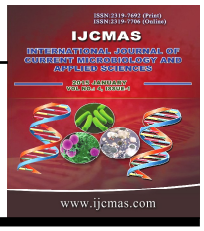
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 515-520

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 515-520**
http://www.ijcmas.com



Original Research Article

Invitro Antioxidant Activity of *Saraca indica* Methanolic Bark Extract

Ardra Asokan[1]*, M.Thangavel[1] and P.Nisha[2]

[1]Department of Microbiology, Sree Narayana Guru College, Chavady, Coimbatore, India
[2]Department of Biosciences, S.S.V.College, Valayanchirangara, Ernakulam, Kerala, India
**Corresponding author*

Keywords

Saracaindica, methanolic, antioxidant, invitro

A B S T R A C T

Saraca indica is an important plant of Indian system of medicine for its chemical constituents and its well-known pharmacological activities. It belongs to the family *Caesalpiniaceae* and is a universal panacea in the Ayurveda medicine. The plant bark tends to possess anti-cancer, anti-menorrhagia, anti-oxytocic, anti-microbial activity and has extended uses in all traditional medicinal formulations. In this study, the methanolic extract of the bark has been studied for its antioxidant properties using various assays. The experiment results demonstrated that the extract had antioxidant properties in all *invitro* models.

Introduction

Free radicals are reactive chemical species contains an unpaired electron spinning on the peripheral layer around the nucleus. These are generated in body during normal metabolic functions (Queen Rosary Sheela and Alex Ramani, 2011). ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals, hydroxyl radicals, non-free radicals and singlet oxygen (Halliwell, 1995) which can cause damage to other molecules by extracting electrons from them in order to attain stability. These species causes cellular damage by reacting with various biomolecules of body such as membrane lipids, nucleic acid, proteins and enzymes and may lead to various disorders. Our body under these oxidative stress antioxidants are used up as body's defense system and interferes with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Many studies have correlated the antioxidant properties of plants role in oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process (Vant Veer *et al.*, 2000; Bokov *et al.*, 2004; Madamanchi *et al.*, 2005). It is reported that foods from plant origin usually contain natural antioxidants and can scavenge free radical (Nohl, 1993).

Saraca asoca Roxb. De Wilde, syn. *S. indica*auct non L. (Ashoka) is an evergreen tree belonging to the Caesalpiniaceae subfamily of the legume family (Sivarajan and Balachandran, 1994). It occurs almost throughout India up to an altitude of 750 m.

Five lignin glycosides, lyoniside, nudiposide, 5-methoxy-9ßxylopyranosyl-(-)-isolariciresinol, icariside E3 and schizanriside, three flavonoids, (-)-epicatechin, epiafzelechin- (4ß-8)-epicatechin and procyanidine B2, together with ß-sitosterol glucoside, were isolated from dried bark (Dhavan *et al.*, 1977).

The bark of the Ashoka tree is used to make a drug, which is reported to possess a stimulating effect on the endometrium and ovarian tissue (http://www.holisticonline.com). The present investigation aims to examine the antioxidant activity of the bark extracts of *Saracaindica* through various assays.

Materials and Methods

Preparation of plant extract

The bark of *Saracaindica* plant specimen was obtained. The bark specimen was washed thoroughly with double distilled water and allowed to shade dry for four weeks until the water content has been completely removed. The dried sample was then pulverized using electric blender and the powdered sample of (150g) was extracted using 500ml of methanol in Soxhlet apparatus for 48 hours at 50°C. The apparatus was intermittently shaken and the extracts were filtered and evaporated to dryness into solid mass devoid of solvent and stored in desiccators until further use.

Chemicals

All the chemicals were purchased from M/S Sigma Chemical Co. Other chemicals and reagents used were of analytical grade. UV-visible spectrophotometer, Shimadzu 1800 was used for recording the spectra.

DPPH radical scavenging activity (Blois, 1958)

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical method. The DPPH solution (0.1mM) in ethanol was prepared and 1.0ml of this solution was added to 4.0 ml of extracts solution (or standard) in distilled water at different concentrations (20-100 µg/ml). Thirty minutes later, 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.

$$\%of\ DPPH = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

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

ABTS radical scavenging activity (Rice Evans *et al.*, 1997)

The ABTS (2, 2′-Azinobis (4-Ethylbenzthiazoline-6-Sulfonate) radical scavenging activity of the extracts were measured by Rice-Evans *et al.* (1997). ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting ABTS solution (7 mM) with 2.45 mM of ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hr before use. Different concentrations (20-100µg/ml) of methanolic extracts or standard (0.5 ml) were added to 0.4 ml of ABTS solution and the final volume was made up to1 ml with ethanol. The absorbance was read at 745nm and the %

inhibition was calculated. The experiment was performed in triplicate.

$$\text{Inhibition percentage}\ (I\%) = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance}_{control}} X\,100$$

$$\text{Percentage of Inhibition} = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance}_{control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) [12]

The method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{4+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ). To 900 µl of FRAP reagent different concentrations of sample solution (20, 40, 60, 80 and 100 µg/ml) was added and the final volume was made upto 1ml. The increase in absorbance at 594 nm was measured at 4 min. $FeSO_4.7H_2O$ was used as a standard. FRAP value was expressed as mmol/100 g on dry weight basis using the calibration curve of Fe^{2+}. Working FRAP reagent was prepared by mixing 25ml of acetate buffer, 2.5 ml of TPTZ solution, and 2.5 ml of $FeCl_3.6H_2O$ solution in the ratio 10:1:1. All the reagents were prepared freshly.

Reducing power activity [13]

The reductive potential of the extract was determined by the different concentrations of extracts and standard in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide $[K_4Fe(CN)_6]$ (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm.2.5 ml of supernatant was mixed with equal volume of distilled water and $Fecl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm. The reductive potential is directly proportional to the absorbance of the reaction mixture. The experiment was carried out in triplicates.

Results and Discussion

In DPPH assay, the IC_{50} values of methanol extract of *S. indica* was found at the concentration of 64.50µg/ml and the standard 41.65 µg/ml (Table 1). At a concentration of 100µg/ml of methanol extract the percentage of inhibition was found to be 69.31%. However, the scavenging activity of ascorbic acid at the same concentration was 80.88% (Fig. 1). DPPH free radical compound has been widely used to test the free radical scavenging ability of various plant extracts; the antioxidant present neutralizes the $DPPH^{\bullet}$ by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by color changes from purple to yellow by read at 517 nm. The methanolic extract of *Saracaindica* demonstrated H-donor activity in this study. Similar DPPH antioxidant assay has been performed by Panchawat *et al.* (2010) with *S.indica* bark extracts and reported an IC_{50} value of ethanolic (90%), ethanolic (60%) and acetone extracts prepared by ultrasonication method were shown to be 139.78, 126.54, 97.82 µg/ml respectively. The ABTS radical scavenging activity of methanolic extract of *S. indica* has been found to increase in a dose dependent manner. The IC_{50} value of methanol extract of *S. indica* was found to be at the concentration of 63.40µg/ml against the 47.35 µg/ml ascorbic acid used as the standard (Fig. 2). In this assay, the pre-formed radical mono cation of 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) is generated by oxidation of ABTS with potassium persulfate (a blue chromogen) and is reduced in the presence of hydrogen donating antioxidants. The discoloration following the sample addition

indicates that ABTS radical cations were quenched or reduced by the antioxidants in the sample was determined by measuring the decrease of absorbance. The methanolic extract of *S. indica* exhibited an IC_{50} of 79.5µg/ml, lower ferric reducing capacity compared to that of standard-ascorbic acid with IC_{50} at 45µg/ml (Fig. 3). The ability of the extract to reduce ferric ions was determined using the FRAP assay. An antioxidant capable of donating a single electron to the ferric TPTZ (Fe (III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. The reducing power of the methanolic extract of *S. indica* was found to be steadily increased in direct proportion to the increasing concentrations of the extract (Fig.4). IC_{50} value in reducing power assay of *S. indica* was found to be 55.98µg/ml comparing with the standard antioxidant ascorbic acid at the concentration of 50µg/ml. The high reducing power is indicative of the hydrogen donating ability of the active species present in the extract.

Table.1 Antioxidant activity of *Saraca indica* bark extract

S. NO	Antioxidant Assays	IC_{50} values(µg/ml)	
		Standard	***S.indica***
1	DPPH radical scavenging activity	41.65	64.50
2	ABTS radical scavenging activity	47.45	63.40
3	Ferric Reducing Antioxidant Power	45.0	79.5
4	Reducing power activity	50.0	55.98

Figure.1 Estimation of DPPH Radical Scavenging Activity of *Saraca indica*

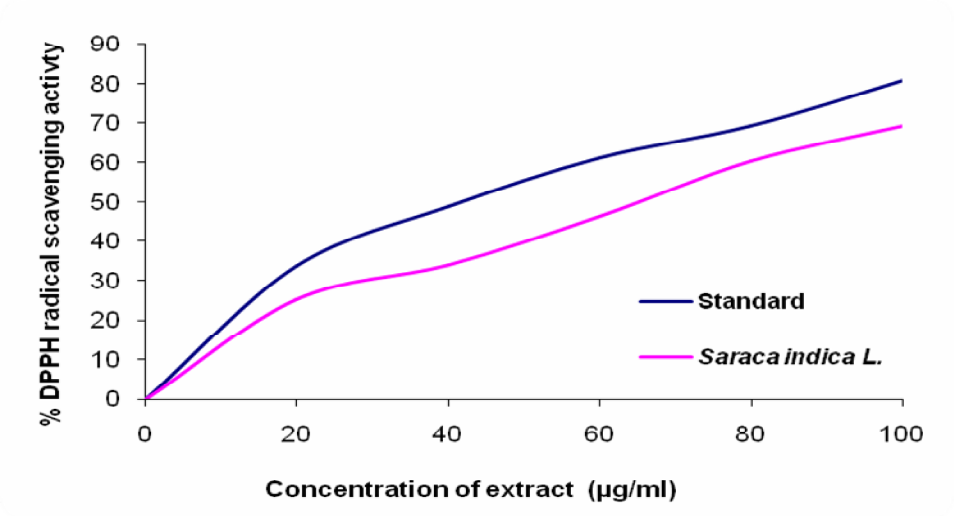


Figure.2 Estimation of ABTS Radical scavenging activity of *Saraca indica*

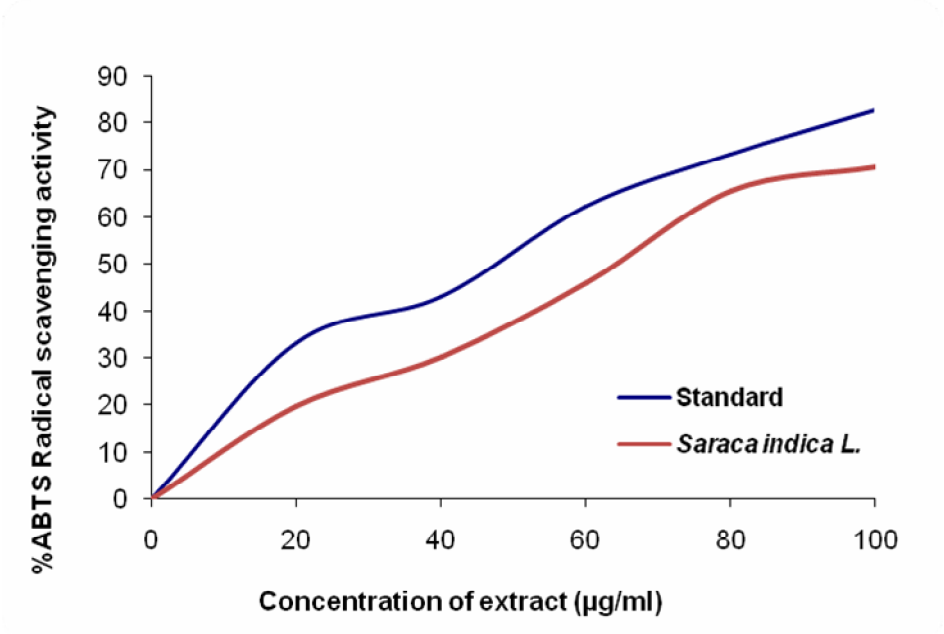


Figure.3 Estimation of Ferric Reducing Antioxidant Power (FRAP) of *Saraca indica*

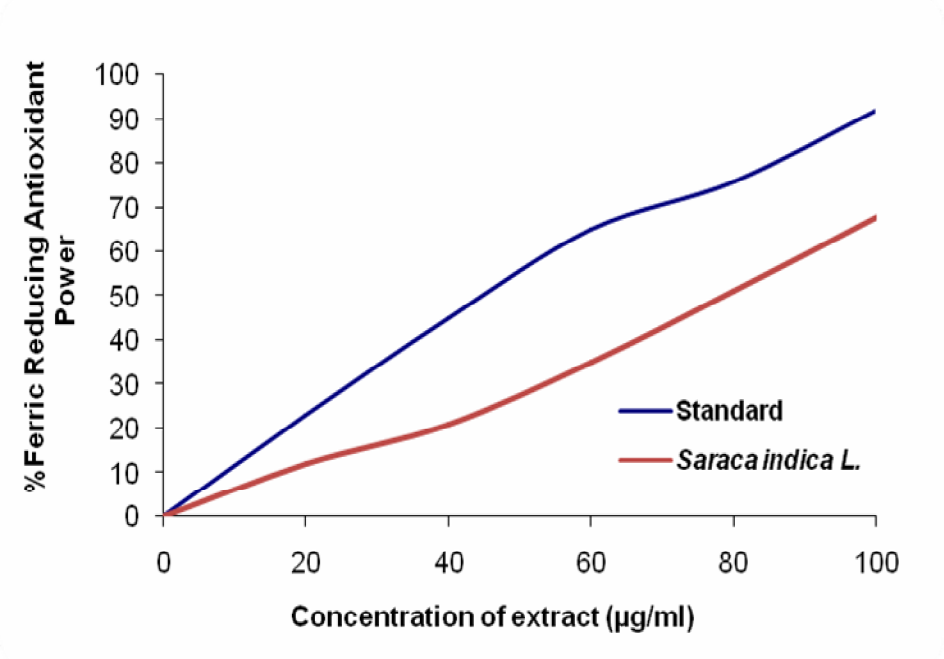
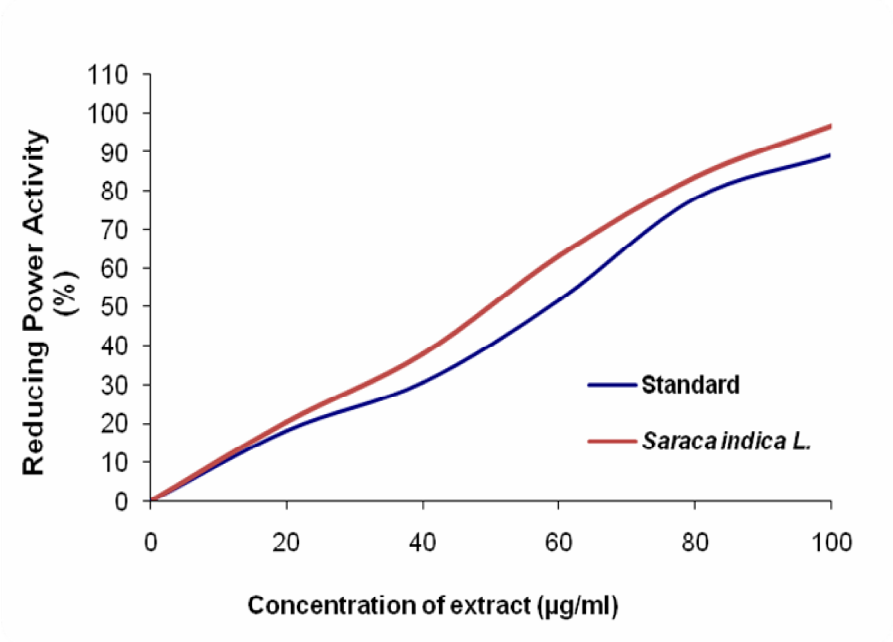


Figure.4 Estimation of Reducing Power Activity of *Saraca indica*



References

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, 29: 1199–1200.

Bokov, A., Chaudhuri, A., Richardson, A. 2004. The role of oxidative damage and stress in aging. *Mech. Ageing Dev.*, 125: 811–826.

Dhavan, B.N., Patnaik, Rastogi, R.P., Singh, K.K., Tandon, J.S. 1977. *Indian J. Exp. Biol.*, 15: 208–219.

Halliwell, B. 1995. How to characterize an antioxidant: An update. *Biochem. Soc. Symp.*, 61: 73–101.

http://www.holisticonline.com

Iris, F.F., Benzie, Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Anal. Biochem.*, 239(1): 70–76.

Madamanchi, N.R., Vendrov, A., Runge, M.S. 2005. Oxidative stress and vascular disease. *Arterioscler. Thromb. Vasc. Biol.*, 25: 29–38.

Nohl, H. 1993. Involvement of free radicals in ageing: A consequence or cause of senescence. *Br. Med. Bull.*, 49: 653–667.

Oyaizu, M. 1986.Studies on products of browning reactions: Antioxidative activities of browning products of browning reaction prepared from glucosamine. *Japan. J. Nutr.*, 44: 307–315.

Panchawat, S., Sisodia, S.S. 2010. Invitro antioxidant activity of *Saracaasoca* stem bark extracts from various extraction processes. *Asian J. Pharm. Clin. Res.*, 3(3).

Queen Rosary Sheela X and Alex Ramani V, 2011.Invitro Antioxidant activity of *Polygonumbarbatum* Leaf Extract. *Asian J. Pharm. Clin. Res.*, 4(suppl. 1): 113–115.

Rice Evans, C.A., Miller, N.J., Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci.*, 2(4): 152–159.

Sivarajan, V.V., Balachandran, I. 1994. Ayurvedic drugs and their plant sources. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. Pp. 57.

Vant Veer, P., Jansen, M.C., Klerk, M., Kok, F.J. 2000. Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutr.*, 3: 103–107.