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Qualitative and Quantitative Phytochemical Estimation of Plant: Cyperus rotundus L.

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Abstract:

In Asia, the use of herbal medicine reflects a long history of human interactions with the environment. The World Health Organization (WHO) reports that over 80% of the world's population depend on traditional medicine for their primary healthcare need. Medicinal plants are defined as those that have positive pharmacological effect on either humans and Animals. Fossils provide evidence that humans have been using plants as medicine for roughly 60,000 years. In many societies ,growing and gathering medicinal herbs has great cultural value because phytoconstituents are lead chemicals in the current drug discovery process. Worldwide more than 2,00,000 compounds, including primary and secondary metabolites have been discovered and extracted from higher plants. Methanolic and Chloroformic extract of leaf and rhizome of C.rotundus were prepared and subjected to qualitative phytochemical analysis. The objective of present study was screening of such phytochemicals in selected medicinal plant i.e., Cyperus rotundus L. after analysis it was found that steroids, Triterpenoids, Alkoloids, Flavonoids, Phenol, Saponin, Proteins found almost both leaf and Rhizome extract. Carbohydrate found only in leaf extract and Tannins found only in rhizome extract. It was concluded that the plant studied was reach in phytochemicals with significant medicine and pharmacological applications.

Keywords - Cyperus rotundus L., Phytochemicals, Pharmacological effect, Flavonoids

Introduction:

In Asia, the use of herbal medicines reflects a long history of human interactions with the environment. The World Health Organization (WHO) reports that over 80% of the world's population depends on traditional medicine for their primary healthcare needs. The plants used in traditional medicine contain a wide range of ingredients that can be used to treat infectious and chronic diseases.

Medicinal plants are defined as those that have positive pharmacological effects on either humans or animals [1]. Fossils provide evidence that humans have been using plants as medicine for roughly 60,000 years.[2] With fewer adverse effects, medicinal plants offer accessible and reasonably priced remedies. In many societies, growing and gathering medicinal herbs has great cultural value.[3] Because phytoconstituents are employed as lead chemicals in the current drug discovery process, plants are also very important in modern medicine. Worldwide, more than 200,000 compounds, including primary and secondary metabolites, have been discovered and extracted from higher plants.

A very varied taxonomic member of the *Cyperus* genus, *Cyperus rotundus* L. forms species complexes and is thought to have originated in Asia. Because of its distinctive reddish-brown-purple spikelet, the perennial herb usually referred to as "puple nut sedge". The plant typically reaches a height of 20 to 50 cm, yet under ideal circumstances it may reach greater

heights. Culms typically have terminal inflorescence and are erect, solitary, smooth, and trigonous. The glossy, somewhat serrated, dark to bright green leaves are typically shorter than the culm.

Materials and Methods:

Collection of Plant:

The plant material of *Cyperus rotundus* was collected from Nanded District, Maharashtra, India. The collected plant was identified and authenticated by Department of Botany, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad.

Powdering and Extraction of Plant Material:

Powdering and extraction of collected plant material was done in reference with the previous literature. [4-8] The leaf and the rhizome were separated and dried in the shade separately for about two weeks, powdered individually in mechanical grinder and stored air tightly for further study. Leaf and stem powder of *Cyperus rotundus* was extracted with different solvents such as Ethanol, Methanol, Ethyl acetate,chloroform by using Soxhlet apparatus assembly for 48h in five times, the extracts obtained were concentrated by simple distillation. Finally, the crude extracts were stored in glass vial for further studies.

Qualitative Analysis Of Plant Extract:

The crude extract of leaves and Rhizome of Cyperus rotundus was subjected to preliminary phytochemical analysis in accordance with the modified procedure in the published literatures. [4-9]

Test for Glycoside:

1. Keller killani test: (Cardiac glycosides) the test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and concentrated sulphuric acid is added from the sides of the test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green in colour.

2. Raymond's test: Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

3. Legal's test: The test solution treated with 1ml pyridine and 1ml sodium nitroprusside gives pink to red colour appears.

Test for Alkaloids:

1. Mayer's test: Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream coloured precipitate.

2. Wagner's test: The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gives brown precipitate.

3. Hager's reagent: The acetic test solution treated with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.

Test for Flavonoids:

1. Ferric chloride test: The test solution with few drops of ferric chloride solution shows intense green colour.

2. Shinoda test: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red colour.

3. Zinc - Hydrochloric acid-reduction test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

4. Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

5. Lead acetate solution test: Test solution with few drops of lead acetate solution (10% w/v) gives yellow precipitate.

Test for Steroids:

1. Chloroform Test: The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (1 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of

steroids.

2. Salkowski's test: The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour.

Test for Phenols:

1. Ferric Chloride Test: A small amount of the ethanolic extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl3) was added. A blue, green, red or purple color is a positive test.

Test for Terpenoids:

1. Salkowaski test: When few drops of concentrated sulphuric acid is added to the test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

2. Liebermann Burchard test: The test solution treated with few drops of aceticanhydride and mixed well. When concentrated sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and the upper layer turns green.

Test for Saponins;

1. Foam test: Saponins when mixed with water and shaken shows the formation of foam which is stable at least for 15 min.

2. Haemolysis test: 2 ml of 18%w/v sodium chloride in two test tubes were taken.To one test tube distilled water and to the other test tube 2 ml of filtrate were added and then few drops of blood was added to both the tubes. Mixed and observed the haemolysis under microscope.

3. Raymond's test: Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

Test for Carbohydrates:

1. Molisch's test: Test solution with few drops of Molisch's reagent and two ml of concentrated sulphuric acid added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.

2. Barfoed's test: Test solution treated with Barfoed's reagent and boiling on a water bath shows brick red precipitate.

3. Benedict's test: Test solution treated with Benedict's reagent and boiling on a water bath shows reddish brown precipitate.

Test for Proteins:

1. Millon's test: Test solution treated with Millon's reagent and heated on a water bath; protein is stained yellow on warming.

2. Xanthoproteic test: Test solution treated with concentrated nitric acid and on boiling gives yellow precipitate.

3. Biuret test: Test solutions treated with 40% sodium hydroxide and dilute copper sulphate solution gives blue colour.

4. Ninhydrin test: Test solution treated with ninhydrin reagent gives blue colour.

Test for Starch:

1. Starch Reagent: Test: 1ml of of extract was added into 10ml of Nacl solution. After heating, starch reagent was added a blue purplish colour is a positive test for the presence of starch.

Test for Tannins:

1. Gelatin Test: Plant Extract is dissolved in 5ml of distilled water and 1% gelatin solution and 10% Nacl. Reaction gives a white precipitate.

2. NaoH Test: 4 ml of 10% NaoH added into the 0.4ml of extract and shaken well formation of emulsion.

Test for Flavanol:

1. Ferric chloride test: The test solution with few drops of ferric chloride solution shows intense green colour.

2. Shinoda test: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red colour.

3. Zinc - Hydrochloric acid-reduction test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

4. Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

5. Lead acetate solution test: Test solution

with few drops of lead acetate solution (10% w/v) gives yellow precipitate.

Test for Anthocynin:

1.Hcl Test: 2ml of plant extract and 2 ml of 2N Hcl were mixed with few ml of ammonia gives the pink red solution turns into blue violet after addition of ammonia.

Quantitative Analysis Of Plant Extract:

Estimation of Total Phenolic content:

The total phenolic content of various extractions was determined with Folin-Ciocalteu method using spectrophotometer.

Leaf and Rhizome extract of *C.rotundus* in the concentration of 200, 400, 600, 800, 1000 µg/ml was used in the analysis. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water in a 25 ml volumetric flask. Then Folin-Ciocalteu phenol reagent was added to the mixture and shaken vigorously. After 5 minutes, 10 ml of 7% sodium carbonate solution was treated to the mixture. The volume was adjusted up to 25 ml. Similarly, a set of standard solutions of Gallic acid (200, 400, 600, 800 and 1000 µg/ml) were prepared in similar manner. Absorbance of test and standard solutions were determined against the reagent blank at 550 nm on spectrophotometer after the incubation period of 90 min at room temperature. The total phenolic content was expressed as µg of Gallic acid equivalent (GAE) per g of extract. The absorbance of test sample was performed in triplicate.

Estimation of Total flavonoids content:

Total flavonoid content was measured by the aluminium chloride colorimetric assay.

Briefly, the reaction mixture contains 1 ml of extract and 4 ml of distilled water in a 10 ml volumetric flask then Add, 0.30 ml of 5% sodium nitrite and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2 ml of 1M sodium hydroxide was treated and diluted to 10 ml with distilled water. A similar set of reference standard solutions of quercetin (200, 400, 600, 800 and 1000 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with spectrophotometer. The total flavonoid content was expressed as µg of quercetin equivalents (QE) per g of extract. The absorbance of test sample was performed in triplicate.

Results:

The result of preliminary qualitative Phytochemical analysis shown in table 1 and table 2. The results revealed the presence of medically active compounds present in plant *C.rotundus*.From the table 1 it could be seen that steroids, alkaloids, Tritepenoids, Glycoside, saponin, flavonoids, tannins, phenols, protein were present in root extract of C.rotundus . By observing table 2 we conclude that steroids, Tritepenoids, Saponin, alkaloids, flavonoids, phenols, proteins present in Leaf extract of *C.rotundus* .

The result of quantitative estimation of TPC and TFC along with standard curve plotted have been depicted in fig A & B.It is clearly evident from the result that TPC in both stem and rhizome extract is greater than than TFC of stem and root extract of *C.rotundus*.

Table No.1 Qualitative Phytochemical Analysis of leaf extract of *C.rotundus* .

Sr.No.	Chemical Constituents	Test	CRLE (Ethanol)	CRLE (Methanol)	CRLE (Chloroform)	CRLE (Ethyl Acetate)
1.	Steroids	Chloroform test	--	--	--	++
		Salkowski test	++	++	--	--
		Sulphur test	--	--	--	--
2.	Triterpenoids	Salkowaski test	++	++	--	--
		Liebermann Burchard test	++	++	--	--
		Biuret test	++	--	++	++
		Ninhydrin test	--	--	--	--
3.	Glycoside	Raymond's test	--	--	--	--
		Keller killiani test	--	--	--	--
		Legal's test	--	--	--	--
4.	Saponin	Foam Test	--	++	--	++
		Raymond's test	--	--	--	--
5.	Alkaloids	Wagner's test	--	++	--	--
		Hager's test	--	--	++	++
		Mayer's test	++	--	--	--
6.	Flavonoids	Lead acetate solution test	++	--	++	--
		Shinoda test	--	++	--	--
		Ferric chloride test	--	++	--	++
		Zinc- Hydrochloric acid reduction test	++	--	--	++
		Alkaline reagent test	++	--	--	++
7.	Phenol	Fecl3 test	++	++	++	++
8.	Proteins	Xanthoproteic Test	--	--	++	--
		Miillion's Test	--	++	--	--
		Ninhydrin Test	--	--	--	--
9.	Tannins	Gelatin Test	--	--	--	--
		NaOH Test	--	--	--	--

Table No.2 Qualitative Phytochemical Analysis of Rhizome Extract of *C.rotundus*.

Sr.No.	Chemical Constituents	Test	CRRE (Ethanol)	CRRE (Methanol)	CRRE (Chloroform)	CRRE (Ethyl Acetate)
1.	Steroids	Chloroform test	++	--	--	++
		Salkowski test	++	++	--	--
		Sulphur test	--	--	--	--
2.	Triterpenoids	Salkowaski test	++	++	--	--
		Liebermann Burchard test	--	--	--	--
		Biuret test	--	--	++	--
		Ninhydrin test	--	--	--	--
3.	Glycoside	Raymond's test	--	--	--	--
		Keller killiani test	--	--	++	--
		Legal's test	--	--	--	++
4.	Saponin	Foam Test	--	--	++	++

		Raymond's test	--	--	--	--
5.	Alkaloids	Wagner's test	++	--	--	--
		Hager's test	--	++	++	++
		Mayer's test	--	--	--	--
6.	Flavonoids	Lead acetate solution test	++	--	--	--
		Shinoda test	++	++	--	--
		Ferric chloride test	--	--	--	++
		Alkaline reagent test	--	++	++	--
7.	Phenol	Fecl3 test	++	++	++	++
8.	Proteins	Xanthoprotic Test	--	--	--	--
		Million's Test	++	++	--	++
		Ninhydrin Test	--	--	--	++
9.	Tannins	Gelatin Test	++	--	--	--
		NaOH Test	--	--	--	--
		Ninhydrin Test	--	++	++	--

Table No.3: Standard: Quercetin

Sr.No.	Quercetin Concentration (µg/ml)	Absorbance at 510 nm
1.	200	0.215
2.	400	0.452
3.	600	0.612
4.	800	0.856
5.	1000	1.203

Table No.4: Estimation of Total Flavonoid

			C.rotundus Leaf		C.rptundus rhizome	
Sr.No.	Sample	Concentration (µg/ml)	Absorbance at 510 nm	TFC (µg QE /g)	Absorbance at 510 nm	TFC (µgQE/g)
2.	Methanolic extract	200	0.055	40.20	0.124	100.22
		400	0.163	110.25	0.204	200.02
		600	0.188	180.26	0.336	308.11
3.	Ethnolic extract	200	0.091	30.25	0.054	41.20
		400	0.124	100.60	0.164	120.30
		600	0.196	190.25	0.233	230.11
4.	Ethyl Acetate extract	200	0.064	40.23	0.154	140.26
		400	0.120	100.26	0.231	210.32
		600	0.228	210.32	0.297	280.1
5.	Chloroformic extract	200	0.084	40.30	0.112	100.20
		400	0.164	120.55	0.194	190.26
		600	0.358	300.85	0.262	250.12

Table No.5: Standard: Gallic Acid

Sr. No.	G.A.Concentration (µg/ml)	Absorbance at 510 nm
1.	200	0.198
2.	400	0.412
3.	600	0.625
4.	800	0.812
5.	1000	0.925

Table No.6: Estimation of total phenolic content

			C.rotundus Leaf		C.rptundus rhizome	
Sr.No.	Sample	Concentration (µg/ml)	Absorbance at 510 nm	TPC (µg GA /g)	Absorbance at 510 nm	TPC (µg GA/g)
2.	Methanolic extract	200	0.093	93.62	0.060	60.23
		400	0.152	156.20	0.153	154.36
		600	0.315	316.02	0.210	210.36
3.	Ethnolic extract	200	0.056	56.32	0.126	125.62
		400	0.152	156.23	0.232	234.20
		600	0.216	261.30	0.425	425.12
4.	Ethyl Acetate extract	200	0.080	80.12	0.112	112.30
		400	0.154	154.32	0.362	362.20
		600	0.231	231.02	0.421	421.30
5.	Chloroformic extract	200	0.022	22.30	0.131	132.30
		400	0.120	120.30	0.266	266.30
		600	0.284	286.32	0.311	311.20

Graph of the Activity:

- Graph plotted concentration of Gallic acid VS absorbance of the Gallic acid at 550nm

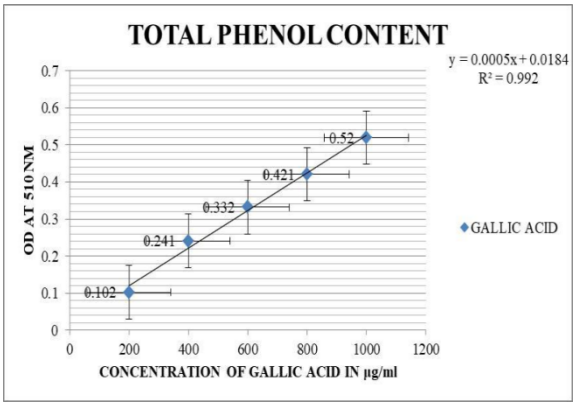


Fig.(A)

- Graph plotted concentration of Quercetin VS absorbance of the Quercetin at 510nm

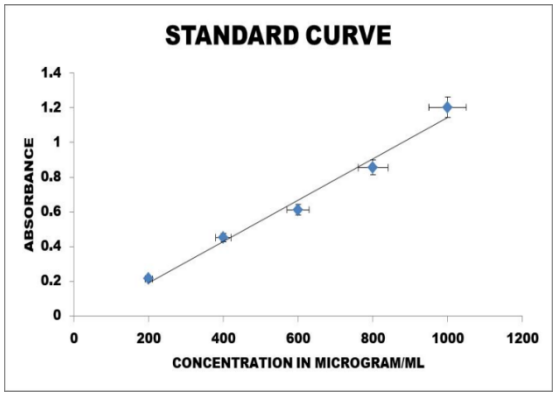


Fig. (B)

Conclusion :

Nature is a unique source of structures of high phytochemical diversity , many of them passesing interesting biological activities and medicinal properties .the choice of the solvent is very essential to extraction of various phytochemicals from plants.From overall scenario ,it is conducted that as the plant studied ,found to rich in phytochemicals ,are full of pharmacological and medicinal significance. Further study is required to find their potential in mentioned biological properties such as antimicrobial ,Antiameobic etc.

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