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ISOLATION AND CHARACTERIZATION OF NATURAL PRODUCT COMPOUNDS FROM RHIZOMES OF *CYPERUS ROTUNDUS* LINN.

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Keywords:

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ABSTRACT: Background: *Cyperus rotundus* Linn. is a traditional medicinal plant appearing among Indian, Chinese and Japanese natural drugs, used for the treatment of spasms stomach disorder and also used as anti-inflammatory, antimalarial, hypotensive, antipyretic, anthelmintic, hepatoprotective agent. Rhizomes of *C. rotundus* have been used in ancient medicine in India for fever, dysentery, pruritis, pain, vomiting and various blood disorders. **Objective:** To isolate active Phytochemicals from rhizomes of title Plant. **Materials and Methods:** Petroleum ether extract of rhizomes was chromatograph over silica gel column and eluted with different solvent systems. Characterization of isolated compounds was done on the basis of spectral studies (IR, ^{1}HNMR, ^{13}CNMR, MS). **Results:** Ellagic acid (I), 6-hydroxy-3, 4-dimethyl coumarin (II), 6-methoxy-7, 8-methylenedioxy coumarin (III) and quercetin (IV) were isolated. **Conclusion:** Rhizomes of title plant are rich source of natural compounds and these compounds have very useful medicinal activities so present work gives a direction for future investigators to get some medicinally important drugs.

INTRODUCTION: *Cyperus rotundus* Linn. is commonly known as nut grass or nagarmotha, belonging to the family Cyperaceae and originated in India as a weed [1]. *C. rotundus* is a traditional medicinal plant appearing among Indian, Chinese and Japanese natural drugs, used for the treatment of spasms stomach disorder and anti-inflammatory diseases [2, 3, 4, 5]. *C. rotundus* have been reported to contain oils, alkaloids, glycosides, saponins, flavonoids, tannins, starch, carbohydrates [6], proteins [7] and traces of Mg, V, Cr, Mn and Co [8].

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Monoterpenes and sesquiterpenes are isolated from this plant and showed *in vitro* antimalarial activity[9]. *C. rotundus* had remarkable hypotensive and antipyretic effects [10] and also exhibits anthelmintic properties [11].

The alcoholic and aqueous extracts of *C. rotundus* also exhibit hepatoprotective activity [12]. Rhizomes of *C. rotundus* have been used in ancient medicine in India for fever, dysentery, pruritis, pain, vomiting and various blood disorders [13]. The methanol extract of the rhizomes has been found to inhibit nitric oxide and superoxide production in murine macrophage cell line [14]. The tubers of the plant are used in folk medicine as sedative, carminative, stimulant, tonic, aphrodisiac, diuretic, stomachic, colic remedy and used to remove renal calculi [15], estrogenic, antiemetic [16], in diarrhoea and women's diseases [17].

Dried tuberous roots is a source of aromatics and useful in perfumery [18]. The rhizomes of the plant have been also recommended for the use in several clinical conditions like fever and arthritis [19]. Rhizomes are cooling, intellect promoting, nervine tonic, diuretic, antiperiodic, and used to treat diarrhoea, leprosy, bronchitis, analgesic and amenorrhea [20, 21].

MATERIALS AND METHODS:

General Experimental Procedures: Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative and quantitative TLC was conducted on aluminium sheet Kieselgel 60 F254 (E. Merck). Silica gel (E. Merck, 60 - 120 mesh, 550 gm) used for column (1.5 m × 4.0 cm) chromatography.

The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 at 300 MHz and 75 MHz on a Brucker NMR instrument, respectively, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 / DA-6000 mass spectrometer using Argon / Xenon as FAB gas.

Plant Material: The plant material, rhizomes have been collected from the surroundings of Jaipur and the authenticity of the plant was done by the Department of Botany, University of Rajasthan, Jaipur (voucher no. RUBL-3092).

Extraction and Isolation: Shade dried rhizomes (5kg) of title plant were powdered and extracted with Petroleum ether on a steam bath for (12 × 3) h. The extract was concentrated under reduced pressure. The syrupy mass so obtained was treated with acetonitrile for the removal of fats to run the column smoothly. The fat free extract was chromatographed over silica gel column and eluted with the solvent of increasing polarity where compounds I to IV were isolated, purified and characterized.

RESULTS:

Ellagic Acid (Compound I): A dark brown solid mass was obtained when column was eluted with petroleum ether which on crystallization with ethyl acetate and methanol yielded light brown crystals, m.p. 278 - 80 °C. It gave a bluish green colour with ferric chloride. IR (cm^{-1}, KBr): 3500, 3300 - 2650, 1699, 1640, 1545, 1509, 1472, 1435, 1395, 1263, 1224, 1111, 1045, 922, 882 and 756. ^{1}HNMR (δppm, $CDCl_3$ + DMSO-d_6): 7.33 (*s*, H-5, 5´), 4.91 (br *s*, 4 × OH). ^{13}C NMR (δppm, $CDCl_3$ + DMSO-d_6): 170.3 (C-7, 7´), 146.3 (C-4, 4´, 3, 3´), 139.5 (C-2, 2´), 121.9 (C-6, 6´), 110.3 (C-1, 5, 1´, 5´). Mass (m/z): 302 [M^+], 272, 256, 228, 212, 200, 184, *etc.* Molecular formula calculated as $C_{14}H_6O_8$.

6-hydroxy-3, 4-dimethyl Coumarin (Compound II): An olive green solid mass was obtained when column was eluted with petroleum ether and benzene (4:1) which on crystallization with ethyl acetate yielded shining green crystals, m.p. 241-43ºC. IR (cm^{-1}, KBr): 3500, 1720, 1615, 1570, 1560, 1130 and 690. ^{1}HNMR (δppm, $CDCl_3$): 7.33 (1H, *d*, H-5), 7.27 (1H, *d*, H-7), 6.77 1H, *d*, H-8), 2.35 (3H, *s*, 4-CH_3) and 2.15 (3H, *s*, 3-CH_3). Mass (m/z): 190 [M^+], 175, 162, 161, 134, 133, 108, 107, 90, 84 *etc.* Molecular formula calculated as $C_{11}H_{10}O_3$.

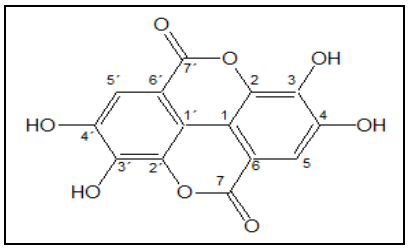
6- methoxy- 7, 8- methylenedioxy Coumarin (Compound III): A pale yellow solid mass was obtained when column was eluted with petroleum ether and benzene (1:1), m.p. 219 - 221 ºC. It appeared as blue fluorescent spot on TLC plate when viewed in UV light. It gave a blue green colour in Lab at test (sulphuric acid and ethanolic gallic acid) and red colour with phloroglucinol and sulfuric acid. IR (cm^{-1} KBr): 1720, 1620, 1590, 1560, 1130 and 655. ^{1}H NMR (δppm, $CDCl_3$): 7.57 (1H, *d*), 7.51 (1H, *d*), 6.58 (1H, *s*), 6.30 (2H, *s*, -O-CH_2-O-), 6.16 (3H, *s*, -OCH_3) and 3.93 (3H, *s*, -OCH_3). Mass (m/z): 220 [M^+], 205, 204, 192, 177, 146, 134, 120,106, 91, 79, 69, *etc*. Molecular formula Calculated as $C_{11}H_8O_5$.

Quercetin (Compound IV): The light brown solid obtained when column was eluted with acetone and methanol (1:1), m.p. 301-302 ºC. It gave blue-green colour with alcoholic $FeCl_3$, reddish brown in Shinoda's test and yellow colour showing light green fluorescence with conc. sulphuric acid. It gave dark bright yellow spot on TLC plate when viewed in UV light. IR (cm^{-1}, KBr): 3450 - 3100 (broad), 3040, 3010, 1660, 1590, 1520, 1230, 1200, 1180, 910, 830, 820 and 790. ^{1}H NMR (δppm, $CDCl_3$ + DMSO-d_6.): 12.31 (OH, s), 10.25 (OH, s), 8.87 (OH, s) 8.68 (OH, s), 8.31 (OH, s) 7.81-7.62. Mass (m/z): 302 [M^+], 301, 285, 284, 152, 137,

135, 132, 105, 95, 89, 77 *etc*. Molecular formula Calculated as $C_{15}H_{10}O_7$.

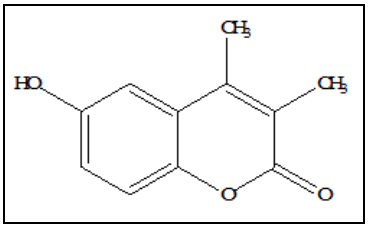
DISCUSSION:

Compound I: The elemental and mass spectral analysis established its molecular formula as $C_{14}H_6O_8$. The appearance of a bluish-green colour with ferric chloride suggested its phenolic nature. In the infrared spectrum important peaks appeared at 3500 (-OH group), 3300-2650 (broad, -OH stretching), 1699, 1640 (C=O stretching), 1545, 1509 and 1472 (C=O stretching) for aromatic system, 1224 (C–O stretching of phenolic OH) and 1111 (C–O–C) cm^{-1}. The ^1H NMR spectrum showed a singlet at 7.33 which was assigned to the presence of two isolated aromatic protons at C-5 and C-5´. The four hydroxyl group appeared as a broad singlet at 4.91. In ^{13}C NMR spectrum, the two conjugated ester carbonyl carbons (C-7, 7´) appeared at 170.3 whilst the C-2, 2´ carbons were observed at 139.5. A signal at 146.3 was assigned to carbons C-4, 4´ and C-3, 3´ with attached –OH groups. A peak at 110.3 appeared for the four carbons (C-1, 5 and C-1´, 5´) where as C-6, 6´ carbons appeared at δ 121.9. In the mass spectrum, the molecular ion peak was observed at m/z 302. The other important fragments were observed at 256, 228, 212, 200,184, *etc*. The above spectral data resembled with those reported values for ellagic acid, it was thus confirmed that Compound I is Ellagic acid.



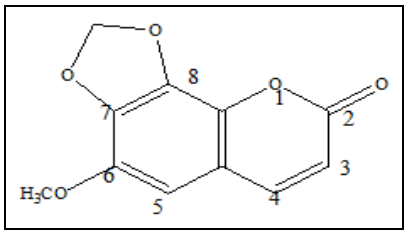
Compound I

Compound II: The elemental analysis and molecular weight determination indicated its molecular formula as $C_{11}H_{10}O_3$. The appearance of blue green colour in UV light indicated its coumarin nature. The important peaks observed in the infrared spectrum were at 3500 (-OH stretching), 1720 (coumarin lactone), 1615, 1560, 1570, (aromatic C=C) and 1130 (O=C-O) cm^{-1}. In the ^1H NMR spectrum the presence of three protons were observed in the aromatic region. A doublet at 6.77 (*J = 8* Hz) corresponded to C-8 proton which was ortho coupled to C-7 proton which appeared as a double doublet at 7.33 (*J = 8, 2* Hz). The third aromatic proton (C-5) was observed as a doublet at 7.33 (*J = 2* Hz) which was placed meta to C-7 proton. In addition to this, two methyl singlets appeared at 2.15 and 2.35 which could be placed at C-3 and C-4 respectively. In the mass spectrum, the molecular ion peak was observed at m/z 190, which served as the base peak also. Prominent peaks appeared at m/z 175 (M^+CH_3), 162[M^+-2CO]. From these spectral data the structure of compound was established as 6- hydroxy- 3, 4- dimethyl coumarin.



Compound II

Compound III: The elemental analysis and molecular weight determination indicated its molecular formula as $C_{11}H_8O_5$. The positive Labat test and colour with acidified phloroglucinol indicated the presence of methylenedioxy group. In the UV light indicated its coumarin nature. The important peaks observed in the infrared spectrum were at 1720 (C=O), 1620, 1590, 1560, (aromatic C=C) and 1130 (O=C-O) cm^{-1}. The ^1H NMR spectrum exhibited signals due to three aromatic protons appearing as two doublets at 7.57 and 6.27 (AB system, *J = 9.6* Hz, C-3, C-4 protons) and a singlet at 3.93 and two proton singlet at 6.16, corresponded to the presence of methoxy and methylenedioxy group in the compound. In the mass spectrum the molecular ion peak was observed at m/z 220. Prominent peak also appeared at m/z 205 [M^+-15], 192 [M^+-28] and 177 [M^+-43]. On the basis of above discussion the structure for the compound was assigned as 6-methoxy-7, 8-methylenedioxy coumarin.

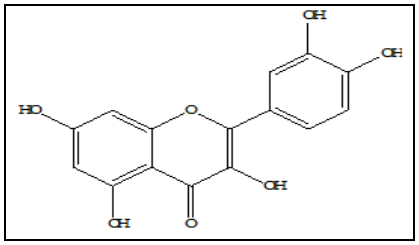


Compound III

Compound IV: This compound was found to be homogenous on TLC. The elemental analysis and molecular weight determination established its molecular formula as $C_{15}H_{10}O_7$. Its solubility in alkali, blue-green colouration with alcoholic $FeCl_3$ and dark reddish-brown colour in shinoda's test indicated its flavanoid nature. It developed green spot when TLC was developed with NP/PGE reagent under UV light at λ_{max} 365 nm. This compound showed R_f value 0.82 and 0.67 in system A and B respectively. System A is a mixture of Ethyl acetate, Formic acid, Glacial acetic acid and water in ratio of 51:11:11:27 and system B is a mixture of Ethyl acetate, Formic acid, Glacial acetic acid, Ethyl methyl ketone and water in ratio of 50:7:3:30:10.

In the infrared spectrum, a broad peak at 3450 indicated the presence of –OH groups. Peaks also appeared at 3010 (aromatic C-H stretching), 1590, 1520 (aromatic C=C stretching), 1230, 1200 and 1180 cm^{-1} (C-O stretching). The ^1H NMR spectrum displayed a set of doublets at 6.32 and 6.50 (*J = 2.5* Hz) for the meta-couple protons on C-6 and C-8 respectively. A doublet centered at 7.00 (*J = 8.5* Hz) could be attributed to C-5' proton. The remaining protons at (C-2' and 6') appeared as an overlapping doublet (*J = 2.5* Hz) and quartet (*J = 2.5*, *8.5* Hz) in the region 7.62-7.81. Singlet at 12.31, 10.25, 8.87, 8.68 and 8.31 due to hydroxyl group were placed at C-5, C-3, C-7 and C-4' respectively.

In the mass spectrum, the molecular ion peak appeared at m/z 302 which corresponded to the molecular formula $C_{15}H_{10}O_7$ indicating the presence of an additional oxygen (in comparison to compound kaempferol). Intense peak appeared at m/z 301 [M^+-H], 285[M^+-OH], 284 [M^+-H_2O], 152 [$C_7H_4O_4$] and 137 [$C_7H_5O_3$].



Compound IV

CONCLUSION: Petroleum ether extract of *Cyperus rotundus* rhizomes have a range of different class of natural compounds. These compounds have very useful medicinal activities so present work gives a direction for future investigators to get some medicinally important drugs.

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CONFLICT OF INTEREST: Nil

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