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Research Article

ANTIOXIDANT ACTIVITY OF *ALSTONIA SCHOLARSIS* EXTRACTS CONTAINING FLAVONOID AND PHENOLIC COMPOUNDS

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ABSTRACT

The present work is carried out to screen for total phenols, flavonoids, and free radical scavenging activity in methanolic extract of leaf, root and bark of *Alstonia scholaris* Linn (Apocynaceae) using *in vitro* tests including 1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging and superoxide anion radical scavenging methods. Significant differences in DPPH scavenging activity were found between methanolic extract of leaf, root and bark were investigated, the highest radical scavenging activity were observed in root extract (33.00±4.62% inhibition). The total phenol content of the investigated results ranged from 34.97± 0.76 to 46.11 ± 0.85mg GAE/g extract, while flavonoid content ranged from 14.43± 2.37 to 22.54 ± 0.98 mg QE/g extract and the antioxidant activity of the methanol extract increased in a concentration-dependent manner.

Keywords: *Alstonia scholaris,* 1, 1-diphenyl-2-picryl-hydrazil (DPPH), Superoxide anion radical scavenging method, Antioxidant activity.

INTRODUCTION

Alstonia scholaris is an antimalarial[1] drug used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The plant *Alstonia scholaris* Linn (R.Br), belongs to the family Apocynaceae and is native to India. It grows throughout India, in deciduous and evergreen forests, also in plains [2]. Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) and free radicals, such as the hydroxyl radical (OH) and superoxide anion (O_2^-) are produced as normal products of cellular metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases and premature aging [3]. Many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other Carotenoids which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [4]. Moreover, plant secondary metabolites such as flavonoids and terpenoids play an important role in defense against free radicals [5]. Antioxidants regulate various oxidative reactions naturally occurring in tissues and are evaluated as a potential anti-aging agent. Hence, antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors. Antioxidants have been widely used as food additives to provide protection from oxidative degradation of foods and oils. Hence, antioxidants are used to protect food quality mainly by the prevention of oxidative deterioration of constituents of lipids. The most extensively used synthetic antioxidants are propylgallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) [6]. The ethanolic extract of leaves of *Alstonia scholaris* Linn had a significant *(p<0.01) in vitro* antioxidant activity [7]. The present work shows maximum *in vitro* antioxidant activity from ethanolic extract of root of *Alstonia scholaris.*

METHOD

Plant material and preparation of the extract

Fresh leaves, root and bark of *Alstonia scholaris* free from disease were collected from local areas in Davanagere district, Karnataka. The plant materials are shade dried and then powdered using a mechanical grinder. 250 grams of pulverized leaf root and bark materials were soaked in 750 ml of methanol (LR grade, Merck, India) separately and kept on a rotary shaker for 24 h. Each extract was filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint color. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was subjected to screening for antioxidant activity.

Antioxidant Activity

Superoxide anion scavenging activity:

Superoxide anion scavenging capacity of was assessed by the method of Nishikimi [8]. Effect of *Alstonia scholarsis* extract for different concentrations (200, 400, 600, 800 and 1000µg/ml) was calculated. The percentage of super oxide anion scavenging was calculated as:

% scavenged of superoxide anion = [(Ablank-ASample) /Ablank] × 100

Where:

Ablank: The absorbance of the blank in absence of sample

ASample: The absorbance in the presence of the sample.

Free radical scavenging activity

Free radical scavenging activity was evaluated using α-tocopherol and BHA as standard antioxidants. The radical scavenging activity was measured using the stable radical DPPH according to the method described by Shimada [9] with some modifications. Various concentrations of the extracts were added to 4 ml of a 0.004% methanol solution of DPPH. The mixture was shaken and left for 30 min at room temperature in the dark, and the absorbance was then measured with a spectrophotometer at 517 nm. All determinations were performed in triplicate. The antioxidant activity was calculated as the percent inhibition caused by the hydrogen donor activity of each sample according to the following:

Inhibition (%) = (1– absorbance of the sample/absorbance of the blank) × 100.

Total Phenolic Content (TPC)

The extraction of total phenolic was performed using the Folin–Ciocalteu assay following the method of Kim [10] with some modifications. In total, 100µl of each extract (1 mg/ml) was added to a test tube containing 50 µl of the phenol reagent (1 M). A further 1.85 ml of distilled deionized water was added to the solution and allowed to stand for 3 min after vortexing; then 300µl Na_2CO_3 (20% in water, v/v) was added and vortexed and the final volume (4 ml) was obtained by adding 1.7 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The final mixture was vortexed, and then incubated for 1h in the dark at

room temperature. The absorbance was measured at 725 nm using a UV-VIS spectrophotometer (V 530; Jasco, Tokyo, Japan). A standard curve was prepared using 0, 65.5, 125, and 250 mg/l gallic acid in methanol: water (50:50, v/v). Total phenolic values are expressed in terms of gallic acid equivalents (GAE) in milligrams per gram plant extract. All determinations were performed in triplicate.

Total Flavonoid Content (TFC)

The total flavonoid content in extracts was determined according to Moreno [11]. A 0.5 ml sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminum nitrate and 0.1 ml of potassium acetate (1M), and 4.3 ml of 80% ethanol was added to make a total volume of 5ml. The mixture was vortexed and the solution was allowed to stand for 40 min for reaction at room temperature. The absorbance was measured spectrophotometrically at 415 nm. All determinations were performed in triplicate. Total flavonoid values are expressed in terms of quercetin equivalents (QE) per gram of plant extract. A standard curve was prepared using 0, 5, 10, and 100 mg/l solutions of quercetin.

Statistical analysis

Antioxidant activity, total phenolic content, and flavonoid content are reported as the mean ±standard deviation (SD). Significance differences for multiple comparisons were determined using one way analysis of variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Percentage inhibition of DPPH and IC50 are parameters widely used to measure antioxidant/free radical scavenging power [12, 13]. DPPH is a reactively stable free radical. The assay is based on the scavenging ability of antioxidants towards the stable radical DPPH. DPPH gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract [14]. The DPPH inhibition of leaf, root and bark of plant extracts is summarized in Table 1. Significant differences ($P < 0.05$) in DPPH scavenging activity were found between in three extracts were ranging from 21.44±1.34% to 33.00±4.62%. This range of antioxidant activity may be attributable to the wide variety of bioactive compounds, such as flavonoids and phenols.

Methanolic extract of roots of *Alstonia scholaris* possessed the highest DPPH scavenging activity (33.00±4.62%) followed by bark extract (29.41±2.08%) and leaves extract (21.44±1.34%).The higher antioxidant activity of *Alstonia scholaris* could be due to the high concentration of total phenolic and flavonoids in the plants. Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties [15]. Root extract of *Alstonia scholaris* were reported to contain maximum flavonoids (22.54±0.98mg QE/g). Phenolic are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as a standard [16]. Bark extract of *Alstonia scholaris* possesses maximum phenol content (46.11±0.85mg QE/g).

The methanol extract appeared to be potent with a maximum inhibition of 72.82 % at 1000 µg/ml concentration (Table: 2; figure: 1). The result of the study clearly indicates that as the concentration of methanolic extract increases percentage of inhibition also shows increasing results.

Table 1: Table shows antioxidant activities, phenolic and flavonoid content of *Alstonia scholaris*

Plant material	Material used	%Inhibition of DPPH	Total Flavanoids (mg QE/g extract)	Total phenols (mg GAE/g extract)
Alstonia scholaris	Leaf	21.44±1.34	14.43±2.37	34.97±0.76
	Root	33.00±4.62	22.54±0.98	45.47±1.07
	Bark	29.41±2.08	20.16±1.11	46.11±0.85

Values are mean±S.E; *P <0.05*

Table 2: The effect of different concentration of *Alstonia scholaris* methanolic extract on scavenging of superoxide anion radical

Concentration in µg/ml	Percentage of Inhibition
200	26.37%
400	39.64%
600	50.13%
800	61.27%
1000	72.82%

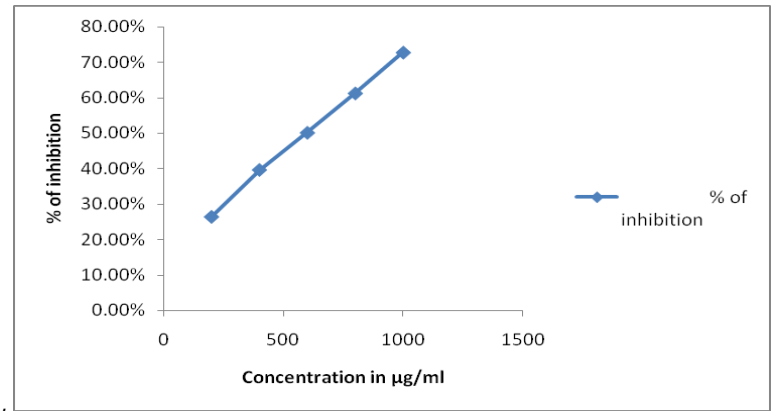

Fig. 1: Effect of methanolic concentration on percentage of inhibition

CONCLUSION

This study suggested that the methanolic extract of *Alstonia scholaris* plant possess antioxidant activity, which might be helpful in preventing of various Oxidative stress- related diseases and also the present work provides the evidence for presence of bioactive compounds like Flavanoids and phenols and hence, it is worthwhile to isolate and elucidate the bioactive principles that are responsible for the anti-oxidant activity that is underway.

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