



Phytochemical profiling of ethanolic extract of aerial and underground parts of *Cyperus scariosus* R.Br.

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ABSTRACT:

The present study was aimed to carry out the phytochemical screening of phytochemical constituents present in ethanolic extract of aerial and underground parts of *Cyperus scariosus* R. Br. In the qualitative phytochemical tests the aerial parts shows the presence of alkaloids, flavonoids, phenolic compounds, tannins, carbohydrates (Starch) and reducing sugars, whereas, alkaloids, flavonoids, phenolic compounds, cardiac glycosides, carbohydrates (Starch), proteins, reducing sugars and saponins were found to be present in underground parts. In the quantitative tests, in the aerial and underground parts of *C. scariosus* the alkaloid content was found to be 43.04 ± 1.07 and 79.31 ± 0.98 mg/g, the flavonoid content was 40.1 ± 1.2 and 42.08 ± 0.91 mg/g and the polyphenols content was 33.06 ± 4.81 and 28.85 ± 3.12 mgGAE/g plant sample.

Keywords: *Cyperus scariosus* R. Br., aerial parts, underground parts, qualitative phytochemical test, quantitative phytochemical test.

1. Introduction:

Cyperaceae a family of monocotyledonous graminoid flowering plants known as sedges and superficially resemble grasses or rushes. This family has approximately 5,500 species described in about 109 genera (Arshiya et.al., 2013). *Cyperus scariosus* R. Br is perennial grass herb standing erect in stagnant water. It is 20-100 cm tall and arises from underground rhizomes and tubers. The stolons of this herb are slender with aromatic blackish tubers (ovoid). The aerial stem is terete/ trigonous at least the lower part, 1.5-03 mm thick (WadoodKhan, 2015). It is extensively used in the traditional systems of medicine.

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 (Savithamma et al., 2011).



Fig 1: Morphology of *C. scariosus* plant

2. Materials and Methods:

2.1: Collection of plants material

Plants of *Cyperus scariosus* R. Br. were collected from field and near damp water of Degloor, Dharmabad and Kinwat of Nanded Dist, Maharashtra state. The plants were identified by Dr. M. A Wadood Khan, Plant taxonomist, M.S.P Mandal's Majalgaon Arts, Science and Commerce College, Majalgaon, Dist. Beed, M.S.

2.2: Qualitative phytochemical tests analysis

The ethanolic extracts of aerial and underground parts of *Cyperus scariosus* R. Br. 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).

3.3: Quantitative phytochemical analysis

The phytochemicals detected in the ethanolic extracts of aerial and underground parts of *Cyperus scariosus* R. Br were quantified using standard procedures as described by Harborne (1973); Krishnaiah et al., (2009) and Nhut et. al., (2020).

1) Estimation of Alkaloids

The 5 g of the sample was weighed, to this 200 mL of 10% acetic acid in ethanol was added, covered and left to stand for 4 hour. It was filtered, and the filtrate was then concentrated to a quarter of its original volume on a water bath. Drop by drop, concentrated NH_4OH was added to the filtrate until the precipitation was complete. After allowing the entire solution to settle, the precipitate was collected, washed with dilute NH_4OH , and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973; Longbap et.al, 2018).

2) Estimation of Flavonoids

For the estimation of flavonoids 10 g of each plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. After that, a Whatman No. 42 filter paper was used to filter the entire solution into a pre-weighed 250 mL beaker. The filtrates were placed in a water bath, allowed to dry completely, and then weighed. (Krishnaiah et al., 2009).

3) Total polyphenol content

All procedures were carried out protected from light. The plant extracts were dissolved with the extraction solvent itself to obtain the experimental solution. The mixture of 0.5ml of the test solution was taken, to this 2.5ml of 10% Folin-Ciocalteu solution was added and mixed well by a vortex machine. After 5 min of rest, 2.0 ml of 7.5% Na_2CO_3 was added to the mixture and incubated for 60 min. Finally, the absorbance of test solutions was measured with blank at 765 nm wavelength using UV-Visible spectrophotometer and evaluated against the standard gallic acid. The total polyphenol content was expressed as mg of GAE/g of extract (Nhut et. al., 2020).

3. Results and Discussion

The qualitative screening is very important to determine the phytochemical compounds present in herbal plants. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation (Kavitha, 2017).

3.1: Phytochemical analysis of ethanolic extract of aerial parts of *Cyperus scariosus*

The preliminary phytochemical screening of ethanolic extract of aerial parts of *C. scariosus* revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, carbohydrates (Starch) and reducing sugars. The other phytocompounds including glycosides, cardiac glycosides proteins and saponins were absent as shown in Table 1.

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

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	-

+ Present - Absent

From the phytochemical investigation it was revealed that the ethanol extract of aerial parts of *Cyperus scariosus* may have anti-inflammatory, antioxidant agents associated with free radical-scavenging action due to the presence of flavonoids and antidiarrheal activity owing to presence of tannins.

3.2: Phytochemical analysis of ethanolic extract of underground parts of *Cyperus scariosus*

The results of preliminary phytochemical screening of ethanolic extract of underground parts of *C. scariosus* are presented in Table 2. The results showed the presence of alkaloids, flavonoids, phenolic compounds, cardiac glycosides, carbohydrates (Starch), proteins, reducing sugars and saponins.

Table 2: Phytochemical analysis of ethanolic extract of underground parts of *Cyperus scariosus*

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	+

+ Present - Absent

In the aerial parts of *Cyperus scariosus* glycosides, cardiac glycosides, proteins and saponins were found to be absent, whereas in the underground parts cardiac glycosides, proteins and saponins were present. The glycosides were found to be absent in both aerial and underground parts of *Cyperus scariosus*.

Many studies have been carried out to determine the bioactive compound of *Cyperus scariosus* using simple phytochemical screening. Nafees et. al., (2022) reported the presence of alkaloids, proteins, carbohydrates, phenols, flavonoids, sterols, glycosides, cardiac glycosides, sterols/terpenes, and volatile oil were all found during the phytochemical screening of *Cyperus scariosus*. Kakarla (2016) reported the presence of alkaloids, flavonoids, glycosides, carbohydrates and phenols in the methanolic extracts of rhizome of *Cyperus scariosus*. Tannins, saponins and proteins were absent. Based on literature search, there has been no research carried out on the ethanolic extracts of aerial parts of *Cyperus scariosus*. The plant was found to be enriched with most of the assessed phytochemicals except glycosides.

3.3: Quantitative phytochemical analysis:

Estimation of Alkaloids and Flavonoids:

The alkaloid content in the aerial and underground parts of *C. scariosus* was found to be 43.04 ± 1.07 and 79.31 ± 0.98 mg/g of plant sample. In the underground parts of *C. scariosus* the concentration of alkaloid was more as compared to aerial parts as shown in Table 3.

The flavonoid content in the aerial and underground parts of *C. scariosus* was found to be 40.1 ± 1.2 and 42.08 ± 0.91 mg/g of plant sample. The alkaloids are natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. Flavonoids tend to be most commonly known with regards to antioxidant nature. They are transformers which alter the body biochemical reactions to carcinogenic chemicals, viruses, and things that trigger allergies (Madhu et. al., 2016).

Estimation of Total polyphenol content:

The polyphenols content in the ethanolic extracts of aerial and underground parts of *C. scariosus* were found to be 33.06 ± 4.81 and 28.85 ± 3.12 mgGAE/g as shown in Table 3. The phenolic compounds are some of the most widespread molecules among plant secondary metabolites and act as natural antioxidants (Madhu et. al., 2016).

Table 3: Quantitative Phytochemical analysis of *C. scariosus* aerial and underground Parts

Plant sample	Alkaloid content (mg/g)	Flavonoid content (mg/g)	Total phenolic content mgGAE/g
Aerial parts of <i>C. scariosus</i>	43.04 ± 1.07	40.1 ± 1.2	33.06 ± 4.81
Underground parts of <i>C. scariosus</i>	79.31 ± 0.98	42.08 ± 0.91	28.85 ± 3.12

Mean \pm SD [n=3]

4. Conclusion

Based on the results obtained in the present investigation, it may be concluded that the ethanolic extract of underground parts of *Cyperus scariosus* contains most of the assessed phytochemicals than aerial parts. The concentration of alkaloids and flavonoids is also found to be more in underground parts of the plant as compared to aerial parts.

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