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Evaluation of Antioxidative Properties of the Different Solvent Extracts of Acacia Nilotica (l.) Leaf

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ABSTRACT

The present study was aimed to investigate the antioxidant properties of the various solvent extracts of the leaves of Acacia nilotica. The antioxidant activities of the extracs have been evaluated by using several in vitro assays and were compared to standard antioxidants such as ascorbic acid, α-tocopherol, curcumin and butylated hydroxyl toluene (BHT). All the extracts showed effective H-donor activity, reducing power, free radical scavenging activity, metal chelating ability and inhibition of β-carotene bleaching. None of the extracts exerted an obvious pro-oxidant activity. The antioxidant property depends upon concentration and increased with increasing amount of the extracts. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extracts. The results obtained in the present study indicate that the leaves of Acacia nilotica are a potential source of natural antioxidant.

1. Introduction

Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA (Fang *et al*., 2002). Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources (Lee *et al*., 2004). A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases (Cuzzocrea *et al*., 2001). There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage and mutagenesis (Grice, 1986; Wichi, 1988). Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals (Formica and Regelson, 1995; Rice-Evans *et al.*, 1997). Hence, nowadays search for natural antioxidant source is gaining much importance. *A. nilotica* is a common, medium sized tree, locally known as 'Babla' belongings to the sub family Mimosaceae. and the most significant genus of family Leguminosae.

The plant is therapeutically used as anti-cancer, antitumor, antiscorbutic, astringent, anti-oxidant, natriuretic, anti-spasmodial, diuretic, intestinal pains, nerve stimulant, cold, congestion, coughs, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis (Sapna *et al*., 2011). The plant is considered to

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be antispasmodic and antidysenteric (Said, 1969). Pods and tender leaves are reported to treat diarrhoea (Nadkarni,1976). The plant has been shown to exhibit antibacterial (Abd *et al*., 1992), anti-inflammatory (Dafallah *et al.,* 1996), antiplatelet aggregatory activity (Shah *et al*., 1997), cestocidal activity (Ghosh *et al*., 1996) antibacterial effects (Sotohy *et al*.,1997),spasmogenic, vasoconstrictor actions (Amos *et al*.,1999), antihypertensive, antispasmodic activities (Gilani *et al*., 1999), inhibitory effect against hepatitis C virus (Hussein *et al*., 2000) and cytotoxic activity (Tezuka *et al*.,2000). There is a limited research has been done to evaluate the potentiality of *A. nilotica* plant.

So, the objective of the present study was to investigate the antioxidant properties of the different solvent extracts of the leaves of *Acacia nilotica*. Therefore, systematic research with medicinal plants may open the new door of many therapeutic choices.

2. Materials and methods

2.1. Collection of plant

The leaves of the plant *A. nilotica* L. were collected from Rajshahi University campus, Bangladesh. It was identified and authenticated in the department of Botany, University of Rajshahi, Rajshahi,Bangladesh.

2.2. Preparation of plant extract

Fresh leaves of the plant were washed under running tap water and air dried for about one week and then homogenized to fine powder and stored in airtight bottle. The powder of leaves (100 gm) were extracted with 100 ml ethanol, methanol, chloroform and petroleum ether using conical flask in a shaking incubator at 28°C for two days. The extracts were filtered and evaporated until dryness. The extracts were stored at 4°C.

2.3. Drugs and chemicals

All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

2.4. Primary Phytochemical screening

Preliminary phytochemical screening of the leaves extracts of *A.nilotica* were carried out.

Antioxidant activity test

2.5. DPPH radical scavenging assay

DPPH assay was carried out according to the method described by Soni *et al.* (2012). The 0.1 ml of extract at various concentration (10, 50, 100 and 100µg/ml) are added to 3 ml of a 0.004% methanol solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Radical Scavenging Activity} = (Ac - As / Ac) \times 100$$

Where Ac= Absorbance of control, As = Absorbance of sample.

Then percentage DPPH radical scavenging activity was plotted against concentration, and from the graph IC_{50} was calculated.

2.6. Reducing power ability

The reducing power was investigated by the Fe^{3+}-Fe^{2+} transformation in the presence of the extracts as described by Fejes *et al*., (2000). The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Meir *et al*., 1995). Butylated hydroxy toluene (6.25-100 µg/ml) was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

2.7. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by the ability of the different extracts of *A. nilotica* leaves to scavenge the hydroxyl radicals generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction) (Halliwell *et al*., 1987; Ilavarasan *et al*., 2005). The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100^0C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Quercetin (6.25-100 µg/ml) was used as a positive control.

2.8. Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the fractions (6.25-100 µg/ml) in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the extracts was determined and the IC_{50} values were compared with the standard, α-tocopherol (Oktay *et al*., 2003).

2.9. Nitric oxide radical scavenging assay

This assay was performed according to the method described by Sreejayan *et al.,* (1997). The absorbance

of the chromophore formed was measured at 546 nm. Inhibition of the nitric oxide generated was measured by comparing the absorbance values of control, extracts and curcumin (6.25-100 µg/ml).

2.10. Thiocyanate method

The peroxy radical scavenging activity was determined by thiocyanate method using α- tocopherol (6.25-100 µg/ml) as standard (Yildirim *et al*., 1999). The percentage scavenging activity was calculated and the IC_{50} values of the extracts were compared with the standard, α-tocopherol.

2.11. Total antioxidant activity

The total antioxidant capacity of the extracts was determined by phosphomolybdate method using α-tocopherol as the standard (Jayaprakasha *et al*., 2002). The total antioxidant capacity was expressed as µg equivalents of α-tocopherol by using the standard tocopherol graph.

2.12. Ferrous chelating ability

The ferrous chelating ability of the extracts was monitored by measuring the formation of the ferrous ion-ferrozine complex. The reaction mixture containing 1.0 ml of different concentrations of the extracts (6.25-100 µg/ml) was mixed with 3.7 ml of methanol, 0.1 ml of 2 mM ferrous chloride and 0.2 ml of 5 mM ferrozine to initiate the reaction and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The percentage chelating effect on ferrozine-Fe^{2+} complex was calculated. The IC_{50} values were compared with ascorbic acid (Huang and Kuo, 2000).

2.13. β-carotene bleaching assay

A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml chloroform and 1.0 ml of this solution was then pipetted out into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was completely evaporated using a vacuum evaporator. Aliquots of 5.0 ml of this emulsion were transferred into a series of tubes containing various concentrations of the extracts (6.25-100 µg/ml) or α tocopherol. The absorbance of the extracts and the standard was measured immediately (t=0) and after 90 min at 470 nm. The tubes were incubated at 50^0C in a water bath during the test. The antioxidant activities (AA) of the samples were evaluated in terms of bleaching of β-carotene using the following formula:

$$AA\ 100 = \frac{1-(A_o - A_t)}{(A_o - A_t)}$$

where A_0 and $A_0^{\bullet}$ are the absorbance values measured at zero time of incubation for test sample and control respectively and A_t and $A_t^{\bullet}$ are the absorbance values of the test sample and control respectively, after incubation for 90 min. (Jayaprakasha *et al*., 2002).

2.14. Pro-oxidant activity

The pro-oxidant activity of the extracts was determined by bleomycin-dependent DNA damage. The reaction mixture (4.5 ml) contained calf thymus DNA (10 µg/ml), 50 µg of 1.0 ml bleomycin sulfate, 1.0 ml of 5 mM magnesium chloride, 1.0 ml of 50 mM ferric chloride and 1.0 ml of different concentrations of the extracts. Ascorbic acid was used as the positive control. The mixture was incubated at 37 ^{o}C for 1 h. The reaction was terminated by the addition of 0.05 ml EDTA (0.1 M). The colour was developed by adding 0.5 ml of 1% w/v thiobarbituric acid (TBA) and 0.5 ml of 25% v/v HCl followed by heating at 37^{o}C for 15 min. After centrifugation, the extent of DNA damage was measured at 532 nm using an UV-spectrophotometer. All the determinations were carried out in triplicate (Ng *et al*., 2003).

2.15. Total antioxidant activity

Total soluble phenolics of the extracts were determined with Folin-Ciocalteu reagent following Gulcin *et al*., (2004) method. The concentration of total phenolic compounds in the extracts was determined as µg pyrocatechol equivalent by using the standard pyrocatechol graph.

2.16. Estimation of total flavonoid content

Total soluble flavonoid content of the extracts was determined with aluminium nitrate following Hsu, (2006) method. The total flavonoid content in the extracts was determined as µg quercetin equivalent by statistical analysis method.

3. **Results**

3.1.Primary phytochemical screening

Primary phytochemical screening of the extracts of the leaves of *A. nilotica* L. revealed the presence of flavonoids, saponins, phenols, tannins, terpenoids, steroids, carbohydrates, glycosides, gum and anthroquinones.

3.2. DPPH assay

All the extracts of *A.nilotica* demonstrated H-donor activity. The highest DPPH radical scavenging activity

was detected in ethanol extract (IC_{50} 0.85 µg/ml), followed by pet-ether, methanol and chloroform extracts (IC_{50} 0.242, 0.245 and 0.278 µg/ml respectively) (Table 1). These activities are less than that of ascorbic acid. The scavenging ability increased towards the pet-ether extract of the solvent.

3.3. Reducing power ability

Table 2 shows the reductive capabilities of different solvent extracts of *A.nilotica* when compared to the standard, BHT. Like the antioxidant activity, the reducing power increased with increasing amount of the extracts. The ethanol extract of *A.nilotica* showed the highest reducing ability (absorbance 22.948667) than all the other extracts tested. However, the activity was less than the standard, BHT (absorbance 3.017). The pet-ether, chloroform and methanol extracts also showed significant activity indicating its reductive ability.

3.4. Hydroxyl radical scavenging assay

Hydroxyl of deoxyribose by the free radicals generated by the Fenton reaction. All the extracts of *A.nilotica* and the standard (quercetin) inhibited the production of hydroxyl radicals. The scavenging activity of the pet-ether extract (IC_{50} 0.141 µg/ml) was higher than that of quercetin (0.308 µg/ml). The IC_{50}s values of the chloroform, methanol and ethanol extracts were 0.348, 0.565 and 0.625 µg/ml respectively (Table 1).

Table 1. Antioxidant activities of the extracts of *Acacia nilotica* L.leaves

Extracts (Solvents and standards)		IC_{50} (µg/ml) DPPH	$OH^{\cdot}$	$NO^{\cdot}$	H_2O_2	Fe^{2+} chelating	Thiocyanate method
Pet-ether	Solvents	0.242	0.141	0.384	0.092	0.393	0.278
Chloroform		0.278	0.348	0.183	0.186	0.276	0.271
Methanol		0.245	0.565	0.553	0.232	0.433	0.405
Ethanol		0.85	0.625	0.370	0.125	0.405	0.460
Ascorbic acid	Standards	0.79				0.017	
Quercetin			0.308				
Curcumin		-		0.078			
α-tocopherol					0.065		0.96

Results are expressed as mean of three parallel measurements. Values within a column followed by ±SD are significantly different (P<0.05).

Table 2. Reducing power ability of different solvent extracts of *A.nilotica* L.leaves

Extracts (Solvents)	Absorbance at 700 nm Concentration (µg/ml) 6.12	12.5	25	50	100
Pet-ether	0.0605±0.0106	0.122±0.0148	0.2625±0.009	0.519±0.0509	0.98±0.028
Chloroform	0.065±0.028	0.0915±0.019	0.182±0.0268	0.2925±0.007	0.499±0.005
Methanol	0.125±0.022	0.306±0.097	0.491±0.149	0.830±0,279	1.479±0.4945
Ethanol	0.533±0.0152	0.833±0.342	1.218±0.3468	1.7136±0.285	2.948±0.914
Standard(BHT)	0.6474±0.0061	1.0533±0.0152	1.5613±0.0807	2.1716±0.0515	3.017±0.0204

Values are expressed as mean± STD of three parallel measurements. *P<0.001 when compared with standard, BHT.

Table 3. Total antioxidant activity (phosphomolybdate method), total phenolic and flavonoid contents of different extracts of *A.nilotica* L. leaves

Extract (Solvents)	Total antioxidant activity(µg vitamin E equivalent/100µg)	Total phenolic content(µg pyrocatechol equivalent/mg)	Total flavonoid content(µg quercetin equivalent)
Pet-ether	54.6±0.989	90.303±0.861	75.539±11.156
Chloroform	36.575±9.510	17.15±6.966	52.906±10.048
Methanol	54.075±3.853	48.951±6.442	106.554±9.162
Ethanol	63.475±0.399	193.958±7.864	114.554±22.114

Values are expressed as mean± STD of three parallel measurements. Values within a column followed by different letters are significantly different (P<0.05).

Table 4. β-Carotene bleaching inhibitory activity of different extracts of *A.nilotica* L. leaves

Extracts (Solvents)	Time of incubation(mm/h)	Absorbance at 470 nm					
		6.12 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	IC_{50}
Pet-ether	0	0.282	0.397	0.562	0.609	0.695	0.068
	90	0.089	0.166	0.295	0.328	0.391	
Chloroform	0	0.075	0.089	0.099	0.119	0.145	0.117
	90	0.047	0.054	0.062	0.080	0.104	
Methanol	0	0.415	0.632	0.713	1.208	1.283	0.099
	90	0.288	0.407	0.447	0.924	0.983	
Ethanol	0	0.515	0.575	0.642	0.715	0.858	0.145
	90	0.455	0.502	0.557	0.622	0.763	
Standard (Ascorbic acid)	0	0.091	0.137	0.189	0.285	0.725	0.140
	90	0.059	0.082	0.138	0.230	0.664	

Values are expressed as mean of three parallel measurements. Values within a column followed by different letters are significantly different (P<0.05).

Table 5. Pro-oxidant activity of different extracts of *A.nilotica* L. leaves

Extracts(Solvents)	Absorbance at 532 nm				
	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Pet-ether	0.153±0.003	0.096±0.001	0.064±0.002	0.044±0.002	0.018±0.001
Chloroform	0.143±0.002	0.083±0.002	0.067±0.002	0.045±0.002	0.035±0.002
Methanol	0.064±0.002	0.045±0.001	0.034±0.002	0.021±0.001	0.017±0.001
Ethanol	0.084±0.001	0.064±0.002	0.041±0.001	0.032±0.001	0.027±0.001
Standard(Ascorbic acid)	0.904±0.002	0.780±0.002	0.511±0.004	0.277±0.005	0.121±0.003

Values are expressed as mean± STD of three parallel measurements. Values within a column followed by different letters are significantly different (P<0.05).

3.5. Hydrogen peroxide scavenging assay

All the extracts of *A.nilotica* scavenged hydrogen peroxide in a concentration-dependent manner (Table-1). The pet-ether extract of *A.nilotica* showed strong H_2O_2 scavenging activity (IC_{50} 0.092 µg/ml) whereas that of the standard, α-tocopherol was 0.065 µg/ml. The ethanol, chloroform and methanol extracts also showed significant scavenging activities (IC_{50} were 0.125, 0.186 and 0.232 µg/ml respectively) when compared to the standard (Table 1).

3.6. Nitric oxide radical scavenging assay

The extracts of *A.nilotica* effectively reduced the generation of nitric oxide from sodium nitroprusside. The chloroform extract showed strong nitric oxide scavenging activity (IC_{50} 0.183 µg/ml) and that of standard curcumin was 0.078 µg/ml. The ethanol extract (0.37 µg/ml), pet-ether extract (0.384 µg/ml) and methanol extract (0.553 µg/ml) also showed good scavenging activities

3.7. Thiocyanate method

The total antioxidant activity of the extracts of *A.nilotica* was determined by the thiocyanate method and compared with the standard, α-tocopherol. The absorbance decreased with the increasing concentrations of the extracts, which indicate that the extracts could effectively decrease the amount of formed peroxides. The total antioxidant activity of the pet-ether and chloroform extracts were almost similar (IC_{50} 0.278 and 0.271 µg/ml respectively) and that of the standard, α-tocopherol was 0.096 µg/ml. The methanol and ethanol extracts also showed good antioxidant activity but at higher concentrations (IC_{50} 0.405 and 0.46 µg/ml respectively).

3.8. Phosphomolybdate method

The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as α-tocopherol equivalents. Among the extracts tested, the ethanol extract contains 63.475µg vitamin E equivalent/ 100 µg. The antioxidant activity increased in the order of ethanol extract > pet-ether extract > methanol extract > chloroform extract.

3.9. Ferrous chelating ability

Addition of the extracts of *A.nilotica* interferes with the ferrous-ferrozine complex and the red colour of the complex decreased with the increasing concentrations of the extracts. All the extracts captured ferrous ions before ferrozine and thus have ferrous chelating ability. Among the extracts tested, the chloroform extract

showed the highest ferrous ion chelating ability (IC_{50} 0.276 µg/ml). The abilities shown by pet-ether, methanol and ethanol extracts were almost similar (IC_{50} 0.393, 0.433 and 0.405 µg/ml respectively). Ascorbic acid (IC_{50} 0.017 µg/ml) showed the highest ferrous ion chelating ability (Table 1).

3.10. β-carotene bleaching assay

Addition of the extracts of *A.nilotica* reduced the discolouration of β-carotene thereby preventing its bleaching. The pet-ether, methanol and chloroform extracts showed strong inhibition on β-carotene bleaching and their IC_{50} values are 0.068, 0.099 and 0.117 µg/ml respectively. These activities were significantly higher than that of α-tocopherol (IC_{50} 0.14 µg/ml). The IC_{50} of the ethanol extract of *A.nilotica* was 0.145 µg/ml, which was similar to that of standard (Table 4).

3.11. Bleomycin-dependent DNA damage

The pro -oxidant activity of the extracts of *A.nilotica* was assessed by their effects on damage to DNA in the presence of a bleomycin-Fe^{3+} complex. The absorbance of all the extracts decreased with increasing concentrations, which proves that none of the extracts exhibited pro-oxidant activity (Table 5).

3.12. Total phenolic and flavonoid content

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the different extracts of *A.nilotica* L.were solvent dependent and expressed as µg pyrocatechol equivalent.. The content of the total phenolics in the extracts decreased in the order of ethanol > pet-ether > methanol > chloroform extracts. On the other hand the total flavonoid content in the extracts was expressed as µg quercetin equivalent. The ethanol extract of *A. nilotica* L.showed highest amount of flavonoids among the extracts tested. The content of total flavonoids in the extracts decreased in the order of ethanol extract> methanol extract > pet-ether extract > chloroform extract.

4. **Discussion**

Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms.

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts (Nanjo *et al.,* 1996). DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. All the extracts showed significantly higher inhibition percentage (stronger hydrogen –donating ability) and positively correlated with total phenolic content.

The transformation of Fe^{3+} into Fe^{2+} in the presence of various extracts was measured to determine the reducing power ability. The antioxidant principles present in the extracts of *A.nilotica* caused the reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

Hydroxyl radical is the most deleterious and reactive among the ROS and it bears the shortest half-life compared with other free radicals. The oxygen derived hydroxyl 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 (Halliwell *et al*., 1987). All the extracts of *A.nilotica* when added to the reaction mixture, scavenged the hydroxyl radicals and prevented the degradation of deoxyribose.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell (Halliwell, 1991). Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the extracts. All the extracts of *A.nilotica* scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralising it into water.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Marcocci *et al.,* 1994). All the extracts of *A. nilotica* leaves decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with $NO^{\cdot}$ thereby inhibiting the generation of nitrite.

The amount of formed peroxides was measured by the thiocyanate method. The extracts were incubated with linoleic emulsion in dark at 37°C and the amount of

peroxides was determined spectrophotometrically by measuring the absorbance at 500 nm (Yen and Chen, 1995). A decrease in absorbance indicated the antioxidant activity of the extracts which might be due to the inactivation of the free radicals and the presence of flavonoid like phytochemicals.

The metal chelating ability of the extracts of *A.nilotica* was measured by the formation of ferrous ion-ferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562 nm (Yamaguchi *et al*., 2000). It was reported that the chelating agents which form σ bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilising the oxidised form of the metal ion (Duh *et al*., 1999). The results of our study demonstrate that the extracts have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal chelating ability of the extracts demonstrated that they reduce the concentration of the catalysing transition metal involved in the peroxidation of lipids.

The β-carotene bleaching assay is a commonly used model to analyze the antioxidant activity of the plant extracts because β-carotene is extremely sensitive to free radical mediated oxidation of linoleic acid. In this assay, oxidation of linoleic acid, an unsaturated fatty acid occurs due to the production of reactive oxygen species formed from halogenated water. The reactive oxygen species will initiate β-carotene oxidation leading to discolouration (Gutierrez *et al*., 2006). All the extracts of *A.nilotica* inhibited β-carotene oxidation, suggesting that the antioxidant activity could be related to high level of phenolic compounds.

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications (Yen *et al*., 1993). The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Flavonoids also a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, anticancer activities. They also inhibit enzymes such as aldose reducatse and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants (Cao *et al.*, 1997). The presence of high phenolic and flavonoid content in the extracts has contributed directly to the antioxidant activity by neutralising the free radicals.

Bleomycin-dependent DNA damage has been adopted as a sensitive and specific method to examine the potential pro-oxidant drugs. Degradation of DNA occur if the samples to be tested reduce the bleomycin-Fe^{3+} to bleomycin-Fe^{2+} resulting in the formation of a product similar to MDA which reacts with TBA to give a pink colour (Liu and Ng, 2000). All the extracts decreased the absorbance and bleomycin-Fe^{3+} is not converted into bleomycin-Fe^{2+}, thereby preventing the DNA degradation. These results confirm that the extracts of *A.nilotica* are devoid of pro-oxidant activity.

5.Conclusions

Based on the results obtained, it may be concluded that all solvent extracts of the leaves of *A.nilotica* L. showed strong antioxidant activity, reducing power ability, free radical scavenging activity, metal chelating ability and inhibition of β-carotene bleaching when compared to standards such as ascorbic acid, α-tocopherol, curcumin, and butylated hydroxytoluene. As the various extracts of *A.nilotica* exhibited different reactive oxygen species scavenging activities, there may be different percentages of phytochemical constituents present in the extracts. Further studies to evaluate the *in vivo* potential of the extracts in various animal models and the isolation and identification of the antioxidant principles in the leaves of *A. nilotica* L. are being carried out.

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