



Research Article

EVALUATION OF TOTAL PHENOLIC, FLAVONOID AND ANTIOXIDANT ACTIVITY OF *SAGITTARIA SAGITTIFOLIA* L.

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ABSTRACT

The present investigation is a study of the Leaves of *Sagittaria sagittifolia* L. with respect to potential as antioxidant in relation to their total content of Phenolic and Flavonoids compounds in five different organic solvents. The amounts of total phenols were analyzed with the Folin- Ciocalteu Reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent. The antioxidant activity of extracts were expressed as percentage of DPPH radical inhibition and IC₅₀ values in percentage ranged from 18.86± 0.23 % to 86.65 ± 0.43 % Maximum phenolic content was found in the methanolic extract (36.4± 0.30) where as maximum flavonoids are detected in ethanolic extract (16.60± 0.01). The high contents of phenolic and flavonoids compounds indicated that these compounds contribute to the antioxidant activity.

KEY WORDS: Phenolic, Flavonoid, Antioxidant, *Sagittaria sagittifolia* L.

INTRODUCTION

Sagittaria sagittifolia L. (Family Alismataceae) commonly known as Arrowhead and Newsa. It is a perennial and herbaceous plant growing in marshes, lakes and paddy field with up to 18 cm long surface runners with 1-5 cm thick rhizome (Root stock) containing starchy root tubers. Leaves, tuber, stems and seeds are biofunctionally active and edible part of the plant having various therapeutic properties, such as antidiabetic, antiseptic, antianemics, anti-inflammatory, antiscorbutic, anthelmintic, antioxidant and prevent from the cardiovascular diseases. There are about more than eight thousand naturally occurring plant phenolics, possess a wide spectrum of biochemical activities such as antioxidant, anticarcinogenic and antimutagenic. Phenolics are the largest group of phytochemical that account for the most of the antioxidant activity in plant or plant products. Plant phenolics are commonly found in both edible and non-edible parts of plant and have been reported to have multiple biological effects, including antioxidant activity.[4, 12]. A polyphenol antioxidant is a type of antioxidant containing a polyphenolic or natural phenol substructure they may affect cell-to-cell signaling receptor sensitivity, inflammatory enzyme activity or gene regulation. Aquatic plants have polyvalent potential for food, fodder, medicine and reservoir of many biofunctional molecules to cure various ailments and diseases. Aquatic plants are used by people since ancient time as natural resources for their day to day requirement directly or indirectly, because of their potential source of different nutrients and biochemical constituents. Antioxidant activity of plants might be due to their phenolic compounds. [13]Synthetic antioxidant like BHA (Butylated Hydroxy anisole), BHT (Butylated Hydroxy toluene), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompted negative health effects. These synthetic antioxidant also show low solubility and moderate antioxidant activity. [2, 7].

Our present investigation is a study of the Leaves of *Sagittaria sagittifolia* L. with respect to potential as antioxidant in relation to their total content of Phenolic and Flavonoids compounds.

MATERIAL AND METHODS

Collection and Preparation of plant Sample

Leaves of *Sagittaria sagittifolia* L. (Newsa) were collected from the watershed region in an around Gorakhpur. Taxonomically, the plant material was authenticated and identified with Voucher Number 103410 form NBRI Lucknow by Dr. L. B. Chaudhary, Senior Principle Scientist and Curator of Herbarium plant diversity, Systematics and Herbarium Division. The specimens also have been submitted at NBRI, Lucknow for further reference in herbarium section. For extraction of phytochemical the leaves were plucked in preflowering stage. The sample was thoroughly washed with running distilled water to remove the extraneous matter and then air dried under shed till constant weight. After complete drying, the leaves were grinded in mixture, the powder was kept in small plastic bag with proper labeling and used for further analysis.

EXTRACTION OR PREPARATION OF EXTRACTS

For extraction, the dried and pulverized plant sample of 20 gm. leaves were extracted using five different organic solvent 250 ml mixture with the help of soxhlet for 48 hour and solvent was evaporated to dryness and concentrated through Rotatory Evaporator. Then the crude extracts were obtained which were used for determination of total phenolic and flavonoid content and their antioxidant activity. [1,3]

PREPARATION OF STANDARD SOLUTION AND REAGENT

For making standard solution 10 mg Gallic acid and Quercetin were weighed into a 50 ml volumetric flask containing 10 ml methanol and solution was made up to 10 ml. with the same solvent.

PROCEDURE FOR DETERMINATION OF TOTAL PHENOLIC CONCENTRATION

UV/Vis double beam spectrophotometric methods were used for the determination of total amount of phenolic in the leaves of *Sagittaria sagittifolia* Linn. with the Folin- Ciocalteu Reagent.

An aliquot (1 ml) of extract or Standard solution of Gallic Acid (100, 200, 300, 400, 500 µg/ml) were prepared in methanol. 0.5 ml of each sample was introduced in to test tube and of mixed with 2.5 ml of a 10 time dilute Folin- Ciocalteu Reagent. The mixture was shaken for 5-7 min afterword 2 ml 7% Na₂CO₃ solution was added to the mixture then volume was mead up to the mark and tubes were covered with parafilm and allowed to stand for incubation for an hour. After incubation absorbance was read at 765 nm by using UV/Vis spectrophotometer against reagent blank and for each sample three replicate assay was performed [8,9,11].

The total phenolic content was calculated as Gallic acid equivalent (GAE) by the equation.

$$T = C \times V/M$$

Where:

T = Total phenolic content in mg/ml.

C = Concentration of Gallic acid established from the calibration curve in mg/ml.

V = Volume of the extract solution in ml.

M = Weight of the extract in gm.

PROCEDURE FOR DETERMINATION OF TOTAL FLAVONOID CONTENT

Concentration of Flavonoids in the Leaves Extract was determined using spectrophotometric method by aluminum chloride assay. Quercetin was used as standarad.[5] About 20, 40, 60, 80, 100 µg/ml was added in volumetric flask containing 4 ml of distilled water 0.3 ml of 5 % NaNO₂ added to the flask and stand for 6 to 10 m and then add 2 ml of 1M NaOH and make a volume up to 10 ml with distilled water. The absorbance was noted against the blank at 510 nm using UV- Vis spectrophotometer.[9,6]

EVALUATION OF ANTIOXIDANT ACTIVITY

Determination of Antioxidant activity of leaves extracts was carried out by using quantitative DPPH (1, 1- diphenyle – 2 picrylhydrazyl). Various concentration of sample extract in methanol was prepared. Gallic acids were used as a positive control. Blank sample were run using 1 ml methanol in place test sample. A solution of DPPH of concentration 0.2 mM was prepared in 70% methanol and kept overnight. Stock solution (1mg/ml) of the extract was prepared in 70% methanol. One ml of 0.2 mM DPPH in methanol was added to 1 ml of test solution or standard and kept at dark place for 30 minutes. Optical density of these samples was measured at 517 nm.[10,11]. The activities of the extracts are measured in terms of percent inhibition (IC₅₀) and calculated by following formula. [13, 15]

$$\text{Percent inhibition} = A-B/A \times 100$$

A = optical density of the blank

B = optical density of the sample

STATISTICAL ANALYSIS: All data were estimated from triplicate procedure expressed as mean ± standard deviation (SD).

Table 1: The Yield of Solid Residue after Extraction and Evaporation from 20 g of Dried Plant Material

Extracts	Yield (mg)
Aqueous	17.5 ± 0.5
Ethanol	29.9 ± 1.1
Methanol	49.7 ± 0.3
Acetone	19.5 ± 0.4
Chloroform	40.8 ± 0.5

Table 2: Phenolic and Flavonoid Content in Leaves of *Sagittaria sagittifolia* L. in Different Extracts.

Solvent Extract	Phenolic mg/g	Flavonoid mg/g
Aqueous	24.6 ± 0.26	10.77 ± .025
Ethanol	30.4 ± 0.25	16.60 ± 0.01
Methanol	36.4 ± 0.30	13.25 ± 0.30
Acetone	19.3 ± 0.20	8.05 ± 0.02
Chloroform	30.4 ± 0.25	6.07 ± 0.02

Table 3: Free Radical Scavenging Activity by DPPH assay of Leaves of *Sagittaria sagittifolia* L. in Different Extracts.

Solvents	Percentage Inhibition of DPPH							IC ₅₀ (µg/ml)
	15.0 µg/ml	30.0 µg/ml	45.0 µg/ml	60.0 µg/ml	75.0 µg/ml	90.0 µg/ml	105.0 µg/ml	
Ethanol	24.3 ± 0.2	31.2 ± 0.3	46.9 ± 0.3	70.3 ± 0.2	77.8 ± 0.1	82.0 ± 0.3	94.0 ± 0.3	45.8
Ethanol 80%	20.3 ± 0.2	32.2 ± 0.3	43.9 ± 0.3	61.3 ± 0.2	70.8 ± 0.1	83.0 ± 0.3	92.0 ± 0.3	47.1
Methanol	34.1 ± 0.01	51.8 ± 0.2	62.2 ± 0.03	69.3 ± 0.1	72.2 ± 0.03	84.5 ± 0.3	93.1 ± 0.2	37.4
Methanol 80%	35.3 ± 0.02	39.2 ± 0.4	50.0 ± 0.08	57.3 ± 0.05	64.2 ± 0.1	77.09 ± 0.2	94.1 ± 0.1	26.3
Dist. Water	31.4 ± 0.1	37.2 ± 0.3	50.0 ± 0.08	60.0 ± 0.2	67.4 ± 0.05	80.2 ± 0.3	90.0 ± 0.1	61.2
Acetone	22.6 ± 0.2	26.7 ± 0.2	38.1 ± 0.1	55.4 ± 0.3	71.5 ± 0.2	76.9 ± 0.3	89.5 ± 0.2	70.6
Ascorbic acid	43.6 ± 0.1	58.2 ± 0.2	69.1 ± 0.02	73.4 ± 0.003	89.2 ± 0.06	94.3 ± 0.03	97.1 ± 0.04	09.2
BHA	42.3 ± 0.05	50.0 ± 0.07	71.21 ± 0.1	82.4 ± 0.2	89.4 ± 0.03	95.6 ± 0.1	95.6 ± 0.1	20.6
BHT	44.11 ± 0.1	52.7 ± 0.09	74.7 ± 0.2	80.5 ± 0.3	87.9 ± 0.1	94.9 ± 0.2	94.9 ± 0.2	16.9

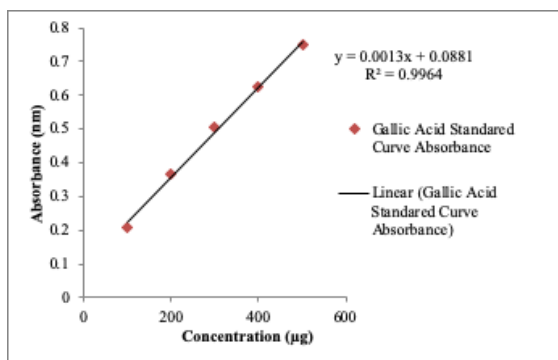


Figure 1: Standard Curve of Gallic Acid

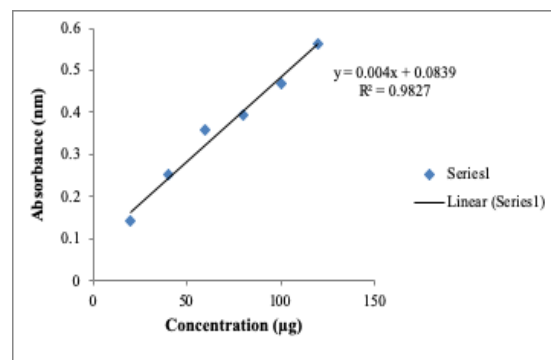


Figure 2: Standard Curve of Quercetin

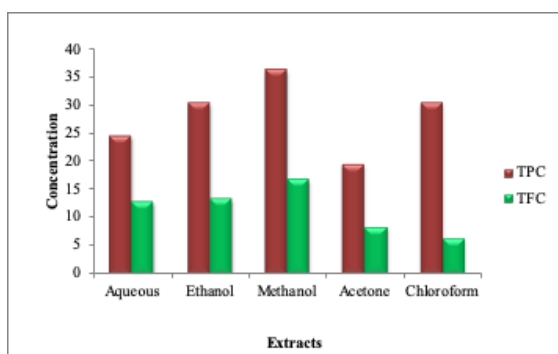


Figure 3: Total Phenolic and Flavonoid Concentration in Leaves of *Sagittaria sagittifolia* L.

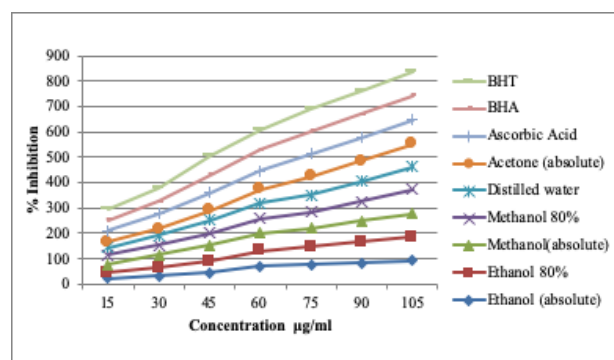


Figure 4: Free Radical Scavenging Activity by DPPH assay of Leaves of *Sagittaria sagittifolia* L. in Different Extracts.



A



B



C



D

Figure 5: (A) Fresh Leaves of *Sagittaria sagittifolia* L., (B) Powder of Leaves, (C) Extraction of Leaves through Soxhlet, (D) Concentrated form of Leaf Extract through Rotatory Evaporator.

RESULTS AND DISCUSSION

The evaluation of concentration of total phenolic, flavonoid and antioxidant activity of *Sagittaria sagittifolia* (Leaves) were examined in different extracts and shown in tables. The result shows that the yield of Leave extract in different organic solvents obtained from 20g of dry leaves powder. Among the various extracts the highest yield of residue was found in methanol extract that is 49.7 ± 0.3 (Table 1). The total phenolic concentration in leaves extract were examined using Folin-Ciocalteus Reagent (FCR) is expressed in terms of Gallic Acid equivalent. The highest value of phenolic content is measured in methanol extract that is 36.4 ± 0.30 and lower in Acetone that is 19.3 ± 0.20 (Table 2). The phenolic content in leaves depends upon the type of extracts that is polarity of solvents. The total flavonoid content was determined using Quercetin as standard compound. The amount of total flavonoid is expressed using $AlCl_3$ reagent in terms of mg/g Quercetin equivalent using standard curve equation. The test revealed that the highest amount of TFC were found in ethanol extract that is 16.60 ± 0.01 and lowest in chloroform extract that is 6.07 ± 0.02 (Table 2). The antioxidant activity of various extracts from leaves of *S. sagittifolia* L. was determine using DPPH, free radical compound that has been used for determining the free radical scavenging activity of sample. The result shown in table-3. Result shows that the antioxidant activity of five different extract of leaves expressed in terms of percentage of inhibition (%) and IC_{50} value ($\mu\text{g/ml}$). The two standard compounds BHA and Ascorbic acid are used. The test revealed that acetone extract of leaves showed lowest antioxidant activity with IC_{50} $70.6 \pm 2.09 \mu\text{g/ml}$. highest antioxidant activity is shown by ascorbic acid with IC_{50} $9.02 \pm 1.53 \mu\text{g/ml}$. Among different extracts used 80 % methanolic extract showed higher antioxidant activity with lowest IC_{50} $26.3 \pm 1.05 \mu\text{g/ml}$.

CONCLUSION

The present investigation revealed the phenolic and flavonoid concentration in leaves of *Sagittaria sagittifolia* L. From the result the amount of phenolic content in the extracts are indicate that they are significant for their antioxidant activity. The plant can be regarded as promising plant having antioxidant activity with high potential value for drug formation. The extensive investigation of phenolic compound will provide additional source of antioxidant and exploration of their pharmaceutical activity in the field of medical. It could be concluded that the leave of *Sagittaria sagittifolia* L. is natural source of antioxidant substance with appropriate amount. It provides the basis for food industries, pharmacy and phytotherapy.

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