Full Length Research Paper

In -vitro and *-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg.

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Strychnos henningsii Gilg is used traditionally for the treatment of various ailments in southern Africa traditional medicine. The antioxidant and free radical scavenging activity of aqueous extract of this plant was investigated both *in -vivo* and *-vitro* using spectroscopic method against 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anions, hydrogen peroxide (H_2O_2), nitric oxide (NO), 2,2'- azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS) and the ferric reducing agent. Total phenols, flavonoid, flavonol and proanthocyanidin were also determined to assess their effects on the antioxidant activity of this plant. Free radical scavenging activity of the plant extract against H_2O_2, ABTS and NO was concentration dependent with IC_{50} value of 0.023, 0.089 and 0.49 mg/ml respectively. However, *S. henningsii* exhibited lower inhibitory activity against DPPH with IC_{50} value of 0.739 mg/ml. The reducing power of the extract was found to be concentration dependent. The administration of the aqueous extract at 250, 500 and 1000 mg/kg body weight to male Wistar rats significantly increased the percentage inhibition of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Whereas, lipid peroxidation level in hepatotoxic rats decreased significantly at the dose of 500 and 1000 mg/kg body weight at the end of 7 days. The extract yielded high phenol content (48 mg/g tannic acid equivalent) followed by proanthocyanidin (8.7 mg/g catechin equivalent) flavonol (5.5 mg/g quercetin equivalent) and flavonoids (4.8 mg/g quercetin equivalent) respectively. A positive linear correlation was observed between these polyphenols and the free radical scavenging activities.

Key words: *Strychnos henningsii*, enzymes, free radicals, CCl_4, antioxidant activity.

INTRODUCTION

Many human diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of pro-oxidants (Hazra et al., 2008). Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite, play a vital role in damaging various cellular macromolecules. These include DNA molecules, proteins along with lipid peroxidation. This damage may result into many diseases, including diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis (Polterat, 1997). However, human cells have an array of protecting mechanisms to prevent the production of free radicals and oxidative damage (Chandra et al., 1994).

These mechanisms include both enzymic and non enzymic antioxidants such as superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol (Niki et al., 1994). The protective roles of these enzymes may be disrupted as a result of various pathological processes and thereby causes damage to the cells. Antioxidant supplement has been reported to reconcile the upshot of these radicals by directly reacting and quenching their catalytic metal ions (Robak and Marcinkiewicz, 1995). Several synthetic antioxidant agents including BHA and BHT are commercially available, however, are reported to be toxic to animals including human beings (Madhavi and Salunkhe, 1995). Furthermore, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, flavonols and proanthocyanidins (Rice-Evans et al., 1995). Today, a vast majority of plants used in traditional medicine in South Africa have not been eva-

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luated for their antioxidant potential. One of such plants is *S. henningsii*. *S. henningsii* Gilg. (Loganiaceae) is a small evergreen tree or shrub with leathery leaves. The bark is crown compact with dark green, glossy foliage and the fruit is oblong which turn brown when ripen. The leaves of the plant have a characteristic aromatic-pungent odour with rough texture. It is one of the most widely distributed species of strychnos in east and southern Africa (Leeuwenberg, 1969).

The bark of *S. henningsii* has been recommended for the treatment of various diseases by the traditional health practitioners in southern Africa. These diseases include rheumatism, gynaecological complaints, abdominal pain, snake bite, gastrointestinal pain, malaria and diabetes mellitus (Hutchings, 1989; Bisset, 1970). In east Africa, the bark has been documented to have significant medicinal uses in the healing of wounds and as a mouth antiseptic (Hutchings, 1989). About five compounds, including indolinic alkaloids, strychnine, brucine, curarine and bitter glycoside have been isolated from this plant (Tits, 1982).

Before the commencement of this work, there was no information in scientific literature on the free radical scavenging and antioxidant activity of the aqueous extract of *S. henningsii* bark both *in vivo* and *in vitro*. Therefore, this study was aimed at providing information on the phytochemicals and antioxidant activities of this plant.

MATERIALS AND METHODS

Plant material

The bark of *S. hennigsii* was collected in February, 2009 from a thick forest in Amathole District (Eastern Cape, South Africa). The plant was identified by its vernacular name and later authenticated by Prof. DS. Grierson of Botany Department, University of Fort Hare. Voucher specimen (Sun MED 2009) was deposited at the Giffen Herbarium of the University.

Preparation of extract

The bark material was air-dried at room temperature in the laboratory. The dried material was then pulverized using an electric blender (Waring Products Division, Torrington, USA). About 60 g of the powdered plant material was extracted in 1 L of cold distilled water maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No 1 filter paper. The filtrate was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 8.4 g of dry extract. The resulting extract was prepared in distilled water to give desired doses of 250, 500 and 1000 mg/ml used in this study.

Animals

Male Wistar rats (*Rattus norvegicus*) with a mean weight of 175 g ± 5.2 were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. They were kept in clean metabolic cages placed in a well ventilated

house conditions (temperature 23 ± 1°C: photoperiod: 12 h light and dark cycle each throughout the experimental period; humidity: 45 - 50%). The rats were allowed free access to food (Balanced Trusty Chunks, Pioneer Foods (Pty) Ltd, and Huguenot, South Africa) and water *ad libitum*. The experiment was carried out after its approval by the Animal Ethics Committee of the University of Fort Hare in accordance with the recommendations for the proper care and use of laboratory animals.

Animal grouping and extract administration

Twenty five male rats were randomized into five groups consisting of five each. Group 1 served as control and was given distilled water alone (0.5 ml) per day for seven days with the aid of oropharyngeal cannula. Groups 2 animals served as hepatotoxic control, treated with CCl_4 in a single dose of 0.5 ml administered orally for seven days, while the animals in group 3 - 5 were treated like the control except that they received 0.5 ml of the extract corresponding to 250, 500 and 1000 mg/kg body weight respectively. Again group 3 - 5 was given 0.5 ml of CCl_4 on the seventh day after 6 h of extract administration. All the animals from each group were sacrificed by ether anesthesia 24 h after their respective 21 daily doses of the extract and distilled water. The liver from each animal was excised, rinsed in ice cold 0.25 M sucrose solution and 10 %w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4°C. The supernatant obtained was used for the estimation of catalase, superoxide dismutase, lipid peroxidation (TBARS) and reduced glutathione.

Estimation of total phenolic compounds

Total phenol

The total phenolic content in the aqueous bark extract of *S. henningsii* was determined spectrophotometrically with Folin Ciocalteau reagent using the modified method of Wolfe et al. (2003). An aliquot of the extract (0.5 ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (75% w/v). The resulting mixture was vortexed for 15 s and incubated at 40°C for 30 min for colour development. The absorbance of the samples was measured at 765 nm using Hewlett Packard, UV/visible light. Total phenolic content was expressed as mg/g tannic acid equivalent from the calibration curve using the equation:

$$Y = 0.1216x, R^2 = 0.936512$$

Where; x was the absorbance and Y was the tannic acid equivalent (mg/g).

The experiment was conducted in triplicate and the results are reported as mean ± SD values.

Total flavonoids

The method of Ordon et al. (2006) was used to estimate total flavonoid contents of the extract solution based on the formation of a complex flavonoid-aluminium. A volume of 0.5 ml of 2 % $AlCl_3$ ethanol solution was added to 0.5 ml of extract solution. After one hour of incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer. All determinations were done in triplicate and values were calculated from calibration curve obtained from quercetin using the equation:

$$Y = 0.0255x, R^2 = 0.9812$$

where x was the absorbance and Y the quercetin equivalent (mg/g).

Total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran (2007). The reacting mixture consisted of 2.0 ml of the sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20°C. Total flavonoid content was calculated as quercetin (mg/g) equivalent from the calibration curve using the equation:

$Y = 0.0255x$, $R^2 = 0.9812$

Where; x was the absorbance and Y the quercetin equivalent (mg/g).

Total proanthocyanidins

Total proanthocyanidins was determined based on the procedure of Sun et al. (1998). To 0.5 ml of 1 mg/ml extract solution was added 3 ml of vanillin-methanol (4 % v/v) and 1.5 ml of hydrochloric acid and then vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve:

$Y = 0.5825x$, $R^2 = 0.9277$

Where; x was the absorbance and Y the catechin equivalent (mg/g).

In vitro antioxidant activity

Determination of reducing power

The reducing power of the extract was evaluated according to the method of Yen and Chen (1995). A volume of 1.0 ml of the extract prepared in distilled water and BHT, Vitamin C and Vitamin E (0 - 5.0 mg/ml) were mixed individually to the mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide $(K_3Fe(CN)_6)$ (1%w/v). The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v), which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1 %, w/v). The absorbance was measured at 700 nm against a blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

DPPH radical scavenging activity

The method of Liyana-Pathiranan and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical in the extract solution. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract prepared in methanol containing 0.025 - 0.5 mg of the plant extracts and standard drugs separately (BHT and rutin). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by the equation:

DPPH radical scavenging activity = {(Abs $_{control}$ − Abs $_{sample}$)/(Abs control)} × 100

Where; Abs $_{control}$ is the absorbance of DPPH radical + methanol; Abs $_{sample}$ is the absorbance of DPPH radical + sample extract or standard.

ABTS radical scavenging activity

The method of Re et al. (1999) was adopted for the determination of ABTS activity of the plant extract. The working solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowed to react for 12 h at room temperature in the dark. The resulting solution was further diluted by mixing 1 ml of freshly prepared ABTS solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm after 7 min using spectrophotometer. The percentage inhibition of $ABTS^{·+}$ by the extract was calculated and compared with that of BHT and rutin using the following equation:

$ABTS^{·+}$ scavenging activity = {(Abs $_{control}$ − Abs $_{sample}$)/ (Abs control)} × 100

Where; Abs $_{control}$ is the absorbance of $ABTS^{·+}$ + methanol; Abs $_{sample}$ is the absorbance of $ABTS^{·+}$ + sample extract or standard.

Scavenging activity of nitric oxide

The method of Garrat (1964) was used to determine the nitric oxide radical scavenging activity of aqueous extract of *S. henningsii*. A volume of 2 ml of 10 mM sodium nitroprusside prepared in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract, BHT and rutin individually at various concentrations (0.025 - 0.5 mg/ml). The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33 % prepared in 20 % glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 540 nm. The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

NO radical scavenging activity = {(Abs $_{control}$ − Abs $_{sample}$) / (Abs control)} × 100

Where; Abs $_{control}$ is the absorbance of NO radical + methanol; Abs $_{sample}$ is the absorbance of NO radical + sample extract or standard.

## Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Ruch et al. (1989). Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

H_2O_2 radical scavenging activity = {(Abs $_{control}$ − Abs $_{sample}$)/ (Abs control)} × 100

Where; Abs $_{control}$ is the absorbance of H_2O_2 radical + methanol; Abs $_{sample}$ is the absorbance of H_2O_2 radical + sample extract or standard.

Determination of catalase activity

The activity of catalase was assayed following the method
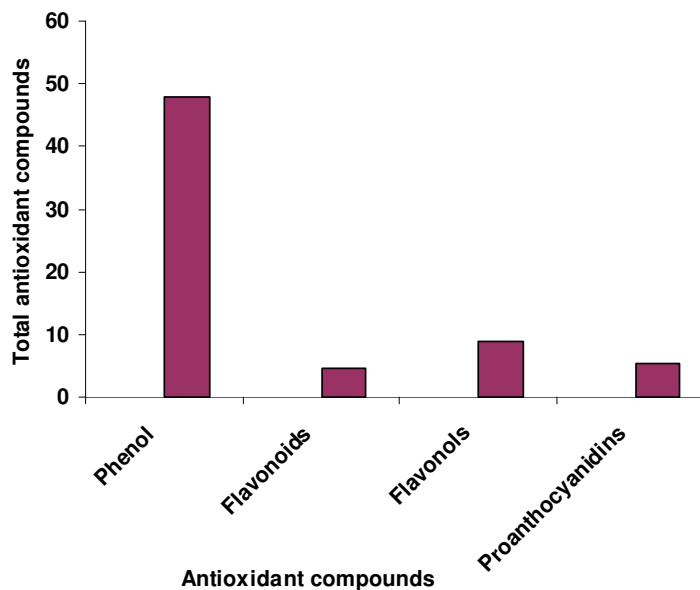
Figure 1. Polyphenol contents of aqueous extract from the bark of *S. henningsii.*

described by Pari and Latha (2004). The percentage inhibition was evaluated following decrease in absorbance at 620 nm. The liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuge at 5000 rpm.

The reaction mixture consisted of 0.4 ml of hydrogen peroxide (0.2 M), 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of liver homogenate (10% w/v). The reaction of the mixture was stopped by adding 2 ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ prepared in glacial acetic acid). The changes in the absorbance was measured at 620 nm and recorded. Percentage inhibition was calculated using the equation:

% catalase inhibition = [(normal activity – inhibited activity) / (normal activity)] × 100%.

Determination of superoxide dismutase activity

Superoxide dismutase was assayed as described by Misra and Fridovich (1972). The assay mixture contained 0.5 ml of hepatic PMS, 1 ml of 50 mM sodium carbonate, 0.4 ml of 25 μm nitroblue tetrazolium and 0.2 ml of freshly prepared 0.1 mM hydroxylamine-hydrochloride. The reaction mixture was mixed quickly by inversion followed by the addition of clear supernatant of 0.1 ml of liver homogenate (10% w/v). The change in absorbance was recorded at 560 nm. Percentage inhibition was calculated using this equation:

% superoxide dismutase inhibition = [(normal activity – inhibited activity)/(normal activity)] × 100%.

Determination of reduced glutathione activity

Reduced glutathione was determined using the modified method of Ellman (1951). An aliquot of 1.0 ml of supernatant of liver homogenate was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm. The percentage inhibition of

GSH was calculated using the following equation:

% reduced glutathione inhibition = $(A_o - A_1)/A_o$ × 100

Where; A_o is the absorbance of the control and A_1 is the absorbance of the sample extract.

Estimation of lipid peroxidation

Lipid peroxidation in the liver was estimated colorimetrically by thio-barbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelsson (1968). In brief, 0.1 ml of liver homogenate (10% w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and cooled. The amount of malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm against reference blank. Percentage inhibition was calculated using the equation:

% lipids Inhibition = $\{A_o - A_1\}/A_o$ × 100

Where; A_o is the absorbance of the control and A_1 is the absorbance of the sample extract.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates and were subjected to paired student's t-test. Significant levels were tested at $P < 0.05$.

RESULTS

The total phenolic contents in the aqueous extract of *S. hennigsii* are shown in Figure 1. The plant extract possessed high phenol contents (48 mg/g tannic acid

equivalent) followed by proanthocyandins (8.7 mg/g catechin equivalent), flavonols (5.5 mg/g quercetin equivalent) and flavonoid (4.8 mg/g quercetin equivalent). Phenolic compounds, especially flavonoids and phenols have been shown to possess significant antioxidant activity.

Figure 3 shows the dose-response curve of DPPH radical scavenging activity of *S. henningsii* compared with rutin and BHT. It was observed that the extract had DPPH scavenging activity with IC_{50} value of 0.739 mg/ml. The scavenging activity of this plant against DPPH was observed as the weakest among other reactive oxygen species evaluated.

S. hennigsii was fast and effective scavenger of ABTS radicals as shown in Figure 4. A comparable scavenging activity of this plant was observed with that of BHT and rutin. The IC_{50} values of the extract, rutin and BHT were 0.089, 0.016 and 0.015 respectively. At 0.5 mg/ml, the plant extract showed higher inhibitory activity in removing ABTS radicals from the reaction system.

The antioxidant potentials of the plant extract was estimated from their ability to reduce Fe^{3+} to Fe^{2+}. This was observed from yellow colour of the test solution that changed to various shades of green and blue depending on the concentration of the plant extract. The reducing value of the extract was significantly lower than that of BHT, Vitamin C and Vitamin E used as reference compounds in this study (Figure 2). At 0.5 mg/ml, the absorbance of plant extract was still low as compared to the reference drugs.

The scavenging activity of aqueous extract of *S. henningsii* compared to BHT and Vitamin C for hydrogen peroxide is shown in Table 1. The results indicated a concentration dependent activity against H_2O_2 with IC_{50} values of 0.023, 0.018 and 0.02 mg/ml for plant extract, BHT and Vitamin C, respectively. The percentage inhibition values at 0.5 mg/ml for plant extract, BHT and Vitamin C were 92.51, 98.46 and 99.82%, respectively.

S. henningsii extract caused a moderate dose-dependent inhibition of nitric oxide with an IC_{50} of 0.49 mg/ml as shown in Table 2. The scavenging activity of BHT and Vitamin C showed IC_{50} values of 0.032 and 0.026 mg/ml and percentage inhibition of 85.96 and 94.22%, respectively, while the percentage inhibition of *S. henningsii* was 50.31%.

Table 3 showed the effect of plant extract on the activities of antioxidant enzymes in the liver of control and experimental rats. There was a marked decreased in the percentage inhibition of superoxide dismutase, catalase and the level of GSH in carbon tetrachloride treated rats when compared with normal control group.

However, the percentage inhibition of SOD, CAT and the level of GSH were significantly increased followed the oral administration of plant extract at 250, 500 and 1000 mg/kg body weight in a dose dependent manner.

In vivo lipid peroxidation study revealed that rats treated with carbon tetrachloride showed a significant
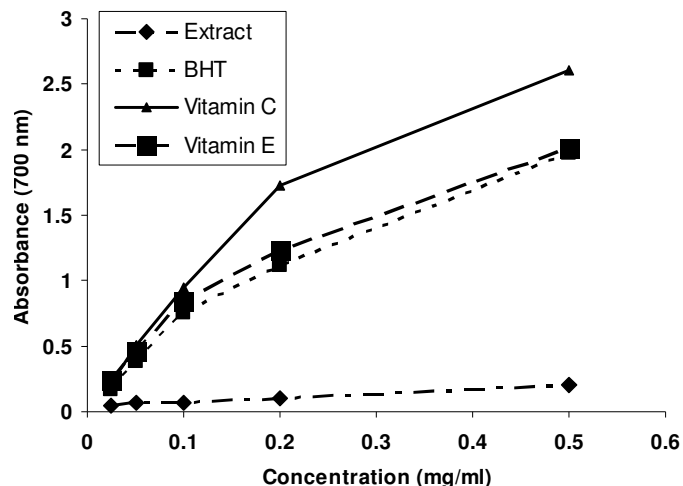
Figure 2. Total ferric reducing potential of aqueous bark extract of *S. henningsii*.

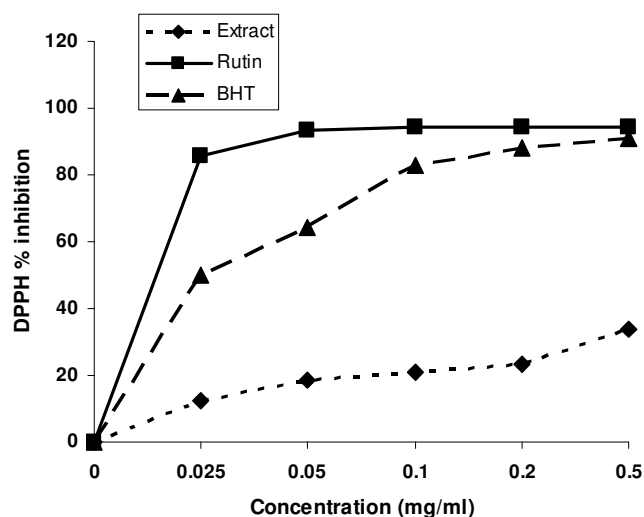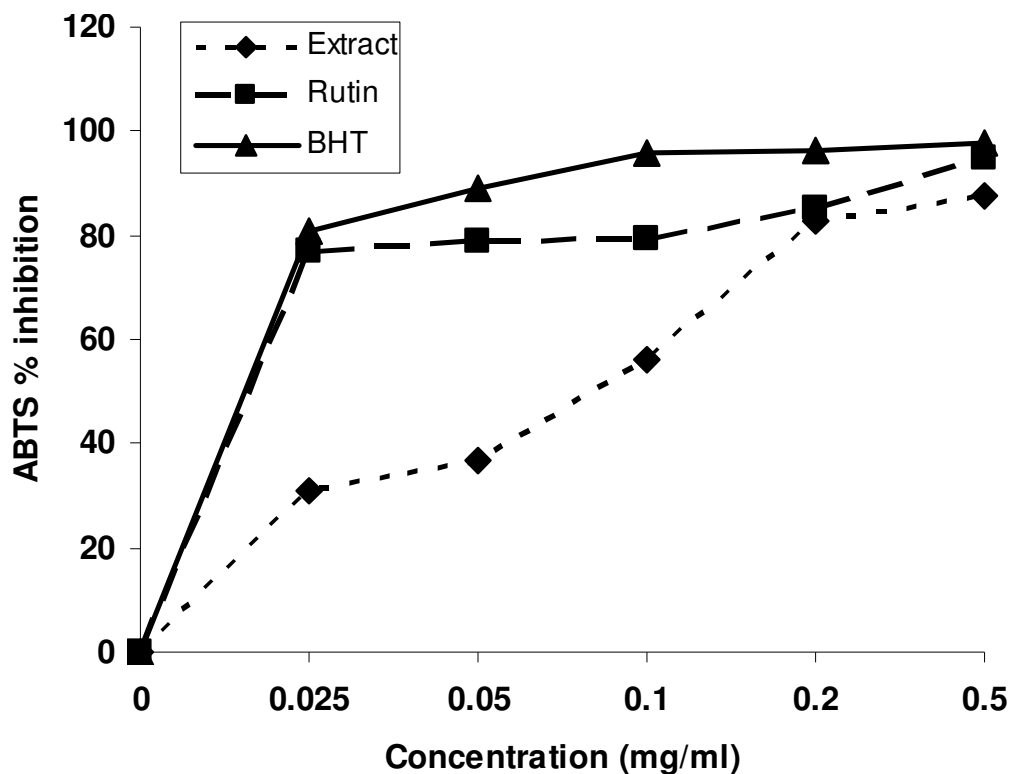**Figure 3**. DPPH radical scavenging activity of the aqueous bark extract of *S. henningsii*.

increase (P < 0.05) in TBARS when compared with normal control group. Treatment with aqueous extract of *S. henningsii* for 8 days was able to lower the rise in TBARS level dose dependently as shown in Table 3.

DISCUSSION

Polyphenols are the major plant compounds with high level of antioxidant activity. This activity could be due to their ability to adsorb, neutralize and to quench free radicals (Duh et al., 1999). Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and

Figure 4. ABTS radical scavenging activity of the aqueous bark extract of *S. henningsii*.

Table 1. Hydrogen peroxide radical scavenging activity of aqueous extract of *S. henningsii* bark.

Sample	Concentration (mg/ml)	% inhibition	IC_{50}	r^2
	0.025	54.82 ± 0.006		
	0.050	77.80 ± 0.004		
Aqueous extract	0.100	85.38 ± 0.007	0.023	0.9962
	0.200	87.52 ± 0.003		
	0.500	92.51 ± 0.005		
Vitamin C			0.020	0.9861
BHT			0.016	0.9952

In each group n = 5 rats, Values are expressed as mean ± SD. r^2— regression co-efficient.

Table 2. Nitric oxide radical scavenging activity of aqueous extract of *S. henningsii* bark.

Sample	Concentration (mg/ml)	% inhibition	IC_{50}	r^2
	0.025	15.80 ± 0.142		
	0.050	16.30 ± 0.423		
Aqueous extract	0.100	28.74 ± 0.074	0.490	0.9982
	0.200	35.56 ± 0.099		
	0.500	50.31 ± 0.174		
Vitamin C			0.026	0.9868
BHT			0.032	0.9946

In each group n = 5 rats, Values are expressed as mean ± SD. r^2 – regression co-efficient.

Table 3. Effect of aqueous extract of *S. henningsii* bark on LPO, antioxidant enzymes and GSH in carbon tetrachloride induced liver damage in male Wistar rats.

Group	CAT	SOD	GSH	TBARS
Control	89.27 ± 0.24	80.22 ± 0.21	83.55 ± 0.33	91.67 ± 0.32
CCl$_4$	31.00 ± 0.22^a	42.60 ± 0.21^a	31.77 ± 0.31^a	45.00 ± 0.35^a
CCl$_4$ + *S. henningsii* (D1)	37.46 ± 0.34^b	59.33 ± 0.24^b	43.33 ± 0.39^b	47.00 ± 0.43^b
CCl$_4$ + *S. Henningsi* (D2)	47.82 ± 0.32^c	62.11 ± 0.29^c	48.44 ± 0.36^c	52.50 ± 0.32^c
CCl$_4$ + *S. Henningsii* (D3)	64.00 ± 0.28^d	67.77 ± 0.26^d	54.00 ± 0.36^d	58.33 ± 0.30^d

[a-d] test values carrying superscripts different from the control across each parameter are significantly different ($P > 0.05$). Each value is mean $\pm$ S.D (n = 5 rats). D1 = 250 mg/kg body wt; D2 = 500 mg/kg body wt and D3 = 1000 mg/kg body wt. Results are expressed as percentage inhibition of the control.

carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans et al., 1995).

In the present study, it was found that the aqueous extract of *S. henningsii* contains high level of phenol content that might account for the strong activity observed against ABTS and H$_2$O$_2$ radicals. This scavenging activity may be due to the presence of hydroxyl groups attached to the aromatic ring structures and thus help to quench the radicals (Vinson et al., 1998). On the other hand, the weak and moderate activity depicted in DPPH and NO radicals may be as a result of lower content of flavonoids which have been reported to possess high antioxidant activity.

The reducing power of *S. henningsii* extract obtained in this study was determined by measuring the transformation of Fe^{+3} to Fe^{+2}.   The observed result obtained showed that the extract possessed antioxidant activity in a concentration dependent manner.   This effect may suggest the ability of *S. henningsii* to minimize oxidative damage to some vital tissues in the body (Kojic et al., 1998 and Weigh and et al., 1999).

The relatively low level of flavonoids might account for the weak activity observed in the DPPH radical scavenging assay as shown in Figure 3. The scavenging of ABTS$^{.+}$ by the plant extract was found to be higher than that of DPPH radical and this could be due to different mechanisms involved in the radical-antioxidant reactions. For instance, solubility of the extracts in different testing system, substrate used and quantitation method may influence the ability of herbs to quench different radicals (Yu et al., 2002). This result corroborate with the report of Wang et al. (1998) who found that some compounds with ABTS$^+$ scavenging activity may not exhibit DPPH scavenging activity. As a result, it may be difficult to compare antioxidant activity based on antioxidant assay because of the different test system and the substrate to be protected (Frankel and Meyer, 2000).

Hydrogen peroxide is highly important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions (Gulcin et al., 2003). This assay shows the ability of *S. henningsii* to inhibit hydrogen peroxide in

the reaction mixture. From the results, it appeared that activities of the plant extract were nearly the same with the reference compounds. This could be due to the presence of phenolic compounds that donate electron to H$_2$O$_2$ and thus neutralizing it to water (Mathew and Abraham, 2006). Nitric oxide (NO) is a reactive free radical generated from sodium nitroprusside in aqueous solution at physiological pH and reacts with oxygen to form nitrite. It is well known that nitric oxide play an important role in various inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Hazra et al., 2008).. The present study revealed that the extract has a moderate activity in scavenging nitric oxide radical by directly competing with oxygen, nitric oxide and its derivative (Marcocci et al., 1994). Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver damage (Lee et al., 2001). The hepatotoxic effects of this chemical is mostly based on membrane lipid peroxidation. Consequently, leads to the induction of trichloromethyl radical that resulted into severe cell damage (Johnson and Kroening, 1998). In this present study, the rats treated with single dose of CCl$_4$ developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the lipid peroxidation. This indicates the inability of antioxidant defense mechanism in the system to prevent the formation of excessive free radicals. The treatment with aqueous extract of *S. henningsii* was able to reduce the level of lipid peroxides in a dose dependent manner as compared with the hepatotoxic group.

Superoxide dismutase has been reported as one of the most important enzymes in the enzymatic antioxidant defense system (Curtis and Mortiz, 1972). It removes superoxide anion by converting it to hydrogen peroxide, and thus diminishing the toxic effect caused by this radical. The observed decrease in percentage inhibition of superoxide dismutase may be due to the hepatocellular damage by CCl$_4$. However, an increased in the percentage inhibition of superoxide after plant extract administration implies an efficient protective mechanism of this plant. Catalase is another antioxidant enzyme widely distributed in the animal tissues. It decomposes

hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance and Greenstein, 1992). The reduction of activity of this enzyme may lead to deleterious effects as a result of superoxide and hydrogen peroxide assimilation. In the present study, the percentage inhibition of catalase was shown to increase after the administration of the aqueous extract in a dose related pattern. This indicates the hepatoprotective ability of this plant against liver damage.

Reduced glutathione (GSH) is a tripeptide, non enzy-matic biological antioxidant present in the liver. It protects cellular proteins against reactive oxygen species generated from exposure to carbon tetrachloride (Arivazhagan et al., 2000). Decreased level of GSH is associated with increase lipid peroxidation which is also confirmed in this study. The ability of plant extracts to reactivate the hepatic glutathione reductase was reflected by decreasing the level of lipid peroxidation. This result corroborated with earlier report of Bhandarkar and Khan (2004).

In conclusion, present results demonstrate that aqueous bark extract of *S. henningsii* has both *in -vivo* and *-vitro* antioxidant activities due to the presence of phenolic compounds. Moreover, the ability of this plant to prevent the process of initiation and progression of liver diseases may be attributed to the observed result.

ACKNOWLEDGEMENT

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