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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON WHOLE PLANT OF *UTRICULARIA RETICULATA*

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ABSTRACT

"Utricularia reticulata" is a medium to large sized probably annual carnivorous plant which is used by the tribes for the treatments of ulcers, wound healing, and neutralizing venoms of snakes, spiders, eye diseases. The study highlights the antibacterial studies of the plant thereby increasing the utilization of this commonly available plant for its medicinal property. The whole plants were collected, dried and subjected to successive solvent extraction. The phtyochemical and pharmacological studies were carried out. Further the extracts were tried for antioxidant activity and antimicrobial studies. Ethyl acetate extracts were shown significant antioxidant and antibacterial properties. Inspite of the data reveled form phytochemical screening and literature survey the phytoconstituents like alkaloids, glycosides, phenolic and flavonoids, flavonones, terpenoids and sterols present in the plant were responsible for the activities.

Keyword: *Utricularia reticulata*, antioxidant, antibacterial.

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INTRODUCTION

The relationship between plants and people started from the creation of human the man has been using herbs and herbal products for curing disease. People who are traditional remedies may not understand the scientific rationale behind their medicines but they know from personal experience that some medicinal plant are highly effective if used at therapeutic dose. The plant *"Utricularia reticulata"* which is used by the tribes of Sri lanka and India for the treatments of ulcers, wound healing, and neutralizing venoms of snakes, spiders, eye diseases. It is a medium to large sized probably annual carnivorous plant grows in marshy grasslands or wet soil over rocks at lower altitudes up to 750 m. It is a common weed found in rural field. The present study highlights the antibacterial studies of the plant thereby increasing the utilization of this commonly available plant for its medicinal property.[1,2]

Fig-1 ***Utricularia reticulata***

MATERIALS AND METHODS (10, 11)

Collection

The whole plant of *Utricularia reticulata* was collected from Kasaragod and dried and powdered

Pharmacognostic Studies[3,4,5,6,7]

Determination of moisture content: Five grams of the plant powder were placed in a tarred evaporating dish. Drying was carried out at 105^0C for 5 hours. The drying was continued at 1 hour interval until difference between two successive weighing corresponded to not more than 0.25%. Constant weight was reached when two consecutive weighing's, after drying for 30 minutes and cooling for 30 minutes in desiccators, showed not more than 0.01gm difference.

Determination of ash value

Total ash: specified quantity of drug ignited at a temperature not more than 450^0C until it was white indicating the absence of carbon. and weighed

Acid insoluble ash: To total ash, 25 ml of 2N HCl was added, covered with a watch glass and boiled for 5 minutes. The watch glasses were rinsed with 5 ml each of hot water and added into the crucible. Collected the insoluble matter on an ashless filter paper and washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to and weighed.

Water soluble ash: To the total ash, 25 ml each of water was added and boiled for 5minutes. The insoluble matter was collected in a sintered glass crucible. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^0C. The content of water soluble ash was calculated**.**

Determination of extractive value

Alcohol soluble extractive value: Macerated 5 grams of coarsely powdered air dried plant of *Utricularia reticulata* with 100ml ethanol in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and then allowed to stand undisturbed for another 18 hours. Filtered rapidly, by taking precaution against loss of alcohol. Then 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^0C and weighed. Calculated the %w/w alcohol soluble extractive with reference to the air dried material.

Water soluble extractive value: The above procedure was repeated by replacing alcohol by chloroform water.

Organoleptic evaluation: Color, size, odour, taste, texture and fracture were examined.

Quantitative Microscopy: The length and width of stained fibers was measured by focusing them on the calibrated eyepiece micrometer.

F] Evaluvation of foreign matter: About 100-500g of powder was weighed spread out as a thin layer. The foreign matter was detected.

Extraction : Successive solvent extraction of whole plant using solvents of increasing polarity viz. petroleum ether, chloroform, acetone, ethyl acetate, methanol, water.

Preliminary Phytochemical Screening[3]*:*

Various chemical tests were carried out using the extract was performed for identify the presence of alkaloids, glycosides, phenolic and flavonoids,flavonones,terpenoids and sterols.

***In-Vitro* Antioxidant Activity**

Hydrogen peroxide scavenging assay[8]: The various extracts of plant (50,100,150, and200 µg/ml)and the standard ascorbic acid (50,100,150 & 200 µg/ml) were dissolved in 3.4ml of 0.1 M phosphate buffer (pH7.4) and mixed with 0.6ml of 40mM solution of hydrogen peroxide. The reaction mixture and blank was incubated for 19 min. Absorbance was measured at 230 nm for each concentration and the IC_{50} value was calculated.

Antibacterial Activity (Agar well diffusion method) [9, 10.]The test organisms was inoculated on Mullen Hinton agar plate. The wells were then punched in to agar medium. The drug and standard (100 µl) were added and incubated for 24hrs at 37^0C. Observations were made for the zone of inhibition around the drug and compared with that of standard.

RESULTS AND DISCUSSION

Pharmacognostic studies. Studies were concentrated on ash value, extractive value, moisture content; water soluble extractive value was found to be more than that of the alcohol soluble extractive value. The results are shown in the table-1.

Table-1 Pharmacognostic studies of the plant

S No	Parameters	Average(%W/W)
1	Moisture Content	3.12±0.95
2	Ash Value Total Ash Acid Insoluble Ash Water Soluble Ash	 19.90±.0.91 19.60±0.82 38.00±1.02
3	Extractive Value Water Soluble Extractive Value Alcohol Soluble Extractive Value	 19.90±212 5.30±1.25
4	Foreign Matter	0.216±0.015

Quantitative Microscopy

Table-2 Length and width of phloem fibers of the plant

S.No	Maximum(µm)	Minimum(µm)	Average(µm)
Length	783	58	420.5
Width	72.5	14.5	43.5

Extraction

Successive solvent extraction method was done using petroleum ether, chloroform, acetone, ethyl acetate, methanol and water. The characteristics of extracts shown in the table-3.

Table-3: Extracts characteristics

Sl no	Solvent used for extraction	colour	Consistency	Percentage yield(%w/w)
1	Petroleum ether	yellow	Semisolid	8.75
2	Chloroform	Greenish	Semisolid	15.3
3	Acetone	yellow	Semisolid	13.8
4	Ethyl acetate	Pale yellow	Semisolid	10.5
5	Methanol	Pale yellow	Semisolid	12.9
6	Aqueous	Yellow Brown	Solid	23.5

Preliminary phytochemical screening

Results of Preliminary phytochemical screening of different extracts of the plant are shown in table-4.

Table-4 Results of Preliminary phytochemical screening of different extracts of the plant.

Sl no:	Phytoconstituents test/Reagents used	Pet.ether extract	Chloroform extract	Acetone extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1	Alkaloids	-	+	++	-	-	-
2	Glycosides	-	+	+	+		-
3	Phenolic Compounds	-	++	++	+	+	++
4	Flavones & Flavonoids	-	+	++	++	+	++
5	Carbohydrates	-	+	++	++	+	+
6	Proteins	-	++	-	-	-	-
7	Terpenoids	+	++	++	+	+	+
8	Sterols	+	-	-	+		
9	Saponins	-	-	-	++	+	+
10	Gum & Mucillages	-	-	-	-	-	-
11	Volatile Oil	-	-	-	-	-	-

***In-vitro* antioxidant activity**

In-vitro anti-oxidant activity studies were done by using hydrogen peroxide scavenging assay using ascorbic acid as standard. Acetone, chloroform, ethyl acetate and alcoholic extracts were shown to have significant antioxidant property. The results are shown in the table -5.

Table-5 IC_{50}values of *Utricularia reticulata* in various extracts

Sl.no	Sample	IC_{50} (µg/ml)
1	Standard	46.68±0.04
2	Acetone	61.37±0.25
3	Chloroform	83.35±0.35
4	Ethyl acetate	90.47±0.12
5	Methanol	123.14±0.20
6	Aqueous	151.01±0.22
7	Petroleum ether	154.10± 0.25

Antibacterial activity

The extracts were subjected to antimicrobial activity using different bacterial strains by agar well diffusion method. The methanolic and ethyl acetate extracts were shown significant antibacterial properties. The results are shown in the table-6 and figure-1,2.

Table-6 Zone of inhibition in bacterial strains

Sl no	Extracts	Diameter of zone of inhibition in mm					
		Stephylococcus aureus			***Escherichia coli***		
		100µg/ml	200µg/ml	Standard (100µg/ml)	100µg/ml	200µg/ml	Standard (100µg/ml)
1	Acetone	10	12	38	10	10	30
2	Ethyl acetate	15	22	47	10	10	30
3	methanol	25	34	44	31	38	40
4	chloroform	20	30	40	22	30	22

(-) indicates no zone of inhibition, (Diameter of zone of inhibition:17 mm & above: Sensitive, 13-16mm: Moderately sensitive, <12 mm: resistant).

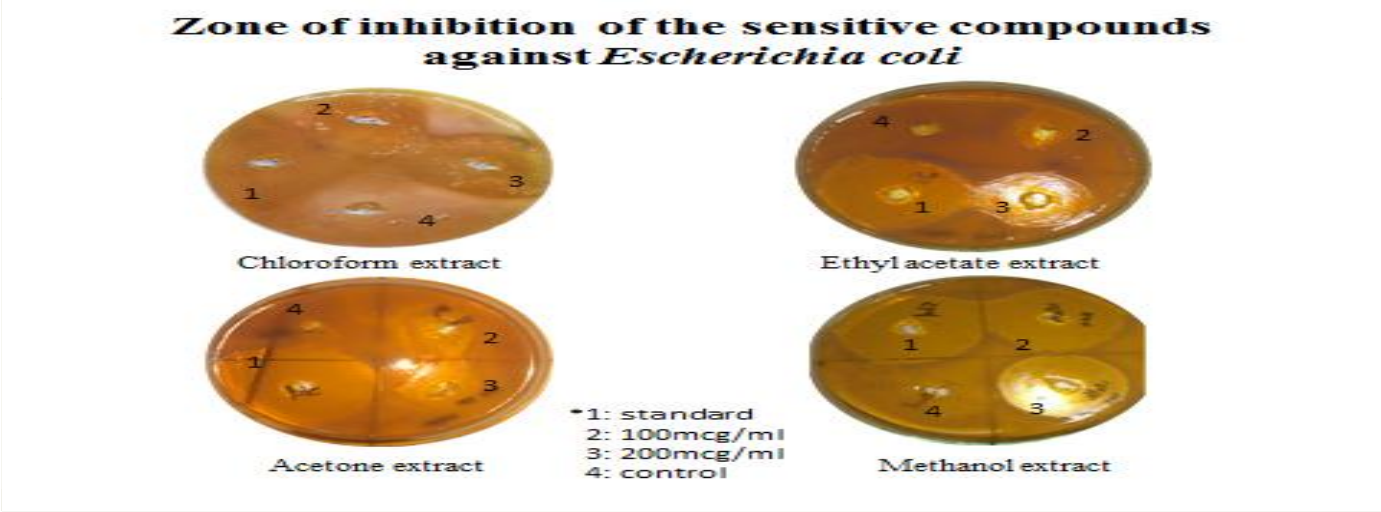

Fig-1 Zone of inhibition of gram negative bacterial strains

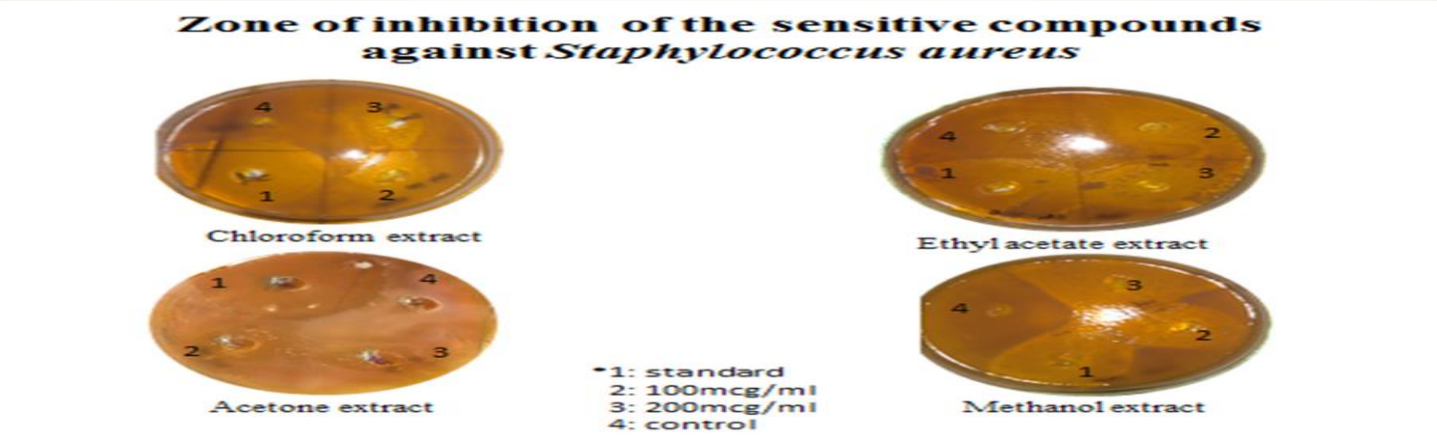

Fig-2 Zone of inhibition of gram possitive bacterial strains

CONCLUSION

"Utricularia reticulata" is a medium to large sized probably annual carnivorous plant which is used by the tribes for the treatments of ulcers, wound healing, and neutralizing venoms of snakes, spiders, eye diseases. Hence the whole plants of *Utricularia reticulate* were collected dried and subjected to extraction. The phtyochemical and pharmacological studies were carried out. Further the extracts were tried for antioxidant activity and antimicrobial studies. Acetone, chloroform, ethyl acetate and alcoholic extracts were shown significant antioxidant activity. The methanolic and ethyl acetate extracts were shown significant antibacterial activity. Inspite of the data reveled form phytochemical screening and literature survey the phytoconstituents like alkaloids, glycosides, phenolic and flavonoids, flavonones, terpenoids and sterols present in the plant were responsible for the antioxidant and antimicrobial properties.

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