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Antioxidant activity of *Saraca asoca* bark extracts: An *in vitro* evaluation

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Abstract
The intension of the present study was to evaluate the *in vitro* antioxidant activity of extracts of *Saraca asoca* bark. The ethanol and aqueous extracts of *Saraca asoca* bark were screened for *in vitro* antioxidant potential using models *viz*, 1,1-diphenly-2-picryl hydrazine (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation scavenging activity, ferric reducing antioxidant power (FRAP) assay and nitric oxide free radical scavenging activity (NOS) reducing power ability using standard procedures. The extracts of *Saraca asoca* bark exhibited strong antioxidant activity with DPPH, ABTS, FRAP and NOS assays. The radical scavenging effect was found to increase with increasing concentrations. The IC50 values of ethanolic and aqueous extracts of in all models *viz*, DPPH, ABTS, FRAP and NOS were found to be 80.09 µg/ml, 38.76 µg/ml, 63.73µg/ml, 9.94 µg/ml and 73.6 µg/ml, 77.15 µg/ml, 51.12µg/ml, 24.86 µg/ml, respectively. The results show that there was an increase in the reducing power of the plant extract as the concentration of the extract increases. Thus, it can be accomplished that the extracts of *Saraca asoca* bark is a potential source of natural antioxidants.

Keywords: *Saraca asoca*, antioxidant, DPPH, ABTS, FRAP, NOS

Introduction

Ayurveda is a traditional medicinal system, where herbal therapies were utilized systematically. Herbal medicine can be used as a complementary and alternative medicine to treat various diseases, because they are easily available and economical with reliable remedy [1]. *Saraca asoca* (family Caesalpiniaceae) is one of the most ancient sacred, legendary evergreen tree of India tropical areas with medicinal properties, exhibiting a number of pharmacological effects.

Antioxidants are very important for human health, which is essential supplementation to provide cellular protection against the deleterious effect of excessive reactive oxygen species (ROS) concentration [2]. Free radicals such as hydroxyl, peroxyl and superoxide radicals produced during normal metabolic function causes damage to the bio-molecules are very transient and highly reactive. This can lead to adverse effects on human health and causes severe diseases [3, 4]. Several studies show that elevated level of free radicals may be associated with carcinogenesis. It is estimated that more than 1300 Indians die due to cancer. Mortality rate due to cancer it was increased up to 6% [5]. ROS is a double edge sword while ROS generation is essential for cell survival, proliferation and progression of cancer cells. In contrast increased ROS utilise as a major mechanism in chemotherapeutic mediated cells death and hence essential mediators of apoptotic mediated cell death [6, 8].

Damage caused by free radicals can results in formation of single and double strand breaks of DNA, and oxidation of purine and pyrimidine bases, leading to genome instability and subsequent carcinogenesis [9, 11]. Therefore, protection of cell from oxidative damage by anti-oxidant supplements is very helpful in prevention and treatment of cancer [12, 14].

Since ancient times, medicinal plants were used as key therapeutic agents all over the globe, especially among the rural communities of developing countries due to the unavailability of an accessible and affordable primary health care system [15, 16]. World Health Organization (WHO) reported that about 80% people across the globe uses medicinal plants. A wide range of biological and pharmacological properties of medicinal plants manifest them a therapeutic potential, for the treatment of various diseases [17, 19].

Saraca asoca (family Caesalpiniaceae) is one of the most ancient plants widely distributed throughout the Indian subcontinent [20, 21]. Various medicinal uses of *Saraca asoca* had been reported in Charaka Samhita (100 A.D.) [22]. The pharmacological effects like anti-hyperglycemic, antipyretic, antibacterial, antihelmintic activities etc. of different parts of plant are well described in literature [23, 26].

A traditional drug Asoka Aristha used for the treatment of menorrhagia is originated from *Saraca asoca* [27]. The therapeutic action of *Saraca asoca* stem bark extracts is mainly due to their secondary metabolites like flavonoids, terpenoid, lignin, phenolic compounds, tannins etc [28, 34]. Chemicals present in natural products like plants might be an age-old science, but with promising pharma formulation in the current century. Present study was designed to evaluate the antioxidant activity of aqueous and ethanolic extract of *Saraca asoca* bark.

Materials and Methods

Collection of Sample

Plant extract preparation the dried stem of *Saraca asoca* were collected and authenticated by Rapinat Herbarium, St. Josephs College, Tiruchirappalli. It was crushed, powdered, and extracted with 95% alcohol, using Soxhlet apparatus.

After the completion of extraction, the extract was filtered and the solvent was removed by distillation under reduced pressure [35].

DPPH (Diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. The DPPH solution (0.1mM) in ethanol was made, and 1.0ml of this solution was added to 3.0 ml of extracts solution (or standard) in water at different concentrations. After 30 minutes, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation [36].

% of DPPH of radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

Where, control was the absorbance of the control reaction and test was the absorbance in the presence of extracts. The mean values were obtained from triplicate experiments.

ABTS Radical scavenging activity

ABTS radical scavenging assay was carried out using the method described by Pai *et al*. 2015 where stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate/ ammonium persulfate solution [37]. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12h at 30ºC in the dark.

The solution was then diluted by mixing 1 mL ABTS solution with 60 ml methanol to obtain an OD of 0.706±0.001 at 734 nm using the spectrophotometer. Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution and the OD was taken at 734 nm after 7 min using the spectrophotometer. All the readings were taken in triplicates.

The ABTS scavenging capacity of the extract was calculated as:

ABTS radical scavenging activity (%) = ABTS control – ABTS sample × 100 ABTS Control ABTS control is the absorbance of ABTS radical + methanol; ABTS sample is the absorbance of ABTS radical + sample extract/standard [38].

FRAP Radical scavenging activity

Ferric reducing antioxidant power (FRAP) assay was carried out according to the method of Jain *et al*. 2014 [39].

FRAP reagent was prepared using acetate buffer (1.6g sodium acetate and 8 ml acetic acid make up to 100mL) (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride solution in proportion of 10:1:1 (v/v) respectively.

The FRAP reagent was prepared fresh and was warmed to 37ºC in oven prior to use. In total of 100 µL extracted samples were added to 3 mL of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using spectrophotometer at 0 min and after 4 min. Standard curve of ascorbic acid was prepared. FRAP reagent was used as a blank for both standard and samples.

FRAP value of sample was obtained using the formula:

%Inhibition = Change in absorbance of sample from 0 to 4 mins × 1000uM Change in absorbance of standard from 0 to 4 mins

Nitric oxide Radical scavenging activity

To measure the nitric oxide free radical scavenging activity, 50 μL of plant extract of different concentrations, dissolved in DMSO, was taken and then methanol was added to make the volume 150 μL. About 2.0 mL of sodium nitroprusside (10 mM) in phosphate buffer saline was added in each tube and they Oxidative Medicine and Cellular Longevity 3 were incubated at room temperature for 150 min. After the incubation, 5 mL of Griess reagent was added to each tube and the absorbance of chromophore formed was measured at 546 nm on spectrophotometer. Same procedure was repeated with ascorbic acid (positive control) and methanol (blank which served as control) [40, 41].

The IC50 values of plant extract and ascorbic acid were calculated as

% Scavenging Reduction = [Absorbance of control −Absorbance of test sample Absorbance of control] × 100.

Result and Discussion

DPPH Radical Scavenging Assay

The antioxidant activity of *Saraca asoca* bark ethanol and aqueous extract was evaluated using the DPPH free radical scavenging method. Ascorbic acid was used as standard compound.

Both extracts showed strong antioxidant activity in the DPPH inhibition assay as evidenced by the IC_{50} values 80.09 µg/ml and 73.6 µg/ml of ethanol and aqueous extracts, respectively (Table.1). Higher phenol and flavonoid content in the plant tissue can also lead to increase in antioxidant activity [42].

Table 1: Percentage of inhibition of DPPH radical scavenging activity of *Saraca asoca* bark extracts

S. No	Concentration	Ascorbic acid		Ethanol bark extract		Aqueous bark extract	
		Absorbance	Percentage inhibition	Absorbance	Percentage inhibition	Absorbance	Percentage inhibition
1	10	0.62	10	0.43	36	0.52	25
2	20	0.584	15	0.386	44	0.472	32
3	40	0.526	24	0.318	54	0.42	39
4	60	0.477	30	0.286	58	0.388	44
5	80	0.402	42	0.232	66	0.342	50
6	100	0.378	46	0.202	71	0.292	58
	Ic_{50} values	48.21 µg/ml		38.76 µg/ml		77.15 µg/ml	

ABTS scavenging activity

ABTS method also depicted antioxidant power of *Saraca asoca* bark extracts. About 38.76 µg/ml of scavenging power was noted in ethanol extract and 77.15 µg/ml of scavenging power was noted in aqueous extract at 100 µg/ml concentration. The extracts efficiently scavenged ABTS radicals generated by the reaction between 2, 2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium Persulfate. Were shown in table 2.

Table 2: Percentage of inhibition of ABTS radical scavenging activity of *Saraca asoca* bark extracts bark extract

S.No	Concentration	Ascorbic acid		Ethanol bark extract		Aqueous bark extract	
		Absorbance	Percentage inhibition	Absorbance	Percentage inhibition	Absorbance	Percentage inhibition
1	10	0.278	28	0.312	19	0.332	14
2	20	0.242	37	0.289	25	0.32	17
3	40	0.206	47	0.274	29	0.28	26
4	60	0.184	52	0.245	38	0.266	31
5	80	0.17	56	0.197	50	0.246	37
6	100	0.152	60	0.171	56	0.226	41
	Ic_{50} values	61.70 µg/ml		80.09 µg/ml		73.6 µg/ml	

Ferric reducing antioxidant activity

The ability of the extract to reduce ferric ions was determined using the FRAP assay. The ethanolic bark extract of *S. asoca* exhibited an IC50 of 63.73µg/ml, lower ferric reducing capacity compared to that of aqueous bark extract with IC50 at 51.12µg/ml.

Table 3: Percentage of inhibition of FRAP radical scavenging activity of *Saraca asoca* bark extracts

S. No.	Concentration	Standard	Ethanol bark extract	Aqueous bark extract
		Percentage inhibition	Percentage inhibition	Percentage inhibition
1	10	0.288	0.326	0.296
2	20	0.348	0.422	0.338
3	40	0.422	0.54	0.376
4	60	0.536	0.62	0.432
5	80	0.638	0.78	0.488
6	100	0.73	0.846	0.532
	Ic_{50} values	12.44 µg/ml	9.94 µg/ml	24.86 µg/ml

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was performed with *Saraca asoca* ethanol and aqueous extract using ascorbic acid as standard compound. In this study it was observed that the extracts have the ability to scavenge nitric oxide radical in dose dependent manner. The value was found to be IC_{50} 9.94 µg/ml for ethanol and 24.86 µg/ml aqueous extracts (Table.4).

Table 4: Percentage of inhibition of NOS radical scavenging activity of *Saraca asoca* bark extracts

S.No.	Concentration	Ascorbic acid	Ethanol bark extract	Aqueous bark extract
		Absorbance	Absorbance	Absorbance
1	10	2.322	2.010	1.196
2	20	2.180	1.836	1.680
3	40	1.944	1.632	1.466
4	60	1.686	1.348	1.284
5	80	1.442	1.086	0.966
6	100	1.166	0.942	0.820
	Ic_{50} values	87.85 µg/ml	63.73 µg/ml	51.12 µg/ml

Conclusion

In summary, the bark of *Saraca asoca* bark extracts may be considered as a good source of phenols and natural antioxidants to treat many diseases. *Ashoka* is ancient and reliable source of medicine with well-known pharmacological activities. The present study was to determine the biopotential active compounds and nutraceutical values present in the bark part of *Saraca asoca*. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *Saraca asoca* should be emphasized for the control of various diseases.

References

1. Navneet Kumar Yadav, Karan Singh Saini, Zakir Hossain, Ankur Omer, Chetan Sharma, Jiaur R *et al*. Gayen, *et al*. Saraca indica bark extract shows in-vitro antioxidant, anti-breast cancer activity and does not exhibit toxicological effects. Oxidative Medicine and Cellular Longevity, 2015.
2. Chen C, Chen Y, Lu P, Lin W, Chen T, Lin C *et al*. Proteus mirabilis urinary tract infection and bacteremia: Risk factors, clinical presentation, and outcomes. J Microbiol Immunol Infect. 2012; 45:228-36.
3. Ganie SA, Dar TA, Hamid R, *et al*. *In vitro* antioxidant and cytotoxic activities of *arnebia benthamii* (wall ex. g. don): a critically endangered medicinal plant of Kashmir valley. Oxidative Medicine and Cellular Longevity, 2014, 8.
4. Sahreen S, Khan MR, Khan RA. Ameliorating effect of various fractions of *Rumex hastatus* Roots against

Hepato- and Testicular Toxicity Caused by CCl4. Oxidative Medicine and Cellular Longevity, 2013, 11.
5. Rajan S, Mahalakshmi S, Deepa M, Sathya K, Shajitha S, Thirunalasundari T *et al*. Antioxidant potentials of Punica granatum fruit rind extracts. IJPPS. 2011; 3(3):82-88.
6. Dreher D, Junod A. Role of oxygen free radicals in cancer development. European Journal of cancer. 1996; 32(1):30-38.
7. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free radical biology and medicine. 1990; 8(6):583-599.
8. Ray G, Batra S, Shukla NK, *et al*. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast cancer research and treatment. 2000; 59(2):163-170.
9. Bravo J, Arbillaga L, de Peña MP, Cid C. Antioxidant and genoprotective effects of spent coffee extracts in human cells. Food and Chemical Toxicology. 2013; 60:397-403.
10. Martin OA, Nowsheen S, Siva S, Aziz K, Hatzi VI, Georgakilas AG. Inflammation and oxidative dna damage: a dangerous synergistic pathway to cancer. Cancer: oxidative stress and dietary antioxidants, 2014, 63-74.
11. Dizdaroglu M. oxidatively induced dna damage and its repair in cancer. Mutation research/reviews in mutation research. 2015; 763:212-45.
12. Yong YK, Tan JJ, The SS *et al*. *Clinacanthus nutans* extracts are antioxidant with antiproliferative effect on cultured human cancer cell lines. Evidence-based complementary and alternative medicine.2013:8.
13. Bennett LL, Rojas S, Seefeldt T. Role of antioxidants in the prevention of cancer. Journal of experimental & clinical medicine. 201; 4(4):215-222.
14. Martinez-Perez C, Ward C, Cook G, *et al*. Novel flavonoids as anti-cancer agents: mechanisms of action and promise for their potential application in breast cancer. Biochemical Society transactions. 2014; 42(4):1017-1023.
15. Tabuti JR, Lye KA, Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. Ethnopharmacol. 2003; 88(1):19-44.
16. Han SR, Han HY, Lee BS, *et al*. Toxicity assessment of *Leonuri herba* aqueous extract orally administered to rats for 13 consecutive week. J Ethnopharmacol. 2013; 149(1):371-376.
17. Debas HT, Laxminarayan R, Straus SE. Complementary and alternative medicine in disease control priorities in developing countries. D.T. Jamison, J.G. Breman, A.R. Measham, *et al*. Eds., Washington (DC), 2006.
18. W.H. Organization guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. World Health Organization, Geneva, Switzerland, 2004.
19. Oumarou BF, Tchuemdem LM, Djomeni PD, *et al*. Mineral constituents and toxicological profile of Jateorhiza macrantha (Menispermaceae) aqueous extract. J Ethnopharmacol. 2013; 149(1):117-122.
20. Pradhan P, JosephL, Gupta V, *et al*. *Saraca asoca* (Ashoka): a review. Journal of chemical and pharmaceutical research. 2009; 1(1):62-71.
21. Bhalerao SA, Verma DR, Didwana VS, Teli NC. *Saraca asoca* (Roxb.), De. Wild: An overview. Annals of Plant Sciences. 2004; 3(7):770-775.
22. Mittal A, Kadyan P, Gahlaut A, Dabur R. Nontargeted identification of the phenolic and other compounds of *saraca asoca* by high performance liquid chromatography-positive electrospray ionization and quadrupole time-of-flight mass spectrometry. ISRN Pharm, 2013, 293935.
23. Kumar S, Narwal S, Kumar D, Singh G, Narwal S, Arya R *et al*. Evaluation of antihyperglycemic and antioxidant activities of *Saraca asoca* (Roxb.) De Wild leaves in streptozotocin induced diabetic mice. Asian Pacific Journal of Tropical Disease. 2012; 2(3):170-176.
24. Sasmal S, Majumdar S, Gupta M, Mukherjee A, Mukherjee P. Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb. Asian Pacific journal of tropical biomedicine. 2012; 2(10):782-786.
25. Shirolkar A, Gahlaut A, Chhillar A, Dabur R. Quantitative analysis of catechins in *Saraca asoca* and correlation with antimicrobial activity. Journal of Pharmaceutical Analysis, 2013, 3(6).
26. Sarojini N, Manjari SA, Kanti CC. Phytochemical screening and anthelmintic activity study of *Saraca indica* leaves extracts. International Research Journal of Pharmacy. 2011; 2(5):194-197.
27. Middelkoop TB, Labadie RP. The action of *Saraca asoca* Roxb. De Wilde bark on the PGH2 synthetase enzyme complex of the sheep vesicular gland. Z Naturforsch C. 1985; 40(7-8):523-526.
28. Suja M, Rajan S, Thiyagarajan BJ, Thirunalasundari T, Thenmozhi S. Pharmacognostical and phytochemical studies of An Ayurvedic drug *Saraca asoca* stem bark. Journal of Pharmacy Research. 2012; 5(2):1119-1121.
29. Tusharkumar D. Chemical investigation of phenolic constituents of two important medicinal plants *Terminalia Chebula* and *Saraca Asoca*, 2017.
30. Saha J, Mukherjee S, Gupta K, Gupta B. High-performance thin-layer chromatographic analysis of antioxidants present in different parts of *Saraca asoca* (Roxb.) de Wilde. Journal of Pharmacy Research. 2013; 7(9):798-803.
31. Maruthappan V, Sakthi Shree K. Antiulcer activity of aqueous suspension of *Saraca indica* flower against gastric ulcers in albino rats. Journal of Pharmacy Research. 2010; 3(1):20
32. Mathew N, Anitha M, Bala T, Sivakumar S, Narmadha R, Kalyanasundaram M *et al*. Larvicidal activity of S*araca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. Parasitology research. 2008; 104(5):1017-1025.
33. Cibin TR, Devi DG, Abraham A. Chemoprevention of two-stage skin cancer *in vivo* by *Saraca asoca*. Integr Cancer Ther. 2012; 11(3):279-286.
34. Cibin T, Devi DG, Abraham A. Chemoprevention of skin cancer by the flavonoid fraction of *Saraca asoka*. Phytotherapy Research. 2010; 24(5):666-672.
35. Mukherjee PK. Quality control of herbal Drugs: an approach to the evaluation of botanicals. New Delhi: Business Horizons, 2002.
36. Blois. Invitro Antioxidants estimation. Food rese internat. 1958; 41:1-15.

37. Upadhya V, Pai SR, Hegde HV. Effect of method and time of extraction on total phenolic content in comparison with antioxidant activities in different parts of *Achyranthes aspera*. Journal of King Saud University, 2015.
38. Arindam Ghatak, Sidhesh Nair, Ambrish Vajpayee, Palak Chaturvedi, Shardool Samant, Ketki Soley, *et al*. Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De.Wilde. International Journal of Advanced Research. 2015; 3(5):318-327.
39. Jain N, Goyal S, Ramawat KG. Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in Rajasthan. Int PharmSci. 2011; 3(3):248-53.
40. Marcocci L, Packer L, Droy-Lefaix M-T, Sekaki A, Gardes-Albert M. Antioxidant action of *Ginkgo biloba* extract EGb 761. Methods in Enzymology. 1994; 234:462-475.
41. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. Journal of Ethnopharmacology. 2007; 113(2):284-291.
42. Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K *et al*. Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De. Wilde. 2015; 3(5):318-327.