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ANTIOXIDANT ACTIVITY OF THE LEAVES OF *Alstonia scholaris R.Br.*

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ABSTRACT

Alstonia scholaris is a large evergreen tree belongs to family Apocynaceae, grows throughout India, in deciduous and evergreen forests and also in plains. The plant is also abundantly found in Mizoram and is known as Thumriat. The latex of the plant is applied to wounds and boils and also mixed with oil to cure earache by mizo tribes. Plants or plant-derived compounds possessing high levels of antioxidant properties also show wound-healing activities as antioxidants reduce the adverse effects of wounds by removing products of inflammation. In the present study, the antioxidant activity of the methanolic extract of leaves of *Alstonia scholaris R.Br.* was estimated by three *In vitro* assay methods namely (1) DPPH free radical scavenging activity (2) Ferric reducing antioxidant potential (FRAP) assay and (3) Total Phenolic content estimation, spectrophotometerically. The observed DPPH free radical scavenging activity was 23.26±0.79 mg TE/gm dry leaves while FRAP activity and total Phenolic content were 6.93±1.04 mg TE and 0.79±0.14 mg GAE /gm of dry leaves respectively.

KEYWORDS: Alstonia schlolaris, DPPH, FRAP, Total phenolic.

INTRODUCTION

Alstonia scholaris is a large evergreen tree measuring 17 to 20 m in height with a straight often fluted and buttressed bole, about 110 cm in diameter. Bark is grayish brown, rough, lanticellate abounding in bitter, white milky latex; leaves 4-7 in a whorl, coriaceous, elliptic-oblong, pale beneath; flowers small, greenish white, numerous in umbellate panicles, corolla

tube short. Very strongly scented; fruits follicles, 30-60 cm long, seeds papillose with brownish hair at each end. The plant belongs to family Apocynaceae, grows throughout India, in deciduous and evergreen forests and also in plains. The plant is found in India in the sub Himalayan region from the Yamuna eastward ascending to 3000 feet above sea level, abundantly found in West Bengal and South India.[1] The plant is also abundantly found in Mizoram and is known as Thumriat. The latex of the plant is applied to wounds and boils and also mixed with oil to cure earache by mizo tribes.[2] The plant is also reported as a stimulant, carminative, stomachic, expectorant and febrifuge. The decoction of the dried bark is used extensively to treat asthma, hypertension, lung cancer and pneumonia, whereas an infusion of the leaves is used to cure fever.[1]

The phytochemical constituents of a plant often determine the physiological action on the human body. For centuries, plants have been used to treat several diseases worldwide, and are still playing a major role in healthcare systems in many developing countries. The use of plant extracts or plant-derived compounds in the treatment and management of wounds has been well documented. Similarly, the presence of effective antioxidants in various plant extracts is well known. It has also been noted that many plants or plant-derived compounds possessing high levels of antioxidant properties also show wound-healing activities. In recent years, the search for "natural remedies" for the treatment of wounds and for novel antioxidants has gained momentum, and a significant body of literature is now available in this area of research.[3] Reactive Oxygen Species (ROS) is produced in high amounts at the site of wound as a defense mechanism against invading bacteria.[4] However, the presence of increased numbers of neutrophils and ROS overwhelm the antiprotease substances that normally protect the tissue cells and the extracellular matrix.[5] At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids.[6] Fibroblasts may be killed and skin lipids will be made less flexible by excess ROS. Because of these, the overall role of antioxidants appears to be significant in the successful treatment and management of wounds. Antioxidants reduce these adverse effects of wounds by removing products of inflammation. They counter the excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect protease inhibitors from oxidative damage.[7] The most likely mechanism of antioxidant protection is direct interaction of the extracts (or compounds) and the hydrogen peroxide rather than altering the cell membranes and limiting damage.[8] Compounds with high radical-scavenging capacity have been shown

to facilitate wound-healing. The antioxidant activities of plant extracts are mainly due to the presence of Phenolic compounds such as flavonoids, Phenolic acids, tannins and Phenolic diterpenss.[9] Hence, the constituents of the extracts such as tannins and flavonoids play a major role in the wound healing by preventing and protecting oxidative damage from free radicals. [10, 11] In the present study, the antioxidant activity of the methanolic extract of leaves of *Alstonia scholaris R.Br.* was estimated by three *in vitro* assay methods namely (1) DPPH free radical scavenging activity, (2) Ferric reducing antioxidant potential (FRAP) assay and (3) Total Phenolic content estimation, spectrophotometerically.

MATERIALS AND METHODS

Plant Material

The plant *Alstonia scholaris R.Br.* was collected from Aizawl area, Mizoram and submitted the herbarium specimens for authentication / identification to the Regional Office, Botanical Survey of India (BSI), Shillong. The BSI, Shillong has authenticated the plants and communicated the identification / authentication report vides letter reference No.BSI/ERC/Tech/2010/052, dated 27.04.2010.

The fresh leaves of the plant were collected, washed and air dried in shade. On complete drying, the dried plant material was ground to powder with Willey / Laboratory Mill and sifted through sieve number 22. The powdered leaves were then subjected to cold maceration using methanol as solvent following the procedure of Manjunatha et al.[12] and Harborne.[13] with slight modifications. Briefly, five hundred (500g) grams of powder was soaked in 2.5 L of methanol (1:5 w/v) in a conical flask for a period of 3 days with intermittent stirring and at the end of 3rd day the content was filtered with muslin cloth followed by Whatmann filter paper No. 1. For complete extraction of the active principles, this process was repeated three times using fresh solvent on each occasion or until the colour of the methanol becomes light. The filtrate obtained was pooled and further subjected to rotary vacuum evaporator. The material was stored at -40ºC in deep freezer in air tight containers till further use.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox), Gallic acid were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate (FeCl3. 6H2O), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained

from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui with slight modifications.[14] DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm in a UV-Visible Spectrophotometer (Thermo- Evolution 201). An aliquot (20 µl) of extract was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals at 517 nm until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. A standard curve was prepared using trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were calculated from the decreased in the absorbance. The free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric Reducing Antioxidant Potential (FRAP) assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to the procedure described by Benzie and Strain with slight modifications.[15] Briefly, 30µl of extract was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture was incubated at 37ºC for 30 min and the increase in absorbance was measured at 593 nm using a UV-Visible Spectrophotometer (Thermo-Evolution 201). The standard curve was prepared using TROLOX (250 -1000µg/ml) and the value of FRAP was calculated from the standard curve. The results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method described by Singleton and Rossi with slight modifications.[16] Briefly, thirty (30) microlitres of the plant extract was added to 1ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 970 µl of sodium carbonate (7.5%) solution. After 1 hr incubation at room temperature, the absorbance was measured at 640 nm using a UV/Visible Spectrophotometer (Thermo-Evolution 201). Different volume (20-

100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

RESULT AND DISCUSSION

The antioxidant activity of the methanolic extract of the *Alstonia scholaris R.Br.* leaves was evaluated by three *in vitro* assay methods viz. DPPH free radical scavenging assay method, Ferric Reducing Antioxidant Potential (FRAP) assay and Total phenolic content assay. The antioxidant activity of the extract based on DPPH free radical scavenging and FRAP assay are expressed as mg trolox equivalent (TE) whereas total phenolic content of the extract is expressed as mg gallic acid equivalent (GAE) per gram of the dry sample. The antioxidant content observed in the present investigation is given in Table1.

Table 1: Antioxidant content of *Alstonia scholaris R.Br.*

Sl. No.	Methods of estimation	Antioxidant content/ g of dry leaves
01	DPPH free radical scavenging method	23.26±0.79 mg TE
02	FRAP assay	6.93±1.04 mg TE
03	Total phenolic content	0.79±0.14 mg GAE

The DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH free radical scavenging was thought to be due to their hydrogen donating ability. The DPPH free radical scavenging activity of *Alstonia scholaris R.Br.* leaves in the present investigation 23.26±0.79 mg TE/gm dry leaves. The ability to scavenge DPPH radicals compliments the total antioxidant capacity of a drug. Pratap and his associates.[17] also reported that Alstonia scholaris had significant (DPPH) free radical scavenging. The ferric reducing antioxidant potential assay is based on the reducing power of a compound (antioxidant). It measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). As the ferric to ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/ Fe^{2+}, the values in the FRAP assay expresses the corresponding concentration of electron donating antioxidants. The FRAP activity in the present investigation was 6.93±1.04 mg TE/gm of dry leaves. Phenolic compounds are herbal substances whose chemical structures may range from quite simple compounds to highly polymerized substances. The capacity of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they possess. Phenols play an important role in antioxidant

activity, because they transfer hydrogen to radicals and produce phenoxide radical, which is stabilized. Therefore, it is important to determine the total amount of phenolics to evaluate the antioxidant capacity of plants. The total phenolic content of *Alstonia scholaris R.Br.* leaves in the present investigation was 0.79±0.14 mg GAE /gm of dry leaves. Pankti and his colleagues (2012) also evaluate the antioxidant potential of different extracts of Alstonia scholaris in various *in vitro* tests and found that dichloromethane (DCM) and ethyl acetate (EA) fractions were found to have significant ($p<0.01$) free radical scavenging and metal ion-chelating properties and on comparison with standard antioxidants such as butylated hydroxyanisole (BHA) and 1-ascorbic acid, it was known that DCM and EA possess powerful in vitro antioxidant activity. This proved the plant as reducing agent, metal chelator, its hydrogen donating ability and effectiveness as scavengers of hydrogen peroxide, superoxide and free radicals. The relationship between the antioxidant properties of the plants and wound healing have been reported in literature. Antioxidants reduce the adverse effects of wounds by removing products of inflammation. They counter the excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect protease inhibitors from oxidative damage.[18] The present finding revealing the high antioxidant content of the Alstonia scholaris suggest that extract of this plant can be used as a potent wound healing material.

CONCLUSION

The methanolic extract of Alstonia scholaris had significant (DPPH) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging and ferric thiocyanate reducing activity. In our study also, considerable content of phenolic compounds and antioxidant activity was observed. Thus Alstonia scholaris leaves can play a pivotal role in the field of wound healing activity, antioxidant and anticancer research. Further, it can be used as a source of the natural antioxidants.

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