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Phytochemical Screening and GC-MS analysis of bioactive compounds present in ethanolic extract of aerial parts of *Cyperus alopecuroides* Rottb.

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ABSTRACT:

In the present investigation the phytochemical screening and gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extract of aerial parts of *Cyperus alopecuroides* Rottb was carried out. The phytochemical screening showed the presence of alkaloids, glycosides, cardiac glycosides, carbohydrates (Starch), proteins and reducing sugars. The GC–MS analysis revealed the presence of 21 phytochemical compounds. The major compounds were identified as 2,3-Dimethyl-4-nitro-1-pyrrolidin-1-yl-butan-1-one (37.3%); Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3-propanediyl ester (14.13%) and Citric Acid (12.54%).

Keywords: *Cyperus alopecuroides* Rottb., aerial parts, phytochemical screening, GC–MS analysis.

1. INTRODUCTION:

The sedge family, Cyperaceae comprises about 4000 species within 90 genera. *Cyperus* is the largest genus that includes about 550 species (Evans, 2002).

Cyperus alopecuroides Rottb. (Foxtail sedge) which belongs to Cyperaceae family, is a sedge plant that has a wide distribution in the tropical regions.

It is robust perennial plant, tufted, 180- 200 cm tall. The rhizome are woody. The stems are acutely trigonous, 4-10 mm wide. Leaves are flat, plicate and shorter than stems. White to glauscent beneath, sheaths coriaceous and 25-30 cm long. The inflorescence compound or decompound (WadoodKhan, 2015).

It is found common, along the banks of rivers, tanks, and lakes and in open marshes, more frequently standing in water.

C. alopecuroides is reported to be tolerant to both waterlogging and salinity (Nawaz et al. 2014).

It is a perennial and rhizomatous plant, usually less than 1 m in height.

Cyperus alopecuroides is a promising aquatic plant for phytoremediation, (Galal et. al., 2021).

Phytochemicals are natural bioactive compounds synthesized in plants that appear to have important physiological impacts in the human body. They cover a broad range of chemical substances such as, alkaloids, flavonoids, polyphenols, saponins, steroids, vitamins, among others. Depending on their role in plant metabolism they are divided into two types viz primary and secondary metabolites (Rex et. al., 2018). Sugars, amino acids, proteins, chlorophyll etc. are examples of primary metabolites whereas, the secondary metabolites includes flavonoids, alkaloids, terpenoids, saponins, tannins and phenolic compounds. The therapeutic properties of plants are due to phytochemicals (Savithramma et al., 2011).

GC-MS combines the separation properties of gas chromatography with the detection feature of mass spectrometry to identify different substances within a test sample depending on their mass.

It is one of the finest technique to identify bioactive compounds such as long chain hydrocarbons, alkaloids, acids, esters, alcohols, steroids, amino and nitro compound etc. (Poornima et. al., 2014; Starlin et. al., 2019; Dineshkumar and Rajakumar, 2016).

GC-MS is widely used in drug detection, environmental analysis, explosives investigation, forensic applications and identification of unknown compounds of plants (Starlin et. al., 2019).

2. MATERIALS AND METHODS:

2.1 Collection of plant material: *Cyperus alopecuroides* plants were collected from field and near damp water of Degloor, Dharmabad and Kinwat of Nanded Dist, Maharashtra state.

2.2 Preparation of plants powder: The aerial and underground parts of *Cyperus alopecuroides* were washed separately with distilled water to remove any external debris. The washed parts were shade dried at room temperature. After complete drying, aerial and underground parts were ground to a fine powder using a blender, packed in an air tight container and stored in a refrigerator at 4^{o}C for further use.

2.3 Soxhlet extraction: 30 grams of both powdered samples was loaded to a blank thimble filter and covered with cotton. 300ml of ethanol was poured into the round bottom extraction flask and the thimble containing the sample was placed into the extraction chamber. The extraction was carried out at 78 ^{o}C. The flask containing solvent and extracted crude was taken after the extraction process (Redfern et al., 2014).

2.4 Phytochemical analysis of plant extracts:

Qualitative phytochemical tests

The ethanolic extracts of aerial and underground parts of all four selected plants were tested for presence and absence of phytochemicals (in qualitative forms), like alkaloids, flavanoids, tannins, saponins, phenolic compounds, glycosides, cardiac glycosides, proteins, carbohydrates using standard procedures and reagents.

1. Test for Alkaloids

a. Wagner's test

For the detection of alkaloids in the plant extracts few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive (Raaman, 2006).

Wagner's Reagent:

This reagent is prepared by dissolving 1.27 gm of iodine and 2 gm of potassium iodide in 100 mL of distilled water.

b. Picric acid test

To the few mL plant extract 3-4 drops of 2% picric acid solution was added. Formation of orange colour confirms the test as positive (Deshpande et. al., 2014; Indhumati et. al., 2018).

2% picric acid solution:

It is prepared by dissolving 2 gm of picric acid in 100 mL of distilled water.

2. Test for Proteins and Amino acids

a) Millon's test:

This test is performed by adding few drops of Millon's reagent to 2mL plant extract.

A white precipitate formation indicates positive test (Silva et. al., 2017; Shaikh and Patil, 2020).

Millon's reagent:

It is prepared by adding 1gm mercury to 9mL fuming nitric acid and equal amount of distilled water was added after completion of reaction.

3. Test for Carbohydrates

Test for starch:

To the plant extract, 5mL 5% KOH solution was added. A cinary colour formation indicates positive test (Audu et. al., 2007).

4. Test for reducing Sugars:

Benedict's test

For the detection of reducing Sugars to the 0.5mL of plant extract 0.5mL of Benedict's reagent was added and boiled for 2 min. The formation of Green/yellow/red colour indicates positive test (Raaman, 2006; Singh and Kumar, 2017).

Benedict's reagent: It is prepared as follows

Solution A: It was prepared by dissolving 173gm sodium citrate and 100gm sodium carbonate in 800 mL of water, boil to make solution clear.

Solution B: It was prepared by dissolving 17.3gm of copper sulphate in 100mL distilled water.

Working solution: Mix solution A and solution B

5. Test for Glycosides:

Aqueous NaOH test

To the ethanolic plant extract 1mL of water was added mixed it properly and few drops of aqueous NaOH solution was added. The formation of a yellow colour indicates positive test (Jagessar 2017).

6. Test for Cardiac Glycosides

Baljet test:

For the detection of Cardiac Glycosides to the 2mL of plant extract, a drop of Baljet's reagent was added. The formation of yellow-orange colour indicates positive test.

(Rahman et. al., 2013; Kumar and Jat, 2018).

Baljet's reagent: It was prepared by mixing 95mL 1% picric acid solution and 5mL 10% NaOH solution

7. Test for Flavonoids:

Ferric chloride test:

This test was carried out by adding few drops 10% ferric chloride solution to the plant extracts. A green precipitate shows positive test (Audu, 2007).

8. Detection of Tannins:

Braymer's test

For the detection of presence or absence of tannins in the plant extracts, to the 1mL of plant extract 3mL distilled water was added. To this 3 drops 10% Ferric chloride solution was added. Blue-green colour formation indicates positive test (Uma et. al., 2017)

9. Test for Phenolic compounds:

Ferric chloride test:

The test for the detection of phenolic compounds was performed by adding few drops of 5% $FeCl_3$ solution to the plant extracts. The appearance of Dark green/bluish black colour indicates positive test (Tiwari et. al., 2011).

10. Test for Saponins:

Foam test:

For the detection of saponins in plant extracts, the 50 mg of plant extract is diluted with distilled water and made up to 20 ml. The suspension is shaken for 15 minutes. The formation of two cm layer of foam shows the presence of saponins (Devmurari, 2010).

2.5 Gas Chromatography-Mass Spectrometry (GCMS) analysis:

GC-MS analysis and characterization of ethanol extracts of aerial and underground parts of *C. rotundus* was done with EI- MS Spectrum scanned at 70 eV at SAIF, IIT Bombay. The relative percentage amount of each component was calculated by comparing its average peak area with the total area.

RESULTS AND DISCUSSION:

Phytochemical analysis of ethanolic extract of aerial parts of *Cyperus alopecuroides*

The ethanolic extract of aerial parts of *C. alopecuroides* showed the presence of alkaloids, glycosides, cardiac glycosides, carbohydrates (Starch), proteins and reducing sugars. The other phytocompounds such as tannins, flavonoids, phenols and saponins were found to be absent as shown in Table 1.

Table 1: Phytochemical analysis of ethanolic extract of aerial parts of *Cyperus alopecuroides*

Sr. no.	Phytochemical	Test	Observation
1	Alkaloids	Wagners test	+
		Picric acid test	+
2	Tannins	Braymer's test	-
3	Flavonoids	Ferric chloride test	-
4	Glycosides	Aqueous NaOH test	+
5	Cardiac glycosides	Baljet test	+
6	Phenols	Ferric chloride test	-
7	Proteins	Millon's test	+
8	Carbohydrates	Barfoed's test	+
9	Reducing sugars	Benedict's test	+
10	Saponins	Foam test	-

Alkaloids, phytochemical compounds found in aerial parts of the studied plant have the greatest significance among the phytoconstituents and have analgesic, antibacterial and antispasmodic activities among several others (Enujugha and Agbede, 2000; Harisaranraj et al., 2009; Uyo et al., 2013). The Glycosides possess antidiarrhoeal

properties (Tiwari et. al., 2011). Cardiac glycosides are used in treatment of congestive heart failure (Ngoci et al., 2011).

GC–MS analysis of *Cyperus alopecuroides* aerial parts:

The GC–MS analysis revealed the presence of 21 phytochemical compounds in ethanolic extract of *Cyperus alopecuroides*. The phytocompounds with their molecular formula, molecular weight (MW), retention time (RT) and concentration (%) are presented in Table 2.

The following bioactive compounds were found in the ethanolic extract Disulfide, isopentyl methyl (1.67%); 2-Methyoxirane-2-carboxylic acid, methyl ester (1.50%);

Citric Acid (12.54%); 1,3,7,9,2,8-Parazabol, 4,5,10,11-tetrabromo-2,2,8,8-tetraethyl-(0.02%); Pentacarbonyltris(trimethylstannyl) Stibinechromium (1.67%); Acetamide, 2-diethylamino-N-[1-(4-hydroxybenzyl)cyclohexyl]- (0.09%); 1,3-Dioxolane-2-acetic acid, 2,4-dimethyl-, ethyl ester (1.27%); 1,3-Dioxolane-2-propanol, 2,4-dimethyl- (1.12%); Ethanethioic acid, S-(2-methylbutyl) ester (0.27%); 1H-Indene, 1-hexadecyl-2,3-dihydro- (0.22%); 2-Pyrrolidinone, 1-[2-oxo-4-(1-pyrrolidinyl)butyl]- (0.84%); Acetic acid, 2-ethylbutyl ester (6.03%); Heptane, 4-ethyl-(0.33%); Benzene, 1,2-bis(hexyloxy)-4-nitro- (6.90%); 1-Decanol, 2-ethyl- (4.52%); 3-Pentanethiol (8.31%); 2,3-Dimethyl-4-nitro-1-pyrrolidin-1-yl-butan-1-one (37.3%); Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3-propanediyl ester (14.13%); Octane, 3,4,5,6-tetramethyl- (1.40%) 2-Methylpropionic acid, 3,4-dichlorophenyl ester (0.90%) and [1-(Diethylamino)ethylidenimino]sulfur pentafluoride (0.46%).

The major compounds were identified as 2,3-Dimethyl-4-nitro-1-pyrrolidin-1-yl-butan-1-one (37.3%); Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3-propanediyl ester (14.13%); Citric Acid (12.54%); 3-Pentanethiol (8.31%); Benzene, 1,2-bis(hexyloxy)-4-nitro- (6.90%) and Acetic acid, 2-ethylbutyl ester (6.03%). The rest of compounds were present by the amount of less than 6%.

Among the compounds identified Disulfide, isopentyl methyl eluted first has the minimum Retention time (7.45 min) whereas, [1-(Diethylamino)ethylidenimino]sulfur pentafluoride eluted lastly and has the maximum Retention time (41.88 min).

The chromatogram of the GC-MS spectral analysis of aerial parts of *C. alopecuroides* reflecting the peaks of the distinct compounds and their retention times are shown in Fig 1.

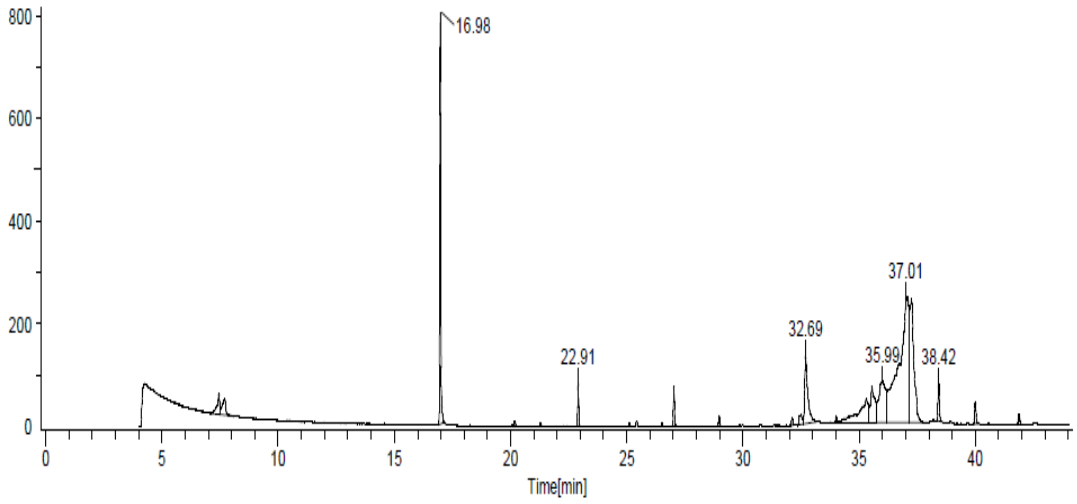

Fig. 1: GC-MS spectrum of ethanolic extract of aerial parts of *Cyperus alopecuroides*

Table 2: GC-MS analysis identified compounds of aerial parts of *Cyperus alopecuroides*

Sr No.	Compound name	Molecular formula	Molecular weight	RT (min)	Peak area/Conc. (%)
1	Disulfide, isopentyl methyl	$C_6H_{14}S_2$	150	7.45	1.67
2	2-Methyoxirane-2-carboxylic acid, methyl ester	$C_5H_8O_3$	116	7.71	1.50
3	**Citric Acid**	$C_6H_8O_7$	192	16.98	**12.4**
4	1,3,7,9,2,8-Parazabol, 4,5,10,11-tetrabromo-2,2,8,8-tetraethyl-	$C_{14}H_{22}B_2Br_4N_4$	584	20.07	0.02
5	Pentacarbonyltris(trimethylstannyl) Stibinechromium	$C_{14}H_{27}CrO_5SbSn_3$	808	20.19	0.12
6	Acetamide, 2-diethylamino-N-[1-(4-hydroxybenzyl)cyclohexyl]-	$C_{19}H_{30}N_2O_2$	318	21.29	0.09
7	1,3-Dioxolane-2-acetic acid, 2,4-dimethyl-, ethyl ester	$C_9H_{16}O_4$	188	22.91	1.27
8	1,3-Dioxolane-2-propanol, 2,4-dimethyl-	$C_8H_{16}O_3$	160	27.02	1.12
9	Ethanethioic acid, S-(2-methylbutyl) ester	$C_7H_{14}OS$	146	28.97	0.27
10	1H-Indene, 1-hexadecyl-2,3-dihydro-	$C_{25}H_{42}$	342	32.11	0.22

11	2-Pyrrolidinone, 1-[2-oxo-4-(1-pyrrolidinyl)butyl]-	$C_{12}H_{20}N_2O_2$	224	32.48	0.84
12	**Acetic acid, 2-ethylbutyl ester**	$C_8H_{16}O_2$	144	32.69	**6.03**
13	Heptane, 4-ethyl-	C_9H_{20}	128	34.02	0.33
14	Benzene, 1,2-bis(hexyloxy)-4-nitro-	$C_{18}H_{29}NO_4$	323	35.30	6.90
15	1-Decanol, 2-ethyl-	$C_{12}H_{26}O$	186	35.54	4.52
16	**3-Pentanethiol**	$C_5H_{12}S$	104	35.99	**8.31**
17	**2,3-Dimethyl-4-nitro-1-pyrrolidin-1-yl-butan-1-one**	$C_{10}H_{18}N_2O_3$	214	37.01	**37.3**
18	**Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3-propanediyl ester**	$C_{16}H_{30}O_4$	286	37.25	**14.13**
19	Octane, 3,4,5,6-tetramethyl-	$C_{12}H_{26}$	170	38.42	1.40
20	2-Methylpropionic acid, 3,4-dichlorophenyl ester	$C_{10}H_{10}Cl_2O_2$	232	40.00	0.90
21	[1-(Diethylamino)ethylidenimino]sulfur pentafluoride	$C_6H_{13}F_5N_2S$	240	41.88	0.46

Based on literature search, there has been no research carried out on GC–MS analysis of the ethanolic extract of aerial parts of *Cyperus alopecuroides.*

Among the different identified compounds in the aerial parts of *Cyperus alopecuroides* the compound 1H-Indene, 1-hexadecyl-2,3-dihydro- possess anti-5-HT, Hallucinogenic, HDL-genic, Helicicide and Hemagglutinator activities. It also acts as antidote for heavy metals. Using Dr. Duke's phytochemical and ethnobotanical database (online), the biological activity of the identified phytocomponents was ascertained.

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REFERENCES:

1. Audu, S.A., Mohammad, I. and H.A. Kaita (2007) Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Sci J. 4(4):75-79.
2. Deshpande, P.K., Gothalwal, R. and A.K. Pathak (2014). Phytochemical analysis and evaluation of antimalarial activity of *Azadirachta indica*. The Pharma Innovation. 3(9):12-16.

3. Devmurari, V. P. (2010). Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb Arch. Appl. Sci. Res. 2(1): 354-359.

4. Dineshkumar G. and R. Rajakumar (2016). Gas Chromatography-Mass Spectrometry analysis of bioactive components from the ethanol extract of *Avicennia Marina* leaves. Innovare Journal of Science. 4 (4): 9-12**.**
5. Duke's Phytochemical and Ethnobotanical Databases: U.S. Department of Agriculture, Agricultural Research Service 1992 [Online]. Available: http://phytochem.nal.usda.gov

6. Enujugha VN and Agbede JO (2000). Nutritional and antinutritional characteristics of African oil bean (*Pentaclethra macrophylla* Benth.) seeds. Appl Tropic Agric., 5: 11- 14

7. Galal, T.M., Shedeed, Z.A., Gharib, F.A. *et al.* (2021). The role of *Cyperus alopecuroides* Rottb. sedge in monitoring water pollution in contaminated wetlands in Egypt: a phytoremediation approach. Environ Sci Pollut Res 28, 23005–23016.

8. Harisaranraj R, Suresh K and Sravanababu S (2009). Evaluation of chemical composition in Rauwolfia serpentine and Ephedra vulgaris. Adv Biol Res., 3:174-178

9. Indhumati, V., Perundevi, S., Vinoja, B.N., Manivasagan, S.K., Ramesh and N.G. Babu (2018). Phytochemical screening and antimicrobial activity of fresh and shade dried leaves of *Azadirachta indica.* Inter J of Inno in Eng and Tech. 11(3):27-32.

10. Jagessar, R.C. (2017). Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae). J Pharmaco and Phyto. 6(6):1714-1721.

11. Kumar, V. and R.K. Jat (2018). Phytochemical estimation of medicinal plant *Achyranthes aspera* root. Int J of Res in Pharma and Pharmaceut Sci. 3(1):190-193.

12. Nawaz T, Hameed M, Ashraf M, SajidM, Ahmad A, Batool R, Fatima S (2014) Anatomical and physiological adaptations in aquatic ecotypes of *Cyperus alopecuroides* Rottb. under saline and waterlogged conditions. Aquat Bot 116:60–68. https://doi.org/10.1016/j.aquabot.

13. Ngoci S.N., Mwendia C.M., Mwaniki C.G., 2011. Phytochemical and cytotoxicity testing of Indigofera lupatana Baker F. Journal of Animal & Plant Sciences, 11(1), 1364-1373.

14. Poornima, K., Perumal, P.C. and V. K. Gopalakrishnan (2014). Protective effect of ethanolic extract of *Tabernaemontana divaricata* (L.) R. Br. against DEN and Fe NTA induced liver necrosis in Wistar Albino rats. BioMed res inter. 2014: 1-9.
15. Raaman, N. (2006). Phytochemical Techniques. New India Publishing Agency, New Delhi, 19-24.

16. Rahman, M.A., Rahman, M.A. and NU Ahmed (2013). Phytochemical and biological activities of ethanolic extract of *C. hirsute* leaves. Bangladesh J of Scienti and Indust Res. 48(1):43-50.

17. Redfern, J., Kinninmonth, M., Burdass, D. and J. Verran (2014). Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Micro Biol Educa. 15(1): 45–46.
18. Rex, J.R.S., Muthukumar, N.M.S.A. and P. M. Selvakumar (2018). Phytochemicals as a potential source for anti-microbial, anti-oxidant and wound healing - a review. MOJ Biorg Org Chem. 2(2):61-70.
19. Savithramma, N., Linga Rao M., and D. Suhrulatha (2011). Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific Researc. 8: 579-84.
20. Shaikh, J.R. and M. Patil (2020). Qualitative tests for preliminary phytochemical screening: An overview. Int J of Chem Stu. 8(2): 603-608.
21. Silva, G.O., Abeysundara, A.T., and M.M. Aponso (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. Ameri J Essen Oils and Nat Prod. 5*:* 29-32.
22. Singh, V. and R. Kumar (2017). Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand Region. Int J of Life Sci Scient Res. 3(6):1451-1458.
23. Starlin, T., Poochi, P., Basanth, T., & V.K. Gopalakrishnan (2019). Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. Bioinformation. 15: 425-429.
24. Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and H. Kaur (2011). Phytochemical screening and extraction: A Review. *Internationale Pharmaceutica Sciencia*. 1(1):98-106.
25. Uma, K.S., Parthiban, P. and S. Kalpana (2017). Pharmacognostical and preliminary phytochemical screening of Aavaarai Vidhai Chooranam. Asi J of Pharma and Clin Res. 10(10):111-116.
26. Uyoh EA, Ita EE and Nwofia GE (2013). Evaluation of the chemical composition of Tetrapleura tetraptera (Schum and Thonn.) Taub. Accessions from Cross River State, Nigeria. Int J Med Aromat Plants., 3 (3): 386-394.
27. W. C. Evans, Trease and Evans Pharmacognosy, 15th Ed., W. B. Saunders, London, 2002, p. 38.