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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (3): 453-457, 2008
ISSN 1028-8880

Antioxidant Activities of Methanolic Extract of *Sapium ellipticum*

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Abstract: The stem bark extract of *S. ellipticum* (Hochst) Pax was investigated for its antioxidant properties in this study. The extract was evaluated for antioxidant activity *in vitro* in terms of its ability to inhibit lipid peroxidation and its free radical scavenging, reducing and metal chelation powers. The total amount of phenolic compounds in the extract was also determined in terms of gallic acid equivalent. The extract produced effective free radical scavenging and reducing activities in a dose dependent fashion. The extract exhibited noticeable inhibition of lipid peroxidation of linoleic acid emulsion. These activities were less than that of ascorbic acid and 2,6-Di-tert-butyl-4-methylphenol used as positive controls. The extract however demonstrated poor iron chelating ability compared to ethylene diamine tetraacetic acid. The total phenolic content of the extract was 50.61±0.08 mg g^{-1} in terms of gallic acid. This study showed that the stem bark extract of *S. ellipticum* exhibits significant antioxidant activity and is a good source of natural antioxidants.

Key words: Antioxidant activity, free radical scavenging power, reducing power, iron chelating power, linoleic acid emulsion, *Sapium ellipticum*

INTRODUCTION

Free radicals and reactive oxygen species such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and peroxy radical ($ROO^{\bullet}$) are always looking for another electron in molecules to pair causing them to be oxidized thus producing damage in several biomolecules such as lipids, proteins and nucleic acid (Lopaczyski and Zeisel, 2001; Block *et al*., 2002; Halliwell and Gutteridge, 2006). They have been implicated in several degenerative and chronic diseases such as inflammation, diabetes, hypertension, aging process and cancer (Greenacre and Ischiropoulos, 2001; Alvarez and Radi, 2003; Klaunig and Kamenduis, 2004). Antioxidants dispose, scavenge and suppress the formation of reactive oxygen species and free radicals or oppose their actions. They also oppose oxidative damage (Record *et al*., 2001; Willcox, 2004; Tsao and Akhtar, 2005). Defence mechanisms in the body help to get rid of excess radicals produced during normal metabolic processes. Degenerative problems may however arise when the balance shift to the side of free radicals (Mau *et al*., 2001; Gulcin *et al*., 2002) thus body needs antioxidant supplements to reduce oxidative damage and retard lipid peroxidation (Gulcin *et al*., 2003a).

Sapium ellipticum (Hochst) Pax (Euphorbiaceae) is tree reaching usually to about 15 m high and is widely distributed from Senegal to West Cameroon and across tropical Africa into Natal. The stem bark is used in Congo as purgative and in Zaire for eczema. In Central Africa, the decoction of the bark is used for scurvy and stomatitis. A preparation of dried leaves is applied to wounds in Tanzania. The leaf preparation is also used for sore-eyes and abdominal swelling (Burkill, 1994). Preliminary investigation of the stem bark extract of *S. eliipticum* in our laboratory showed the presence of phenolic compounds. Phenolics have been implicated in antioxidant activities (Phan *et al*., 2001; Qian and Nihorimbere, 2004) thus it was considered rational to determine the antioxidant properties of the extract.

MATERIALS AND METHODS

Chemicals: Folin-Ciocalteu phenol reagent, 2,6-Di-tert-buytl-4-methylphenol (BHT), linoleic acid, ammonium thiocyanate, [4,4'- [3-(2-pyridinyl-1,2,4-triazine-5,6-diyl]bisbenzenesulfonic acid] (ferrozine), ethylene diamine tetraacetic acid (EDTA), 90% 1,1-diphenyl-2-picrylhydrazyl (DPPH), $FeCl_2$ tetrahydrate, gallic acid, anhydrous sodium carbonate, anhydrous ferric chloride, potassium ferricyanide and trichloroacetic acid were purchased from Sigma Chemical Company (St. Louis, MO). Ascorbic acid and all other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

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Plant material: The stem bark of *S. ellipticum* was collected at Ibadan, in Oyo, Nigeria and authenticated by Mr. K.I. Odewo of Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria after comparing with voucher specimen with number FHI 70686. The barks were broken into small pieces, dried at 30°C and milled to produce fine powder.

Extraction: About 500 g of powdered bark was extracted with methanol (3 L) using Soxhlet apparatus for 48 h. The extract was concentrated under reduced pressure until a semi-solid sticky mass was obtained. The yield was 15.63% w/w.

Determination of total phenolic content: A determination of total phenolic content measured as gallic acid equivalents was made using the Folin-Ciocalteu reagent using the method of Singleton *et al.* (1999). Gallic acid equivalents were determined from a calibration concentration curve. The extract (100 mg mL^{-1}, 1.0 mL) was mixed thoroughly with 5 mL Folin-Ciocalteu reagent (diluted ten-fold) and after 5 min, 4.0 mL of sodium carbonate (0.7 M) was added and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance was measured at 765 nm in a spectrophotometer. All determinations were carried out in triplicate.

Determination of radical scavenging activity: The free radical scavenging activity of stem bark extract was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of Yen and Chen (1995). The extract (1.0 mL, 0.1-2.0 mg mL^{-1}) was diluted to 20% of the original concentration with methanol and 1.0 mL of methanolic solution of DPPH (1 mM) was added. The mixture was shaken vigorously and allowed to stand at 20°C in dark for 30 min. The absorbance was then measured at 517 nm using spectrophotometer. Ascorbic acid and BHT were used as positive controls and deionized water in place of extract or the controls was used as blank. The decrease in absorbance of the reaction mixture indicated higher free radical scavenging activity.

Determination of reducing power: The reducing power of *S. ellipticum* extract was determined using the method of Lai *et al.* (2001). The extract (1.0 mL, 0.1-1.5 mg mL^{-1}) in phosphate buffer (2.5 mL, 0.2 M, pH 6.6) was mixed with potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture and centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm against a blank in the spectrophotometer. Ascorbic acid and BHT were used as positive controls. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of Fe^{2+}-chelating ability: The chelation of ferrous ion by the extract and EDTA used as positive control was measured using the method described by Decker and Welch (1990). The *S. ellipticum* extract (1 mL, 0.1-10.0 mg mL^{-1}) was diluted to 20% of the original concentration with water, mixed with $FeCl_2$ (0.1 mL, 2.0 mM) and after 30 min, ferrozine (0.2 mL, 5 mM) was added. The resulting mixture was shaken vigorously and left to stand for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. The lower the absorbance of the reaction mixture, the higher the Fe^{2+}-chelating ability. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated from the absorbance ratio to that of the blank without any sample.

Antioxidant activity: The antioxidant activity of the extract was measured using ferric thiocyanate according to the method of Chang *et al.* (2002). The extract (0.5 mL, 0.5-7.5 mg mL^{-1}) was mixed with linoleic acid emulsion (2.5 mL, 0.56 % w/v, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0) then incubated at 60°C in the dark for 12 h to accelerate oxidation. Ethanol (4.5 mL, 75%), ammonium thiocyanate solution (0.2 mL, 4 M), sample solution (0.1 mL) and ferrous chloride (0.2 mL, 20 mM in HCl) were mixed in sequence and after 3 min the absorbance for the red colour was measured at 500 nm. The level of lipid peroxidation inhibition (%) by the extract was calculated from the absorbance ratio to that of the blank without any sample. Ascorbic acid and BHT were used as positive controls.

Statistical analysis: All data were expressed as mean±standard deviation. Analysis of variance was performed by ANOVA procedures and $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The total phenolic content of the *S. ellipticum* extract measured by Folin Ciocalteu reagent in terms of Gallic Acid Equivalent (GAE) was found to be 50.61±0.08 mg g^{-1}. Antioxidant properties of medicinal plants have been shown to be due to high content of phenolic compounds (Vinson *et al.*, 2001; Meyers *et al.*, 2003). Polyphenols have a high redox potential which allows them to act as reducing agents, hydrogen donors

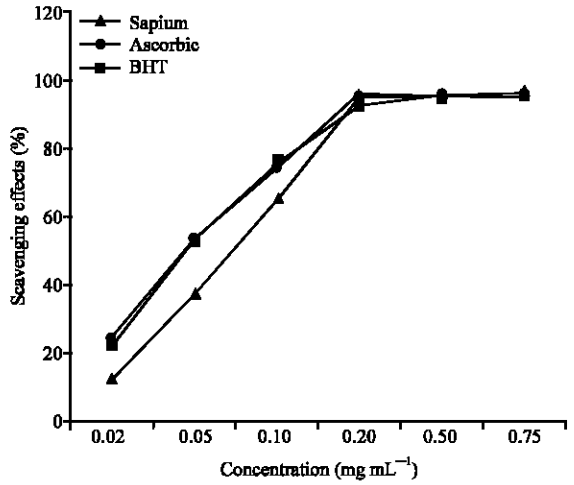

Fig. 1: Scavenging effects of *S. ellipticum* leaf extract, ascorbic acid and BHT on DPPH radical. Each value represents mean±standard deviation (n = 3)

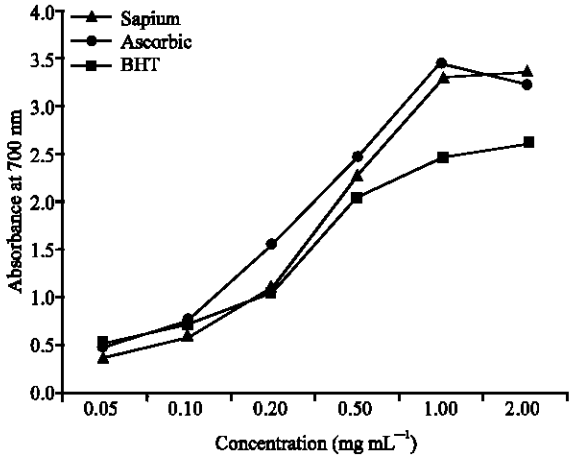

Fig. 2: Reducing power of *S. ellipticum* leaf extract, ascorbic acid and BHT. Each value represents mean±standard deviation (n = 3)

and singlet oxygen quenchers (Kahkonen *et al*., 1999). The delocalization of electrons over the phenolics and stabilization by the resonance effect of the aromatic nucleus prevents the continuation of the free radical chain reaction (Tsao and Akhtar, 2005). The antioxidant effects of the extract may be due to its phenolic content.

DPPH radical is commonly used to determine the free radical scavenging power of antioxidants (Chang *et al*., 2002). The radical changes colour from purple to yellow after reacting with antioxidants which scavenge the radical by hydrogen donation (Mokbel and Hashinaga, 2006). Phenolic compounds donate electrons to unstable and highly reactive free radicals thus acting as radical quenchers (Tsao and Akhtar, 2005). Figure 1 shows decrease in concentration of DPPH radical due to scavenging power of *S. ellipticum* extract, ascorbic acid and BHT. The extract and positive controls used demonstrated a dose-dependent activity. The radical scavenging effects increased with increasing concentration. The activities decreased in the following order: ascorbic acid > BHT > extract. The radical scavenging activity of the extract is less than that of BHT and ascorbic acid but there is no significant difference between them $p>0.05$. The concentration of *S. ellipticum* extract required to reduce the absorbance of DPPH control solution by 50% (EC_{50}) was calculated to be 0.19 mg mL^{-1} and for BHT and ascorbic acid were 0.11 and 0.10 mg mL^{-1}, respectively.

In the reducing power assay, the antioxidants in the extract would cause the conversion of Fe^{3+}/ferricyamide complex to the ferrous form (Fig. 2). The reducing power increased with increasing concentration of the extract and the standards. The reducing power of the extract of *S. ellipticum* and standard compounds follow the order of ascorbic acid > Sapium > BHT. This shows that the reducing ability of *S. ellipticum* extract was evident. This suggests that the bark extract is an electron donor and could neutralize free radical (Zhu *et al*., 2001).

The chelating effect of extract of *S. ellipticum* (Fig. 3). The extract demonstrated chelating effect in dose dependent manner and reached 32.4% at a concentration of 3.0 mg mL^{-1} then decreased as the concentration increased. EDTA used as positive control showed a better chelating ability than the extract. These results suggested that metal chelation plays very little role in antioxidant properties of the extract. Iron chelation has been reported to prevent oxidative damage by minimizing formation of radicals and lipid peroxidation (Gulcin *et al*., 2003b).

The antioxidant activity was measured using thiocyanate method. They demonstrated noticeable antioxidant effects at all tested concentration (Fig. 4). The antioxidant activity increased with increasing concentration. The antioxidant activity increased in the order sapium< ascorbic acid < BHT. The inhibition of lipid peroxidation in the linoleic acid system by the extract was less than that of BHT and ascorbic acid but there is no significant difference ($p>0.05$) between them. One of the major consequences of increased oxidative stress in lipid peroxidation is the oxidative degradation of lipids which eventually produces injuries (Chang *et al*., 2002). Thus agents like *S. ellipticum* bark extract which inhibit peroxidation of lipids could be useful as preventive agents against such injuries.

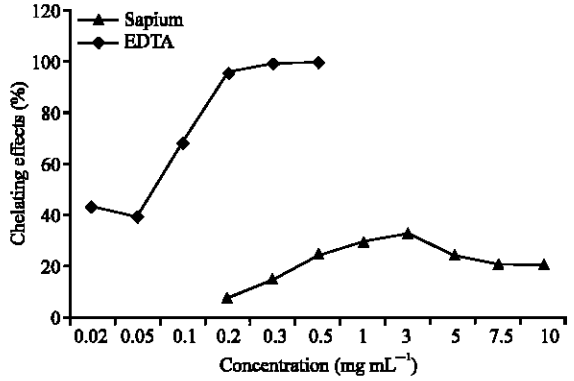

Fig. 3: Chelating effect of *S. ellipticum* leaf extract on Fe^{2+} ion. Each value represents mean±standard deviation (n = 3)

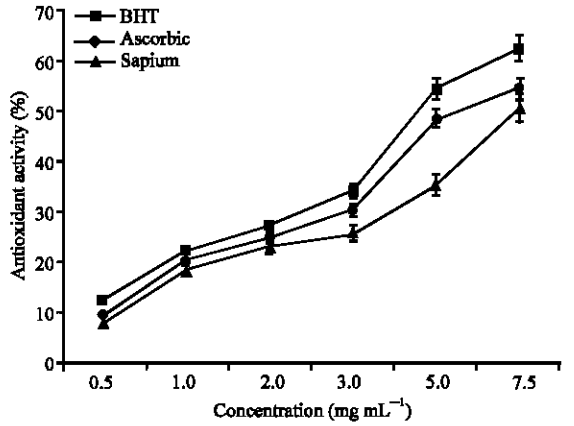

Fig. 4: Antioxidant activity of *S. ellipticum* extract, ascorbic acid and BHT in linoleic acid emulsion. Each value represents mean±standard deviation (n = 3)

CONCLUSION

The methanolic extract of *S. ellipticum* bark demonstrated strong antioxidant activities *in vitro* but this is less that of BHT and ascorbic acid used as positive controls. The antioxidant activities may be due to its phenolic contents, free radical scavenging and hydrogen donating ability. Thus extract of *S. ellipticum* bark could be described as a good source of natural antioxidants. However, further studies are necessary to establish these activities *in vivo* and to isolate and identify agents responsible for them.

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