Research Article

Total Phenolics, Flavonoids and Antioxidant Evaluation in the Leaves of *Argyreia nervosa* Burm.

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ABSTRACT

Argyreia nervosa Burm (Convolvulaceae), also known as elephant creeper has hepatoprotective, hypoglycemic, anticonvulsant, aphrodisiac, antioxidant, antiviral, nematicidal, antimicrobial, immunomodulatory, analgesic and anti-inflammatory activity. In the present work we evaluate the qualitative, quantitative and antioxidant potential of the water extract of *Argyreia nervosa* leaf with physical constants and extractive values. Preliminary phytochemical screening on the leaves was also studied. The determination of these characters will help further researches in their phytochemical as well as pharmacological analyses of this species. Total phenolics, flavonoids contents and antioxidant capacity were evaluated according to standard procedures. The results of this study showed that the water extract of *A. nervosa* leaf has significant amount of total phenolic (110.8 ± 0.19 mg GAE/g) and flavonoid contents (76.60 ± 0.36 mg RE/g) whereas total antioxidant capacity was found 1.16 ± 0.11 mg GAE/g when compared with gallic acid as standard. Hence, it is concluded that the *A. nervosa* leaf extract contain remarkable amount of total phenolic and flavonoid content can be used as a powerful herbal antioxidant.

Keywords: *Argyreia nervosa*, Convolvulaceae, Total antioxidant capacity, phytochemicals.

INTRODUCTION

Argyreia nervosa Burm is a climbing shrub with woody tomentose stem belongs to family Convolvulaceae. It is commonly known as elephant creeper in English and samundar-ka-pat, Samudra sok in India.[1,2] It is widely distributed in tropical regions of the world.

In India it is found mainly in East slopes of the West Ghats at an altitude of 900 m^3 often cultivated native in India from Assam and Bengal to Karnataka.[4,5] It is generally growing in slightly moist localities like river banks, edges of lakes etc. and as undergrowth in semidecidous forests.[6]

Leaves are traditionally used by Rajasthani tribes to prevent conception.[7] Seeds possess hypotension, spasmolytic activity[8], and anti-inflammatory activity.[9] Roots are used as an appetiser, brain-tonic, cardiotonic; expectorant, anti–inflammatory; in anaemia, aphrodisiac, cerebral disorders, diabetes, obesity, syphilis, tuberculosis, ulcers and wounds.[10] Chemical analysis revealed the presence of triterpenoids, flavanoids, steroids and lipids.[11] The leaves of *A. nervosa* contain anthocyanins, β-sitosterol, quercetin, kampferol and its glycosides kampferol 3-O-L rhamnopyranoside, 7, 8, 3', 4', 5'-pentahydroxy flavone, 5-O-α-D-rhamnopyranoside, 5-O-β-D-glucopyranoside.[10] Seeds of *Argyreia nervosa* contain Argyreioside (24R-ergost-5-en-11-oxo-3 beta-ol alpha –D glucopyranoside xylose).[12]

Present research work has been made to study the qualitative, quantitative phytochemical parameters and antioxidant activity of the leaves of *Argyreia nervosa* Burm.

MATERIALS AND METHODS

Plant Material

The plant material was collected from the Uttar Pradesh, India in June 2013. The plant was identified and authenticated by Dr. J. K. Tiwari, Dept of Botany, H.N.B. Garhwal (A Central) University, Srinagar Garhwal, Uttarakhand; India. A herbarium was preserved in the department of Pharmaceutical Chemistry for further reference.

The leaves were separated, dried, coarsely powdered passed through sieve no 40 and stored in a closed container for further use. All chemicals and reagents used were of analytical grade obtained from S.D. Fine Chemicals Ltd., Mumbai.

Methods

The ash values and extractive values with various reagents were determined according to Indian Pharmacopoeia.[13]

Extractive values were performed with various solvents like petroleum ether, chloroform, ethyl acetate, alcohol and water. Preliminary phytochemical tests were carried out for ethanol, aqueous and powder with water type of extracts to identify the presence of various chemical constituents like alkaloids, phytosterols, carbohydrates, terpenoids, saponins, flavonoids, phenolic compounds etc. using specific reagents through standard procedures.[14,15]

Extraction

Defined quantities of plant material were collected; shade dried at room temperature, pulverized and extracted

different solvents like petroleum ether, chloroform, ethyl acetate, alcohol and water in a Soxhlet extractor. All extracts was concentrated and dried using rotary flash evaporator. It was kept in desiccators until further used.

Total Phenolic Content (TPC)

The concentration of total phenolic compounds of the water extract was determined by the Folin-Ciocalteu method[16] using the extracts at a dilution of 1:100 in water. The absorbance of the samples was measured at 765 nm. The results are expressed as mg of gallic acid equivalent (GAE)/g of each sample.

Total Flavonoid Content (TFC)

The total flavonoid contents of the *A. nervosa* (water extract) was measured using the aluminium chloride assay.[17] Briefly, ethanol extract (10 mg) of *A. nervosa* leaf was dissolved in H_2O (1 mL) in a test tube, to which 5 % (w/v) $NaNO_2$ (60 µL) was added. After 5 min, a 10 % (w/v) $AlCl_3$ solution (60 µL) was added. After 6 min, 1 M NaOH (400 µL) was added and the total volume made up to 2 mL with H_2O. The solution was mixed well and the absorbance measured at 510 nm against a reagent blank. Concentrations were determined using a rutin standard curve. Mean total flavonoid contents (n = 3) were expressed as milligrams rutin equivalents (RE) per g (mg RE/g dry).

Total Antioxidant Activity

Total antioxidant activity of *A. nervosa* (water extract) was determined according to the method of Prieto.[18] Briefly 2 mL of sample was taken at different concentrations (50, 10, 250, 500 and 1000 µg) and mixed with 1 mL of standard reagent 0.6 M Sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Then Reaction mixture was incubated at 95° C for 90 min. Absorbance of all the sample was measured at 635 nm.

RESULTS AND DISCUSSION

The various physical constants such as total ash value (5.2 %), acid insoluble ash (2.0 %) and water soluble ash (4.12 %) were determined as shown in Table 1 (Figure 1). The extractive values of the powder with different solvents were determined and its result was reported in Table 2 which indicates the nature of constituents present (Figure 2).

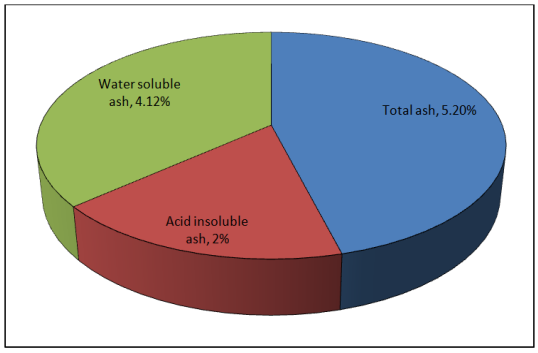

Figure 1: Ash Values of *Argyreia nervosa* Burm

Table 1: Determination of Ash Values of *Argyreia nervosa* Burm.

S. No.	Ash type	Percentage of Ash
1.	Total ash	5.2% w/w
2.	Acid insoluble ash	2.0% w/w
3.	Water soluble ash	4.12% w/w

Table 2: Determination of Extractive Values *of Argyreia nervosa* Burm.

S. No.	Extracts	Percentage of extractive
1.	Petroleum ether	2.99% w/w
2.	Chloroform	0.90% w/w
3.	Ethyl acetate	1.97% w/w
4.	Ethanol	1.23% w/w
5.	Water	7.68% w/w

Table 3: Preliminary phytochemical screening of *Argyreia nervosa* Burm.

S. No.	Tests	Powder + Water	Ethanol extract	Water extract
1.	**Alkaloids**			
	Dragendroff's test	+ ve	+ ve	+ ve
	Mayer's test	+ ve	+ ve	+ ve
	Hager's test	+ ve	+ ve	+ ve
	Wagner's test	+ ve	+ ve	+ ve
2.	**Carbohydrates**			
	Fehling's test	+ ve	+ ve	+ ve
	Molish test	+ ve	+ ve	+ ve
3.	**Gums/Mucilage**			
	Water	-ve	- ve	- ve
	Alcohol	-ve	- ve	- ve
4.	**Tannins**			
	Aq. $FeCl_3$ Test	+ ve	+ ve	+ ve
	Alc. $FeCl_3$ Test	+ ve	+ ve	+ ve
5.	**Flavonoids**			
	Lead acetate test	+ ve	- ve	+ ve
	Shinoda test	+ ve	- ve	+ve
	Mg/Hcl	+ ve	- ve	+ ve
6.	**Saponins:**			
	Foam Test	+ ve	+ ve	- ve
	Lead acetate test	+ ve	+ ve	+ ve
7.	**Sterols:**			
	Salowaski test	+ ve	+ ve	+ ve
	Libberman Burchad test	+ ve	+ ve	+ ve

The various qualitative chemical tests (Table 3) have shown the presence of triterpenoids, saponins, sterols, flavanoids, carbohydrates phenols and tannins in large amount whereas aromatic acids, gums, mucilage and volatile oils were totally absent in the water extract of this plant part. Qualitative chemical test confirms that the water extract showed maximum phytoconstituents (including flavonoids mostly responsible for antioxidant

activity) in the leaves of *A. nervosa*. Hence, water extract was used for the estimation of total phenolic, flavonoid and antioxidant capacity.

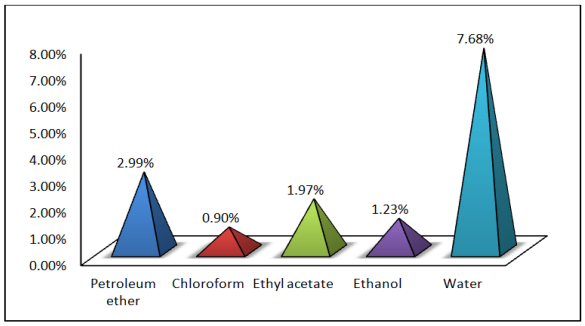

Figure 2: Extractive Values *of Argyreia nervosa* Burm.

Total Phenolics, Flavonoids Contents and Antioxidant Capacity

The results showed that total phenolic content of *A. nervosa* water extract was 110.8 ± 0.19 mg GAE/g whereas the concentrations of total flavonoids in the same extract was found to be 76.60 ± 0.36 mg RE/g. It is well known that the qualitative and quantitative composition of polyphenolic compounds depends on numerous factors, particularly plant chemotype and growth conditions (rain, soil, temperature, etc.). The progressive increase of total polyphenolic compounds found discards the probable presence of chemotypes. However the influence of ecological factors may explain the wide variation of concentrations detected in the water extracts. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. More polar solvent contain more flavonoid content.[19] The water extracts of *A. nervosa* leaf showed significant total antioxidant capacity which was found to be 1.16 ± 0.11 mg GAE/g when compared to gallic acid as standard (Table 4) (Figure 3).

Table 4: Determination of Total Phenolic, Flavonoid Content and Total Antioxidant Capacity *of Argyreia nervosa* Burm.

S. No.	Water Extract of *A. nervosa* Leaves	Values
1.	Total Phenolic Content	110.8 ± 0.19 mg GAE/g
2.	Total Flavonoid Content	76.60 ± 0.36 mg RE/g
3.	Total Antioxidant Capacity	1.16 ± 0.11 mg GAE/g

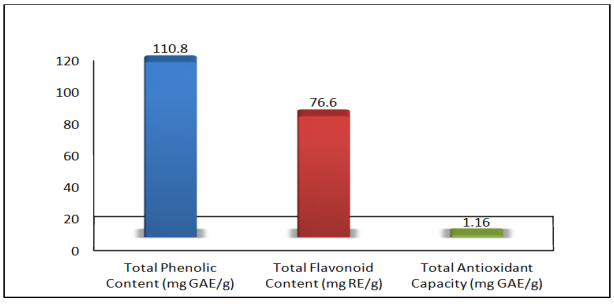

Figure 3: Total Phenolic, Flavonoid Content and Antioxidant Capacity *of A. nervosa* Burm.

CONCLUSION

In this research, we have made an attempt to provide the antioxidant potential and phytochemicals present in the *A. nervosa*, a medicinal plant found in tropical and sub-tropical countries. All the results indicate that flavanoids presented in the plant part could be an important source of antioxidant molecules. The antioxidant capacity of flavanoids is based on their molecular structure. The hydroxyl group position and other characteristics in the chemical structure of flavanoids are more important for their antioxidant and free radical scavenging actions. Plant phenolics in general are effective free radical scavengers and antioxidants. In conclusion the remarkably strong *A. nervosa* leaf extract can be used as a powerful herbal antioxidant. The antioxidant activity should be regarded as an additional health promoting value for use as phytonutrients. This plant has diversified pharmacological potential and was used since ancient times. It has a strong future in the field of herbal medicine, thus the plant should be cultivated in a large scale particularly in unutilized and wasteland which will helpful the financial upliftment of the farmers along with the development of research in the field of herbal medicine. Furthermore, systemic and scientific research is required to explore the maximum pharmacological potential of the plant.

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