Full Length Research Paper

Antidenaturation and antioxidative properties of phytochemical components from *Spondias mombin*

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The antidenaturation and antioxidant properties of *Spondias mombin* Linn (Anacardiaceae) methanol leaf extract (SMC) and fractions prepared from it were evaluated in this study. SMC and its fractions: ether (SME), saponin-rich (SMS) and flavonoid-rich (SMF) were phytochemically screened and evaluated for total antioxidant activity (TAA), ability to inhibit deoxyribose degradation (DEO), lipid peroxidation inhibitory activity (LPIA), 2,2 - diphenyl-1-picryl hydrazyl scavenging activity, ability to chelate ferrous ions and protein denaturation inhibitory activity (PRO). The antioxidant and antidenaturation activities were in the order SME > SMF > SMC > SMS. TAA strongly correlated with DEO, LPIA and PRO. The results indicate that *S. mombin* contains a diverse array of phytochemicals with potent antioxidant and bio-preservative properties which can serve as candidates for food preservation and drug development.

Key words: *Spondias mombin*, phytoconstituents, antioxidant activity, protein denaturation.

INTRODUCTION

Recent studies have shown that many diseases are due to oxidative stress resulting from an imbalance between formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA. This leads to damage to these biomolecules for example protein denaturation, DNA degradation and lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular and inflammatory diseases and ageing (Braca et al., 2002; Aukrust et al., 2005). Preservation of the integrity of these

molecules is essential for optimum health. Human cells protect themselves against free radical damage by enzymatic antioxidants such as superoxide dismutase (SOD) and catalase, or non-enzymatic ones such as ascorbic acid, tocopherol and glutathione. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements may be vital to combat oxidative damage (Rahman, 2007). *Spondias mombin* Linn belongs to the family Anacardiaceae. The leaves have been reported to possess abortifacient, antidiarrhoeal, antimicrobial,

antiviral and wound-healing properties and to be rich in vitamin C (Ayoka et al., 2006; Ayoka et al., 2008). The aim of the present study was to evaluate the protective ability of methanol extract and fractions of *S. mombin* leaves against oxidative stress and protein denaturation *in vitro.*

MATERIALS AND METHODS

Chemicals and reagents

DPPH radical (2,2 - diphenyl-1-picryl hydrazyl), thiobarbituric acid (TBA) and 2-deoxyribose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trisodium orthophosphate was obtained from BDH Chemicals Ltd. (Poole, England). Ethylenediaminetetraacetic acid was obtained from Fisher Scientific Ltd (UK). Other chemicals and reagents used were of analytical grade.

Sample extraction and fractionation

The leaves of *S. mombin* were obtained from Ore town, Ondo State, Nigeria and were authenticated at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria (voucher number IFE-16747). Air dried leaves were powdered and 923.2 g macerated in 3.5 L of 80% methanol for 48 h. The mixture was filtered and the filtrate obtained was concentrated using a rotary evaporator and then lyophilized to obtain the methanol crude extract (SMC) (4.93% yield). The extract (15 g) was defatted with n-hexane (3 × 50 ml) and then partitioned between ether and water (1:1, 3 × 100 ml). The ether portion was concentrated to give the ether fraction (46.67% yield). The aqueous portion was extracted with n-butanol (2 × 50 ml) and the butanol extract partitioned with KOH (2 × 50 ml). The butanol portion was collected and concentrated to give the saponin-rich fraction (2.67% yield). The potassium hydroxide portion was partitioned between HCl and butanol (1:1, 2 × 50 ml). The butanol portion was collected and concentrated to yield the flavonoid-rich fraction (1.33% yield).

Animal handling and care

Male Wistar rats (200 - 250 g) were used. The animals were handled in accordance with the international guide for the care and use of laboratory animals (Committee for update of the guide for the care and use of laboratory animals, 2011).

Phytochemical screening

Phytochemical tests were carried out on samples as previously described (Edeoga et al., 2005; Sofowora, 2006).

Total antioxidant activity

Total antioxidant activity (TAA) was determined as previously described (Umamaheswari and Chatterjee, 2008). A reagent solution containing 0.6 M H_2SO_4, 4 mM ammonium molybdate and 28 mM sodium phosphate was prepared. Mixtures of the samples and reagent solution in tubes were incubated at 95°C for 90 min and absorbance was read at 695 nm against a blank containing distilled water in place of the samples. Results were expressed in µg/ml ascorbic acid equivalent (AAE).

Ability to inhibit deoxyribose degradation

The ability of extracts and fractions to inhibit deoxyribose degradation (DEO) was assessed using the method of Kumar et al. (2009). Briefly, different concentration of samples were added to a reaction mixture containing deoxyribose (20 mM), phosphate buffer (500 mM, pH 7.4), hydrogen peroxide (20 mM) and $FeSO_4.7H_2O$ (0.5 mM). The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the successive addition of 2.8% TCA and 0.6% TBA solution. The tubes were subsequently incubated in boiling water to develop the pink coloured $MDA-(TBA)_2$ adduct. The absorbance was measured at 532 nm in a spectrophotometer.

Lipid peroxidation inhibitory activity

Evaluation of Fe^{2+}- induced lipid peroxidation inhibitory activity (LPIA) of extracts and fractions in the brain (LPB), liver (LPL) and testes (LPT) of rats was performed following the method described by Sabir and Rocha (2008). Rats were sacrificed by administration of an overdose of anaesthetic. The tissues (brain, liver, testes) were quickly removed and placed on ice. A 10% homogenate of each tissue was prepared in 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged for 10 min at 3000 rpm to yield a pellet that was discarded and a low speed supernatant which was used for thiobarbituric acid reaction.

DPPH radical scavenging activity

DPPH antiradical activity (DRS) of extracts and fractions was evaluated using a spectrophotometric method (Mensor et al., 2001). DPPH (1 ml, 0.3 mM) was added to 1 ml of extract or standard and allowed to react at room temperature. The absorbance was read after 30 min at 517 nm and converted into percentage antioxidant activity.

Iron chelating ability

The ability of extract and fractions to chelate ferrous ions (ICA) was evaluated as previously described (Puntel et al., 2005). EDTA was used as the reference compound.

Evaluation of protein denaturation inhibitory activity

The antidenaturation activity (PRO) was evaluated using a modified method of William et al. (2002) with egg albumen as protein source. Egg albumen (0.5 ml) in 0.2 M phosphate buffer (pH 7.4) and 0.5 ml of extract or fractions were incubated at 27°C for 15 min. Thereafter, the mixture was heated at 60°C for 10 min. The absorbance was read at 660 nm after cooling. Results were expressed as percentage inhibition of protein denaturation.

Statistical analysis

Data are presented as mean ± SEM (n = 3) and were analysed statistically by one-way ANOVA, followed by Duncan's multiple range test. The bivariate correlation analysis was performed, quoting the Pearson correlation coefficients and test of significance. Significance was accepted at $P < 0.05$.

RESULTS

Table 1 presents the phytochemical groups detected in

Table 1. Phytochemical constituents of extract and fractions of *Spondias mombin.*

Test	Sample			
	SMC	SME	SMF	SMS
Saponins	++	-	-	+++
Flavonoids	++	-	+++	-
Steroids	+++	-	++	-
Terpenoids	++	-	-	+++
Tannins	+++	++	-	-
Alkaloids	+	-	-	-
Cardiac glycosides	+++	++	-	+

- indicates absence and + indicates presence.
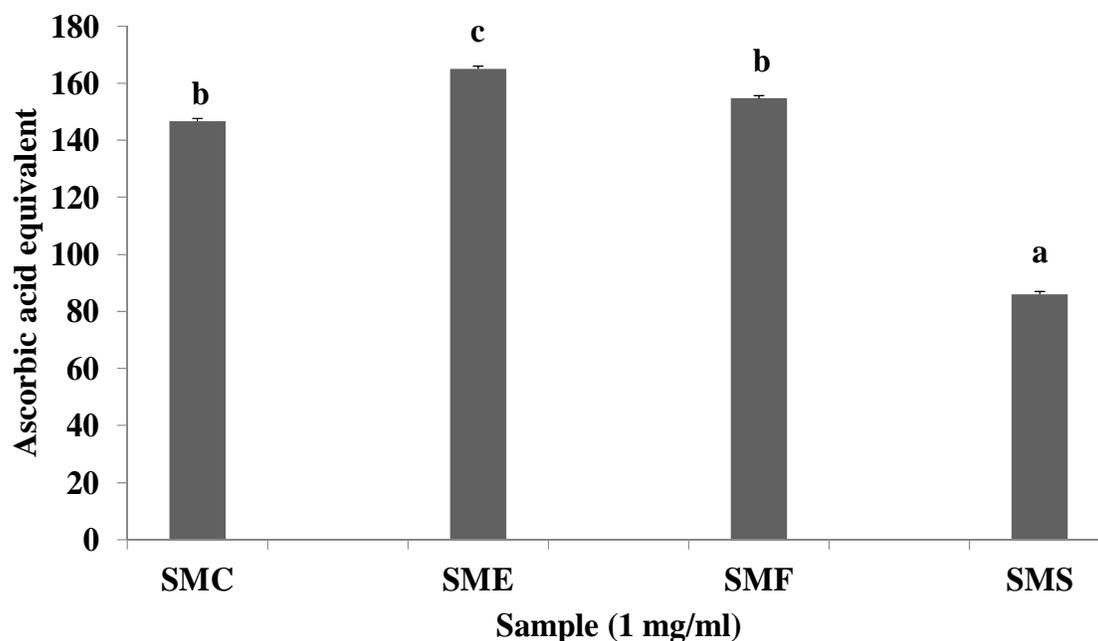
Figure 1. Total antioxidant activity of extract and fractions. Values are given as mean ± SEM (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

the samples. Fractionation resulted in the localization of phytochemicals in the fractions based on solvent properties. SMF and SMS were enriched in flavonoids and saponins respectively. The seeming contaminants may be due to hybrid phytochemicals. The ether fraction was enriched in tannins and cardiac glycosides.

SME had the highest TAA and SMS the least (Figure 1). However, SMC demonstrated the highest DEO followed by SMF while SMS had the least with IC_{50} values of 48.01 ± 0.21, 103.81 ± 0.58 and 270.59 ± 13.89 µg/ml respectively (Figure 2).

Samples, in general, had comparable LPB. SMF and SME showed good LPL while SME also demonstrated

remarkable LPT - with respect to the reference standards employed (Figure 3a to c). DRS was highest in SME (Figure 4) while ICA was comparable in the fractions at 50 - 250 µg/ml (Figure 5). PRO followed the same trend as TAA (Figure 6) with SME demonstrating the best antidenaturation activity.

Table 2 presents the relationship between total antioxidant activity of extract and fractions and their inhibitory potentials. The correlation of TAA with PRO, DEO and LPIA are shown in Figures 7a to c. The total antioxidant activity (TAA) correlated strongly with PRO (r^2 = 0.966), DEO (r^2 = 0.801) and inhibition of lipid peroxidation in rat organs (r^2 = 0.901).
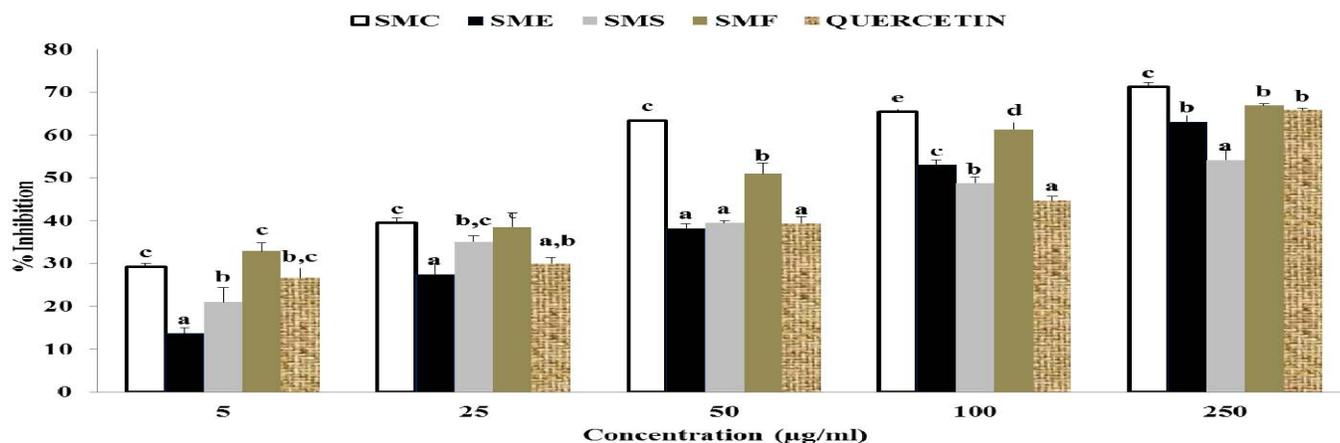
Figure 2. Ability of extract and fractions to inhibit the degradation of 2-deoxyribose. Values are given as mean ± SEM (n = 3). Values with the same alphabet for each sample concentration are not significantly different.
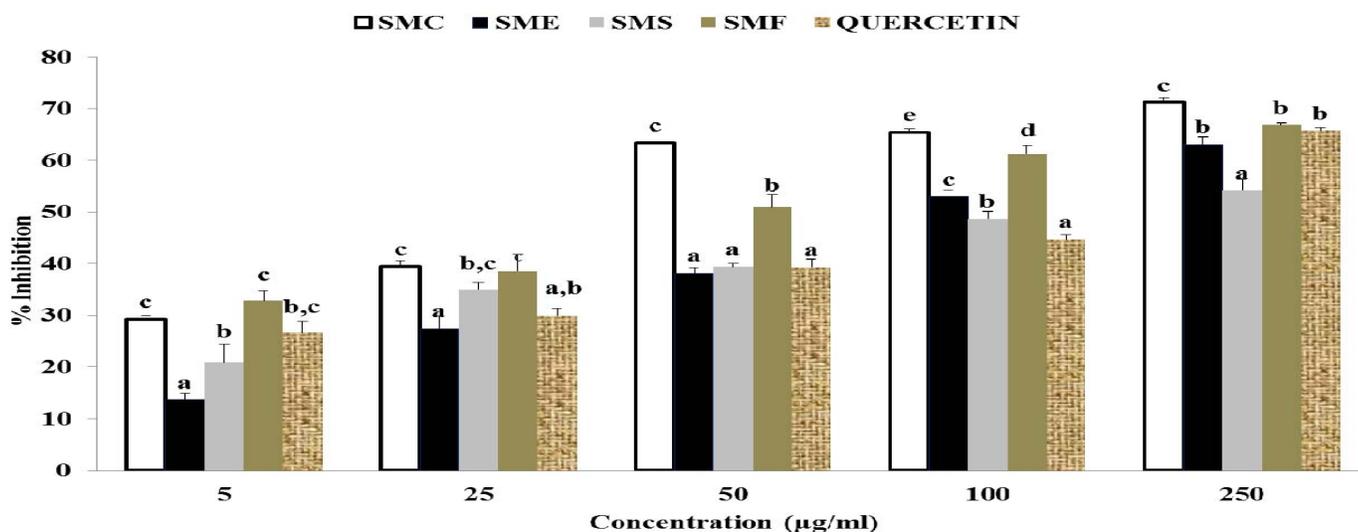

Figure 2. Ability of extract and fractions to inhibit deoxyribose degradation. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

DISCUSSION

Herbal medicine is becoming increasingly popular worldwide and scientific evidence of efficacy of these herbs is continually emerging from controlled preclinical and clinical trials. The medicinal value of herbs lies in their unique content of phytochemicals that elicit definite physiological actions in the mammalian body. Flavonoids and polyphenols are potent water-soluble antioxidants and free radical scavengers which can prevent oxidative cell damage and can lower risk of various pathologies (Urquiaga and Leighton, 2000). The mechanism of action of flavonoids is through scavenging or chelation of free radicals. The hydroxyl groups of flavonoids and other phenolic compounds confer scavenging ability (Cook and Samman, 1996; Yildrim et al., 2000). Flavonoids and other phenolic derivatives with antioxidant and anti-aging properties have been identified in *S. mombin* leaves (Corthout et al., 1992). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity (Gwotmut and Uwafor, 2001) and the presence of these phytochemicals may be associated with the reported oxytocic and abortifacient activities of the plant's leaf extract (Offiah and Anyawu, 1989). These phytochemicals may also account for the plant's reported anti-microbial, anti-bacterial, molluscicidal (Corthout et
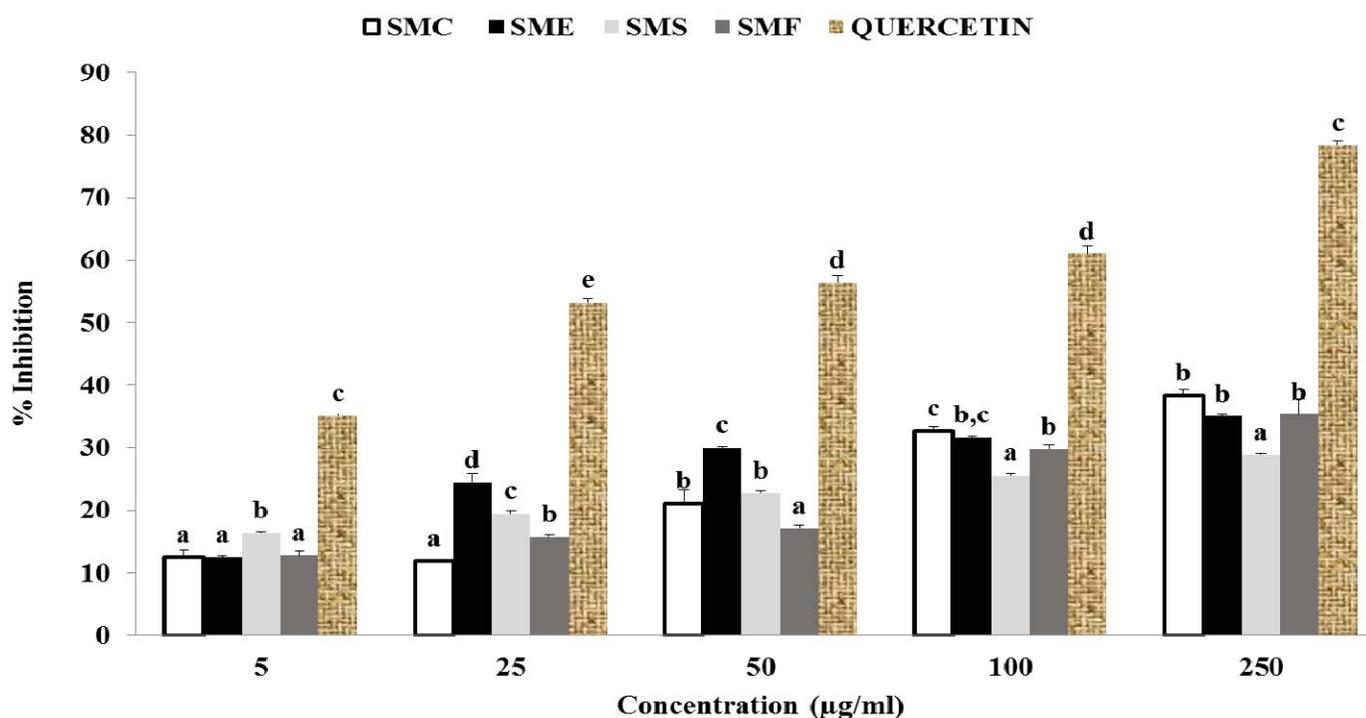
Figure 3a. Lipid peroxidation inhibitory activity of extract and fractions in rat brain. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.
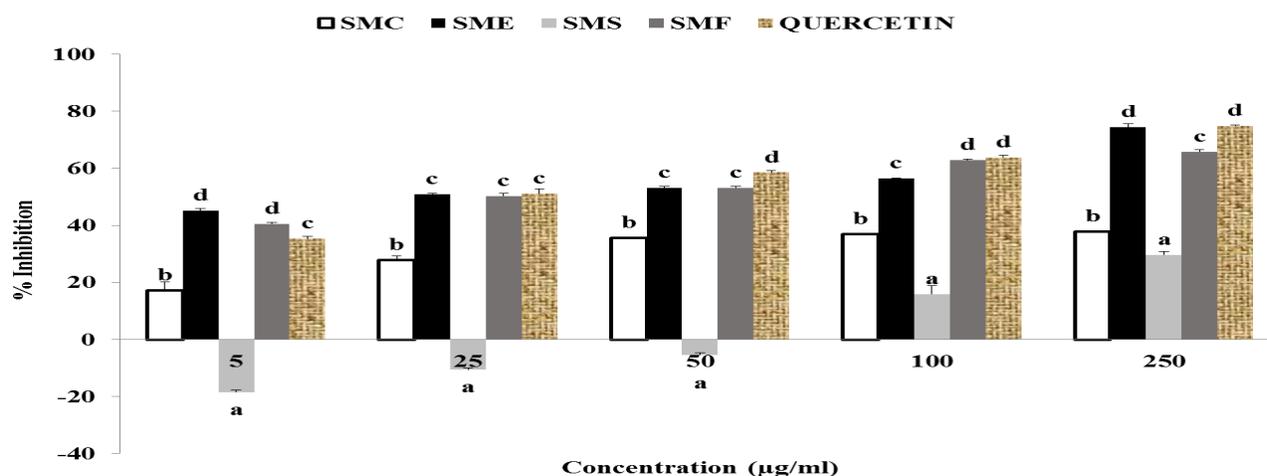

Figure 3b. Lipid peroxidation inhibitory effect of extract and fractions in rat liver. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

al., 1994), anti-viral (Corthout et al., 1992), anti-malarial (Caraballo et al., 2004) and anti-helminthic (Ademola et al., 2005) properties.

The total antioxidant activity shown by *S. mombin* extract and fractions (Figure 1) reflects free radical scavenging ability, reductive potential and the ability of the extracts to deactivate by chelation, the transition metals involved in initiation of free radical induced macromolecular damage. It is a reflection of the aggregate overall activity shown in multiple antioxidant assays (Figures 2 to 5).

The extract and fractions showed ability to inhibit heat-induced protein denaturation (Figure 6). When a protein is denatured, the secondary and tertiary structures are
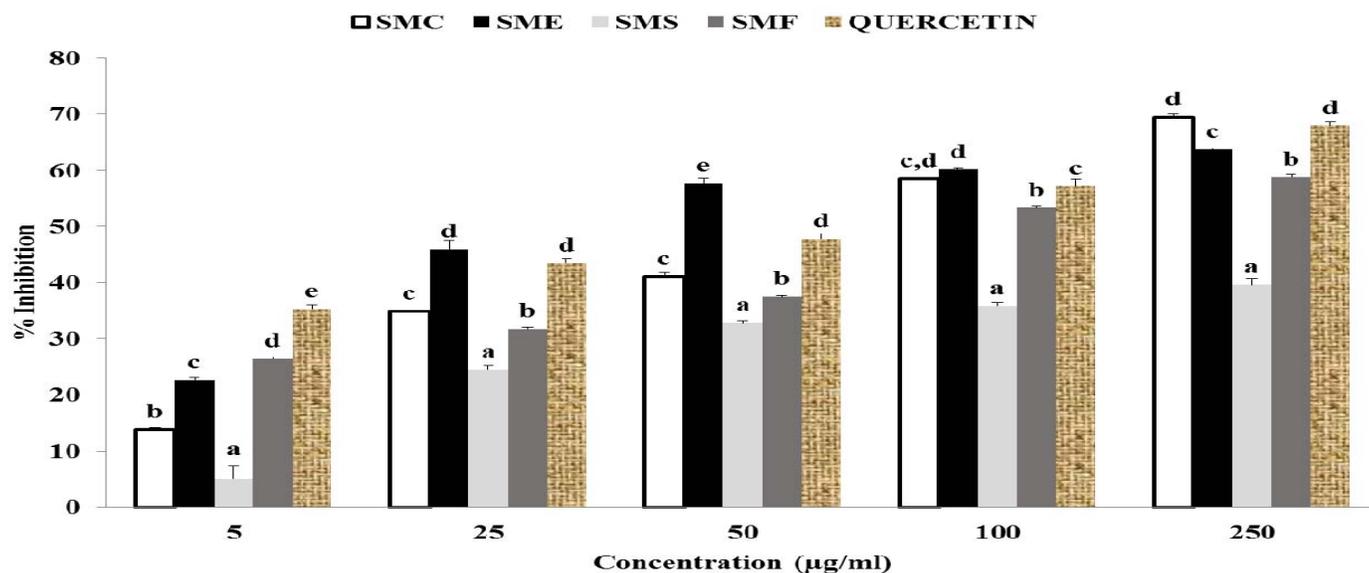
Figure 3c. Lipid peroxidation inhibitory effect of extract and fractions in rat testes. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.
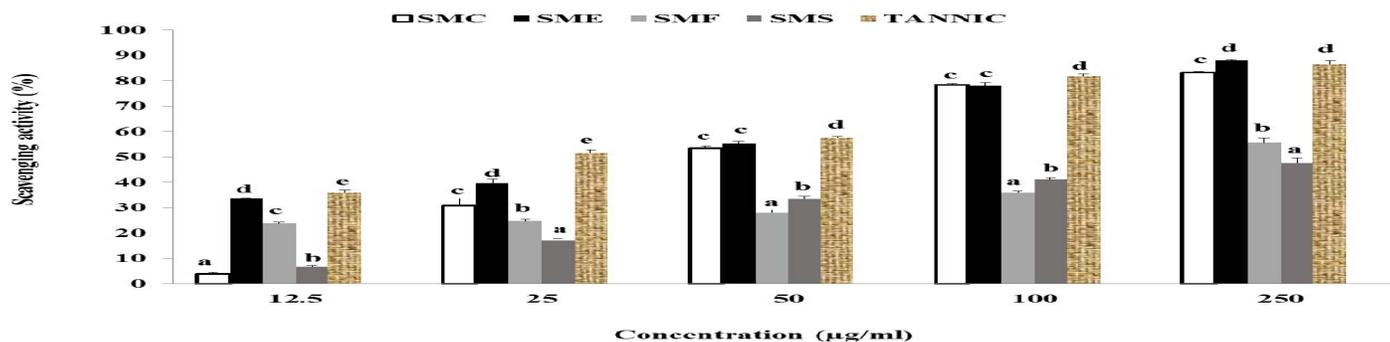

Figure 4. DPPH scavenging activity of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

altered but the peptide bonds of the primary structure between the amino acids are left intact. Since all the structural levels of the protein determine its function, the protein can no longer perform its function once it has been denatured. *S. mombin* extract and fractions may inhibit protein denaturation by preventing the disruption of hydrogen bonds and non-polar hydrophobic interactions responsible for the secondary structure and tertiary structure of proteins. They may also compete with water molecules in the interaction with amide nitrogen and carbonyl oxygen thus helping to stabilize the helical structure of the protein.

S. mombin extract and fractions also demonstrated the ability to inhibit the degradation of deoxyribose (Figure 2). Hydroxyl radicals, produced through iron-catalyzed decomposition of deoxyribose molecule can cause

severe deleterious effect on biological macromolecules (Graf et al., 1984).

The extract and fractions inhibited lipid peroxidation in the brain, liver and testes of rats *in vitro* as shown in Figures 3a to c. The overproduction of ROS can result in a direct attack on the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Iron can stimulate lipid peroxidation by the Fenton reaction and can also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction (Halliwell et al., 1991). Free radical induced formation of lipid peroxides is thought to play an important role in the etiology and pathogenesis of a number of oxidative stress related diseases. As a result of lipid peroxidation, a number of unstable intermediary metabolites (mainly aldehydes
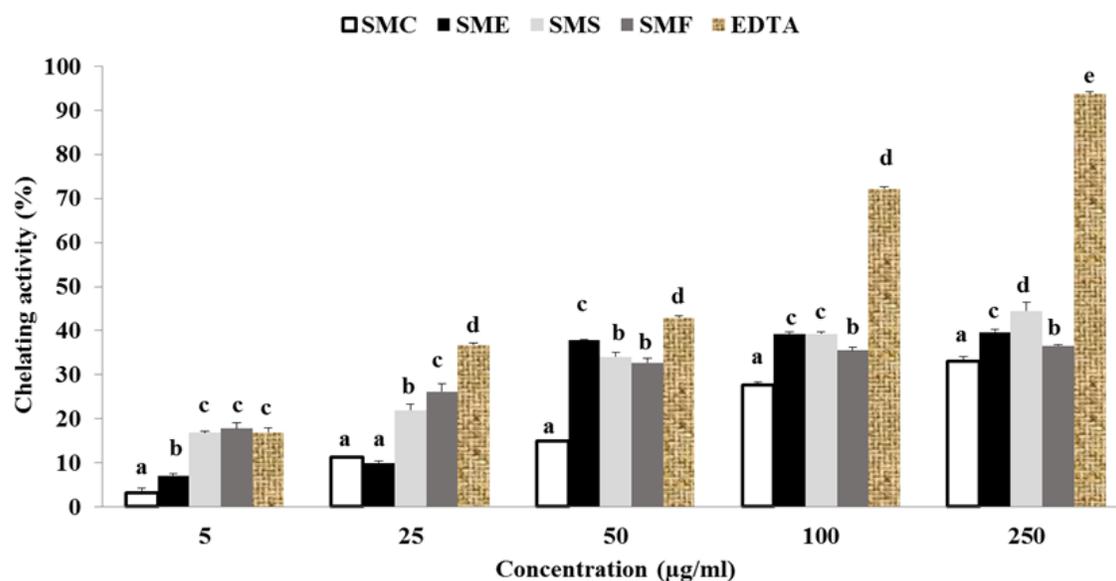
Figure 5. Chelating ability of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

Figure 6. Protein denaturation inhibitory effect of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.
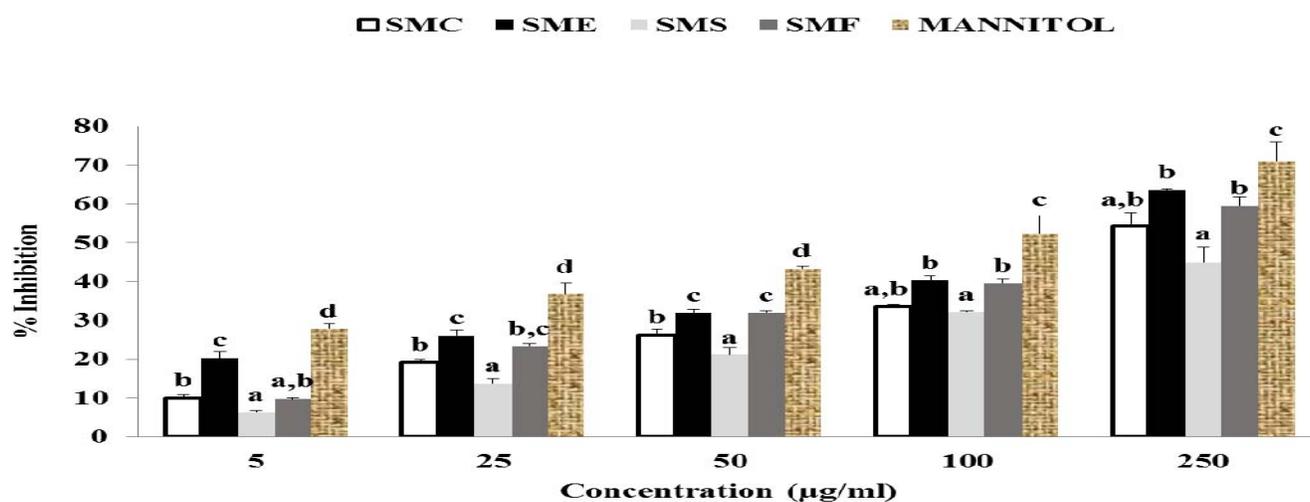
such as MDA and 4-HNE) are produced from cellular molecules (Halliwell, 1993; Erba et al., 2005).

As observed in Table 2 and Figures 7a to c, the total antioxidant activity of *S. mombin* extract and fractions correlated with DEO, LPIA and PRO. This suggests that the antioxidant property is a major underlying factor of the protective properties shown by the plant.

In general, SME had the highest activity followed by SMF, SMC and SMS. The high activity shown by SME may be due to the presence of high amounts of tannins. Tannins are metal ion chelators (Karamać, 2009), and have shown potential antiviral (Lü et al., 2004),

antibacterial (Akiyama et al., 2001), and antiparasitic effects (Kolodziej and Kiderlen, 2005). Souza et al. (2006) reported that tannins have anti-inflammatory and antiulcer activity in rodents. The activity exhibited by SMF was close to that of SME and this may be as a result of the presence of flavonoids and phenolic compounds.

Free radicals are constantly generated in the living system and they can cause extensive damage to tissues and biomolecules leading to various diseases, especially degenerative diseases. Agents with antioxidant properties have been found to be useful in treating these disorders. Therefore, a huge body of scientific research is

Table 2. Relationship between total antioxidant activity and inhibitory potentials of extract and fractions.

Test	Sample			
	SMC	SME	SMS	SMF
PRO	0.972**	0.971**	0.969**	0.976**
DEO	0.734	0.944**	0.881*	0.887*
LPB	.908*	0.939**	0.952**	0.971**
LPL	0.853*	0.971**	0.967**	0.904*
LPT	0.833*	0.839*	0.785	0.940**

*Correlation is significant ($P < 0.05$); **Correlation is significant ($P < 0.01$). SMC, Crude extract; SME, Ether fraction; SMS, Saponin-rich fraction; SMF, Flavonoid-rich fraction; PRO, protein degradation inhibitory activity; HRS, hydroxyl radical scavenging activity; LPB, lipid peroxidation inhibitory activity in rat brain; LPL, lipid peroxidation inhibitory activity in rat liver; LPT, lipid peroxidation inhibitory activity in rat testes.
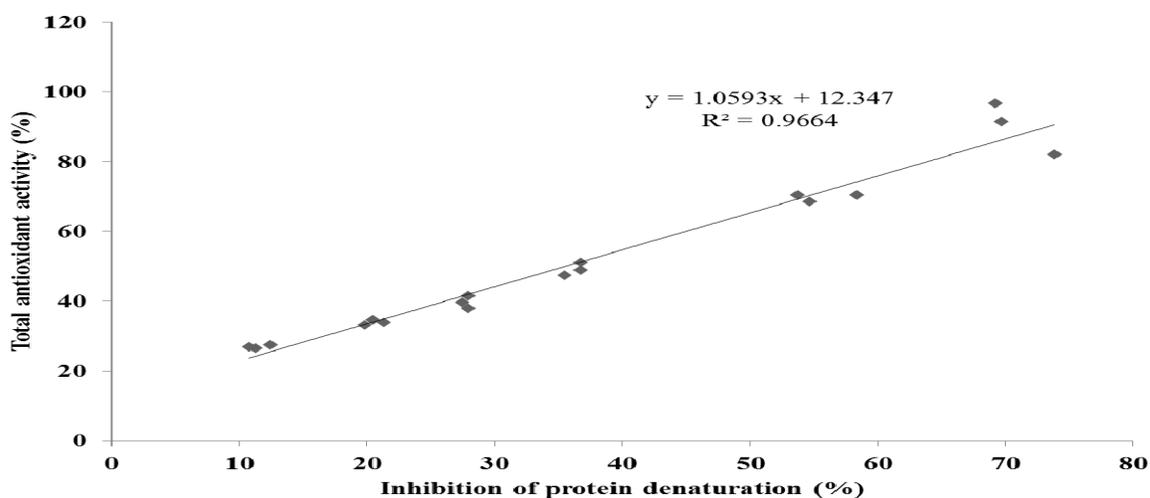
Figure 7a. Relationship between total antioxidant activity and protein denaturation inhibitory potential. Values are given as mean ± SD (n = 18).
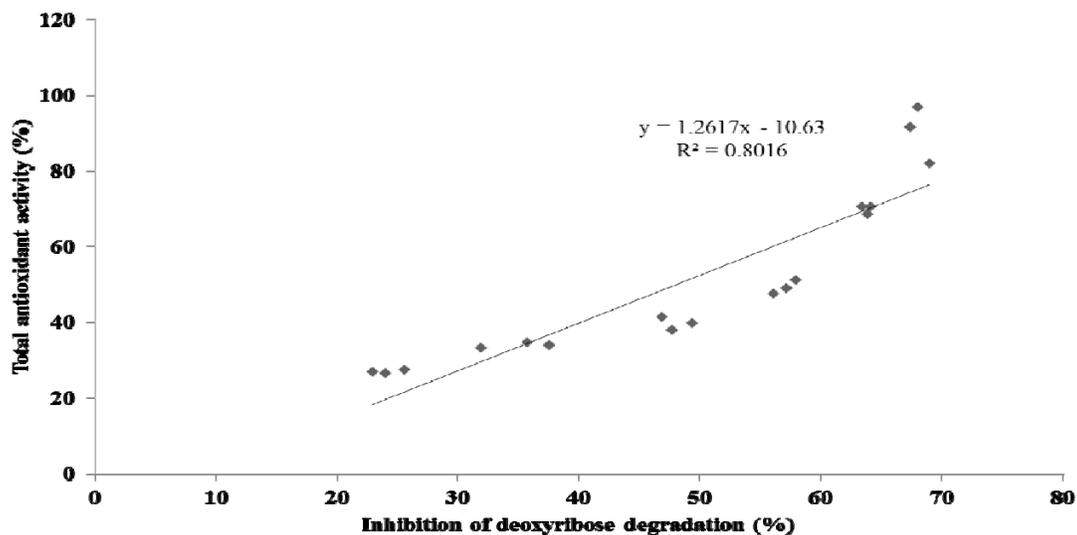
Figure 7b. Relationship between total antioxidant activity and Hydroxyl radical scavenging activity of extract and fractions. Values are given as mean ± SD (n= 18).
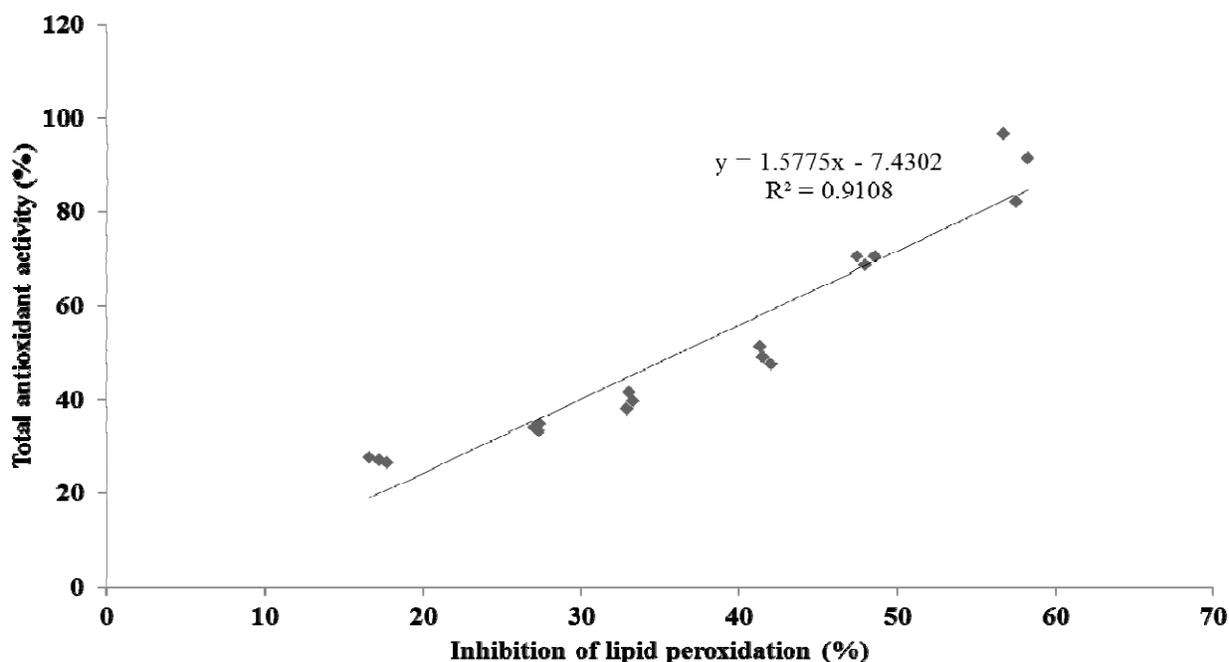
Figure 7c. Relationship between total antioxidant activity and lipid peroxidation inhibitory potential. Values are given as mean ± SD (n= 18).

focused on exploring for safe and effective antioxidants and to encourage the consumption of natural antioxidants from food supplements and traditional medicines (Souza et al., 2006; Yazdanparast and Ardestani, 2007) and, many natural antioxidants have been isolated from different parts of plants (Yazdanparast et al., 2008). The results obtained in this study indicate that extract and fractions from *S. mombin* are significant source of antioxidants, which might be helpful in combating oxidative stress and assaults on biomolecules from various sources.

Conflict of Interest

The authors declare that there are no conflicts of interests.

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