

Comparative Study of *in vitro* Antioxidant Activity of the Methanolic Extracts of *Salvia splendens* and *Pterospermum acerifolium*

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Abstract: Background: *Salvia splendens* and *Pterospermum acerifolium* (L.) Willd are two different plant species widely available in Jharkhand region. Literature review and phytochemical investigations prompted the possible antioxidant property of these plants. **Materials and methods:** The plant materials were collected, shade dried and individually subjected to extraction with methanol. The methanolic extract of *Salvia splendens* (MESS) and *Pterospermum acerifolium* (MEPA) were subjected to various *in vitro* antioxidant tests-DPPH radical scavenging activity, scavenging of hydrogen peroxide and superoxide anion scavenging activity. **Results:** There was a significant decrease in the DPPH radical, hydrogen peroxide and superoxide anion in various concentrations tested. The results observed were comparable to that of standards l- ascorbic acid and BHA. However, MESS had similar but quantitatively lesser activity than MEPA. The observed antioxidant activity is attributed to the phytoconstituents terpenoids, anthocyanins in *Salvia splendens* and flavonoid, kaempferol and luteolin in *Pterospermum acerifolium*.

Key words: *Salvia splendens*, *Pterospermum acerifolium* (L.) Willd, *In vitro* antioxidant activity, free radical scavenging

INTRODUCTION

Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radicals which can form dangerous chain reactions. Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Antioxidants terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. They are substances when present at low concentrations compared with that of an oxidisable substrate delays or prevents oxidation of that substrate. They may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species which are linked to a variety of diseases including cancer, diabetes, shock, arthritis and acceleration of the ageing process (Shimada *et al.*, 1992). Natural antioxidants like anthocyanins, phenolics and flavonoids are gathering considerable attention and hence focus is towards identification of plants with antioxidant ability that may be used for human consumption. This study is an effort to compare the possible antioxidant abilities of two plants

Salvia splendens and *Pterospermum acerifolium* (L.) Willd since both the plants are reported to have some of the above-mentioned phytoconstituents.

Salvia splendens (Lamiaceae/Labiatae), is commonly known as Red Salvia, Scarlet Salvia or Scarlet Sage. It is an ornamental plant native to Brazil, available through out the world and widely available in Jharkand region. The leaves of the plant is traditionally used for dressing of wounds and also applied to itchy skin; dried leaves in the form of tea are used to treat diabetes. It is also used to treat skin sores and to rid the skin of warts. Seed of this plant serves as emetic, to treat dysentery, haemorrhoids and colic disorders. Roots are used in cold and cough whereas the whole plant is used to treat inflammation, arthritis, headache and as diuretic (Lowell, 1997; Edward and Teresa, 1999). According to the ancients, *Salvia* procured immortality, relieved fatigue and preserved the teeth. *Salvia* has multifarious curative effects. It was praised by Hippocrates, Paracelsus, St. Hildegade as relieving cough, as a diuretic, promotor of menstruation, as a wound healing agent, a remedy of sequels to catarrhs, especially of the throat and pharynx: against festering ulcers and as preservative of the teeth. It is also

an antihidrotic and abortifacient. Intravenous injection of sage extract increases the secretion of bile. The leaves have been used in popular medicine in angina, menstruation disorders, cystitis, chronic liver and kidney disease and for checking the secretion of the mammary glands (Bobbi, 2003; Erv, 2003; Lowell, 1997). It has been reported to have anticoagulant (Qureshi *et al.*, 1989), antimicrobial (Khan and Saeed, 1998), insecticidal (Pavela, 2004) and wound healing (Mazumder *et al.*, 2008) activities.

Salvia splendens is reported to contain clerodane diterpenoids, (salviarin, splendidin and splenolides A and B) (Fontana *et al.*, 2006), diterpenes-splenolide A, splenolide B, splenolide C, from the methanol extract of aerial parts of *Salvia splendens* (Hu *et al.*, 1997), pentacyclic tri terpene acids-ursolic acid and oleanolic acid from acetone extract of aerial parts of *Salvia splendens* (Passannanti *et al.*, 1983). The flowers were reported to have anthocyanin -5-o glucoside-6"-malonyl transferase (Suzuki *et al.*, 2001, 2003, 2004), dimalonated anthocyanins- (Pelargonidin-3-caffeoyl glucoside-5-dimalonyl glucoside, pelargonidin-3-p-coumaroyl glucoside-5-dimalonyl glucoside) and malonated anthocyanins (Tomas-Barberan *et al.*, 1987).

Pterospermum acerifolium (L.) Willd: Commonly known as "Dinner Plate tree" which is found in the sub-Himalayan tract and outer valleys from Yamuna eastwards to West Bengal and in Assam and Manipur, up to an altitude of 1200 m, extending southwards into Ramnagar hills of Bihar and in Western ghats of Konkan and North Kanara; it is also common in the Andamans. The tree is also found in Chittagong and Khasia hills. The tree is found in a variety of situations such as swamp forests of Dehra Dun, evergreen forests of North Kanara and along the riverbanks in the sub-Himalayan tract (Wealth of India, 1969; Kirtikar and Basu, 2002; Agarwal, 1964). The leaves are used to stop bleeding in wounds i.e., used as haemostatic and as antimicrobial agent (Wealth of India, 1969; Agarwal, 1964). The flower is sharply bitter, acrid; tonic, laxative, anthelmintic; removes "kapha"; inflammation, abdominal pains, ascites; cures ulcers, leprosy, urinary discharges and tumors (Ayurveda). The flowers are used as a general tonic (Wealth of India, 1969; Chatterjee and Prakash, 1994). Flowers and bark charred and mixed with Kamala applied in suppurating small pox (Wealth of India, 1969; Agarwal, 1964).

The traditional healers of Bagbahera region specially recommend the patients having the problem of Bawasir (piles) to consume a curry prepared by using Muchkund flowers. In combination with other herbs, the traditional

healers use Muchkund flower in the treatment of diseases related to respiratory system. The flowers of *P. acerifolium* are also used in ear ache (Kala, 2005). The flowers were used in Ayurvedic anticancer treatment (Balachandran and Govindarajan, 2005).

Every part of *P. acerifolium* is used to reduce blood glucose level in Indian sub-continent and its hypoglycemic effect on leaves and bark has been scientifically studied till now but hypoglycemic effect of its flowers have not yet been scientifically studied. The leaf extract is reported to lower the glucose level, in type II diabetic model in rats and the bark extract lowers hyperglycemia and hyperlipidemia in type II diabetic model in rats (Kala, 2005).

Leaves and fresh flowers of the plant is reported to have Kaempferol-3-o- β -D-galactoside, a major flavonoid, while the other flavonoids luteolin, luteolin-7-o- β -D-glucoside and luteolin 7-o- β -D-glucuronide were also found in the leaves (Anjaneyulu and Raju, 1987). Kaempferol and Kaempferide-7-glucoside were also reported from the alcoholic extracts of the dried flowers (Varshney *et al.*, 1972).

MATERIALS AND METHODS

Plant material and extraction: The plants were collected from Ranchi, taxonomically identified by the Botanical Survey of India (BSI), Howrah and the voucher specimens [CNH/I/I(44)/2006/Tech.II (*Salvia splendens*), CNH/I/I(44)/2006/Tech.II (*Pterospermum acerifolium* L. Willd) were retained in Department of Pharmaceutical Sciences, B.I.T. Mesra, Ranchi, for future reference. The dried and powdered plant material of *Salvia splendens* and dried leaves of *Pterospermum acerifolium* were individually subjected to extraction in a Soxhlet apparatus using methanol. The solvents were removed from the respective extracts under reduced pressure to obtain a semisolid mass and vacuum dried to yield solid residues. The methanolic extract of *Salvia splendens* was named as MESS and the methanolic extract of *Pterospermum acerifolium* was named as MEPA. The extracts were subjected to preliminary phytochemical investigation using standard methods (Trease and Evans, 1989; Harborne, 1984).

Chemicals and reagents: α -tocopherol, nicotinamide adenine dinucleotide (NADH) butylated hydroxyanisole (BHA), l- ascorbic acid, nitroblue tetrazolium (NBT), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine

(Ferrozine) and trichloroacetic acid (TCA) were obtained from Sigma Aldrich, US. All other chemicals used were analytical grade and obtained from Merck, US.

DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity: The free radical scavenging activity of the MESS and MEPA were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) using the method described by Shimada (Shimada *et al.*, 1992). Briefly 0.1 mM solution of DPPH in ethanol was prepared; 1 mL of the solution was added to 3 mL of sample solution at different concentrations (25-50 $\mu\text{g mL}^{-1}$). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer (Shimadzu UV-Vis 1700). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the standard or test.

Scavenging of hydrogen peroxide: The ability of MESS and MEPA to scavenge hydrogen peroxide was determined according to the method of Ruch (Ruch *et al.*, 1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm (Shimadzu UV-Vis 1700). MESS and MEPA (25-50 $\mu\text{g mL}^{-1}$) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide:

$$\text{The percentage of scavenging of hydrogen peroxide} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance of standard or test.

Superoxide anion radicals scavenging activity: Measurement of superoxide anion radicals scavenging activity of MESS and MEPA was based on the method described by Liu *et al.* (1997). Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT. In these experiments, the superoxide radicals were generated in 3

mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and sample solution of EEAS (25-50 μg) in water. The reaction started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, the absorbance was read at 560 nm by spectrophotometer (Shimadzu UV-Vis 1700) against blank samples using l- ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 was the absorbance of the control (l- ascorbic acid) and A_1 was the absorbance in the presence of standard or test.

Statistical analysis: Experimental results were Mean \pm SEM of three parallel measurements. Analysis of variance was performed by ANOVA followed by Newmans-Keul multiple comparison test. $p < 0.05$ were regarded as significant.

RESULTS

Preliminary phytochemical investigations of MESS showed the presence of anthocyanins, flavonoids, glycosides and terpenoids whereas MEPA showed the presence of alkaloids, flavonoids, glycosides, terpenes and tannins.

DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical scavenging activity: The potential decrease in the concentration of DPPH radical due to the scavenging ability of MESS and MEPA showed significant free radical scavenging activity: 50.99 and 67.77 % of inhibition, respectively, at 500 $\mu\text{g mL}^{-1}$. The free radical scavenging by standards BHA and l-ascorbic acid at 500 $\mu\text{g mL}^{-1}$ was 71.24 and 68.26 %, respectively (Fig. 1). The IC_{50} (The inhibitory conc. at which there is 50% decrease of free radical) of MESS and MEPA was found to be 460 $\mu\text{g mL}^{-1}$ and 242.50 $\mu\text{g mL}^{-1}$, respectively.

Scavenging of hydrogen peroxide: The scavenging of hydrogen peroxide by MESS and MEPA were concentration dependant. There was a marked scavenging of hydrogen peroxide with the maximum inhibition being 57.15 and 61.97% at 500 $\mu\text{g mL}^{-1}$ of MESS and MEPA, respectively which was comparable to the standards BHA and l-ascorbic acid 62.64 and 58.84%, respectively (Fig. 2).

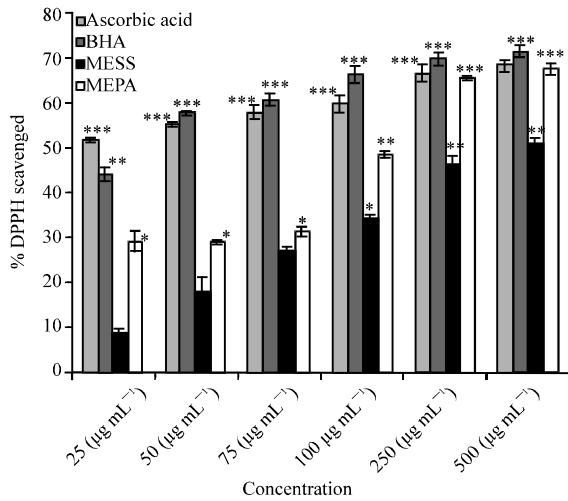


Fig. 1: DPPH Free Radical Scavenging activity of *Salvia splendens* and *Pterospermum acerifolium* (L.) Willd all values are represented as Mean±SEM (n = 3) ANOVA followed by Newmans-Keul multiple comparison test, *p<0.05, **p<0.01, ***p<0.001 compared to control

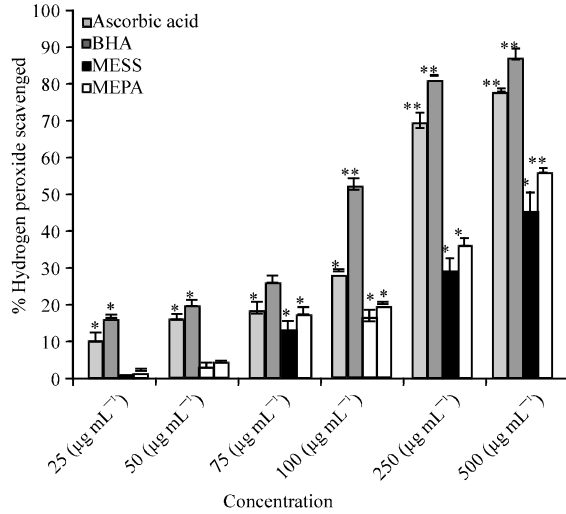


Fig. 3: Superoxide anion radical scavenging activity of *Salvia splendens* and *Pterospermum acerifolium* (L.) Willd all values are represented as Mean±SEM (n = 3) ANOVA followed by Newmans-Keul multiple comparison test, *p<0.05, **p<0.01 compared to control

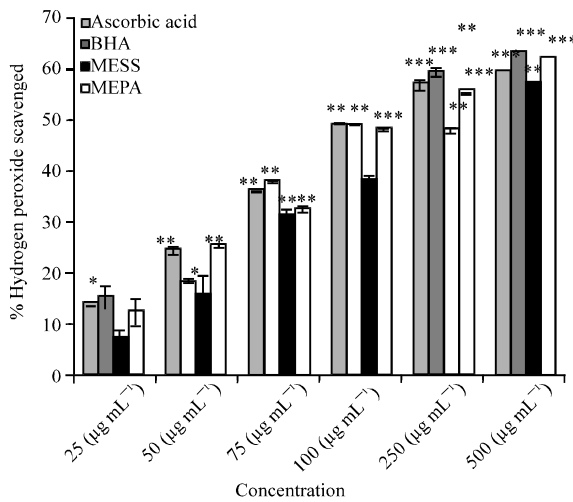


Fig. 2: Hydrogen Peroxide Scavenging activity of *Salvia splendens* and *Pterospermum acerifolium* (L.) Willd all values are represented as Mean±SEM (n = 3) ANOVA followed by Newmans-Keul multiple comparison test, *p<0.05, **p<0.01, ***p<0.001 compared to control

The IC₅₀ (The inhibitory conc. at which there is 50% scavenging of hydrogen peroxide) of MESS and MEPA was found to be 358 µg mL⁻¹ and 307 µg mL⁻¹, respectively.

Superoxide anion radicals scavenging activity: A moderate and concentration dependant inhibition of superoxide radical was observed with 500 µg mL⁻¹ of MESS and MEPA (45.43 and 56.51 %), respectively. The superoxide scavenging of standards BHA and l-ascorbic acid at the same concentration was 88.68 and 78.82 %, respectively (Fig. 3). The IC₅₀ of MESS and MEPA was found to be 527 and 242 µg mL⁻¹, respectively.

DISCUSSION

Oxidative stress is one of the earliest responses to stress in which large quantities of reactive oxygen species are formed. These reactive oxygen species has a role in disease and aging of animals (Melov, 2002). Antioxidant systems offer protection against oxidative damage. However, there are restrictions on synthetic antioxidants, such as BHT because of their carcinogenic potential (Singh *et al.*, 2002). Evolution of naturally occurring antioxidants must have been an important factor that permitted life to continue and to prosper. Natural antioxidants, therefore, have gained importance. These protective agents fall into two broad and overlapping categories-First, catalytic antioxidant enzymes such as catalase, superoxide dismutase and peroxidases-that are able to effectively deactivate potentially hazardous oxidizing agents and, in some cases, even make use of

them for the cell's own purpose. A second class of antioxidants are termed as stoichiometric antioxidants. These are usually small molecules such as flavonoids, isoflavonoids, phenolic acids, lignans, curcumin, hydroquinones that a plant cell can rapidly produce and transport (Larson, 1997).

Reports and phytochemical investigations showed the presence of diterpenoids, triterpene acids, terpenoids and anthocyanins in *Salvia splendens* and presence of tannins, flavonoid, kaempferol and luteolin in *Pterospermum acerifolium* which prompted us to perform comparative study.

DPPH is a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation. Results demonstrate that the MESS and MEPA are free radical inhibitor or scavenger acting possibly as primary antioxidants. The results also demonstrate that MESS and MEPA are effective H₂O₂ and superoxide anion scavengers. The observed effects may be attributed to the presence of its phytoconstituents such as flavonoids, kaempferol, luteolin and anthocyanins which may trail any of the following mechanisms.

Flavonoids have been shown repeatedly to react with O₂-generated by variety of methods. The reactions were oxidations of the flavonoids. Many flavonoid phenoxy radicals appear to undergo subsequent one-electron oxidations, possibly with molecular oxygen/peroxy radicals to afford quinines. These products may be oxidised further to ring opened products. Flavonoids appear to possess a variety of mechanisms of actions which include radical scavenging and metal ion complexation. Flavonoids having greater number of hydroxyl groups or hydroxyl groups localized ortho to one another, are more effective antioxidants. (Larson, 1997). Flavonoid compounds having both o-hydroxylation in the B ring and multiple hydroxylation in the A ring such as quercetin, robinetin, luteolin and myricetin prove to be effective antioxidants compared to compounds lacking these features like kaempferol, naringenin and apigenin which are usually not nearly as efficient (Larson, 1997).

Anthocyanins are cationic polyphenols normally considered to be a class of flavonoids which usually occur as glycosides. There are indications of antioxidant activity for certain compounds of this series. The possible mechanism of their antioxidant activity would be the formation of salt like complexes at the surface of the micelle between the anthocyanin cation and the superoxide anion. These complexes would exhibit high concentration of readily oxidized polyphenol moieties at

the interface where, presumably the generation of reactive oxidants take place (Larson, 1997).

In our present study we observed that the *in vitro* antioxidant property of MEPA was as comparable to that of the standards tested whereas, MESS had similar but quantitatively lesser activity than MEPA. However, decreased potency of MESS compared to MEPA has to be established.

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