Antioxidant Activity of *Saraca Indica* (Roxb.) De. Wilde Methanolic Leaf Extract

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Abstract
India is home to one of the most significant indigenous medicinal herb called *Saraca asoka,* an IUCN (International Union for Conservation of Nature) red listed tree species. The goal of the current study was to assess the antioxidant capacity of *Saraca indica* leaf extract. The findings indicated that certain active phytochemicals with antioxidant properties were present in the extracts. The maximum percentage for scavenging the DPPH radical was 60.50% at a concentration of 0.0825 mg/ml. The methanolic extract of Ashoka leaf has an IC50-DPPH value of 0.064 mg/ml.
The total flavonoid value was found to be 2.41±0.004 mg of quercetin equivalent per gram of flavonoid in the dry extract sample, and the total tannin value was found to be 4.16±0.003 mg per gram in the dry extract sample. The FRAP value of the methanolic leaf extract was found to be 25.2±0.004 mg ferrous sulphate hexahydrate equivalent per gram of dry weight. The methanolic leaf extract of *Saraca indica* at a concentration of 0.2 mg/ml has an absorbance value of 0.332±0.004 for the reduction power assay.

Keyword: *Saraca asoca,* antioxidant activity, FRAP, flavonoid, tannin, DPPH, reducing power

1. Introduction

Saraca asoca (Roxb.), De. wild, or *Saraca indica*, also known as Ashok briksh, Ashoka(Hindi, Bengali, Gujarati, Marathi), Sita ashok, Karkeli (Sanskrit), Sitashok, Asogam (Tamil), Oshok (Bengali), Ashokmu, Vanjulamu (Telugu), and Asok (Kannada). Ashoka is Sanskrit for" without anguish" or" that which doesn't sorrow" (1, 2). It is stated in Hindu litrature that the Indian thinker that established Buddhism, Gautama Buddha (563 – 483B.C.), is said to have been born under this tree. Ashoka is specifically pious to the Hindu deity of love, Kamadeva, for whom it is worshipped every year on December 27 [3].

Saraca indica has been classified as endangered by the IUCN [4]. It is a significant native medicinal plant that may be found all throughout India. The plant's astringent bark has historically been used to treat blood disorders, biliousness, colic, piles, ulcers, fractures, and as an astringent, anthelmintic, demulcent, emollient, and stomachic metropathy, dyspepsia, visceromegaly, and menstrual bleeding. Flowers and bark are both helpful for stomachalgia, syphilis, hyperdipsia, inflammation, dysentery, haemorrhoids, and scabies in children [5, 6, 7]. The bark's medicinal properties as a "female tonic" are attributed to the tannin, catechol, flavonoides, sterol, glucosides, alkaloids, and other organic calcium compounds it contains. It is good for naturally purifying the blood, removing toxins from the body, and preventing skin allergies. Vesicle calculi and bone fractures are treated with seeds. The herb is used to treat tumors, blood problems, indigestion, and dyspepsia [8, 9].

The *Saraca asoca* plant's bark, leaves, and flowers contain a variety of antioxidant substances, including flavonoids, catechin, beta-sitosterol, and lignin glycosides, which aid in stabilizing free radical molecules linked to the emergence of cancer conditions [10]. Free radicals are produced in human bodies by a variety of endogenous systems, exposure to various physicochemical circumstances, and pathological situations. They are primarily made of oxygen (reactive oxygen species, or ROS) and nitrogen (reactive nitrogen species, or RNS). Loss of enzyme

activity can occur as a result of free radical damage to proteins. Mutagenesis and carcinogenesis are both possible outcomes of DNA damage [11, 9].
For proper physiological function, there must be a balance between free radicals and antioxidants. Oxidative stress occurs when the body's capacity to manage free radicals is exceeded, which can lead to the spread of a variety of human disorders [12]. The excess of free radicals can cause structural and functional damage to cell membranes, which in turn can cause degenerative diseases and ailments like Alzheimer's disease, macular degeneration, acute hepatotoxicity, cardio-vascular disease, renal dysfunction, impaired glucose tolerance, rheumatoid arthritis, and oxidative damage to DNA that can result in cancer development [13].

Humans have evolved a very complicated antioxidant defense mechanism that interacts and works in concert to neutralize free radicals in order to protect the body's cells and organ systems from reactive oxygen and nitrogen species. Free radicals can be stabilized or rendered inactive by these antioxidants before they damage cells. Toxic peroxides are eliminated by antioxidant enzymes including catalase, glutathione peroxidase, and superoxide dismutase. Non-enzymatic compounds, in addition to antioxidant enzymes, are crucial components of antioxidant defense mechanisms. These exogenous, non-enzymatic components come from foods. They consist of micronutrients like zinc and selenium as well as tocopherol, carotene, and ascorbic acid [14].

More than 80% of individuals in impoverished countries hardly have enough money for the most basic medical treatments, medications, and vaccinations. Surprisingly many people in industrialized countries choose procedures and goods for which there is few or no evidence of their usefulness or safety. These procedures are collectively referred to as complementary and alternative medicine (CAM) or traditional medicine (TM) [15].

The aim of the current study was to assess the antioxidant capacity of *Saraca indica* leaf extract (SIE). The findings of this study will be useful in understanding how *Saraca indica* leaf extracts are used to prevent and cure various ailments.

2. Materials And Methods

2.1. Collection Of Plant

The plant material such as leaves sample was collected from residential area of Noida, India.

2.2. Drying Of Leaves

The materials were entirely air dried in a clean, well-ventilated space. The samples were cleaned with water, allowed to air dry at room temperature for seven days, and then dried in an oven at 40°C to get rid of any remaining moisture. For later usage, the dried leaves were powdered and kept in airtight containers.

2.3. Preparation Of Plant Extract

The in vitro assay for antioxidants uses methanol, a polar solvent. Methanol was used to extract an equivalent amount of powdered Ashoka leaf samples over the course of three days at room temperature. At a temperature of 60°C, extraction was carried out. The dried sample, which weighed about 3 gm, was put into test tubes with 20 ml of solvent and extracted at room temperature. The sample was homogenized with extraction buffer. The supernatant was collected after three rounds of extraction. Extracts were filtered using Whatman No.1 paper and the filtrates were concentrated using rotary evaporator at 40°C [16]. Extracts were re-suspended in each extracting solvent to achieve a stock solution of 10 mg/ml.

2.4 Chemicals

Sigma Chemical Co. was used to purchase all of the chemicals. Analytical-grade chemicals and reagents were employed throughout. The spectra were taken using a Systronic double beam-UV-2201.

3. Antioxidant assay:

3.1 DPPH radical assay:

The stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical technique was used to evaluate the extracts' free radical scavenging

activity in terms of hydrogen donating or radical scavenging capacity [17]. 3.0 ml of the DPPH solution in methanol (0.24 mg/ml) was added to plant extracts at various concentrations (0.0165-0.0825 mg/ml). After 30 minutes, the absorbance was evaluated at 517 nm. Every experiment was carried out twice. Higher free radical scavenging activity is shown by the reaction mixture having a lower absorbance. The efficiency of absorption of the DPPH radical was determined using the following equation:

% DPPH scavenging effects = $(Ac - At)/Ac \times 100$

Where Ac = Absorbance of the control; At = Absorbance of the test drug/ extracts.

3.2 Ferric reducing antioxidant power (FRAP):

The technique is based on reducing a ferric 2, 4, and 6-tripyridyl-s-triazine complex (Fe4+-TPTZ) to the ferrous form (Fe2+-TPTZ). As a reference solution, different quantities of iron (II) sulphate heptahydrate (FeSO4.7H2O) (27 mg/ml) were added to 3.6 ml of FRAP reagent, and the final volume was brought up to 1 ml. After 5 minutes, the increase in absorbance at 593 nm was seen. A working FRAP reagent was produced by mixing 10:1:2.5 ml of TPTZ solution, 2.5 ml of FeSO4.7H2O solution, and 25 ml of acetate buffer. The reagents were all freshly made. Every experiment was carried out twice [18, 19].

3.3 Total flavonoid content estimation

The total flavonoid content of extracts was assessed using a modified version of the aluminium chloride colorimetric technique [20]. First, in water bath at 25 °C for 5 minutes, 0.5 ml of the extracts were combined with 0.3 ml of 5% sodium nitrate (50 mg/ml). The combination was then given a final addition of 0.3 ml of a 10% aluminium chloride (100 mg/ml) solution. For 6 minutes, the mixture was incubated at 25 °C. After that, 0.5 ml of distilled water and 2 ml of 1 M sodium hydroxide (40 mg/ml) were added to the mixture. Different amounts of quercetin (0.027, 0.054, 0.081, 0.108, and 0.135 mg/ml) were employed as standards.

3.4 Total Tannin Estimation

A strong mixture of alkaline convert phosphotungstomolybdic acid and tannin-like compounds produces a vivid blue solution whose intensity is proportional to the amount of tannins. Add 100 ml of distilled water, 0.1 M ferric chloride, 0.008 M potassium ferrocyanide, and 0.1 N hydrogen chloride to create solution 1. Add 0.05 g of tannic acid (1 mg/ml) diluted in 50 ml of distilled water to make a standard tannic acid solution. Take the (0.02, 0.04, 0.06, and 0.08 mg/ml) of workable tannic acid solutions from the standard and place them in four test tubes. Then, add distilled water to the test tubes to make a total volume of 5 ml.

3.5 Reducing Power Antioxidant Assay

The sample concentration of 0.2 mg/ml and the aliquots of various standard concentrations (0.01, 0.05, and 0.1 mg/ml) were taken, and the volume was made up to 1 ml using methanol. Aliquots were combined with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M phosphate buffer (pH 6.6). For 20 minutes, the mixture was incubated in a water bath at 50°C. The mixture was mixed with aliquots of 2.5 ml of 10% trichloroacetic acid, and the combination was then centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the solution's upper layer was mixed with 0.5 m of freshly prepared 0.1% ferric chloride solution [21]. In a UV spectrometer, the absorbance was measured at 700 nm (Systronic double beam-UV-2201). Without adding extract, a blank was made.

4. Results and discussion

4.1 DPPH assay

In 1958, Marsden Blois developed the DPPH technique [22]. The capacity to neutralize free radicals was evaluated by measuring the reduction of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) scavenging at 517 nm absorbance. This test evaluates the extracts' capacity to neutralize free radicals [17]. A violet solution is produced by the antioxidant assay known as the DPPH free radical technique, which relies on electron transfer. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is reduced, producing a colorless solution. Results were displayed as a graph of

concentration against percentage inhibition using the 50% inhibition concentration (IC_{50}) determined from the regression equation. The methanolic Ashoka leaf extract used in this study had an IC_{50} value of 0.064 mg/ml. Concentration-dependent free radical scavenging was detected in the DPPH radical scavenging activity of the methanol extracts (0.0165–0.0825 mg/ml). The greatest percentage of inhibition was found to be 60.50% at a concentration of 0.0825 mg/ml of methanol extract, while the minimum percentage of inhibition was found to be 20.47% at a concentration of 0.0165 mg/ml of methanol extract (refer to Table 1).

Table 1. DPPH scavenging activity of methanol extract of leaves of *Saraca indica*

Concentration of plant extract (mg/ml)	DPPH scavenging activity in percentage	Average absorbance at 517 nm ± Standard deviation
0.0165	20.47	0.439±0.004
0.033	34.23	0.363±0.001
0.0495	42.93	0.315±0.002
0.066	49.45	0.279±0.004
0.0825	60.5	0.218±0.002

4.2 Ferric reducing antioxidant power (FRAP) assay

Benzie and Strain carried out the FRAP assay for the first time in 1996 [23]. A standard curve was created by combining the FRAP reagent with various known concentrations of solutions containing Fe^{2+}. This allowed for the assessment of antioxidant capacity by estimating the Fe^{2+} content of the samples [24]. In contrast to other assays that evaluate the suppression of free radicals, the FRAP test is the only one that identifies the presence of antioxidants in a sample. The data from the FRAP assay show the concentration of antioxidants that donate electrons when ferric iron (Fe^{3+}) is reduced to ferrous ions (Fe^{2+}) [25]. Fe^{3+}-TPTZ complex is reduced to blue-colored Fe^{2+}-TPTZ, which absorbs at 593 nm at a low pH of around 3.6. Maximum absorbance was found to be 0.264±0.005 at a concentration of 0.243 mg/ml of iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$), while the minimum percentage of inhibition was found to be 0.022±0.004 at a concentration of 0.027 mg/ml of $FeSO_4.7H_2O$. *Saraca asoca* leaf extract has an absorbance value of 0.278±0.04. (refer to Table 2). FRAP value of methanolic leaf extract was found to be 25.2±0.004 mg iron (II) sulfate heptahydrate equivalent per gram of dry weight.

Table 2. FRAP assay determination

Concentration of Iron(II) sulfate heptahydrate (mg/ml)	Average absorbance at 593 nm ± Standard deviation
0.027	0.022±0.004
0.081	0.083±0.002
0.135	0.140±0.004
0.189	0.197±0.007
0.243	0.264±0.005

4.3 Total flavonoid content estimation

Flavonoids were estimated using the aluminium chloride ($AlCl_3$) colorimetric method. Quercetin and other flavonoids in the flavone and flavonol groups can be detected using the $AlCl_3$ colorimetric approach. The fundamental idea behind the aluminium chloride colorimetric approach is that aluminium chloride forms acid-stable complexes with flavone and flavonol C-4 keto groups as well as either C-3 or C-5 hydroxyl groups [26]. A flavonoid's orthodihydroxyl group in the A and B ring groups may react to form a number of acid-labile complexes. These flavonol complexes, which also include kaempferol, rutin, quercetin, and quercitrin, were discovered recently [27, 28]. The highest absorbance of quercetin was found to be 0.463±0.002 at a concentration of 0.135 mg/ml, while the minimum absorbance was found to be 0.022±0.004 at a dosage of 0.027 mg/ml.

Table 3. Total flavonoids content determination

Concentration of Quercetin (mg/ml)	Absorbance at 510 nm ± Standard deviation
0.027	0.093±0.0035
0.054	0.175±0.021
0.081	0.260±0.003
0.108	0.378±0.001
0.135	0.463±0.002

4.4 Total tannin estimation by ferric chloride method

All aerial plant tissues contain tannic acid, a polyphenol that occurs naturally in plants. It was formerly applied locally to cure skin burns, anally to treat unidentified rectal ailments, and orally to alleviate diarrhea [29]. Tannins may precipitate proteins on enterocytes, which lessens peristaltic movement and intestinal secretion and can have an anti-diarrheal effect [30]. The Van-Buren and Robinson (1981) technique [31] at a UV wavelength of 605 nm is used to quantify tannins. The highest absorbance was found to be 0.201±0.003 at a tannin

concentration of 0.08 mg/ml, while the minimum absorbance was found to be 0.04±0.002 at a tannin concentration of 0.02 mg/ml (refer to Table 4).

Table 4. Total tannin content determination by $FeCl_3$ method

Concentration of tannin (mg/ml)	Absorbance at 605 nm ± Standard deviation
0.02	0.040±0.002
0.04	0.091±0.004
0.06	0.138±0.004
0.08	0.201±0.003

3.5 Reducing Power Antioxidant Assay

The reducing power assay is based on the idea that compounds with a high reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric-ferrous complex with a maximum absorption at 700 nm [21]. Depending on the reducing power of each molecule, the test solution's yellow hue changes to various shades of green and blue in this assay. The highest ascorbic acid absorbance was found to be 0.444±0.004 at a concentration of 0.1 mg/ml, while the minimum absorbance was found to be 0.082±0.003 at a concentration of 0.02 mg/ml (refer to Table 4). The methanolic leaf extract of *Saraca indica* has an absorbance value of 0.332±0.004 at a concentration of 0.2 mg/ml.

Table 5. Reducing ability of ascorbic acid at various concentrations

Concentration of ascorbic acid (mg/ml)	Absorbance at 700 nm ± Standard deviation
0.01	0.082±0.003
0.05	0.219±0.004
0.1	0.444±0.004

Conclusion

The phytochemical research concluded that *Saraca asoca* plants have tannins, flavonoids, antioxidant activity, reducing power, and free radical scavenging activity. It's crucial to test the anti-oxidant activity using different radicals and oxidation systems in order to understand the medical benefits of prospective plant sources. Natural goods are recommended because they have fewer or no side effects, are more affordable, and prevent the emergence of antibiotic resistance. This study is crucial for employing medicinal plants as a substitute source for treating a variety of ailments. Future research must clarify how to increase the food consumption of the medicinal plants used in this study and how to make them more bio-available following different processing steps.

References

1. Warrier PK, Nambiar VPK, Ramankutty C, Nair RV. Indian Medicinal Plants: A Compendium of 500 Species. Madras: Orient Longman. 1993-1996.
2. Nayak C, Siddiqui VA, Rajpal Vinay Kr, Singh Hari, Pal Ramender, Singh Vikram, Roy RK, Prakash S, Rai Yogender, Das KC. Saraca indica: a multicentric double blind homoeopathic pathogenetic trial. Indian J Res Homoeopathy. 2009;3(2):32–36.
3. Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, Verma R, Bajpai A. Saraca indica (Ashoka): A review. J chem and pharmaceutical research. 2009; 1(62).
4. IUCN (2011) IUCN red list of threatened species. Version 2011.1. www. iucnredlist.org.
5. Nadkarni AK. Dr. K.M. Nadkarni's Indian Effect of Morus alba L. (mulberry) leaves on anxiety in mice Materia Medica, Bombay Popular Prakashan, Mumbai. 2005; 1:1104-1106.
6. Kashyapa K, Chand CR. The Useful Plants of India. National Institute of Science Communication and information Resources. 2006.
7. Yadav G, Garg VK, Thakur N, Khare P. Locomotor Activity of Methanolic Extract of Saraca indica Bark. Advances in Biological Research. 2013; 7 (1): 01-03.
8. Kirtikar KR, Basu BD. Indian medicinal plants. Delhi: M/s Periodical Expert Book Agency.1981; 883–884.
9. Yadav NK, Saini KS, Hossain Z, et al. Saraca indica bark extract shows in vitro antioxidant, antibreast cancer activity and does not exhibit toxicological effects. Oxidative Med Cell Longevity. 2015; 205360.
10. Ahmad SR, Pritha Ghosh. A systematic investigation on flavonoids, catechin, β-sitosterol and lignin glycosides from Saraca asoca (Ashoka) having anti-cancer & antioxidant properties with no

side effect. Journal of the Indian Chemical Society. 2022; 99(1): 100293.
11. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. Japi. 2004 Oct;52(794804):4.
12. Alkadi H. A review on free radicals and antioxidants. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2020; Feb 1; 20(1):16-26.
13. Abdel-Hameed ESS (2009): Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. Food Chemistry.2009; 114: 1271–1277.
14. Aqil F, Ahmad I, Mehmood Z. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turkish Journal of Biology. 2006; 30: 177-183.
15. https://pubmed.ncbi.nlm.nih.gov/21250367/
16. Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K, Kudale S, Neetin Desai N (2015).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.
17. Asokan A, Thangavel M, Pallath N. Original Research Article in-vitro Antioxidant Activity of Saraca indica Methanolic Bark Extract. 2015, 4(3): 515-520.
18. Catherine RE, Nicholas M, George P. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997, 2(4): 152-159.
19. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 239(1), 70–76.
20. Safafar H, Van Wagenen J, Møller P, Jacobsen C. Carotenoids, Phenolic Compounds and Tocopherols Contribute to the Antioxidative Properties of Some Microalgae Species Grown on Industrial Wastewater. Marine Drugs. 2015; 13(12):7339-7356.
21. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. Ayu. 2013 Apr;34(2):209-14.
22. Blois, M.S. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181, 1199-1200.
23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996 Jul 15;239(1):70-6.
24. Payne AC, Mazzer A, Clarkson GJ, Taylor G. Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. Food Sci Nutr. 2013 Nov;1(6):439-44.
25. Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerød H, Andersen LF, Moskaug Ø, Jacobs DR Jr, Blomhoff R. A systematic screening of total antioxidants in dietary plants. J Nutr. 2002 Mar;132(3):461-71.
26. Ahmed F, Iqbal M. Antioxidant activity of Ricinus Communis. Organic & Medicinal Chem IJ. 2018; 5(4): 555667.
27. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. (Springer-Verlag, New York, U.S.A, 1970).
28. Tristantini D, Amalia R. Quercetin concentration and total flavonoid content of anti-atherosclerotic herbs using aluminum chloride colorimetric assay. AIP Conference Proceedings 2193, 030012 (2019).
29. Yu YL, Leung LK, Bi YR. Antioxidant activity of flavonoids isolated from Scutellaria rehderiana. J Am Chem Soc. 2000;77:807–813.
30. Nair R, Ghakker N, Sharma A. Spectrophotometric Estimation of Tannins in Raw and Processed Form (Paan Masala) Of Areca Nut. Intr J of Edu and Sci Res Rev. 2015; 2(1).
31. Buren JVP, Robinson WB. Formation of complexes between protein and tannic acid. J Agri Food Chem 1969; 17 (4): 772-777