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Analgesic and anti-inflammatory activity of the extracts from *Cyperus rotundus* Linn rhizomes

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ABSTRACT

The rhizome part of *Cyperus rotundus* has been shown to contain 5-hydroxymethyl furfural (HMF) (1), methyl ferulate (MF) (2), (E)-ferulaldehyde (FA) (3), and N-trans-feruloyl tyramine (NTF) (4).These known compounds are being reported for the first time from this plant and their structures were determined by physical properties and spectroscopic analyses. The present study was designed to investigate the analgesic and anti-inflammatory activities of methanol, chloroform and ethyl acetate extracts of *C. rotundus*. All the extract displayed significant analgesic effect in acetic acid and hot plate pain models in a dose dependent manner. As compared with the other extracts, the ethyl acetate extract (500 mg/kg) was the most effective in the analgesic test similar to standard drugs. In writhing test, ethyl acetate extract (500 mg/kg) showed significantly, inhibiting pain by 73.44 %, similar to aspirin which showed 76.47 % inhibition at the dose of 100 mg/kg i.p. In hot plate test, ethyl acetate extract (500 mg/kg) produced maximum possible analgesia of 12.72 ± 1.15 sec at 90 min, whilst morphine sulfate (5 mg/kg) showed 13.83 ± 1.15 sec. Similarly, carrageenan-induced paw volume was significantly reduced by ethyl acetate extract (500 mg/kg) at 2.20 ± 1.18 4 h after administration similar to that of diclofenac sodium which showed 2.24 ± 1.18 at the dose of 100 mg/kg. The data justify the traditional use of *Cyperus rotundus* as medicinal plant which has a potential source of bioactive molecules to treat inflammatory diseases.

INTRODUCTION

Cyperus rotundus Linn, sedge of the family Cyperaceae and order cyperales, is widely distributed in the Mediterranean basin areas (Uddin *et al.*, 2006). It grows under a variety of soil conditions like in waste lands; gardens open area etc. but usually prefer a moist and somewhat sandy soil. The tuber part is one of the oldest known medicinal plants used for the treatment of dysmenorrhea and menstrual irregularities (Yu *et al.*, 2004). It is also traditional medicinal plant appearing among the Indian, Chinese and Japanese natural drugs .It is used in the treatment of spasms, stomach disorder and inflammatory diseases (Gupta *et al*., 1971; Seo *et al*., 2001; Singh *et al*., 1970). Other Pharmacological investigations have indicated that it has remarkable hypotensive (Li, 1992) and antipyretic effects (Vedavathy and Rao 1990). The phytochemical investigation of has revealed the presence of polyphenols, flavonol glycosides, alkaloids, saponins, sesquiterpenoids and essential oils (Nagulendran *et al*., 2007; Venkatsubramanian *et al*., 2010). The major compounds isolated from the extracts of *C. rotundus* rhizome are α and β-cyperone, α and β-rotunol, β-pinene, β-selinene, camphene, cyperene, cyperenone, cyperol, cyperotundone, depoxyguaiene, gamma-cymene, limonene, linolenic-acid, myristic-acid, oleic-acid, pcymol, pectin, polyphenols, rotundene, rotundenol, rotundone, sugeonol, triterpenes including oleanolic acid and sitosterol, as well as flavonoids, sugars and minerals (Thebtaranonth *et al*., 1995; Jeong *et al*., 2000; Sonwa and König 2001).

The present study describes the isolation and structural elucidations of known compounds: 5-hydroxymethyl furfural (**1**), methyl ferulate (MF) (**2**), (E)-ferulaldehyde (**3**), and *N-trans*-feruloyltyramine (**4**) obtained for the first time from the rhizomes of *C. rotundus*.

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The compounds obtained were identified by spectroscopic evidences and by comparison with literature data. To find out the ingredients responsible for the efficiency of this plant, the analgesic and anti-inflammatory activities of different extracts were also studied.

MATERIALS AND METHODS

Chemistry

A melting point was determined on a Fisher Scientific melting point apparatus and was uncorrected. UV spectrum was recorded on Ultraviolet spectrophotometer (UV2550, Shimadzu, Japan) and IR spectrum was recorded on FT-IR spectrometer (Perkin Elmer Spectrophotometer, USA) with KBr tablets from 4000 to 400 cm^{-1} with 2 cm^{-1} resolution. NMR experiments was performed on a Bruker AMX 400 instrument (Bruker Company, Faelladen, Switzerland) with standard pulse sequences running of ^{1}H and ^{13}C NMR (400 and 100 MHz, respectively). Chemical shift values are in δ (ppm) with TMS as internal standard material and the coupling constants (*J*) are in Hz. GC-MS analyzes was performed on a Perkin Elmer Clarus 500 GC-MS systems. The fused-silica HP-5 MS capillary column (30 m - 0.25 mm ID, the film thickness of 0.25 mm) was directly coupled to the MS. Column chromatography (CC) was performed on silica gel 60 as stationary phase (particle size 0.04-0.036 mm, 230-400 mesh, ASTM E. Merck, Germany) and activated by heating at 110°C for one hour before use.

Plant material

Fresh rhizomes of *C. rotundus* growing in wild was randomly collected in the month of December from the river basin of Cauvery at Kumbakonam, Thanjavur District, Tamilnadu (India) and authenticated by Prof. N. Ramakrishnan, (Department of Botany) and voucher specimens (GACBOT-202) was deposited in the Herbarium of Botany Department at Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Extraction and Isolation

The dried rhizomes of *C. rotundus* (750 g, wet weight) were extracted with 90% methanol (MeOH) (4 X 500 ml) under reflux. The obtained methanol extract was concentrated on a rotatory evaporator under reduced pressure at a temperature of 45ºC for complete solvent removal, yielding crude methanol extract. The latter was suspended in methanol: H_2O (9:1) and was subjected to column chromatography packed with silica gel (column grade) and eluted with chloroform and ethyl acetate respectively. These fractions have collected and the solvent recovered by simple distillation, concentrated *in vacuo* and left in an ice-chest for a week. The chloroform fraction (37 g) was evaporated in vacuo and chromatographed on a silica gel 60 as stationary phase (particle size 0.04-0.036 mm, 230-400 mesh) with 25% of acetone in chloroform to yield compound **1** (5.8 g). Further elution with a hexane: ethyl acetate gradient (2:1), yielded compound **2** (3.4 g). The EtOAc fraction was concentrated under vacuum to afford a residue (10.9 g), which was subjected to column chromatography packed with silica gel (6.0 × 5.5 cm, 200−300 mesh) and eluted with a gradient of petroleum ether/acetone (10:1) to get compound **3** (3.2 g) and further eluting with a gradient of $CHCl_3$/MeOH (10:1) gave compound **4** (5. 6 g).

***5-hydroxymethyl furfural* (1)**

Dark-yellow liquid; bp 112-114ºC and or yellow powder; mp 33 – 34ºC; UV λ_{max}^{MeOH} (log ε) 220 - 285 nm; IR ν_{max} (KBr) : 3340, 2920, 1680, 1590, 1450, 1260, 1020 cm^{-1}; ^{1}HNMR [(400 MHz, $CDCI_3$) δ (ppm): 9.57 (1 H s,), 7.23 (1 H d, J = 3.6 Hz,), 6.52 (1 H d, J =3.6 Hz), 4.71 (2 H s)]; ^{13}C NMR [(100 MHz, $CDCI_3$) δ (ppm): 178.1 (CHO), 152.0 (C-2), 123.1 (C-3), 110.1 (C-4), 161.9 (C-5),56.8 (C-6).

***Methyl ferulate* (2)**

Pale yellow gum; λ_{max}^{MeOH} (log ε) 235, 290 - 320 nm; IR ν_{max} (KBr) : 3320, 2900, 2650, 2560, 1740, 1670 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.75 (3H, s, $COOCH_3$), 3.90 (3H, s, $ArOCH_3$), 6.37 (1H, d, J) 15.9 Hz, H-2′), 6.82 (1H, d, J) 8.1 Hz, H-5), 7.10 (1H, dd, J) 2.0, 8.1 Hz, H-6), 7.16 (1H, d, J) 2.0 Hz, H-2), 7.59 (1H, d, J) 15.9 Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD) δ 52.3 ($COOCH_3$), 56.4 ($ArOCH_3$), 112.1 (C-2), 114.8 (C-2′), 116.7 (C-5), 124.1 (C-6), 128.2 (C-1), 147.4 (C-1′), 150.2 (C-3), 151.5 (C-4), 170.6 (CO).

***(E) - feruldehyde* (3)**

Brown oil λ_{max}^{MeOH} (log ε) 245, 310, and 340 nm; IR ν_{max} (KBr) : 3360, 2880, 2720, 1660, 1610, 1480, 1350, 1230, 1050, 760, 620 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.36 (1H, *d*, *J* = 2.0 Hz, H-2), 6.82 (1H, *d*, *J* = 7.0 Hz, H-5), 7.16 (1H, *dd*, *J* = 2.0 & 7.0 Hz, H-6), 7.62 (1H, *d*, *J* = 16.0 Hz, H-7), 6.60 (1H, *dd*, *J* = 16.0 & 8.0 Hz, H-8), 9.60 (1H, *d*, *J* = 8 Hz, H-9), 3.80 (3H, *s*, OMe); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 125.73 (C-1), 110.18 (C-2), 150.15 (C-3), 146.88 (C-4), 115.74 (C-5), 124.10 (C-6), 154.12 (C-7), 115.30 (C-8), 195.16 (C-9), 55.34 (-OMe).

***N - trans-feruloyltyramine* (4)**

White amorphous solid; mp 90 - 92 °C; λ_{max}^{MeOH} (log ε) 220, 290, 320; IR ν_{max} (KBr) : 3340, 1660, 1580, 1450, 1260, 1030 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 3.37 (2H, t, J = 6.89 Hz, H-1), 2. 59 (2H, t, *J* = 7.6 Hz, H-2), 6.98 (1H, d, H-4, H-8), 6.72 (1H, d, *J* =8.63Hz, H-5, H-7), 6.48 (1H, d, *J* = 15.8Hz, H-2'), 7.41 (1H, d, *J* = 7.2 Hz, H-3'), 7.16 (1H, d, *J* = 1.63Hz, H-5′), 6.81 (1H, d, *J* = 8.3Hz, H-8′), 7.01 (1H, dd, *J* = 8.0Hz, H-9′), 7.98 (1H, t, *J* = 5.6Hz, NH), 3.81 (3H, s, 6-OCH3), 9.29 (1H, s, 7'-OH), 9.38 (1H, s, 6-OH); ^{13}C NMR (100 MHz, $CDCl_3$): 41.1 (C-1), 34.6 (C-2), 125.9 (C-3), 114.8 (C-4), 130.1 (C-5), 156.2 (C-6), 130.2 (C-7), 114.9(C-8), 167.4 (C-1′), 122.0 (C-2′), 148.3 (C-3′), 138.2 (C-

4′), 111.0 (C-5′), 148.9 (C-6′), 164.2 (C-7′), 118.1 (C-8′), 123.8 (C-9′), 56.4 (6′-OCH_3).

Biology

Animals

Male albino mice (30 - 40 g) and male albino rats (180 - 220 g) of Wistar strain were procured from the animal house, Government Arts College (Autonomous), Bharathidasan University, Kumbakonam, Tamilnadu, India. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of six animals. They housed in cages and maintained under standard conditions at 26 ± 2°C and relative humidity 44 - 56 % and with 10 h light and 14 h dark cycles per day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet, and water adlibitum. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

Analgesic activity by hot-plate method

Evaluation of analgesic activity of the plant extract was carried out using hot plate method (Eddy and Leimback 1953). Experimental animals of either sex were randomly selected and divided into eight groups consisting of six mice in each group for control, standard and test groups respectively. The control group was treated with 1% DMSO at the dose of 10 ml / kg body weight; test groups were treated at the dose of 250 and 500 mg/kg and the standard was treated with morphine (5 mg/kg) administered via intraperitoneal (i.p.) route. All animals were lowered onto the surface of a hot plate (55 ± 0.5°C) enclosed with cylindrical glass and the time for the animal to jump or lick the forelimb was noted as the reaction time. A cutoff period of 30 seconds was observed to avoid damage to the paw. The observations were made before and after administration of respective drugs at 30 min, 60 min, and at the end of 120 min. The reaction time of the test and standard groups were compared with the control.

Acetic acid induced writhing test

The analgesic activity of the samples was evaluated using acetic acid induced writhing method (Koster *et al*., 1959). Albino mice (30 - 40 g) were divided into eight groups each consisting of six animals. The first group served as control and received 1% DMSO at the dose of 10 ml / kg body weight. The second group served as standard (received aspirin at the 100 mg/kg) while third and fourth groups served as tests and received plant extracts at the doses of 250 and 500 mg/kg orally. Acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. Writhing in animals was produced by i.p. administration of 300 mg/kg acetic acid solution (3%). The writhing movements were observed and counted for every 30 min after acetic acid administration. The number of writhes of test groups at different dose levels along with standard was compared with the control. The percent inhibition of writhing count of the treated group was calculated from the mean writhing count of the control group. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \{ (W_c - W_t) \times 100 \} / Wc$$

Where, W_c = No. of writhes in control group, W_t = No. of writhes in test group

Anti-inflammatory activity by carrageenan induced rat paw edema

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method suggested by Diwan *et al*., 1989. Extracts of *C. rotundus* rhizome were administrated to the animals in the test groups at the dose of 250 and 500 mg / kg by oral route. Animals in the standard group received Diclofenac sodium at the dose of 100 mg / kg, by oral route. Control group animals were received 1% DMSO at the dose of 10 ml / kg body weight. The acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% carrageenan in the right paw. Paw volume was measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, and 3 hrs intervals (Kouadio *et al*., 2000). The efficacy of different drug was tested on its ability to inhibit paw edema as compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

Statistical analysis

The experimental results were expressed in multiple comparisons of Mean ± SEM and was carried out by one-way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test and statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Chemistry

The chloroform and ethyl acetate soluble fractions from the methanol extract of *C. rotundus* rhizome were repeatedly separated by column chromatography. The structure of the isolated compounds was analyzed using UV, IR, ^{1}H- and ^{13}CNMR and MS spectra. Finally, the structure was confirmed by comparison with the reference data, and the compounds were identified as 5-hydroxymethyl furfural (HMF) (1), methyl ferulate (MF) (2), (E)-ferulaldehyde (3), and N-*trans*-feruloyl tyramine (NTF) (4) (Figure 1). On the basis of this discovery, the phytochemical isolation of these compounds from the chloroform and ethyl acetate extracts of *C. rotundus* rhizome rhizomes was reported for the first time and the biological activities were evaluated.

Many reports related to 5-HMF have provided significant proof for its potential importance in anticancer activities, applications as nerve medication, protection of the myocardium, treatments of cardiovascular diseases damage to striated muscles and viscera by combining to protein, and decreasing the accumulation of poisons in the body (Fu *et al*., 2008).

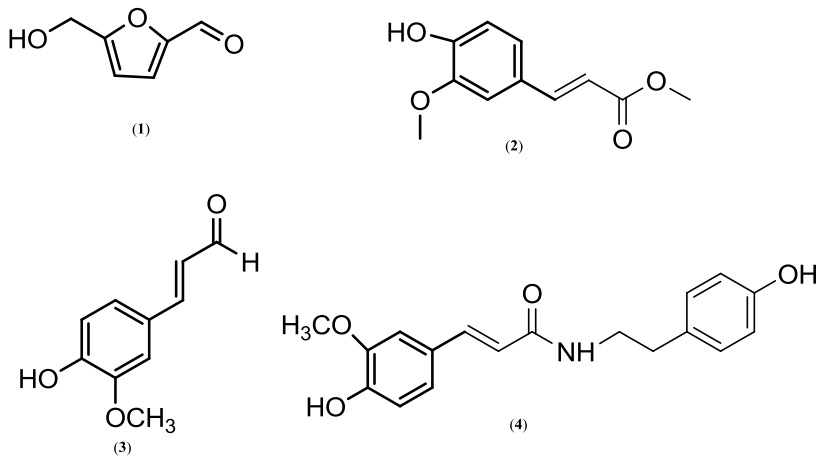

Fig. 1: Chemical structure of compounds presence of *Cyperus rotundus*

Compounds 2 and 3, having similar chemical structure, the methoxy, and hydroxy groups terminate the free radical chain reaction due to their electron donating capacity, and C-C double bonds can provide the attack sites for free radicals. The structural characteristic of ferulic acid and its reduced forms, methyl ferulate (MF) and ferulaldehyde (FA) similar structure, the difference is the one functional group and the presence of the reactive aldehyde group (Kim *et al*., 1999) and a methoxy group. Ferulic acid and its derivatives have been reported to be effective anti-oxidant, anti-microbial, anti-inflammatory, hepatoprotective, neuroprotective, anti-diabetic and anticarcinogenic compounds (Paiva *et al*., 2013; Sultana, 2012). N-*trans*-feruloyl tyramine (NTF), one of the phenylpropanoids, (Dixon and Pavia, 1995) has been reported to show bioactivities such as antimicrobial and anti- HIV activities (Okuyama *et al*., 1986; Wu *et al*., 2005). However, analgesic and anti-inflammatory activity of above-isolated compounds have not been well documented.

Compound **1**, isolated as dark yellow liquid (b.p. 112-114ºC) or yellow powder (m.p 33 - 34ºC), has molecular formula $C_6H_6O_3$ with a molecular ion peak at m/z 126.0. Moreover, the fragment ion peaks such as m/z 109.00 [M - OH] $^+$, 97.00 (100) [M -OHC] $^+$, and 81.00 [M - OH - C - O]$^+$, in the mass spectrum provided more evidence for the final structure elucidation. The UV absorptions at 220 and 285 nm indicate a furfural chromophore. FT-IR spectra of the HMF give the carbon-carbon stretching vibrations at 1590 and 1450 cm^{-1} which is attributed to furan ring structure. The absorptions at 1260 and 1020 cm^{-1} are due to conjugated and unconjugated C-O stretching respectively. The absorption at 3340, 2920 and 1690 cm^{-1} are attributed to -OH, methylene (− CH_2 −) and C=O (aldehyde) stretching, respectively. In the ^{1}H NMR spectrum, five signals were observed which can be attributed to one aldehyde proton at δ H 9.57 (s), two aromatic methine protons at δ H 7.23 (d, J = 3.6 Hz) and 6.52 (d, J = 3.6 Hz) appeared as an AB spin system, which suggested the characteristics of 2,5-disubstituted furfuran ring. The appearance of the downfield proton signal at δ 4.71 ppm (2H, s, H-6) indicated a methylene group attached to a hydroxyl group. The ^{13}C spectra showed six carbon signals consisting of one aldehyde carbon (178.1 ppm), one methylene carbon attached to a hydroxyl group (56.8 ppm), two aromatic methines (110.1, and 109.9 ppm), and two quaternary carbon atoms (152.0 and 161.9 ppm). On the comprehensive spectral analysis, compound **1** was elucidated as a known furan derivative, 5-hydroxymethyl-2-furfural (5-HMF) (Hearn, 1976; Miyazawa *et al*., 2003). Compound **2**, was obtained as pale yellow gum. The GC-MS spectrum showed a molecular ion peak at m/e 208 which is in accordance with the molecular formula of $C_{11}H_{12}O_4$. The UV spectrum showed absorption bands at 320 nm indicating a highly unsaturated chromophoric system and IR spectrum showed absorption bands for the phenolic group (3380 cm), α, β-unsaturated esters (C=O, 1690 cm^{-1}, C=C, 1620 cm^{-1}) and aromatic moiety (1600 - 1510 cm^{-1}). The ^{1}H NMR spectral data of **2** showed the presence of an (E)-p-methoxy cinnamic acid moiety, as represented by three protons of substituted aromatic rings of which one is doublet of doublet at δ 7.16 (dd, H-2), with the coupling constant of J=2.0 Hz should be affected by proton of meta position and at 6.82 (d, H-5) and 7.10 (1H, dd, H-6), two trans olefinic protons at δ 6.35 (d, H-α), 7.64 (d, H-β), and six protons of two methoxy groups at δ 3.75 (3H, s, $COOCH_3$); 3.90 (3H, s, $ArOCH_3$) and hydroxyl group at δH 6.21. The ^{13}C-NMR spectra showed 11 signals, five sp^2 -CH, three sp^2 quaternary carbon atoms and one due to carbonyl which was confirmed by the resonance at δC 193.8 and the last two due to methoxy group. These data agreed well with the data previously reported (Menon *et al*., 1999; Tawata *et al*., 1996). Hence, compound **2** was identified as methyl (E)-3-(4-hydroxy-3-methoxyphenyl) - prop-2-enoate (methyl ferulate). Compound **3** was obtained as a pale yellow liquid; the GC-MS spectrum showed a molecular ion peak at m/e 178.353 which in accordance with the molecular formula of $C_{10}H_{10}O_3$. The UV spectrum displayed absorption band at 245, 310, and 340 nm which was very similar to ferulic acid (Ishak *et al*., 1972). IR spectroscopic analysis showed the presence of phenolic group at 3370 cm^{-1}, absorption bands at 1670 cm^{-1} consistent with α, β, unsaturated carbonyl group and C-H of aldehyde at 2900 cm^{-1}. The ^{1}H-NMR spectrum showed the presence of three aromatic protons, each integrated for one proton signal, which appeared at δ 7.36 (d, J = 2.0 Hz), 6.82

(d, J = 7.0 Hz), and 7.16 (dd, J = 2.0 & 7.0 Hz), and was assigned for H-2, H-5 and H-6 of 1,3,4 - trisubstituted aromatic ring. This was further confirmed by ^{13}C-NMR spectrum, which displayed signals for substituted carbons at δ 125.73 (C-1), 150.15 (C-3), 146.88 (C-4). The downfield shifts of C-3 and C-4 in comparison to C-1 indicated that this carbon bears oxygen functions. The ^{1}H- and ^{13}C-NMR spectrum displayed the presence of methoxyl group at δ 3.81 and δ 55.73 ppm, respectively. In addition to these, the ^{1}H-NMR spectrum displayed a doublet at δ 7.62 (J = 16.0 Hz, H-7), a double doublet at δ 6.60 (J = 16.0 & 8.0 Hz, H-8), and a doublet at δ 9.60 (J = 8 Hz, H-9), each for one proton that indicated the presence of olefinic double bond substituted with aldehyde group (-CH=CH-CHO). The large coupling constant showed the *trans* configuration of the double bond. The ^{13}C-NMR showed 10 signals, including carbonyl which was confirmed by the resonance at δC 193.8 and one at 147.0 due to methoxy group. According to the above data compound **3** was characterized as (E)-ferulaldehyde; ((E) - 4 - hydroxy - 3 - methoxy cinnamaldehyde) by comparison with the literature (Barakat *et al*., 1987; Kelleys *et al*., 1976).

Compound **4** was obtained as colorless crystals. The mass spectrum showed molecular ion peak at m/z 314 [M + H] +, consistent with $C_{18}H_{19}O_4N$. The UV spectrum had three kinds of the band as λmax (MeOH): 225, 292, and 320 nm, indicating a highly conjugated system. The IR spectrum of compound 4 showed a large absorption band at 3340 cm^{-1} (N-H bending) and at 1660 cm^{-1} (C=O stretch) suggesting an amide function. The aromatic skeleton was evidenced by the absorptions between 15800 and 1450 cm^{-1}. The ^{1}H NMR spectrum revealed an aromatic ABX system in the ferulic acid moiety: δ 7.16 (1H, d, J = 1.63Hz), 6.81 (1H, d, J = 8.3Hz), 7.01 (1H, dd, J = 8.0Hz) assigned H-5′, H-8′ and H-9′ respectively and a typical AA`BB` system in the tyramine moiety: δ 6.98 (1H, d, J = 8.6 Hz), 6.72 (1H, d, J = 8.6 Hz) assigned H-4, H-8 and H-5, H-7 respectively. Furthermore, two triplets integrated for two protons are indicating an ethylic chain at δ 3.37 (H-1) and 2.59 (H-2) and olefinic *trans* protons appear as two doublets at δ 6.48 (1H, d, J = 15.8Hz, H-2'), 7.41 (1H, d, J = 7.2 Hz, H-3'). In addition, one methoxy group resonating at δ 3.81 is also present. ^{13}C NMR experiment for compound **4** showed 18 carbon resonance signals corresponding two methylene, nine methines, six quaternary carbons, one carbonyl signal and one methoxyl group at δ 56.4. It could be deduced as an amide since the compound showed the negative reaction with ninhydrin but positive after hydrolysis with hydrochloric acid, in addition to the signal appearing at δ 167.4 (s) in the ^{13}C NMR spectrum confirmed the previous fact. These data were in agreement with those reported for the N-*trans*-feruloyl tyramine (**4**) (Dellagreca *et al*., 2009; Sarker *et al*., 2000).

Biology

Analgesic activity

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases (Weitzmann *et al*., 1990). Hot-plate test is a widely used model for neurologic pain, and centrally acting analgesic agents can increase reaction time in hot-plate test through their action at the spinal cord level (Wigdor and Wilcox 1987). Morphine sulphate used as the standard in this study, acts through binding with opioid receptors (μ, δ and κ) present in presynaptic and postsynaptic membrane. The reaction time following the oral administration of different doses of plant extracts and standard drug were presented in Table 1. At 90 minutes, the maximum reaction time of two different doses (250 and 500 mg/kg body weight) was 6.42 ± 1.11 & 8.60 ± 1.00 for methanol extract and 8.89 ± 1.12 & 10.67 ± 1.10 for chloroform extract of *C. rotundus* respectively. In ethyl acetate extract of two different doses (250 and 500 mg/kg body weight) the maximum reaction time was 10.34 ± 1.14 & 12.72 ± 1.15 sec respectively, while morphine showed the maximum reaction time of 13.83 ± 1.15 sec at 90 min at the dose of 5 mg/kg (Table 1). The results indicated that the extract significantly ($p < 0.001$) raised pain threshold as compared to control and the activity was persistent throughout the entire observation period. The result of hot plate test indicates that the extract also possesses the ability to reduce centrally mediated pain. Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. In acetic acid induced method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and nonsteroidal anti-inflammatory drugs (Colier *et al*., 1968). The acetic acid-induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria and Abdul Gani 2008). The plant extract showed positive results for alkaloids and it is well-known that many alkaloids including caffeine, cocaine, cathinone, nicotine, and yohimbine, possess central stimulant effect (Davis *et al*., 2003). Therefore, the identified polyphenols and alkaloids might contribute in the observed central stimulant effect of *C. rotundus* extract. The significant pain reduction of (Table 2) both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. In this test, after oral administration of methanol extract at doses of (250 and 500 mg/kg body weight), the percent inhibition was 45.73 and 55.38 % respectively. On the other hand chloroform and ethyl acetate extract showed maximum inhibition of the writhing such as 58.64 & 66.17 % and 64.5 & 73.44 % whereas the reference drug aspirin displayed 76.47 % inhibition at the dose of 100 mg/kg as compared to the control, and the results were statistically significant ($p < 0.001$). The analgesic effect of the plants in both models suggests that they have been acting through a central and peripheral mechanism (Sabina *et al*., 2009). It was found that the observed analgesic activity in *C. rotundus* was demonstrated by the active constituents, isolated from the plant extract through a peripherally acting mechanism similar to the non-steroidal anti-inflammatory agents.

Table 1: Effect of *C. rotundus* in hot-plate test in mice.

Treatment	Reaction time (in sec) (M±SD)			
	0 min	30 min	60 min	90 min
Control (1% DMSO)	4.33 ± 0.55	4.37 ± 0.58	4.49 ± 0.50	4.62 ± 0.60
Standard (Morphine Sulphate + 5 mg/kg)	4.67 ± 0.80	8.64 ± 0.50	11.02 ± 1.00	13.83 ± 1.15
MeOH extract 250 mg/kg	4.60 ± 1.10	4.92 ± 1.07	5.23 ± 1.08	6.42 ± 1.11
500 mg/kg	5.00 ± 1.00	5.33 ± 0.58	6.33 ± 1.15	8.60 ± 1.00
$CHCl_3$ extract 250 mg/kg	4.66 ± 1.12	6.18 ± 0.56	7.01 ± 1.13	8.89 ± 1.12
500 mg/kg	4.65 ± 1.15	6.33 ± 0.45	8.33 ± 1.05	10.67 ± 1.10
EtOAc extract 250 mg/kg	4.69 ± 0.15	6.66 ± 0.75	8.18 ± 1.15	10.34 ± 1.14
500 mg/kg	4.58 ± 0.50	7.33 ± 0.70	9.67 ± 1.03	12.72 ± 1.15

Data expressed as Mean ± SEM, n = 6 in each group done by one way ANOVA followed by Dennett's test.

Table 2: Effect of *C. rotundus* on acetic acid induced writhing in mice.

Treatment (n =6)	Dose (mg/kg)	No. of writhes (Per 30 Min)	% inhibition
Control (1% DMSO)	10 mL/Kg	34.00 ± 0.46	-
Aspirin	100 mg/kg	8.00 ± 0.28	76.47
MeOH extract	250 mg/kg	18.45 ± 0.46	45.73
	500 mg/kg	15.17 ± 0.43	55.38
$CHCl_3$ extract	250 mg/kg	14.06 ± 0.25	58.64
	500 mg/kg	11.50 ± 0.26	66.17
EtOAc extract	250 mg/kg	12.07 ± 0.27	64..5
	500 mg/kg	9.03 ± 0.29	73.44

Data expressed as Mean ± SEM, n = 6 in each group by one way ANOVA followed by Dennett's test.

Table 3: Determination of paw volume of rats for *C. rotundus* extracts.

Groups	Initial paw volume	Paw volume at different time interval (in ml)			
		1h	2h	3h	4h
Control (1% DMSO)	1.86 ± 1.15	1.92 ± 1.14	1.98 ± 1.17	1.99 ±1.14	1.98±1.16
Diclofenac Sodium (100 mg/kg)	1.86 ± 1.22	1.98 ± 1.23	2.16 ± 1.22	2.32 ± 1.25	2.24 ± 1.18
Methanol extract(250 mg/kg)	1.88 ± 1.19	1.94 ± 1.22	2.03 ± 1.24	2.14 ± 1.28	2.03 ± 1.14
(500 mg/kg)	1.87 ± 1.15	1.97 ± 1.24	2.06 ± 1.28	2.18 ± 1.26	2.09 ± 1.16
Chloroform extract(250 mg/kg)	1.87 ± 1.20	1.96 ± 1.26	2.08 ± 1.26	2.19 ± 1.24	2.11 ± 1.19
(500 mg/kg)	1.88 ± 1.23	1.97 ± 1.25	2.11 ± 1.23	2.24 ± 1.20	2.15 ± 1.15
Ethyl acetate extract(250 mg/kg)	1.87 ± 1.24	1.99 ± 1.24	2.13 ± 1.24	2.27 ± 1.26	2.16 ± 1.16
(500 mg/kg)	1.86 ± 1.24	1.98 ± 1.24	2.15 ± 1.22	2.30 ± 1.25	2.20 ± 1.18

Data expressed as Mean ± SEM, n = 6 in each group by one way ANOVA followed by Dennett's test.

Anti-inflammatory activity

The anti-inflammatory activity of ethyl acetate, chloroform and methanol extract of *C. rotundus* was determined in carrageen induced paw edema of rats and found to be significant compared to diclofenac sodium used as standard (Table 3). Carrageenan-induced paw edema was tested on male albino rats (weighing 180 - 220 g) in suitable experimental animal model for evaluation of anti- edematous effect on natural products (Winter *et al*., 1962). As shown in Table 3, the rats were divided into eight groups (six animals each) served as control and tested animals. Preferably, two groups are used as control and standard. Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan in normal saline in the left hind paw of the rats, one hr after the oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0 hr and 3hr after carrageenan injection. All tested groups decreased the thickness of edema of the hind paw compared to the control group. The decrease in the paw volume in the group of animals treated with *C. rotundus* methanolic extract 500 mg was 2.09 ± 1.16 and for the chloroform extract 500 mg/kg was 2.15 ± 1.15 and ethyl acetate 500 mg/kg was 2.20 ± 1.18 at 4 h. The paw volume was compared with that of standard diclofenac sodium 100 mg/kg and showed percentage paw volume decrease of 2.24 ± 1.18. The ethyl acetate extract exhibited maximum activity in comparison with chloroform and methanol extracts. It can be seen that group VI & VIII showed pronounced anti-inflammatory effects after three hours of injection. This effect may be due to phenolic derivatives and alkaloid composition in the plant extracts.

CONCLUSION

The present study has demonstrated that rhizome part of *Cyperus rotundus* has been shown to contain 5-hydroxymethyl furfural, methyl ferulate, (E)-ferulaldehyde, and N-*trans*-feruloyl tyramine and are being reported for the first time. Bioactive substances from this plant can, therefore, employed to develop drugs for the treatment of various inflammatory diseases. The chloroform and ethyl acetate extracts showed pronounced anti-inflammatory effects after three hours of injection. The data

support the folk traditional use of *Cyperus rotundus* to treat inflammatory diseases that are associated with pain.

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