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Evaluation of *in vitro* Antioxidant Activity of *Streblus asper* Bark

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Abstract: The objective of the present study is to evaluate the *in vitro* antioxidant activity of methanol extract of stem bark from *Streblus asper* Lour (MESA). *In vitro* antioxidant activity of MESA was assessed by DPPH, nitric oxide, hydroxyl radical, peroxynitrite and superoxide radical scavenging methods. MESA exhibited marked and concentration dependent free radical scavenging effect in all five models. The IC_{50} values of the tested MESA for DPPH, nitric oxide, hydroxyl radicals, peroxynitrite and superoxide radicals were found to be 98±8.96, 151.95±4.24, 24.92±2.10, 220.53±11.90, 76.27±6.12 µg/ml respectively. The results from the present work demonstrated that *S. asper* bark possesses promising antioxidant effect *in vitro*.

Key words: *Streblus asper* · Antioxidant Activity · Free Radical · DPPH · Traditional Medicine

INTRODUCTION

Our body system is exposed to a large number of foreign chemicals in day to day life [1] most of which are man-made and our incapability to properly metabolize them negatively affects our health by generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells [2]. Oxygen consumption inherent in cells growth leads to the generation of sequence of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumor (Prostate and colon cancers) and coronary heart diseases [3].

Various reactive species include superoxide anions, hydroxyl, nitric oxide and peroxynitrite radicals, which play an important role in oxidative stress related to the pathogenesis of various diseases [4]. These species cause the cellular damage by reacting with various biomolecules such as proteins, membrane lipids, enzymes and nucleic acid [5]. This damage is the major contributor in the production of free radicals for healthy individuals and is balanced by the antioxidative defense arrangement.

Antioxidants are important in the prevention of various free radical mediated human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing [6-10]. The screening for antioxidant properties of medicinal plants have been performed increasingly in the last few decades; hoping for finding an efficient remedy for several present day diseases and means to delay aging symptoms [11].

Streblus asper Lour (Moraceae) is commonly known as Siamee Rough Brush in English, *Sakhotaka* in Ayurveda and *Piraayan* in Siddha system of medicine which is widely distributed in most parts of Asia. Various parts of this plant are used as traditional medicine [12, 13]. Roots are used for ulcer, sinuses, antidote for snake bite, epilepsy and obesity; stem used for toothache; stem bark is used in fever, dysentery, diarrhoea, stomach ache, urinary track complaints, piles, oedema and wounds. Leaves are used for eye complications, seeds in epistaxsis and diarrhoea [14]. The Ayurvedic Pharmacopoeia of

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India recommends the use of stem bark in *Raktapitta* (Haemorrhage), *Apaci* (Tubercular adenitis), *Gamdamala* (cervical lymphadenitis) and many other aliments (*Arsa, slipada, prameha, kustha*) [15]. The Central Drug Research Institute (CDRI), Lucknow, India, has developed an antifilarial drug from the crude extract of stem bark to cure filarial lymphangitis, lymphoedema, chyluria caused by filariasis [12]. This plant is also use for cancer and inflammatory swellings [16, 17]. In the present study, we have aimed to evaluate the *in vitro* antioxidant activity of *S. asper* stem bark extract by different antiradical assay models.

MATERIALS AND METHODS

Plant Material and Preparation of Extract: The stem bark of *Streblus asper* Lour (Moraceae) was collected from West Bengal, India, during March 2010 and identified by the Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (No. CNH/I-I/29/2010/Tech.II) has been preserved at our laboratory for future reference. Air-dried stem bark (500 g) material was powdered by mechanical grinder and successively extracted with petroleum ether (60-80°C), then followed by methanol using Soxhlet extraction apparatus. The methanol extract was completely dried under reduced pressure to remove the solvent and the dry extract (MESA, yield: 19% w/w) stored in vacuum desiccator for use in the present study.

Reagents and Chemicals: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), sodium nitroprusside (SNP), napthyl ethylene diamine dihydrochloride (NED), ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), potassium nitrite (KNO_2), Sodium hypochlorite (NaClO), potassium ferricyanide [$K_3Fe(CN)_6$]. Diethylene triamine pentaacetic acid (DTPA) and 5, 5 ´-dithiobis 2 nitrobenzoic acid (DTNB) was obtained from Spectrochem, Mumbai, India. Folin-Ciocalteu's phenol reagent (FCR) was purchased from SISCO Research Laboratories, Mumbai, India. Manganese dioxide (MnO_2) was obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals and solvents were used of high analytical grade.

Evaluation of *in vitro* Antioxidant Activity

DPPH Radical Scavenging Activity: DPPH stable free radical scavenging activity was determined based on the previously described method Chanda and Dave [18]. The absorbance of the various concentrations of extract was measured at 517 nm. The inhibition percentage was calculated as:

Radical scavenging activity (%) = (Abs. control – Abs. sample)/ (Abs. control) × 100.

Scavenging Activity Against NO and OH• Radicals: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions, which can be estimated using Griess Illosvoy reaction [19]. Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as per the above formula.

The OH• scavenging activity of the test plant extract (MESA) was measured according to previously described method Poullain *et al*. [20]. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The percentage of OH• scavenging activity (MESA) was calculated by the above stated formula.

Peroxynitrite Scavenging Activity: Peroxynitrite (ONOO) is a cytotoxic intermediate produced by the reaction between the superoxide anion (O^2) and nitric oxide $(NO)^{\cdot}$ Synthesis of $ONOO^-$ was carried out according to the described method by previous researchers [21]. The concentration of $ONOO^-$ was measured spectrophotometrically at 302 nm (_=1 670 M^{-1} cm^{-1}). An Evans blue bleaching assay was used to measure peroxynitrite scavenging activity. The assay was performed by propose method of previous worker with slight modifications [22]. The percentage scavenging of $ONOO^-$ was calculated by using the above mentioned formula.

Superoxide Anion Scavenging Activity: Superoxide radicals are generated in a PMS-nicotinamide adenine dinucleotide (Reduced form, NADH) system by oxidation of NADH and assayed by the reduction of NBT. Measurement of superoxide anion scavenging activity was done based on the previously described method Bhattacharya and Haldar [23]. The percentage inhibition of superoxide was calculated by

the above mentioned formula. Ascorbic acid served as reference in all these five assays.

Statistical Analysis: Results are expressed as mean ± standard error of mean (SEM), 50% inhibition concentration (IC_{50}) were calculated by plotting the data in the graph as concentration versus percentage inhibition using Graph Pad Prism ver. 5.

RESULTS

In the present study, the percentage of scavenging activity of methanol extract of *S. asper* bark (MESA) was observed and compared with reference compound ascorbic acid on the above mentioned five free radical scavenging assay methods. The extract showed the concentration dependent activity in all above mentioned *in vitro* free radical scavenging test models (Figures 1- 5). Inhibition concentrations (IC_{50}) of MESA and reference ascorbic acid for DPPH, nitric oxide, hydroxy radical, peroxynitrite and superoxide anion were found to be 98±8.96 and 13.38±1.79 µg/ml; 151.95±4.24 and 41.7 6±1.90 µg/ml; 24.92±21 and 9.10±4.0 µg/ml, 220.53±11.90 and 58.87±3.93 µg/ml, 76.27±6.1 and 11.48±0.81 µg/ml respectively (Figure 6).

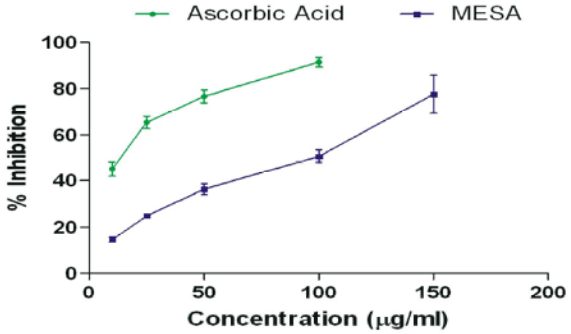

Fig. 1: DPPH scavenging activity of MESA and ascorbic acid. Each value represents mean ± SEM (n = 3).

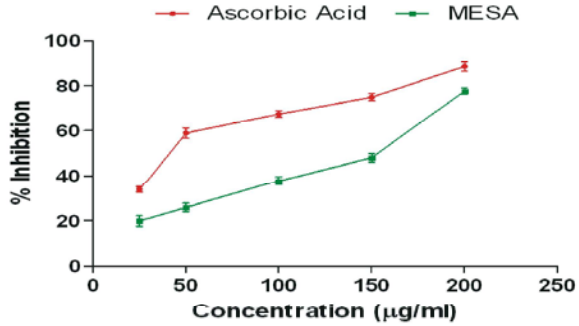

Fig. 2: The nitric oxide radical scavenging activity of MESA and ascorbic acid. Each value represents mean ± SEM (n = 3).

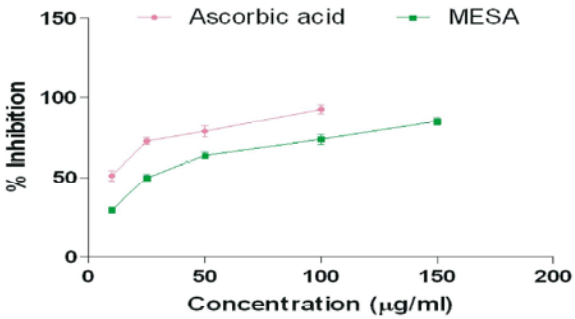

Fig. 3: Hydroxyl radical scavenging activities of MESA and ascorbic acid. Each value represents mean ± SEM (n = 3).

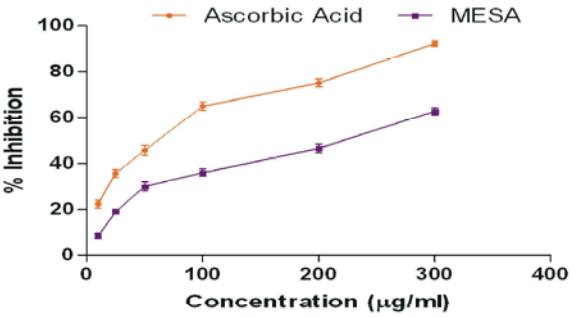

Fig. 4: The peroxynitrite anion scavenging activity of MESA and ascorbic acid. Each value represents mean ± SEM (n = 3).

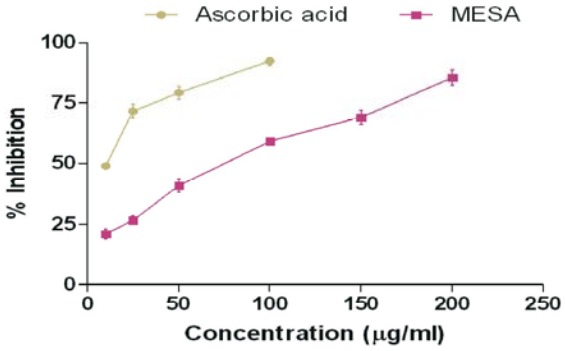

Fig. 5: Superoxide radical scavenging assay of MESA and ascorbic acid. Each value represents mean ± SEM (n = 3).

DISCUSSION

Free radical scavenging activity plays a vital role in a biological system. Many secondary metabolites which include flavonoids, phenolic compounds etc serves as sources on antioxidants and exhibited free radical scavenging activities [11, 24]. In our present study, it was observed that *S. asper* possess effective antioxidant activity.

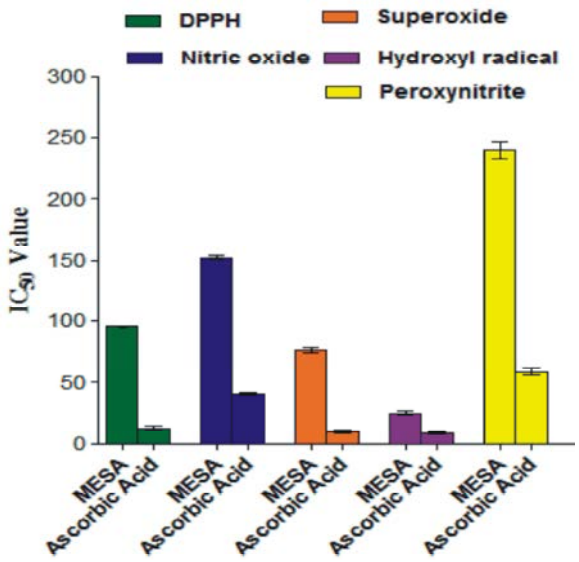

Fig. 6: IC_{50} values (µg/ml). The IC_{50} values of the MESA and ascorbic acid for DPPH, nitric oxide, hydroxyl radical, peroxynitrite and superoxide scavenging activity. All the values are Mean ± SEM of three experiments.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1diphenyl-2-picrly hydrazine and the degree of discoloration indicates the scavenging activity of the chemical [25]. The degree in absorbance of DPPH radical caused by antioxidant is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant activity [26]. In the present study the extract of *S. asper* has significant effect on the DPPH radical with a dose dependant manner.

It is a well known fact that nitric oxide plays an imperative role in disperate systemic process, higher levels of these radical are toxic to tissue and contributes to vascular collapse, carcinoma and ulcerative colitis. The toxicity of nitric oxide increases when it reacts with superoxide radical forming highly reactive peroxy nitrate anion (ONOO-) [22]. MESA shows a declining effect of nitrite generated from the decomposition of sodium nitroprusside *in vitro*, which may be due to the inhibition of nitrite generation by competing with oxygen to react with nitric oxide.

Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous metabolic damage in a biological system. This method involves *in vitro* generation of $OH^{\bullet}$ radicals using Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. The oxygen derived hydroxy radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid. Upon the addition of MESA to the reaction mixture, it removed the $OH^{\bullet}$ from the sugar and prevent the reaction. Peroxynitrite ($ONOO^{-}$) leads to oxidative damage to tissue [22, 23]. Peroxynitrite bleaches Evans Blue by oxidizing it. In the present study, MESA acts in a dose dependant manner in scavenging peroxynitrite.

Superoxide anion is very harmful to cellular components and produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamines [23]. Superoxide dismutase catalyzes the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide. The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT [18]. With decrease in the absorbance at 560 nm, MESA exhibited the ability to quench superoxide radicals in the reaction mixture.

Antioxidant activity has been attributed to be responsible for several biological effects of medicinal plants [27]. From the present investigation, it can be concluded that the *S. asper* stem bark's methanol extract shows promising antioxidant property *in vitro* thus the plant can serve as a natural source of antioxidant agent(s) that can be used in pharmaceuticals field for the prevention and cure of free radical mediated diseases.

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