International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 04, 2020
ISSN: 1475-7192

IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF BARK OF ALSTONIA SCHOLARIS

Jaspreet Kaur Sodhi*[1], Avneet Kaur[2], Prof. Birendra Shrivastava[3], Hardarshan Singh Lamba[4], Jaya Sharma[5]

ABSTRACT

Alstonia scholaris belonging to family Apocynaceae is commonly known as Devil's tree or Saptaparni. It is an evergreen tropical tree native to Indian sub-continent and South East Asia. It contains various phytoconstituents like alkaloids, triterpenoids, flavonoids, steroids and phenolic acids which have shown promising therapeutic potential. The present research was subjected to evaluate the in vitro antioxidant activity of ethanolic extract of bark of Alstonia scholaris using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The extract showed significant antioxidant activity when compared to ascorbic acid. The results of this research work are promising thus indicating the utilisation of the bark of Alstonia scholaris as a significant source of natural antioxidant.

Keywords: *Alstonia scholaris, antioxidant activity, DPPH free radical scavenging activity*

I. INTRODUCTION

Due to their versatile applications, plant derived substances are gaining great interest and there has been an increased focus on plant research all over the world. *Alstonia scholaris* belonging to family Apocynaceae is popularly known as "Devil's tree" or "Saptaparni" and is attaining attention of researcher's for its pharmacological activities. The plant has been used in Ayurvedic, Unani, Homeopathy and Folklore system of medicine to treat various types of disorders.[1,2] It is widely distributed in dried forests of India, Western Himalayas, and Western Ghats as well as in the Southern India[3]. It has been reported to possess antimalarial[4-5], antimicrobial[6-7], free radical scavenging and antioxidant[8-11], anti-diabetic[12-14], analgesic and anti-inflammatory[15-16], anticancer and cytotoxicity[17-22], radioprotective[23-25], CNS activity[26-27],

[1]*HR Institute of Pharmacy, Ghaziabad, Uttar Pradesh, India*
[2] *G D Goenka University, Gurugram, Haryana, India*
[3] *School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India*
[4]*HR Institute of Pharmacy, Ghaziabad, Uttar Pradesh, India*
[5] *School of Pharmacy, Apex University, Jaipur, Rajasthan, India*

immunostimulating[28-29], antifertility[30], antidiarrheal[31-32], bronchodilatory[33], anti-tussive and anti-asthmatic[34] activities.

Phytoconstituents have been reported in different parts of the plant such as bark;[35-36] leaves;[37-38] roots;[39] flowers[40] and fruits[41]. *Alstonia scholaris* is known to be a rich source of alkaloids (about 180 alkaloids) [42-43] like Echitamine[35,44], Scholarine[45], Picrinine[46] , corialstonine and corialstonidine[47]. Isolation of a new secoiridoid glucoside alstonoside, together with two known isoflavone apioglucosides, formononetin 7-0-β-D-apiofuranosyl(1-6)-β-D-glucopyranoside and biochanin α-7-0-β-D apiofuranosy–(1-6)-β-D-glucopyranoside has also been reported[48]. Among the other constituents, Isookanin-7-o-alpha-l-rhamnopyranoside, a new flavanone glycoside[48] and Alstonoside, a secoiridoid glucoside[49] has been recorded. Iridoids, coumarins, flavonoids, leucoanthocyanins, reducing sugars, simple phenolics, steroids, saponins and tannins were also found in the plant[50]. Total phenolics, flavonoid and tannin contents were found to be significantly ($P<0.05$) higher in ethanolic extracts of *Astonia scholaris*[51-52].

The aim of this paper is to analyze the antioxidant activity of *Alstonia scholaris* bark. Antioxidants are molecules that are capable of stabilizing, inhibitin, and deactivating free radicals before reacting[53-54]. They are divided into endogenous antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) that play an important role in maintaining good cellular function and health; and exogenous antioxidants. However, because of oxidative stress, body needs exogenous antioxidants to compensate it[54-55]. These are further divided into natural antioxidants and synthetic antioxidants. As the use of synthetic antioxidants (BHA, BHT, propyl gallate) are not recommended in pharmacology due to safety concerns[56] thus many natural antioxidants from fruits and plants need to be developed. Compounds that act as natural antioxidants are vitamins, phenolics, flavonoids, carotenoids and thiols [57-58].

II. MATERIALS AND METHODS

Chemicals and plant material

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals Co., USA. Ascorbic acid and all other chemicals and solvents used were of analytical grade.

Collection of plant part

The mature bark from woody trunk portion of *Alstonia scholaris* was procured from local area of Ghaziabad, India and the species of the plant was authenticated by the Botanist of Raw Material Herbarium and Museum (RHMD), Delhi (taxonomic reference number: RHMD-3653-54)

Preparation of extract

The collected bark was cleaned, washed, air dried and coarsely powdered. The weight of the dry powder was taken. The coarsely powdered bark (100 gm) was taken in a 500 ml soxhlet apparatus and extracted with 85% ethanol for around 24 hours. After extraction, the ethanol extract was dried free of solvent in a rotary evaporator at low temperature (40°C). This dried extract was tested for antioxidant activity.

Antioxidant assay

DPPH free radical scavenging assay

The antioxidant activity was measured using the standard DPPH method. The capacity of the extracts to scavenge the stable free radical DPPH was monitored according to the procedure described by Amarowicz et al[59]. DPPH being a stable free radical accepts an electron or hydrogen atom to become 1,1-diphenyl-2-picrylhydrazine molecule. The purple colour of free DPPH changes to yellow upon reaction with hydrogen donors because of reaction between hydrogen donors and DPPH free radical that leads to it's reduction to corresponding hydrazine. Various concentrations (2, 4, 8, 16, 32, 64 and 128 µg/ml) of the extract were added to 4 ml of a 0.004 % ethanol solution of DPPH. The mixture was shaken and left for 25-30 mins at room temperature in the dark. The reduction of DPPH radical was determined by reading the decrease in absorbance at 517 nm using an ultraviolet-visible spectrophotometer. 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 formula:

(%) Inhibition = (1 − Absorbance of the sample/Absorbance of the Blank) × 100

III. RESULT AND DISCUSSION

From table 1, it was observed that the ethanolic bark extract of *Alstonia scholaris* showed the highest antioxidant activity of 80.3% as compared to the standard ascorbic acid, 73.6% by DPPH free radical scavenging method (figure 1)**.** This study showed that the ethanolic extract has proton donating ability and could serve as a free radical inhibitor or scavenger and act as a natural antioxidant.

Table 1: DPPH free radical scavenging activity of *Alstonia scholaris* bark extract

Sample No.	Concentration (µg/mL)	%Inhibition	
		Ethanolic extract *A. scholaris*	Ascorbic acid
1.	2	42.6	40.4
2.	4	46.3	50.2
3.	8	50.8	58.3
4.	16	57.4	62.5
5.	32	66.7	65.8
6.	64	72.4	68.2
7.	128	80.3	73.6

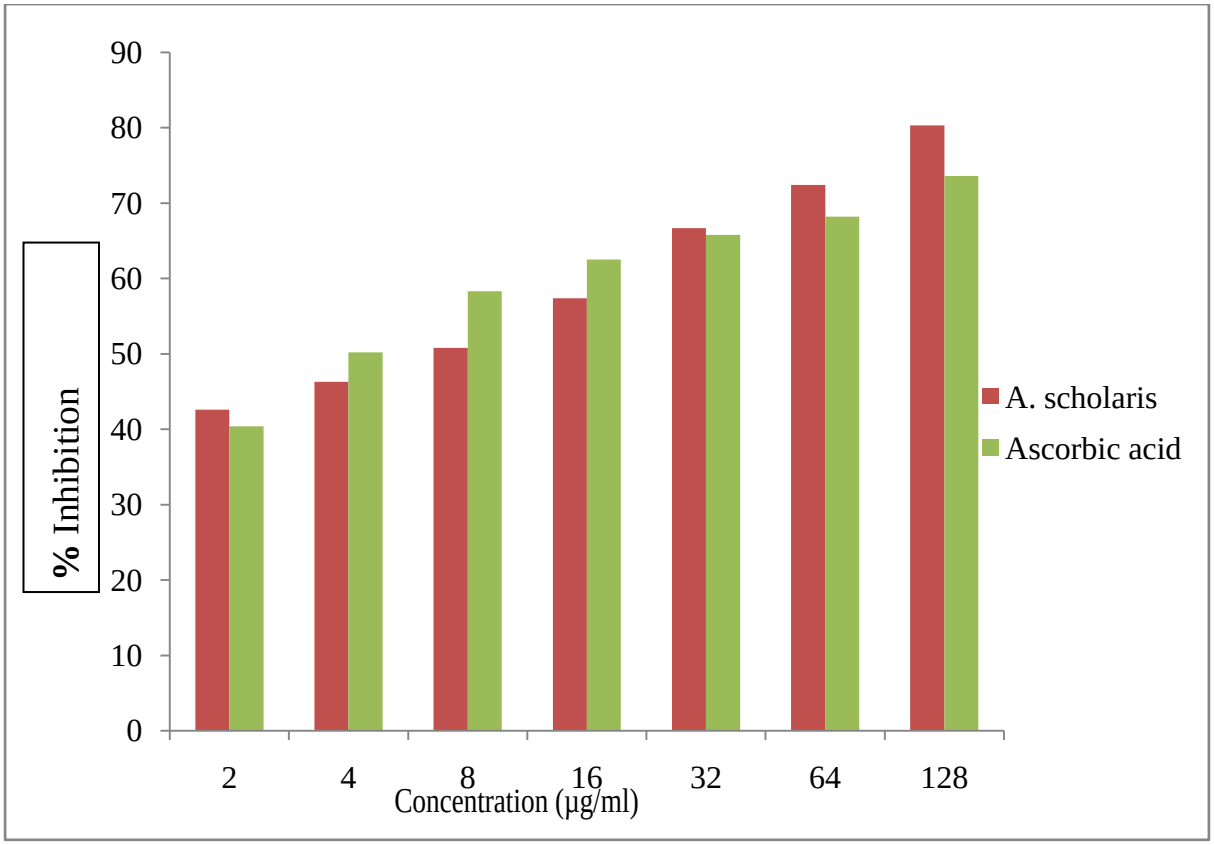

Figure 1: Percentage of free radicals scavenged by ethanolic extract from bark of *A. scholaris* using Ascorbic acid as standard (DPPH free radical scavenging method)

IV. CONCLUSION

In vitro antioxidant activity of the bark extract of *Alstonia scholaris* was performed. The results of DPPH assay shows that the extract has antioxidant activity when compared to the standard ascorbic acid indicating that this extract can be a significant source of natural antioxidants, which might be helpful in preventing the diseases involving free radicals and can be further used for the benefit of mankind.

CONFLICT OF INTEREST

No conflict of interest lies between Authors.

REFERENCE

1. Khare CP. Indian Medicinal Plants: An illustrated Dictionary, New York; Springer: pp. 38-39.
2. Joshi SG. Medicinal Plants, New Delhi; Oxford and IBH Publishing Co. Pvt. Ltd.: 2000.
3. Nadkarni AK. Indian Materia Medica. 3rd Edition, Popular Book Depot, Mumbai, India: 1976.
4. Gandhi M and Vinayak VK. Preliminary evaluation of extracts of *Alstonia scholaris* bark for *in vivo* antimalarial activity in mice. Journal of Ethnopharmacology.1990; 29(1):51–7.

5. Keawpradub N, Kirby GC, Steele JC, Houghton PJ. Antiplasmodial activity of extracts and alkaloids of three *Alstonia* species from Thailand. Planta Medica. 1999;65(8):690–4.
6. Misra CS, Pratyush K, Lipin Dev MS, James J, Veettil AT and Thankamani VA. Comparative study on phytochemical screening and antibacterial activity of *Alstonia scholaris* with the root, leaves and stem barks. International Journal of Research in Pharmacology and Phytochemistry. 2011;1(2):77–82.
7. Singh B and Sangwan P. Taxonomy, ethnobotany and antimicrobial activity of *Alstonia scholaris* (L.) R.Br., *Carissa carandas* L. and *Catharanthus roseus* (L.) G. Don. International Journal of Biotechnology and Biosciences. 2011;1(1):102–12.
8. Arulmozhi S, Mazumder PM, Ashok P and Narayanan LS. *In Vitro* Anti- oxidant and free radical scavenging activity of *Alstonia scholaris* Linn. R. Br. Iranian Journal of Pharmacology and Therapeutics. 2007;6(2):191–6.
9. Ravi Shankar K, Ramesh KVRNS and Naveena P. Free radical scavenging activity of the flower and fruit extracts of *Alstonia scholaris*. Biosciences Biotechnology Research Asia. 2008;5(1):493–4.
10. Kumar A, Kaur R and Arora S. Free radical scavenging potential of some Indian medicinal plants. Journal of Medicinal Plants Research. 2010;4(19):2034–42.
11. Ramachandra YL, Ashajyothi C and Padmalatha Rai S. Antioxidant activity of *Alstonia scholaris* extracts containing flavonoid and phenolic compounds. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(3):424-6.
12. Anurakkun NJ, Bhandari MR and Kawabata J. α-Glucosidase inhibitors from Devil tree (*Alstonia scholaris*). Food Chemistry. 2007;103(4):1319–23.
13. Arulmozhi S, Mazumder PM, Lohidasan S and Thakurdesai P. Antidiabetic and anti-hyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R.Br. European Journal of Integrative Medicine. 2010;2(1):23–32.
14. Bandawane D, Juvekar A and Juvekar M. Anti-diabetic and anti-hyperlipidemic effect of *Alstonia scholaris* Linn. bark in streptozotocin induced diabetic rats. Indian Journal of Pharmaceutical Education and Research. 2011;45(2):114–20.
15. Arulmozhi S, Mazumder PM, Sathiyanarayanan L and Thakurdesai PA (2012) Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris.* Pharmacologia. 2012;3(5):132–7.
16. Karawya MS, Ammar NM, Hifnawy MS, Al-okbi SY, Doha AM and El-Anssary AA. Phytochemical study and evaluation of the anti-inflammatory activity of some medicinal plants growing in Egypt. Medical Journal of Islamic World Academy of Sciences. 2010;18(4):139–50.
17. Kamarajan P, Sekar N, Mathuram V and Govindasamy S. Anti-tumor effect of echitamine chloride on methylcholonthrene induced fibrosarcoma in rats. Biochemistry International. 1991;25(3):491–8.
18. Keawpradub N, Houghton PJ, Eno-Amooquaye E and Burke PJ. Activity of extracts and alkaloids of Thai *Alstonia* species against human lung cancer cell lines. Planta Medica. 1997;63(2):97–101.
19. Saraswathi V, Ramamoorthy N, Subramaniam S, Mathuram V, Gunasekaran P and Govindasamy S. Inhibition of glycolysis and respiration of sarcoma-180 cells by echitamine chloride. Chemotherapy. 1998;44(3):198–205.

20. Jagetia GC and Baliga MS. Evaluation of anti-cancer activity of the alkaloid fraction of *Alstonia scholaris* (Sapthaparna) *in vitro* and *in vivo.* Phytotherapy Research, 2006;20(2):103–9.
21. Sharma V, Mallick SA and Tiku AK. Anticancer activity of Devil tree (*Alstonia scholaris* Linn.) leaves on Human cancer cell lines. Indian Journal of Agricultural Biochemistry. 2010;23(1):63–5.
22. Surya Surendren P, Jayanthi G and Smitha KR. *In vitro* evaluation of the anticancer effect of methanolic extract of *Alstonia scholaris* leaves on mammary carcinoma. Journal of Applied Pharmaceutical Science. 2012;2(5):142–9.
23. Gupta U, Jahan S, Chaudhary R and Goyal PK. Amelioration of radiation- induced haematological and biochemical alterations by *Alstonia scholaris* (a Medicinal Plant) extract. Integrative Cancer Therapy. 2008;7(3):155–61.
24. Gupta U, Chaudhary R and Goyal PK. Post-treatment effects of *Alstonia scholaris* extract against radiation-induced biochemical alterations in Swiss albino mice. Iranian Journal of Radiation Research. 2010;8(3):169–77.
25. Chakraborty S, Mistry D and Pithawala M. Pilot observation on possible ameliorative effect of aqueous extracts from bark of *Alstonia scholaris* on Bleomycin induced chromosomal damage in *in vitro* cultured human lymphocytes. Electronic Journal of Pharmacology and Therapy. 2011;4:9-13.
26. Arulmozhi S, Mazumder PM, Kangralkar VA, SathiyaNarayanan L and Thakurdesai P. Anti- anxiety activity of *Alstonia scholaris* Linn. R. Br. Pharmacologyonline. 2008;3:761–75.
27. Kulkarni MP and Juvekar AR. Effect of *Alstonia scholaris* (Linn.) R. Br. on stress and cognition in mice. Indian Journal of Experimental Biology. 2009;47(1):47–52.
28. Lin SC, Lin CC, Lin YH, Supriyatna S and Pan SL. The protective effect of *Alstonia scholaris* R. Br. on hepatotoxin-induced acute liver damage. The American Journal of Chinese Medicine. 1996;24(2):153–64.
29. Iwo MI, Soemardji AA, Retnoningrum DS and Sukrasno UM. Immuno stimulating effect of pule (*Alstonia scholaris* L.R. Br., Apocynaceae) bark extracts. Clinical Hemorheology and Microcirculation. 2000;23(2-4):177–83.
30. Gupta RS, Sharma R, Sharma A, Bhatnager AK, Dobhal MP, Joshi YC and Sharma MC. Effect of *Alstonia scholaris* bark extract on testicular function of Wistar rats. Asian Journal of Andrology. 2002;4(3):175–8.
31. Patil S, Juvekar AR, Joglekar SN, Shamkuwar PB and Nimbkar SR. Study of antidiarrhoeal activity of *Alstonia scholaris* bark. Indian Drugs, 1999;36:463–5.
32. Shah AJ, Gowani SA, Zuberi AJ, Ghayur MN and Gilani AH. Antidiarrhoel and spasmolytic activities of them ethanolic crude extract of *Alstonia scholaris* L. are mediated through calcium channel blockade. Phytotherapy Research. 2010;24(1):28–32.
33. Channa S, Dar A, Ahmed S and Atta-ur-Rahman. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. Journal of Ethnopharmacology. 2005; 97(3):469–76.
34. Singh PK, Kumar Vinod Tiwari RK, Sharma A, Rao CV and Singh RH. Medico-Ethnobotany of 'Chatara' block of District Sonebhadra, Uttar Pradesh, India. Advances in Biological Research. 2010;4(1):65–80.

35. Manohar H, Ramaseshan S. The structure of echitamine iodide major alkaloid from bark of *Alstonia scholaris*. Curr Sci. 1961;30:5-7.
36. Feng T, Cai XH, Zhao PJ, Du ZZ, Li WQ, Luo XD. Monoterpenoid indole alkaloids from the bark of *Alstonia scholaris*. Planta Med. 2009;75(14):1537-41.
37. Banerji A, Banerji J. Isolation of Pseudo Akuammigine from the leaves of *Alstonia scholaris*. Indian J Chem B Org. 1977;15:390-1.
38. Rahman AU, Alvi KA, Muzaffar A. Isolation and 1H/13C NMR Studies on 19,20-Dihydrocondylocarpine: An alkaloid from the leaves of *Ervatamia coronaria* and *Alstonia scholaris*. Planta Med. 1986;(4):325-6.
39. Boonchuay W, Court WE. Minor alkaloids of *Alstonia scholaris* Root. Phytochemistry, 1976b; 15.
40. Dutta SC, Bhattacharya SK, Ray AB. Flower alkaloids of *Alstonia scholaris*. Planta Med, 1976; 30(1): 86-90.
41. Wongseripipatana S, Chaisri L, Sritularak B, Likhitwitayawuid K. Indole alkaloids from the fruits of *Alstonia scholaris*. Thai J Pharm Sci, 2004; 28: 173-80.
42. Dutt AT. Total alkaloids in *Alstonia scholaris*. Sci Cult. 1944;9:555-6.
43. Dai YY, Yang W, He R, Zhou FL, Mao YL. Resource status, seed germination characteristics and alkaloids contents of *Alstonia scholaris* in Yunnan. Journal of West China Forestry Science. 2008;1(1):73-6.
44. Govindachari TR, Rajappa S. Echitamine. Tetrahedron. 1961;15(1-4):132-43.
45. Banerji A. Scholaricine, an alkaloid from *Alstonia scholaris*. Phytochemistry. 1981;20(3):2771-3.
46. Ghosh R, Roychowdhury P, Chattopadhyay D, Iitaka Y. The structure of an alkaloid, picrinine from *Alstonia scholaris*. Acta Cryst. 1988;44:2151-4.
47. Hadi S. Mataranine a and b: a new diastomeric indole alkaloid from *Alstonia scholaris* R.Br. of Lombok Island. Indonesian J Chem. 2009;9(3):466-9.
48. Chauhan JS, Chaturvedi R, Kumar S. Isookanin-7-O-Alpha-LRhamnopyranoside a new flavanone glycoside from the Roots of *Alstonia scholaris*. Indian J Chem B Org. 1985;24(2):219.
49. Thomas PS, Kanaujia A, Ghosh D, Duggar R, Katiyar CK. Alstonoside, a secoiridoid glucoside from Alstonia scholaris Indian J Chem B Org. 2008;47:1298-02.
50. Khyade MS, Vaikos NP. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. Afr J Biotech. 2009a;8(22):6434-36.
51. Al-Daihan S, Aldbass A, Alotebi L, Bhat R. Antioxidant and antimicrobial activity of whole seed extracts of Perseaamericana Mill. IJPBR, 2016;4(04):15-8.
52. Verma PK, Raina R, Sultana M, Prawez S and Singh M. Polyphenolic constituents and antioxidant/antiradical activity in different extracts of *Alstonia scholaris* (Linn.). Afr J Biotechnol. 2015;14(47):3190-7.
53. Leena Seasotiya, Priyanka Siwach, Sheema Bai, Anupma Malik, Pooja Bharti, & Sunita Dalal. Free radical scavenging activity, phenolic contents and Phytochemical analysis of seeds of trigonella foenum graecum. *Asian Pacific Journal of Health Sciences*,2014; 1(3);219-226.
54. Sisein EA. Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences. 2014;2:110-8.

55. Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxid Med Cell Longev*. 2012;2012:756132.
56. Lu JM, Lin PH, Yao Q and Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. Journal of Cell Molecular Medicine. 2010;14:840-60.
57. Bhattacharya M and Chakraborty S. Free radicals and naturally occurring antioxidants. Journal of Pharmacognosy and Phytochemistry. 2015;3(3):1-7.
58. Nimse SB and Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. The Royal Society of Chemistry. 2015;5:27986–28006.
59. Chang W, Choi CKS, Hwang SS, Bong KC, Hye JA, Min YL, Sang HP and Kim SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. Plant Science. 2002;163:1161-8.