cyclopentanemethanamine, cyclopentane, 1-nitro-2- (2-propenyl)-cis, i-Propyl-3- (phenylamino)-2- (phenylseleno)-3- (phenyl) propanoate, 1-cyclopropyl-2, 3-diazabicyclo [2.2.1] hept-2-ene. The 1 (2)-Acetyl-3 (5)-styryl-5 (3)- methylthiopyrazole was detected for the first time from the ACRE [Figure 4].

DISCUSSION

Different solvents are used to extract the secondary metabolites from plant materials. The most widely used solvents for extraction are water, ethanol, methanol, acetone, and solvent mixtures of different ratios with water, with or without acid.^[24,30-32] The extraction of the secondary metabolites or phenolic constituents is based on the solvent used and its polarity. In this study, CRR extract was prepared using eight solvents based on their increasing order of polarity. Since EARE, ACRE, ETRE, and MERE extracts showed the best quantitative results, they were used for *in vitro* antioxidant and free radical scavenging activity studies.

The phenolics being the major group of secondary metabolites, also act as primary antioxidants and free radical inhibitors. Flavonoid, a polyphenolic compound, is one of the most numerous phenolics and widely spread in the plant kingdom.^[33] Various reports have shown the diverse functions of flavonoid such as UV protection, disease resistance, pigmentation, and nitrogen fixation stimulation in the nodules.^[4,34,35] Proanthocyanidins, a subclass of the most complex flavonoids, are the nonpolar, condensed tannins, and polymer of flavan-3-ols. ACRE extract contained the highest TPC, TFC, and TPAC, whereas PERE contained the least TPC and HERE contained the lowest TFC and TPAC. The principle antioxidants such as 3-hydroxy-4-methoxy-benzoic acid, galloylquinic acid, ferulic acid, quercetin, luteolin, afzelechin, and catechin are the phenolic compounds present in *C. rotundus* L. as reported by Kilani-Jaziri *et al.*^[36] Boeing *et al.*^[37] have reported 70% acetone as an efficient solvent for extracting phenolic compounds which is similar to the results obtained in the present study. Singh *et al.*^[10] had reported that total oligomeric flavonoid extract of *C. rotundus* L. possessed a broad spectrum of pharmacological properties such as antioxidant, antimutagenic, antigenotoxic, antimicrobial, anticancer, and neuroprotective properties.

Further, the effect of CRR on free radical scavenging was investigated using DPPH assay. Among different solvent extracts, ACRE showed better and higher radical scavenging activity than other extracts. The increased scavenging activity of the ACRE extract may be accredited to its potent hydrogen donating ability.^[28] The IC₅₀ values of the EARE, ACRE, ETRE, and MERE were 2.7, 0.8, 1.9, and 2, respectively. The scavenging activity is inversely proportionate to the IC₅₀ value.^[6] The lowest IC₅₀ value of ACRE indicated its high free radical scavenging activity, indeed an indication of high antioxidant activity. *In situ*, Viuda-Martos *et al.*^[38] have shown a linkage between polyaromatic hydrocarbon cations and carcinogenesis. Thus, ACRE can also exhibit

in vivo free radical scavenging activity similar to *in vitro* DPPH free radical scavenging activity.

FRP assay is a reliable and simple method for determining the reducing power of antioxidants. In the presence of an antioxidant, potassium ferricyanide and ferric chloride are converted into potassium ferrocyanide and ferrous chloride, respectively. ACRE showed the highest reducing power than other solvent extracts. The reducing power is dependent on the concentration of the extracts. Phenolic content (118.9 µg GAE/mg extract)

Table 3: Quantitative phytochemical analysis of rhizome of *Cyperus rotundus* L. extracts

Extract	TPC (µg GAE/mg)	TFC (µg QE/mg)	TPAC (µg CE/mg)
HERE	0.896±0.045	7.196±0.359	13.115±0.656
PERE	0.0358±0.002	32.429±1.621	14.426±0.721
EARE	55.627±2.781	126.822±6.341	22.131±1.106
CHRE	1.075±0.054	33.364±1.668	17.213±0.861
ACRE	118.924±5.946	200.654±10.032	45.901±2.295
ETRE	57.383±2.869	109.065±5.453	25.0±1.250
MERE	53.584±2.679	93.177±4.658	19.672±0.983
AQRE	4.050±0.202	26.822±1.341	18.032±0.902

Values represented as Mean±SD of triplicate determination. *C. rotundus*: *Cyperus rotundus*; HERE: Hexane *C. rotundus* L. rhizome extract; PERE: Petroleum ether *C. rotundus* L. rhizome extract; EARE: Ethyl acetate *C. rotundus* L. rhizome extract; CHRE: Chloroform *C. rotundus* L. rhizome extract; ACRE: 70% acetone *C. rotundus* L. rhizome extract; ETRE: 70% ethanolic *C. rotundus* L. rhizome extract; MERE: 70% Methanolic *C. rotundus* L. rhizome extract; AQRE: Aqueous *C. rotundus* L. rhizome extract; GAE: Gallic acid equivalent; QE: Quercetin equivalent; CE: Catechin equivalent; SD: Standard deviation; TPC: Total phenolic content; TFC: Total flavonoid content; TPAC: Total proanthocyanidin content

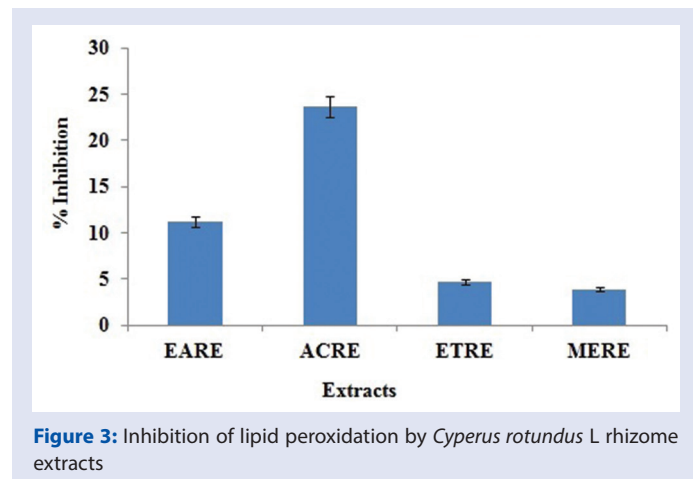


Figure 3: Inhibition of lipid peroxidation by *Cyperus rotundus* L rhizome extracts

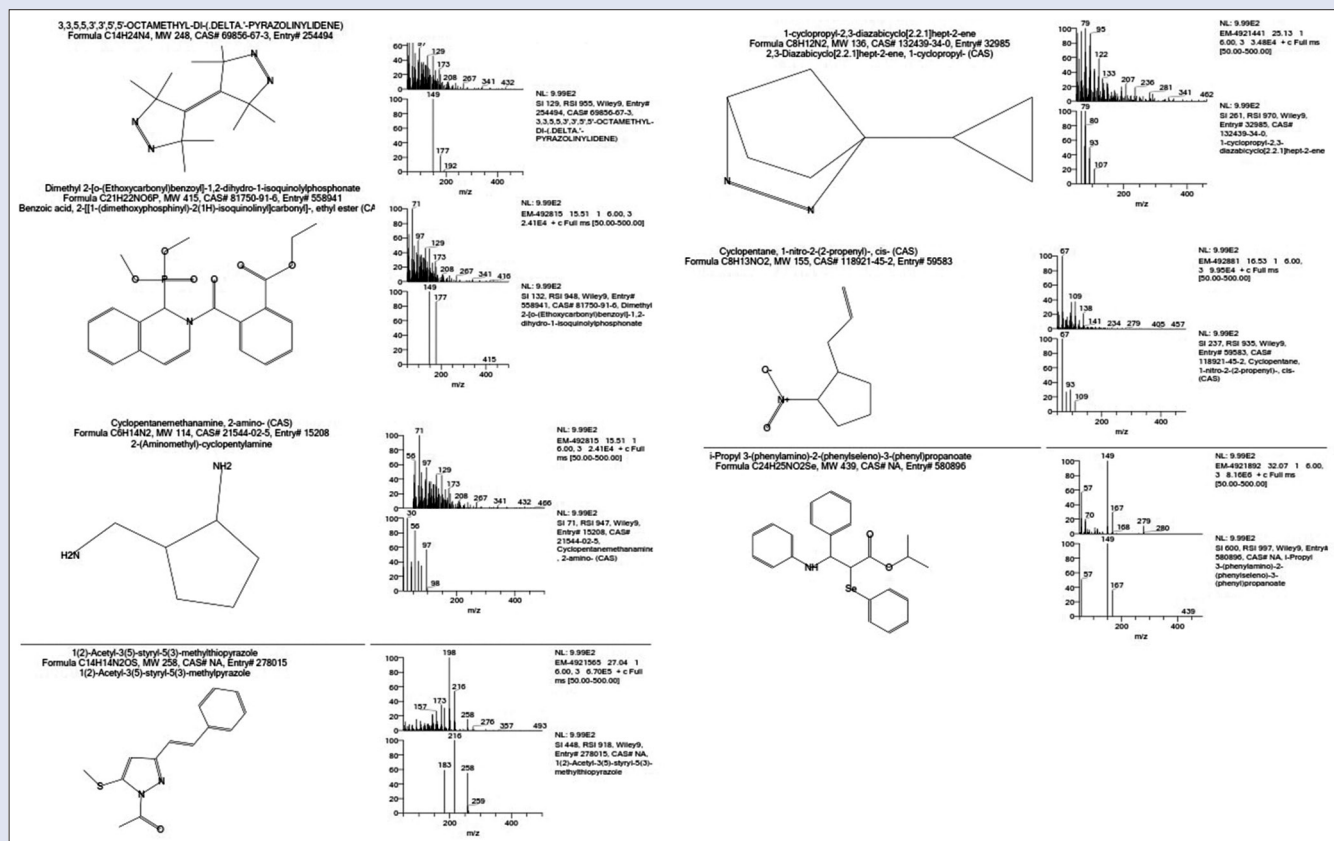


Figure 4: Gas chromatography-mass spectrometry analysis of phytochemicals from acetone rhizome extract of *Cyperus rotundus* L

may be responsible for the ferric reducing ability of ACRE extract in accordance with Siddhuraju *et al.*^[39] Goyal *et al.*^[31] reported that the FRP could be mainly due to the antioxidative compounds.

Total flavonoids contents (200.6 µg QE/mg extract) were found to be more than phenols in this study. Since flavonoids are polyphenols, it is usually observed that the phenolic content is greater than the flavonoid content in the given plant extract. However, sometimes, flavonoid content may be greater than that of the phenols. This may be due to the formation of complex ring structures between various phenolic compounds which may not be measured in the assay for phenols and thus goes unaccounted. These results are seen to be in compliance with the results as reported by Murugan *et al.*^[40]

Lipid peroxides formed as a product of oxidative stress in the body are unstable reactive products. MDA is frequently used as an indicator of lipid peroxidation. Under conditions of oxidative stress, the reactive oxygen species produced, cause lipid peroxidation in cell membranes, particularly through oxidation of polyunsaturated fatty acids.^[4] ACRE exhibited a stronger inhibitory effect on lipid peroxidation, while MERE exhibited a lower inhibitory effect.

GC-MS analysis of ACRE has revealed the presence of alkaloids such as 3,3,5,5,3',3',5',5'-Octamethyl-di-(delta-pyrazolinylidene), dimethyl-2-[o-(Ethoxycarbonyl) benzoyl]-1,2-di hydro-1-iso quinolylphosphonate, 2-amino-cyclopentanemethanamine, cyclopentane, 1-nitro-2-(2-propenyl)-cis,i-Propyl-3-(phenylamino)-2-(phenylseleno)-3-(phenyl)propanoate, 1-cyclopropyl-2,3-diazabicyclo [2.2.1]hept-2-ene. The 1(2)-Acetyl-3(5)-styryl-5(3)-methylthiopyrazole was detected for the first time from the CRR. There are no reports found associated with all these compounds previously. Ambiguity found in earlier reports prompted this study on the antioxidant activity of CRR. Hence, it is very tricky to compare our findings with that of prior studies. The consumption of CRR may prevent human ailments associated with free radicals.

CONCLUSION

This work is an effort to understand the medicinal potential of *C. rotundus* L. cultivated in Karnataka region, India, for the first time. Successive soxhlation of *C. rotundus* L was carried out to identify the suitable solvent system for the extraction of numerous secondary metabolites and acetone was found to be a better solvent. The crude extract of the rhizome of CRR was observed to harbor substantial antioxidant activity, and the ACRE extract showed remarkable activity. The novel compound 1 (2)-Acetyl-3 (5)-styryl-5 (3)-methylthiopyrazole, an alkaloid is reported for the first time. This work could provide a better understanding of phytochemicals present in *C. rotundus* and its antioxidative properties.

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Conflicts of interest

There are no conflicts of interest.

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