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Phytochemical screening and Antioxidant Potential of *Alstonia scholaris* Linn leaf Extracts

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Abstract

Medicinal plants play important roles in our daily life to treat many diseases and ailments. Research in medicinal plants reflects the recognition of the validity of many herbal products. *Alstonia scholaris* (L.) R. Br. (*A. scholaris,* Apocynaceae) commonly called as Indian devil tree has been used as folklore medicines, possesses different pharmacological activities and potentially used as antimalarial drug. In alternative medicinal systems it is effective against different ailments such as asthma, malaria, fever, dysentery, diarrhoea, epilepsy, skin diseases and snakebite. The aim of the present study was to evaluate *in vitro* antioxidant activities, qualitative and quantitative phytochemical analysis of leaves of *A. scholaris* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of methanolic extract of the leaves was assessed against DPPH radical scavenging assay methods using standard protocols. Phytochemical analysis revealed the presence of phenols, diterpines, flavonoids, proteins, carbohydrates and saponins. The total phenol and flavonoids content of methanolic leaves extract of *A. scholaris* was found to be 0.876, 0.757mg/100mg respectively. The activities of methanolic extracts against DPPH assay method were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *A. scholaris* L, Apocynaceae, Antioxidant activity, DPPH assay method.

INTRODUCTION

Plants have been the basis of traditional medicines from time immemorial throughout the world and continue to provide new targets for remedies for many afflictions of mankind. The past couple of decades have seen considerable change in opinion regarding ethno-pharmacological therapeutic applications of phytochemicals. A great deal of effort therefore still focuses on identifying and using these phytochemicals as source of novel therapeutic molecules. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's disease, mongolism, ageing process and perhaps dementia[1]. Antioxidant based drugs or formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer have appeared during the last three decades. This has attracted a great deal of research interest in natural antioxidants. The plant kingdom has been described as a reservoir of many novel biologically active molecules of medicinal value[2]. Recently there has been a surge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury. *A. scholaris* is popularly known as Saptaparni or Devil's tree. It is widely distributed in dried forests of India, Western Himalayas and Western Ghats as well as in the southern India[3]. It is a medium to large tree, about 40 m high with a corky grey to grey-white bark. The outer blaze is cream to yellow with abundant, milky latex that flows rapidly when cut. Leaves are in whorls of 4-8 in the upper exiles, upper surface is dark green, the lower green-white. The tip of the leaf is rounded or shortly pointed and tapered towards the base[4]. Greenish white flowers are umbrellately branched. They are 7-10 mm long. *A. scholaris* is a well-known remedy for the treatment of various types of disorders in the folk and Ayurvedic system of Indian medicine. It has been reported to possess antimalarial, antimicrobial, free radical scavenging and antioxidant, anti-diabetic, analgesic and anti-inflammatory, anticancer and cytotoxicity, radioprotective, CNS activity, immunostimulating, antifertility, antidiarrheal, bronchodilatory, anti-tussive and anti-asthmatic activities[5]. The objective of the present study was to screen the phytochemicals and assess the antioxidant activity of the solvent extracts of leaf of *A. scholaris*. Free radical scavenging ability of the extracts was tested using antioxidant assays, viz., DPPH assay.

MATERIAL AND METHOD

Plant material

Leaves of *A. scholaris* were collected from botanical garden of Vindhya Herbals Bhopal, Madhya Pradesh. Plant material (leaves part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried

plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

50 gram shade dried powder of leaves of *A. scholaris* was extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhletion method

Marc of leaves of *A. scholaris* was exhaustively extracted with methanol solvent by soxhletion method. The extract was evaporated above their boiling points. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis[6].

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures[7, 8]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenol determination

The total phenolic content was determined using the method of Dutta *et al*[9]., 2011 A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Dutta *et al*[9]., 2011 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Antioxidant activity

DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Dutta *et al*[9]., 2011. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

RESULTS AND DISCUSSION

The leaves of *A. scholaris* were collected from the botanical garden of Vindhya Herbals Bhopal, MP, India. Air-dried and extracted by soxhletion extraction process. The crude extracts so obtained after each of the soxhletion extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants was founds to be 6.34% w/w. The results of qualitative phytochemical analysis of the crude powder leaves of *A. scholaris* were shown in Table 1. Methanolic extracts of leaves sample of *A. scholaris* showed the presence of phenols, diterpines, flavonoids, proteins, carbohydrates and saponins. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.015x - 0.001, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.035x + 0.009, R^2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The total phenolic and flavonoids estimation of methanolic extracts of leaves of *A. scholaris* showed the content values of 0.876 and 0.757 respectively Table 2. DPPH radical scavenging assay measured hydrogen donating nature of extracts[10, 11]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *A. scholaris* methanolic leaves extract was found to be 77.67µg/ml as compared to that of ascorbic acid (26.72µg/ml). A dose dependent activity with respect to concentration was observed Table 3.

Table 1: Phytochemical evaluation of *A. scholaris* leaves extracts

S. No.	Constituents	Methanolic extract
1.	**Alkaloids** Hager's Test:	 +Ve
2.	**Glycosides** Legal's Test:	 -Ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +Ve +Ve
4.	**Diterpenes** Copper acetate Test:	 +Ve
5.	**Phenol** Ferric Chloride Test:	 +Ve
6.	**Proteins** Xanthoproteic Test:	 +Ve
7.	**Carbohydrate** Fehling's Test:	 +Ve
8.	**Saponins** Froth Test:	 +Ve
9.	**Tannins** Gelatin test:	 +Ve

+Ve = Positive, -Ve= Negative

Table 2: Results of total phenol and flavonoids content

S. No.	Extract	Total phenol content	Total flavonoids content
		mg/100mg	
1	Methanolic	0.876	0.757

Table 3: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	40.5	24.2
2	20	46.7	25.4
3	40	51.9	35.2
4	60	66.4	40.6
5	80	74.8	56.7
6	100	88.6	59.4
IC 50		**26.72**	**77.67**

CONCLUSION

The results obtained from the leaves of *A. scholaris* Linn extract revealed that has highest phytochemical constituents and potential antioxidants activity. The present study suggested that the purified methanolic extract of *A. scholaris* could be a potential source of antioxidants and phytochemical and thus useful as therapeutic agent against oxidative stress related degenerative disease and disorders in future this study extended to isolation, characterization of bioactive compound.

REFERENCES

1. Polterait O. Antioxidants and free radical scavengers of natural origin. Current Org Chem. 1997; 1:415-440. https://doi.org/10.2174/1385272801666220126162734
2. Amoo SO, Finnie JF, Van Staden J. In vitro pharmacological evaluation of three Barleria species. J Ethnopharmacol. 2009; 121:274-277. https://doi.org/10.1016/j.jep.2008.10.035
3. Nadkarni AK. Indian Materia Medica. 3rd Edition, Popular Book Depot, Mumbai, India, 1976.
4. Meena AK, Nitika G, Jaspreet N, Meena RP, Rao MM. Review on ethnobotany, phytochemical and Pharmacological profile of Alstonia scholaris. Int Res J Pharm. 2011; 2(1):49-54.
5. Mistry D, Parekh B, Pithawala M. Studies on phytochemical constituents and antioxidant activity of Alstonia scholaris, Int J Life Sci. 2016; 4 (4):529-538.
6. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
7. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of Salmalia malabarica. Pharmacologyonline. 2019; 1:21-6.
8. Rani D, Kharkwal H, Jha M, Rai N. Assessment of the total flavonoid, phenol, alkaloid content and sun protection factor in grewia abutilifolia leaf extract. J Pharm Res Int.2021; 33(49A):42-51. https://doi.org/10.9734/jpri/2021/v33i49A33300
9. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. J Adv Sci Res. 2020; 11(03):176-82.
10. Hudson BJ. Food antioxidants. In: Gordon MH, editor. The Mechanism of Antioxidant Action in Vitro. London: Elsevier Applied Science; 1990.
11. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Estimation of total phenolic, total flavonoids and total protein content of hydroalcoholic extract of Anacyclus pyrethrum. Pharmacologyonline. 2019; 1:27-33.