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Research Article

STUDIES ON PHYSICOCHEMICAL PARAMETERS, QUANTIFICATION OF PHYTOCONSTITUENTS AND IN VITRO ANTIOXIDANT STUDY OF *Myxopyrum* SPECIES

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ABSTRACT

Objective: The present study objective is to carry out physicochemical parameters, quantification of secondary metabolites and *in vitro* antioxidant activity of *Myxopyrum serratulum* A.W Hill. Methods: Powdered drug was subjected to physicochemical analysis. The various extracts were subjected to carry out Quantification of secondary metabolites by standard procedure and invitro scavenging assay by DPPH method and nitric oxide assay of selected medicinal plant. Results: Quantification of secondary metabolites indicates the higher percentage of flavonoids, phenols, tannins in ethanol when compared to other solvent extracts. Invitro antioxidant by DPPH method showed significant activity with % inhibition as 78.19% at 1000 µg/ml and Nitric oxide showed significant activity with% inhibition as 75.84% 1000 µg/ml. Conclusion: The present investigation of invitro antioxidant activity proved to exhibit significant activity which is comparable against standard drug vitamin C.

Keywords: DPPH, Nitric oxide, Flavonoid, Phenol, Tannin

INTRODUCTION

All over the world traditional medicine is nowadays revealed by an extensive activity of researches on different medicinal plant and their therapeutic principles. It is a large woody climbing shrub belonging to the family Oleaceae. It is a climbing shrub with small yellow flowers commonly known as 'chathuramulla'. It is widely distributed in Kerala, evergreen forest of Western Ghats, Sikkim, Assam, Chittagong and South Deccan Peninsula, The whole plant has considerable ethnobotanical uses in the treatment of head ache, asthma, cough, fever, nerves, otitis, anti-inflammatory, rheumatism and wounds. Literature review reported for the presence of anti inflammatory activity, wound activity, anti microbial activity, antipyretic activity [1-2] etc. The present work is undertaken to determine physicochemical parameters, quantification of secondary metabolites and *invitro* antioxidant activity in *Myxopyrum serratulum.*

MATERIALS AND METHODS

Physicochemical analysis

The present study on physicochemical characteristics provides necessary tools which may guide the author for proper authenticating of genuine plant. Plants contain a wide variety of chemical compounds. These natural constituents play an essential role in maintenance of health condition.

Preparation and extraction of plant materials

The medicinal plant chosen for the study (aerial parts) were shade dried and cut into small pieces and coarsely powdered. Successive solvent extraction was carried out using cold maceration process based on increasing polarity. All extracts were stored in desiccators.

Quantitative Estimation of Total Phenols [3-4]

Principle

This estimation depend on the transfer of reducing equivalents from phenolic components to phosphomolybdic and phosphotungstic acid which complexes in alkali medium. The blue color chromogen was observed which was determined using Double beam spectrophotometer at 765 nm.

Procedure

The reaction mixture contains (1mg/ml) which was prepared in methanol, 5 ml of 50 % FC reagent in water and 4 ml of 7.5 % Sodium bicarbonate. The samples were incubated at room temperature for 45 min. At 765 nm using Double beam spectrophotometer the sample absorbance was read. Triplicate analysis was carried out. The standard used was Gallic acid. The phenolic content in extracts was expressed as (mg of GAE/gm of extract).

Quantitative Estimation of Tannins

Principle

Tannin-like compounds reduce the phosphomolybdic and phosphotungstic acid in alkaline solution and produces a highly colored blue complex, the intensity of which is proportional to the amount of tannins. At 700 nm using Double beam spectrophotometer samples was read.

Procedure

The reaction mixture contains (1mg/ml) which was prepared in methanol, 0.5 ml of 50 % FC reagent in water and 5 ml of 35 % sodium bicarbonate. For five minutes at room temperature incubation was done for the sample solution. Using

spectrophotometer the absorbance was read at 700 nm. Triplicate analysis was carried out. The standard solution is tannic acid and same procedure was repeated. The tannins content in extracts was expressed as(mg of TAE/gm of extract)

Quantitative Estimation of Flavonoids

Procedure

The reaction mixture contains (1mg/ml) which was prepared in methanol), 4 ml of purified water and 0.3 ml of sodium nitrite (10 %). After 5 min add 0.3 ml of $AlCl_3$ (10 %) to make it alkaline add 2 ml of sodium hydroxide solution (1 %). Without delay after systematic mixing at 510 nm the absorbance of the samples was read out. Using quercetin calibration graph was prepared. The results were articulated as quercetin equivalents as (mg QE/gm of extract).

In vitro Antioxidant Activity

DPPH radical scavenging activity [5]

DPPH assay is measured to assess scavenging activity of antioxidants, since the radical compound is steady and does have to be generated as in other radical scavenging assays. The free radical scavenging activity of aerial parts was measured by in vitro DPPH method.

Procedure

A stock solution contains about 8 mg of DPPH (200µM) was dissolved in 100ml of ethanol, 0.1ml of sample compounds dissolved in various solvents were added at different concentrations (10, 50, 100, 200, 400, 800 and 1000µg/ 0.1ml with 1.9ml DPPH solution. After 90 minutes of incubation in dark. The decrease in absorbance of test mixtures were read at 517nm. Vitamin C was used as the standard drug. The % inhibition was calculated and expressed as percent scavenging of DPPH radical.

$$\text{Percentage inhibition} = \left(\frac{\text{OD of Control - OD of Test}}{\text{OD of Control}}\right)x100$$

Nitric oxide scavenging activity[6-7]

3ml of reaction mixture containing sodium nitroprusside (10mM) in phosphate buffered saline (PBS) and various concentrations of test extracts (10, 50, 100, 200, 400, 800, 1000µg/ml) of *Myxopyrum serratulum.* Incubation was carried out for 4 hrs. In identical manner control was prepared without test compound. After incubation 0.5ml of Griess reagent was added. At 546nm the sample absorbance was measured using Double beam spectrophotometer. The percentage inhibition of nitric oxide was measured.

$$\text{Percentage inhibition} = \left(\frac{\text{OD of Control - OD of Test}}{\text{OD of Control}}\right)x100$$

RESULTS AND DISCUSSION

Table 1: Physiochemical analysis

S. No	Parameters	Values (% w/ w)
1	Ash Value	
	Total ash	4.85 %
	Water soluble ash	1.36 %
	Acid insoluble ash	0.97 %
2	Extractive Values	
	Alcohol	1.84
	Water	2.46
3	Crude fiber content	8
4	Loss on drying at 105º C	10
5	Foreign organic matter	≤2

Ash value is an important quantitative tool used to determine the purity of drug. Percentage weight of loss on drying or moisture content was found to be 10 %. The less value of moisture content could prevent microbial growth. Crude fiber content of aerial parts was found to be 8 %, and it is used to determine adulteration

Table 2: Quantitative determination of Secondary metabolites of *Myxopyrum serratulum*

S.No	Phytochemical Content	Chloroform	Ethyl acetate Extract	70 % Ethanol
1	Total phenols (mg of GAE/g of extract)	14.67±0.21	18.43±0.34	23.53±0.25
2	Total flavonoids (mg of QE/ g of extract	17.70±0.28	19.25±0.25	21.94±0.08
3	Tannins (mg of TAE/ g of extract)	15.93±0.15	17.92±0.13	22.09±0.32

Quantitative analysis of secondary metabolites revealed higher percentage of flavonoids, tannins and phenolic compounds in 70% ethanolic extract when compared to the other solvents extract

Table 3: DPPH radical scavenging activity of *Myxopyrum serratulum*

Conc. (µg/ml)	% inhibition				
	Pet.ether extract (40-60°C)	Chloroform extract	Ethyl acetate Extract	70%Ethanol extract	Standard VITAMIN C
10	13.48 ± 0.43	16.94 ± 0.24	21.68 ± 0.12	30.56 ± 0.18	49.85 ± 0.25
50	15.76 ± 0.13	19.96 ± 0.23	32.02 ± 0.23	45.49 ± 0.22	67.41 ± 0.34
100	17.11 ± 0.21	22.81 ± 0.28	38.67 ± 0.19	49.9 ± 0.28	79.22 ± 0.44
200	19.28 ± 0.15	24.82 ± 0.19	49.23 ± 0.21	60.68 ± 0.35	80.98 ± 0.24
400	20.59 ± 0.21	25.79 ± 0.21	63.84 ± 0.14	70.14 ± 0.05	81.99 ± 0.26
800	21.94 ± 0.32	31.45 ± 0.12	64.89 ± 0.25	75.11 ± 0.33	82.05 ± 0.52
1000	20.04 ± 0.22	33.78 ± 0.22	68.18 ± 0.44	78.19 ± 0.62	80.22 ± 0.42

The proton radical scavenging action is well-known as an essential device of anti oxidation. 1,1-Diphenyl-2-picrylhydrazyl hydrate was used as a free radical to estimate the antioxidative action of medicinal plants. The plant extracts reduce the color of DPPH due to the power of hydrogen donating ability. The results revealed that all the extracts showed dose dependent activity.

The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lesser the IC_{50} value, the higher the antioxidant activity of samples.

Among the various extracts studied the pet ether and chloroform extracts of *showed* IC_{50} value greater than 1000 µg/ml. whereas the IC_{50} value of ethyl acetate extract was calculate to be 412 µg/ml. Out of the 4 extracts studied the ethanolic extracts showed the minimum IC_{50} value 149 µg/ml. The observed IC_{50} value showed that ethanolic extract exhibited highest antioxidant activity; it exhibited maximum activity of 78.19 % at the concentration of 1000µg/ml which is comparable with that of standard vitamin C . Hence the results of DPPH assay reveals that the ethanolic extract having better antioxidant activity when compared to other solvent extracts.

The use of DPPH provides a convenient method to evaluate antioxidants. It is reported that the number of DPPH molecules that are reduced seems to be correlated with the number of available hydroxyl group.

Table 4: Nitric oxide radical scavenging activity of *Myxopyrum serratulum*

Conc. (µg/ml)	% inhibition				
	Pet.ether extract (40-60°C)	Chloroform extract	Ethyl acetate extract	70% Ethanol extract	Standard Vitamin C
10	16.32 ± 0.22	18.04 ± 0.18	21.55 ± 0.09	30.38 ±0.25	40.62 ± 0.23
50	22.43 ± 0.13	23.16 ± 0.22	31.84 ± 0.23	48.71 ± 0.14	63.89 ± 0.34
100	25.05 ± 0.23	29.76 ± 0.22	44.03 ± 0.19	49.25 ± 0.28	72.21 ± 0.24
200	28.97 ± 0.25	31.89 ± 0.29	60.48 ± 0.21	59.14 ± 0.36	75.07 ± 0.24
400	30.29 ± 0.08	34.42 ± 0.24	62.21 ± 0.14	71.18 ± 0.25	76.52 ± 0.26
800	32.54 ± 0.23	41.82 ± 0.12	63.15 ± 0.25	73.27 ± 0.33	77.07 ± 0.22
1000	33.86 ± 0.12	43.26 ± 0.42	64.42 ± 0.44	75.84 ± 0.09	78.07 ± 0.08

Nitric oxide (NO) is an essential element generated by endothelial cells which is prime for the regulation of a variety of physiological processes. Nitric oxide inhibitors have beneficial effects on the treatment of inflammation and tissue damage seen in inflammatory condition. Modern experiments bare that injure caused by Nitric Oxide in cells is multiplied if it reacts with the superoxide radical to yield peroxynitrite ($ONOO^-$), an extremely reactive radical. The nitric oxide scavenging activity of extracts showed a concentration dependent activity.

Among the four extracts studied for Nitric oxide radical scavenging activity the pet ether and chloroform extract showed IC_{50} value greater than 1000 µg/ml. whereas the IC_{50} value of ethyl acetate extract was calculate to be 384 µg/ml. Out of the 4 extracts studied, the ethanolic extracts showed the minimum IC_{50} value 140 µg/ml. The observed IC_{50} value showed that ethanolic extract exhibited elevated antioxidant activity. Among the four extracts, the ethanolic extracts showed maximum percentage inhibition of 75.84 % at the concentration of 1000µg/ml which is comparable with that of standard drug vitamin C which showed the percentage inhibition of 78.07 % at the concentration of 1000µg/ml.

CONCLUSION

The various extracts of *M. serratulum* were screened for its antioxidant potential using *in vitro* models like DPPH and Nitric oxide radical scavenging assay. The ethanolic extract showed highest activity compared to other extracts. In fact, many natural sources contain huge quantity of antioxidants like poly phenols and flavonoids. Numerous phytoconstituents hold significant antioxidant capacity that are related with lower mortality rates to treat various heath ailments So the presence of phytoconstituents such as phenols, tannins, flavonoid in the ethanolic extracts may be responsible for the antioxidant potential.

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