Original Article

Phytochemical and Pharmacological Exploration of *Cyperus articulatus* as a Potential Source of Nutraceuticals and Drug Ingredients

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ABSTRACT

Introduction: *Cyperus articulatus* rhizome has been used in folk medicine by different inhabitants belonging to tropical and subtropical regions. But its metabolite profile and potential pharmacological and food applications were hardly explored. Evaluation of biological activities of *Cyperus articulatus* metabolites was the objective of the present study. **Materials and Methods:** *In vitro* biological studies concerning radical scavenging, reducing activity, food (meat and β-carotene) protection, biomolecule (DNA and Protein) protection and Acetylcholinesterase inhibitory activity were carried out for the rhizome extracts. Chemical constituents of the bioactive rhizome extract were analyzed through HRLC-MS/MS. **Results:** The rhizome acetone extract showed the highest antioxidant activity and protected DNA and protein from degradation at the lowest concentrations compared to all the six different solvent extracts tested. It significantly inhibited β-carotene bleaching, controlled the TBARS values during meat oxidation and significantly inhibited the Acetylcholinesterase enzyme. The major compounds detected in HRLC-MS/MS were dihydroquercetin, mycophenolic acid, embelin, quercetrin, meptazinol, koparin-2-methyl ether, venpocentine along with other phenolics and polyhydroxy compounds. **Conclusion:** The study explored *Cyperus articulatus* rhizome as a pharmacologically important source for nutraceuticals and drug ingredients and suggested further safety and efficacy studies of the detected metabolites.

Key words: Secondary metabolites, Food model, DNA protection, Enzyme inhibition, Dihydroquercetin.

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INTRODUCTION

The potential of plants to prevent or cure many diseases of humans and animals are identified long back in history, and its utilization evolved differently in different parts of the world. Similarly, Indian traditional medications were derived from the Atharva Veda, which mentions many herbs and plant species against different ailments.[1] Reactive oxygen species (ROS) and other free radicals cause oxidative reactions such as lipid oxidation, protein oxidation and nitration, DNA damage, alteration of function of cellular organelles and enzyme dysfunction.[2] Again some neurological disorders like Parkinson's and Alzheimer's are also initiated by free radical-induced oxidative damages.[3] Chlorogenic acid, caffeic acid, carotenoids, flavonoids and tocopherols are natural antioxidants compounds that protect cells and cellular components against oxidative stress-related diseases and disorders.[4,5] Similarly, secondary metabolites of plant origins have been reported to control the target enzymes' expression with lesser adverse effects.[6] Among the neuroprotective drugs, many plant-based metabolites are well-reported and preferred over synthetic drugs to manage the adverse effects.[7]

Cyperaceae family having high distribution in the world's tropical and subtropical regions are generally long grass-like in appearance and are usually found in aquatic habitats or marshy soil.[8] The essential oil from *Cyperus rotundus* has been extensively studied for its antioxidant, bio-molecular protection, and antimicrobial properties and have practical applications in the pharmaceuticals and cosmetics industries.[9,10] *Cyperus* species plants possess diverse secondary metabolites belonging to flavonoid, alkaloid and terpenoid categories. Rhizome extracts of some *Cyperus* plants were previously reported to have many health-beneficial properties.[9-11] *Cyperus articulatus* was not studied much for its biological or pharmacological importance as compared to other *Cyperus* species. Traditionally the use of *Cyperus articulatus* rhizome to treat malaria, epilepsy and dysentery by inhabitants of different countries was reported.[12] The extract of the matured rhizome was studied to have high α-Glucosidase inhibiting activity indicating the occurrence of potential antidiabetic compounds.[13] The rhizome essential oil having major constituents such as monoterpenoids and sesquiterpenoids were extensively studied to have antimicrobial, anticonvulsant, anti-onchocerca and anti-malaria properties.[14-16] However, the studies are limited to the essential oil, composition and antimicrobial studies. The present work is aimed at exploring the phytochemical importance of rhizome bioactive metabolites of *Cyperus articulatus* through a range of *in vitro* assays and validate it as a source of food antioxidants, nutraceuticals, natural food preservative, biomolecular protecting ingredients and enzyme (Acetylcholinesterase) inhibitors and to explore the potential application of the rhizome in food, pharmaceutics and medicine.

MATERIALS AND METHODS

Plant material and Metabolite extraction

Naturally grown *Cyperus articulatus* plants were collected from The Cauvery basin, Karnataka region, India. The plant identity was confirmed at the Botanical Survey of India (BSI), Kolkata, India. For extraction of metabolites, Soxhlet apparatus was used where the dried matured rhizomes in course powdered form were solvent-extracted with six solvents (order: hexane, chloroform, ethyl acetate, acetone, methanol, water). All these rhizome extracts (REs) were dried using a Rota evaporator (Buchi R-205, Switzerland). The initial sample solutions and their double dilutions (in mg/mL) were prepared in methanol. Only the water extract stock was prepared in water. For enzyme inhibition and biomolecule protection experiments, all the extracts were diluted in Millipore water.

Antioxidant activity

Total antioxidant activity (Phosphomolybdenum method)

Different REs and ascorbic acid standard solutions were added to ammonium molybdate reagent solution in a ratio of 1:10 in test tubes. The solutions were incubated at 95°C for about 1.5 h. Then each solution 200μL was transferred to a 96-well microtiter plate (WMP). A spectrophotometric study (at 695 nm) of samples was carried out to express the total antioxidant activity as ascorbic acid equivalents (μg AE/mg crude extract).[17]

DPPH radical scavenging

10μL RE and the standards Butylated hydroxyanisole (BHA) and Gallic acid (GA) were added to 250μL DPPH (0.2mM) solution in methanol in a 96-WMP. The absorbance of 517 nm was recorded after incubation in a dark chamber at 30°C for 15 min.[18] The DPPH radical scavenging potential was calculated using the equation as follows and the results were represented in IC_{50}.

$$I\,(\%) = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

ABTS cation radical scavenging

To 200μL of ABTS solution (2.5mM $K_2S_2O_4$ and 7mM ABTS mixture) in a 96-WMP, 10μL of RE was added. Similar samples were made for standard GA and BHA. The absorbance was recorded at 734 nm after incubation for 30 min at 30°C.[19] The IC_{50} values of crude extracts were calculated as per the above equation.

Superoxide anion radical ($O_2^{-\cdot}$) scavenging

A reaction mixture was prepared by mixing riboflavin (10μL, 0.1mg/mL), phosphate buffer (100μL, 50mM, pH 7.8), methionine (50μL), Nitro blue tetrazolium (NBT) (5μL, 1mg/mL) and EDTA (10μL, 12mM) in a 96-WMP. RE (25μL) and BHA were added to the reaction mixture. Then the 96-WMP containing the whole solution mixtures was illuminated under a 20Wt fluorescent lamp for 15 min. A blank for this experiment was maintained as an unilluminated reaction mixture containing all the reagents. The absorbance was recorded at 560 nm for both the sample and blank.[17] The scavenging results of the samples were expressed in IC_{50} values.

Cupric ion reducing antioxidant capacity (CUPRAC) assay

A reaction mixture containing 10mM $CuCl_2$ (60μL), 7.5mM neocuproine (60μL,) in 95% ethanol and 1 M pH 7.0 NH_4Ac buffer (60μL,) was added to 25μL RE/BHA in a 96-WMP. A blank was maintained similarly except adding $CuCl_2$. The solution mixtures were incubated for 30 min at 30°C before the absorbance was recorded at 450 nm.[17] The results were reported as μg BHAE/mg crude extract.

Ferric ion reducing antioxidant power (FRAP) assay

In a 96-WMP 10μL RE/GA was added to 240μL of FRAP reagent as methods earlier.[20] The sample solutions were incubated for 30 min at 37°C before the absorbance was read at 593 nm. The results are expressed as μg GAE/mg of crude extract.

Metal chelating activity

$FeCl_2$ solution (2mM, 10μL) was added to 200μL RE/EDTA in a 96-WMP. After 5 min incubation, 5mM ferrozine (20μL) was added to initiate the reaction. Similarly, a blank was maintained without adding ferrozine. The absorbance of solutions was read at 562 nm after 10 min incubation of the mixtures at 30°C.[17] The metal chelating potential was expressed as EDTA equivalents (μg EDTAE/mg crude extract).

Antioxidant activity in food and biological model systems

Antioxidant activity in a β-carotene linoleic acid model system

To 2 mL of β-carotene solution (0.5mg/mL in chloroform) in a round bottom flask, Tween 40 (400mg) and of linoleic acid (40mg) were added. The solution was mixed properly and then dried in a vacuum evaporator at 40°C to remove chloroform. Then 50 mL of distilled water was added to the mixture and the whole sample was vigorously shaken. 3.5 mL of this sample solution was taken in a test tube and 500μL of RE (100ppm and 200ppm GA equivalent phenol) was added to it. After 15 min incubation at 50°C, the absorbance was read at 470 nm in 15 min time interval up to 105 min.[21] The standard, BHA was used as the positive control. The reaction mixture devoid of both RE and standard was taken as the negative control. The antioxidant activity or inhibition % was determined as per the following equation:

$$\text{Antioxidant activity (\%)} = 1 - \frac{(S_0 - S_t)}{(C_0 - C_t)} \times 100$$

(S_0 and S_t are the absorbances of test samples measured at zero min and after each 15 min reading respectively; C_0 and C_t are the absorbances of the control at zero min time and after incubation, respectively).

Antioxidant activity in a meat model system (TBARS value)

Ground Pork (40 g), 10 mL of Millipore water and 1 mL of RE (150ppm or 300ppm GA equivalent phenol) were mixed properly, homogenized and cooked at 80°C in a water bath for 40 min. BHT was taken as the positive control. A blank was prepared by adding all the reagents except the RE and BHT. Once the cooked meat samples were cooled and brought to room temperature, the contents were again homogenized and kept in zip-lock plastic bags. The contents were kept for 7 days in a cold chamber at 4°C.[22] Meat sample supernatants were read at 532 nm on 0^{th}, 3^{rd}, 5^{th}, and 7^{th} days for determining the number of oxidative products using the TBARS test as described below.[17,23]

Meat sample (1g) and 2 mL trichloroacetic acid (10%, w/v) were mixed in a centrifuge tube and vortexed. Then 2 mL of 0.02 M TBA reagent was added to it. The whole sample mixture was centrifugated for 10 min (at 3000g). The supernatants were filtered (Whatman-3) and warmed at 95°C in a water bath for 45 min. Once the temperature of the sample was brought down to room temperature, the pink MDA-TBA complex absorbance was recorded at 532 nm. TBARS values were calculated in the presence and absence of RE and the results were interpreted using 1,1,3,3-tetramethoxypropane standard curve. The activities were expressed as mg MDA equivalents/kg samples.

DNA protection assay

RE activity against Fe(II) assisted OH radical-induced DNA degradation was tested using agarose gel electrophoresis of CT DNA.[17] 250ng of DNA in 10μL TE buffer (pH 8) was added to 10μL of RE and vortexed to mix. The sample was then incubated for 5 min at 30°C. 8μL Fenton's reagent (0.5mM $FeSO_4$ and 50mM H_2O_2) was then mixed and the sample mixture was kept for 2 h incubation at 30°C. The CT DNA in buffer without adding RE and Fenton's reagent was taken as the positive control. In 1% agarose gel the degraded/protected DNA was electrophoresed followed by staining with Ethidium bromide. Then with UV illumination, the DNA degradation pattern was photographed/documented.

Protein oxidation prevention

BSA (0.5mg/mL) solution was prepared in pH 7.3 phosphate buffer and was mixed with 50mM AAPH (a peroxyl radical generating species) in the presence or absence of REs as described in the previous literature.[17] After 2 h incubation at 30°C, the SDS-PAGE electrophoresis was carried out for the protein samples. The SDS-PAGE gel was stained with 0.2% Coomassie Brilliant Blue R-250 and then disdained with methanol-acetic acid before it was documented.

Enzyme (Anticholinesterase) inhibitory assay

An acetylcholinesterase (AChE) inhibition study was carried out spectrophotometrically.[24] First 20μL RE and 20μL of AChE (1 U/mL) were mixed in 150μL of phosphate buffer (0.1 M) and incubated for 10 min at 30°C. DTNB solution10mM (15μL) was added, followed by the addition of 14mM ATCI (15μL). The mixture was incubated for 20 min at 30°C before the absorbance of the sample read at 412 nm. The AChE inhibitory activity was plotted against RE concentrations.

HR-LCMS/MS analysis of RE

HR-LCMS analysis of the acetone extract was performed using 6200 series Q-TOF (Q-Exactive Plus Biopharma-High Resolution Orbitrap) mass spectrometer coupled to HPLC equipped with UV–Vis detector. Hypersil gold 3-micron 100 x 2.1 mm column was used. The mobile phases were 0.1% formic acid in water (A) and 90% acetonitrile in water with 0.1% formic acid (B).[25] The LC conditions were set following ac method described in previous literature.[13] 5% B for first 3 min, then a linear increase to 20%, 40%, 50% and 95% B during 3–25, 25–40, 40–55 and 55–63 min respectively. Injection volume was 8μL and a flow rate of 0.4 mL/min was maintained. Both positive and negative mode analysis was done with scan resolution 30,000 and the mass (*m/z*) range in 50–1,500.

Statistical Analysis

The experimental results were analyzed in one-way ANOVA using SPSS V16 software (SPSS Inc., Chicago Ill., USA). The significance was as obtained by Tukey's test (p<0.5). All the tests were performed in triplicates and expressed in Mean ± Standard deviation.

RESULTS

Antioxidant activities of rhizome extracts

The antioxidant potentials of REs extracted using ethyl acetate, acetone, methanol and water are represented in Table 1. Acetone extract showed the significantly lowest IC_{50} values of 12.15μg/mL, 16.35μg/mL and 83.25μg/mL for DPPH, ABTS and O_2^- radicals, respectively in comparison to other RE's tested. Standard GA showed significantly lowest IC_{50} values for all three assays, whereas the BHA activity was almost equivalent to the acetone extract.

Acetone extract showed the highest reducing power of 407.2μg BHAE/mg extract in CUPRAC assay, 155.8μg GAE/mg extract in FRAP assay, and total antioxidant activity (311.4μg AEs/mg extract). Similarly, in the case of the metal-chelating assay acetone extract showed the significantly highest chelating activity of 44.96μg EDTA/mg extract followed by water extract (27.57μg EDTA/mg extract) (Table 1). Surprisingly, the aqueous extract also showed significant metal chelating activity though it contains the lowest total phenol (4.14μg GAE/mg extract) and flavonoid content (0.8μg QCTE/mg extract), which indicates the presence of some non-phenolic metal chelators.

Activities in the food model systems

The acetone extract protected the β-carotene up to 91.35% (at 200ppm) and 72.42% (at 100ppm) from the damaging effect of free radicals and hydroperoxides compared to the protecting effect of control (11.51% protection) and BHA (85.38% protection) over 120 min incubation period (Table 2). Similarly, in the Ground pork meat model, acetone extract decreased the level of MDA to 2.17 and 0.485mg MDA eq./kg at 150ppm and 300ppm, respectively, which was significantly lesser than BHT (4.25 MDA eq./kg) and control (8.72 MDA eq./kg) (Table 3). The 0^{th} and 7^{th} day results (Supplementary Figure: Figure S1) showed the protection of the meat sample by the rhizome acetone extract by inhibiting MDA-TBA chromogen formation.

Results are mean values of three determinations ± SD. Means in a column sharing the same roman superscript are significantly (P < 0.05) different from one another.

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Activities in the biological model systems

The ability of acetone extract to protect DNA from and metal-assisted hydroxyl radical is shown in Figure 1. It shows complete protection of DNA at 11.16μg/mL (lane 12) compared to the control sample, which was completely degraded by Fenton's reagent action (lane 2). Similarly, the acetone extract showed protein oxidation

Table 1: Total phenolics and flavonoids, and antioxidant activities of different extracts of *Cyperus articulatus* rhizome and standards.

Rhizome extracts and standards	Total Antioxidant activity$^{\Psi}$	DPPH*	ABTS*	Superoxide*	CUPRAC$^{\#}$	FRAP$^{\$}$	Metal chelating$^{@}$
EA	115.2±13.58^{b}	40.27±3.85^{c}	25.98±3.2^{d}	95.92±5.21^{b}	119.5±8.52^{b}	96.43±4.73^{b}	12.25±3.2^{c}
AC	311.4±15.34^{a}	12.15±2.32^{b}	16.35±2.15^{c}	83.25±4.56^{a}	407.2±10.46^{a}	155.8±8.91^{a}	44.96±3.56^{a}
ME	89.28±10.36^{c}	98.82±8.32^{d}	96.33±9.15^{e}	245.1±21.56^{c}	23.45±4.85^{c}	22.1±3.24^{c}	14.87±2.58^{c}
WA	29.58±6.92^{d}	NA	NA	NA	NA	NA	27.57±6.84^{b}
GA	ND	2.56±0.54^{a}	2.14±0.35^{a}	ND	ND	-	ND
BHA	ND	10.84±1.5^{b}	9.857±1.22^{b}	80.15±4.48^{a}	-	ND	ND

$^{\Psi}$µg AE/mg extract *IC_{50} in µg/mL; $^{\#}$µg BHAE/mg extract; $^{@}$µg EDTAE/mg extract;; GA: Gallic acid; BHA: Butylated hydroxy anisole; ND-Not determined, NA: No/Little activity measured; EA: Ethyl Acetate; AC: Acetone; ME: Methanol; WA: Water.
Note: Activity of Hexane and Chloroform extracts were not shown because their activity were not significantly different from controls.

Table 2: Inhibitory effect of acetone extract of *Cyperus articulatus* and standard (BHA) against β-carotene oxidation in a β-carotene-linoleate model system at 50°C for 2 h.

Sample	% Inhibition
Control$^{\#}$	11.51 ± 1.12^{c}
Acetone extract (200 ppm)*	91.35 ± 4.82^{a}
Acetone extract (100 ppm)*	72.42 ± 4.75^{b}
BHA (200 ppm)	85.38 ± 3.40^{a}

*The ppm values in parenthesis for Rhizome extract indicates GA equivalent phenol.
$^{\#}$ Control was maintained without the addition of antioxidants.

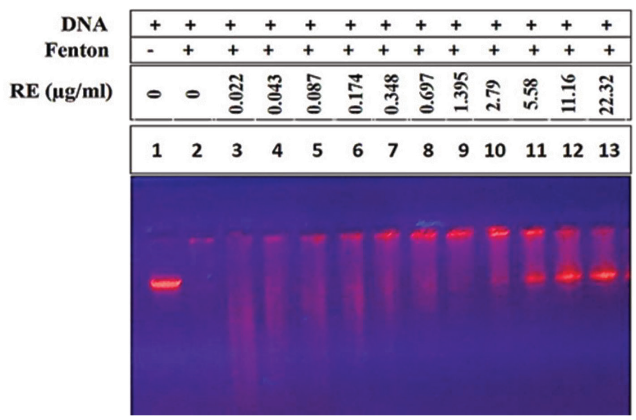

Figure 1: Visualization of the damage induced by hydroxyl radicals on genomic DNA in the presence and absence of acetone extracts from *Cyperus articulatus* by agarose gel electrophoresis.

Lane 1. DNA incubated without Fenton's reagent; Lane 2. DNA incubated with Fenton's reagent; Lanes 3-13, DNA incubated with Fenton's reagent in the presence of 0.022, 0.043, 0.087, 0.174, 0.348, 0.697, 1.395, 2.79, 5.58, 11.16 and 22.32 µg/mL of Acetone extract respectively (Final concentrations).

Table 3: TBARS values (mg MDA eq. per kg) in a meat model system over a 7-day period in presence of acetone extract of *Cyperus articulatus* and standard (BHT).

Sample	Storage Period (Days)			
	0	3	5	7
	TBARS values (mg MDA eq. per kg)			
Control$^{\#}$	1.17± 0.00^{c}	5.53± 0.21^{c}	6.99± 0.24^{d}	8.72± 0.30^{d}
Acetone extract (300 ppm)*	0.04± 0.00^{a}	0.16± 0.01^{a}	0.36± 0.02^{a}	0.48± 0.04^{a}
Acetone extract (150 ppm)*	0.12± 0.01^{b}	0.68± 0.20^{b}	1.67± 0.09^{b}	2.17± 0.06^{b}
BHT (300 ppm)	0.13± 0.01^{b}	0.55± 0.02^{b}	2.52± 0.06^{c}	4.25± 0.06^{c}

*The ppm values in parenthesis for Rhizome extract indicates GA equivalent phenol.
$^{\#}$ Control was maintained without the addition of antioxidants.

disease, neurodegenerative diseases, aging, hypertension and many metabolic disorders.[2,3,26] A strong correlation has been established between antioxidant properties and total phenolic and flavonoids in several plants and their products. The plants like *Cyperus alternifolius*,

prevention at 166.4µg/mL (Figure 2) against AAPH induced radical reaction. In the present study, among all the extracts, acetone extract showed a significant acetylcholinesterase inhibition with an IC_{50} value of 25.22µg/mL (Figure 3).

DISCUSSION

Antioxidant properties and application in food and biological models

Antioxidant phyto-molecules are reported to protect biomolecules against the ROS and other free radicals generated in the body that leads to oxidative stress-related diseases like cardiovascular diseases (CVDs), chronic obstructive pulmonary disease, chronic kidney

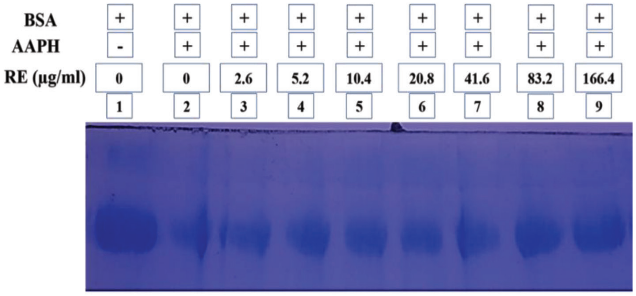

Figure 2: The protective effect of *Cyperus articulatus* rhizome acetone extract on AAPH (50 mM) induced protein oxidation of BSA analyzed by SDS polyacrylamide gel electrophoresis.
Lane 1: BSA incubated without AAPH. Lane 2: BSA with AAPH, Lane 3 to 9: BSA with AAPH in presence of 2.6, 5.2, 10.4, 20.8, 41.6, 83.2, and 166.4 μg/mL, of extract respectively.

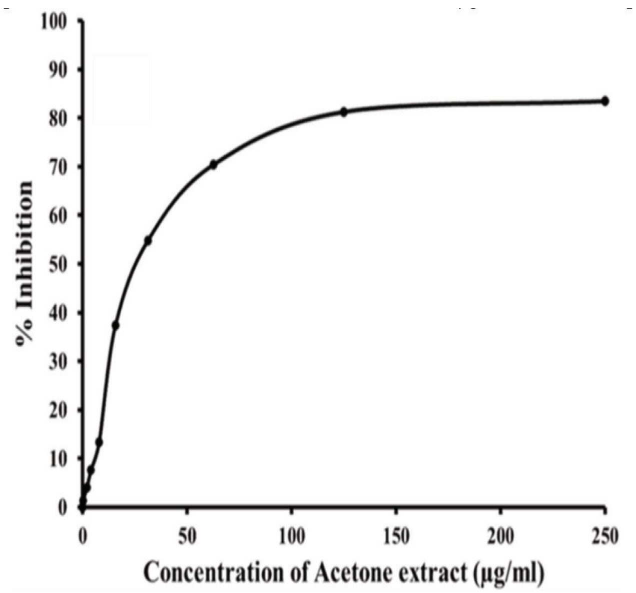

Figure 3: Acetylcholinesterase inhibition by acetone extract of *Cyperus articulatus* with increasing concentrations of extract. (Mean values of three data sets were taken for making plot).

Cyperus rotundus and *Canna indica* are studied and found that the phenolic compounds are the basis on which the antioxidant power (radical scavenging, DNA protection, metal ion reduction, peroxide and nitrous oxide scavenging, etc.) of the extracts correlated with the test results.[27,28]

The acetone extract showed the highest radical scavenging property comparable with the standard BHA indicating high bioactive phenolics in the RE. The reducing power against oxidized metal ions and protecting effects on the biomolecules (DNA and protein) in different drastic mediums have supported the health-beneficial antioxidant effects of the rhizome metabolites. The acetone extract of *Cyperus articulatus* was reported in our earlier study to have the highest phenolics (207.5μg gallic acid equivalent phenol in 1mg extract) and flavonoids (105.6μg quercetin equivalent phenol in 1mg extract) compared to other solvent extracts when extracted in different solvents of increasing polarity order.[13] Thus, the activities are in good agreement with the phytochemical composition in each extract. The oxidative damage of DNA leads to cancer initiation, and this damage is usually caused by OH radicals (which also arise due to peroxide cleavage).[29,30] The present study is the first to report the activity of rhizome extract of *Cyperus articulatus* in protecting the peroxide-induced radical-mediated DNA damage and could, therefore, be used in cancer prevention studies. Earlier studies supported the role of natural antioxidants like phenolics and flavonoids in countering the AAPH radical and thus act against hemolysis, protein oxidation and lipid peroxidation.[31] Similarly, the high radical scavenging activity due to the phenolics and flavonoids in the acetone extract could have protected the BSA protein from oxidation by AAPH radicals. Major compounds identified in acetone extract through HR-LCMS/MS were quercetrin, dihydroquercetin, mycophenolic acid, meptazinol, c16-sphinganine, deoxyelephantopin, phytosphingosine, colforsin, venpocentine along with several other phenolics and non-phenolic compounds (Supplementary Figure: Figure S2 and S3). Some of the compounds were previously isolated from different sources and reported for potential biological activities (Table 4).

The antioxidant properties of the extracts reported above may be attributed to phytochemical constituents' presence endowed in them. The HR-LCMS/MS analysis of acetone extract (Supplementary Figure: Figure S2 and S3) revealed the presence of many compounds which were reported to be of antioxidant character (Table 4). The compounds such as quercetrin, dihydroquercetin, mycophenolic acid, monobenzone, embelin, meptazinol, and koparin-2'-methylether along with other metabolites, possibly play a major role in radical scavenging, metal-reducing and metal chelating activity of acetone extract.

Food antioxidants control the human endogenous free radicals. The antioxidant additives also protect the food from spoilage. In food conservation research, natural food preservatives have been attracting pharmaceutical researchers for food conservation technology.[32] The usage of preservatives, such as benzoic acid and sulphites, causes allergies. Nitrites, nitrosamines, BHA and BHT were reported to be the cause of cause carcinogenicity when used as food additives or preservatives.[33] The food

Table 4: List of the major secondary metabolites identified through HRLC-MS/MS in Acetone extract of *Cyperus articulatus* rhizome and their reported biological importance.

Secondary Metabolites [mw]	Rt (min)	Biological Importance/Activities	Reference(s)
Monobenzone* [200.09]	1.0	Anti-melanoma immunity	38
Monoacetyldapsone (MADDS)* [290.07]	6.2	Anti-leprosy drug	39
Stearic acid* [356.12]	28.5	α- Glucosidase inhibitor	40,41
Embelin* [294.18]	42.2	Drug against some chronic disease	42
Mitotane* [317.95]	42.1	Adrenolytic and anti-cortisolic drug	43
Chloramphenicol 3-acetate* [364.02]	42.5	Antibacterial and anticancer agent	44
Oxprenolol** [265.17]	1.0	β1-selective blocker	45
Melibiose** [342.12]	1.1	Phenolic compound (possible antioxidant)	
Racepinephrine** [183.09]	1.2	Racepinephrine (https://www.drugbank.ca/drugs/DB11124)	--
3-isobutyl-1-Methylxanthine (IBMX)** [322.11]	3.8	A nonspecific cyclic nucleotide phosphodiesterase inhibitor	46
Marmesin** [246.09]	7.7	Biologically active marker and analogous of Coumarin	47
Quercetrin* [448.1]	8.5	Glycoside formed from the flavonoid quercetin, possible antioxidant and hypoglycemic agent	
Dihydroquercetin** [304.06]	8.9	Antioxidant, α-glucosidase inhibitor, Enhances the health-promoting benefits of vitamin C	48–51
5-O-Methylvisamminol** [290.1]	9.1	Antipyretic, analgesic, and anti-inflammatory properties	52
Meptazinol** [233.18]	10.1	Bioactive metabolite: Morphine Cholinergic Simulation	53
2,4,7-tridecatrienal** [192.15]	10.7	Potential antifungal agent	54
Deoxyelephantopin** [344.12]	11.6	Antitumor agent, Wound healing property, multifunctional agent	55–57
Gemfibrozil** [250.16]	12.3	Possible drug against diabetic, antimicrobial property, platelet enhancing effect and multifunctional agent	58–61
C16 Sphinganine** [273.26]	14.4	Antibacterial activity, bioactive metabolite of many natural sources having health beneficial effects	62,63
Phytosphingosine** [317.29]	16.4	Anti-microbial and anti-inflammatory activity	64
Colforsin** [410.23]	16.8	Anti-inflammatory property	65
Dihydrosphingosine** [301.3]	17.4	Sphingonoid compound	--
N-(2-hydroxyethyl) palmitamide** [299.28]	24.9	Anti-inflammatory activity	66
Vinpocetine** [350.2]	25.2	Anti-inflammatory activity	67

* HR LCMS in Negative mode
** HR LCMS in Positive mode

model system results showed that the extract efficiently protected the meat even better than the standard (BHT) after 7th day. The significant inhibition of β-carotene bleaching compared to the standard (BHA) was also the benchmark of the rich and diverse class of antioxidant compounds making the rhizome a possible natural food source of antioxidants and preservatives. Thus, the study revealed the rhizome ingredients' possible utilization as a food preservative, aiming its potential pharmaceutical applications.

Enzyme inhibition activity of rhizome extracts

One of the upcoming trends in the management of Alzheimer's diseases (a major cause of dementia in humans) is suppressing the activity of enzymes (β-secretase (BACE1) and Acetylcholine esterase (AChE) involved in the disease development process. AChE plays a pivotal role in normal signal transmission by degrading acetylcholine (ACh) at cholinergic synapses. The rate of ACh secretion and degradation at cholinergic synapses is well balanced under normal conditions. However, under diseases conditions, ACh degradation is drastically increased, leading to the accumulation of degraded products at the synapse and impeding the normal signal transmission.[34] Though several synthetic compounds are reported and in use to manage the different type of dementia and cognitive dysfunction, considering their adverse effect on the function of the body and their bioavailability use of these compounds are still questioned.[35,36] In this regard, metabolites from a natural source like medicinal plants are considered potential candidates that can prevent/slow down the disease's progress. Similar observations were made by Hemanth Kumar *et al.* in the rhizome extracts of *Cyperus rotundus* which showed high inhibition against AChE.[11] Further, Sharma and Gupta showed that the methanolic extract of *Cyperus rotundus* rhizome inhibits the 50% enzyme (Acetylcholinesterase) activity at 0.5mg/mL concentration.[37] In the present study, the lowest IC_{50} 25.22μg/mL shown by acetone extract indicated the potential of *Cyperus articulatus* rhizome as an enzyme inhibitor source targeting Alzheimer's disease. Our earlier preliminary study on the *Cyperus articulatus* rhizome extract's antidiabetic property reported that acetone extract was rich in α-glucosidase inhibitors where the phenolic and non-phenolic compound fractions have shown significant inhibitory activity against the enzyme α-glucosidase.[13] This study's results, along with the reports of all the previous literature on the study of *Cyperus articulatus* revealed the potential pharmaceutical or medicinal values ranging from antioxidant, antibacterial, antidiabetic, anti-Alzheimer's biomolecular protection and food preservative properties. Above all, the plant is widely distributed in tropical and subtropical regions of the world, and thus the present study may be a suggestive platform for the agricultural practice of *Cyperus articulatus* and its effective utilization.

CONCLUSION

The study revealed that *Cyperus articulatus* rhizome is a rich source of nutraceuticals and ingredients for the drug formulation against various diseases and disorders relating to oxidative stress. The metabolite profile of the rhizome advocated the pharmacological importance of the plant as the major phytochemicals have proven health beneficial bioactivities. Its biological activities analyzed through various assays indicated its potential as a source of antioxidants that protect biomolecules, and also it may exert possible application in food industries as food antioxidants and preservatives. Further acetylcholinesterase inhibition shown by acetone crude extract revealed the plant *Cyperus articulatus* as a possible source of drug molecule against Alzheimer's disease. Further pre-clinical studies on individual bioactive metabolites concerning biological interactions and efficacy may explore *Cyperus articulatus* as a source of natural ingredients for drug formulations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AAPH: 2,2-azobis(2-amidinopropane)dihydrochloride; **ABTS:** 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt; **ANOVA:** Analysis of Variance; **ATCI:** Acetylthiocholine iodide; **BHA:** Butylated hydroxy anisole; **BHT:** Butylated hydroxy toluene; **BSA:** Bovine serum albumin; **CT DNA:** Calf Thymus DNA; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl; **GA:** Gallic acid; **MDA:** Malondialdehyde; **Q-TOF:** Quadrupole time-of-flight; **RE:** Rhizome extract; **TBA:** Thiobarbituric acid; **TBARS:** Thiobarbituric acid reactive substances; **TPTZ:** 2,4,6-tri(2-pyridyl)-s-triazine; **MDA:** Malondialdehyde.

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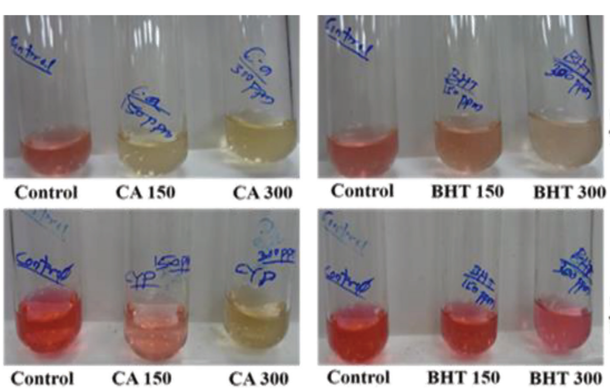


Figure S1: The effect of *C. articulatus* acetone extract and standard (BHT) on the formation of TBA-MDA complex. The results of 0th and 7th day are shown. CA: *Cyperus articulatus*, 150 and 300 ppm phenol (GA equivalent). Standard: BHT, 300 ppm.

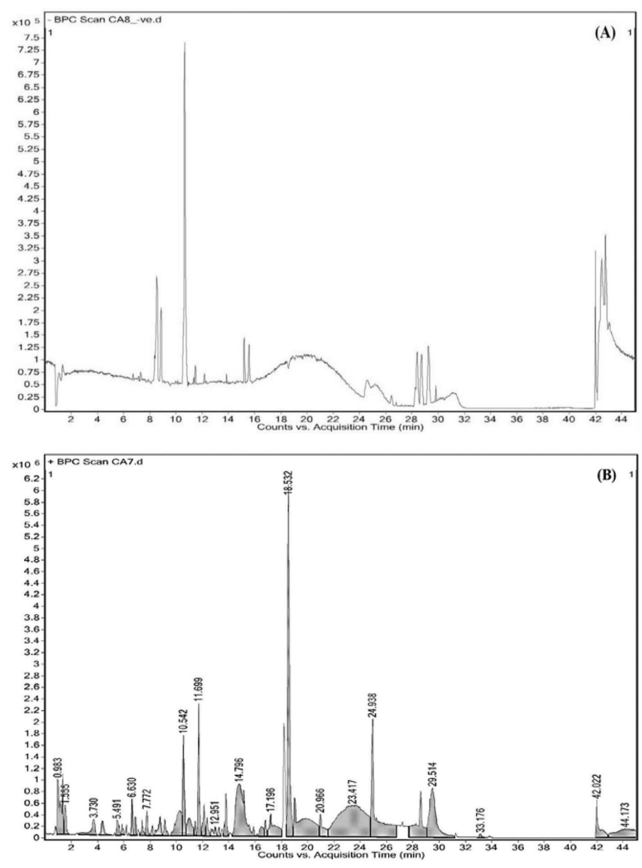


Figure S2: Total Ion Chromatogram (LC-MS BPC scan) of *Cyperus articulatus* rhizome Acetone extract in negative mode (A) and positive mode (B).

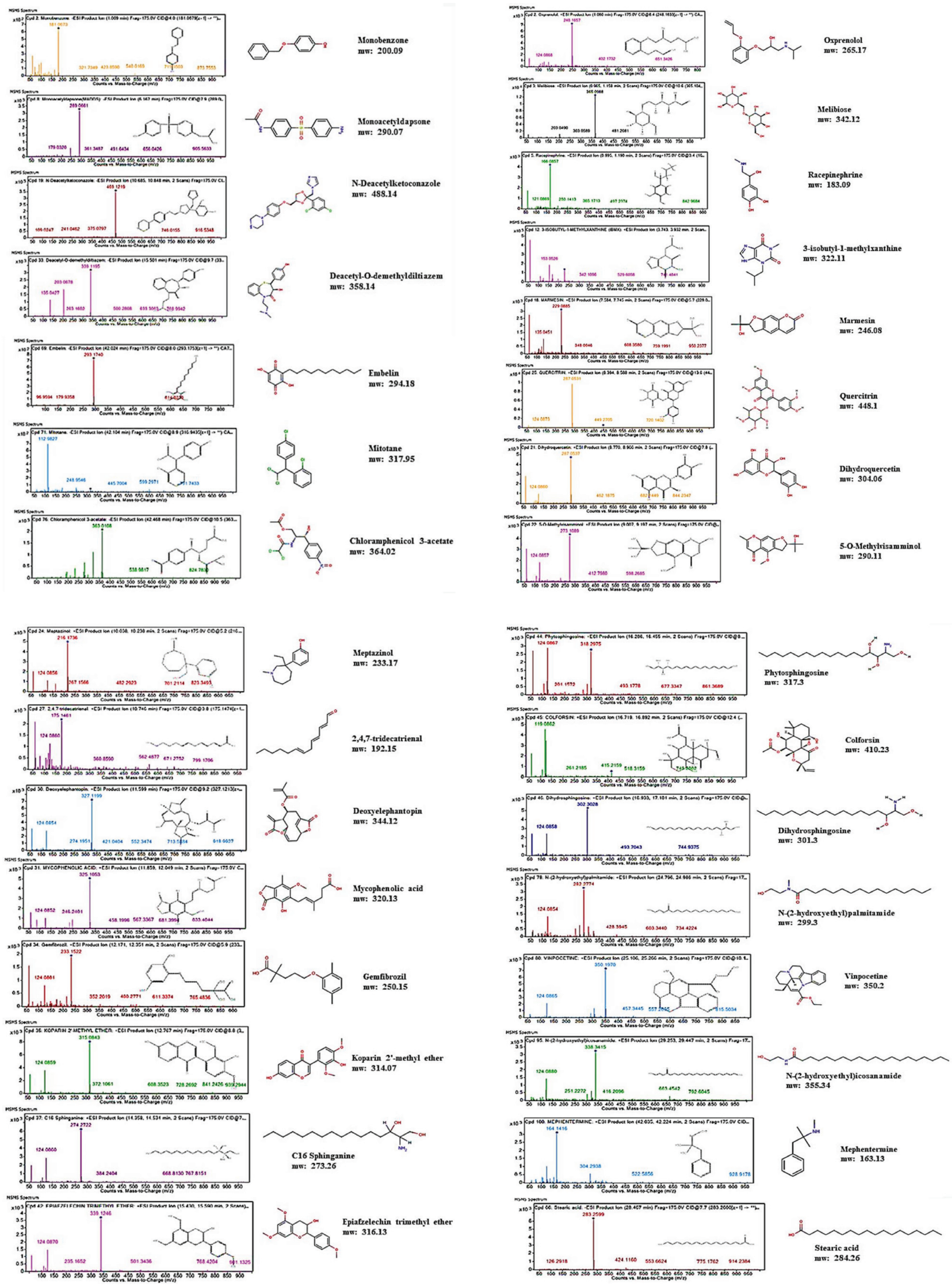
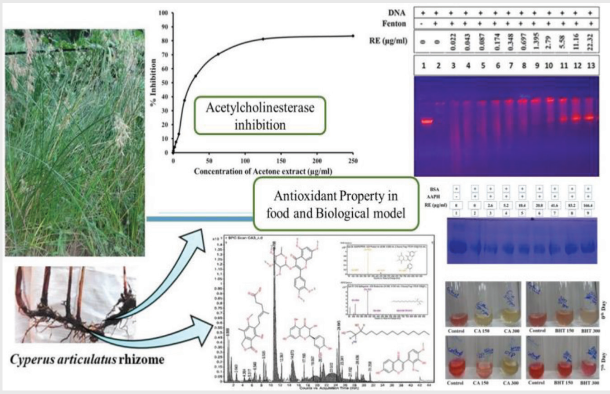


Figure S3: MS/MS peaks and list of respective compounds analyzed in HR-LCMS/MS of *C. articulatus* rhizome extract.

PICTORIAL ABSTRACT



SUMMARY

The present study showed that *Cyperus articulatus* rhizome is rich in antioxidant and nutraceuticals. The acetone extracted metabolites have high antioxidant activities and thus protected DNA and protein from degradation and oxidation. Major metabolites such as quercetrin, dihydroquercetin, mycophenolic acid, embelin, meptazinol and phytosphingosine were detected in HRLC-MS/MS analysis of bioactive rhizome acetone extract. The extract inhibited the bleaching of β-carotene and oxidative degradation of meat sample in food model systems envisaging the rhizome metabolites as potential food preservative. The rhizome extract also possesses Acetylcholinesterase inhibitory metabolites and could be further studied as a possible source of drug ingredient for Alzheimer's diseases.

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