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Short Communication

In vitro evaluation of peroxyl radical scavenging capacity of water extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del

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The present study was planned to confirm the peroxyl radical scavenging capacity of water extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del. in lipid peroxidation assay and results were compared with standard antioxidant (butylated hydroxytoluene). The bark powder of the plant was extracted with different solvents of increasing and decreasing polarity by maceration extraction method and then the water extract was further partitioned with ethyl acetate and water. The scavenging activity of extract was found to increased on fractionating the extract.

Key words: *Acacia nilotica*, lipid peroxidation, peroxyl radicals, phytochemicals.

INTRODUCTION

With the possession of the unpaired electrons, free radicals are usually unstable and highly reactive. Peroxyl radical is a key step in lipid peroxidation and is an important cause of cell membrane destruction and thus tissue damage (Halliwell, 1995). It has also been suggested that oxidative modification of low-density lipoprotein (LDL) is the main cause of atherosclerosis, which is highly related to peroxyl radical formation (Brown and Goldstein, 1983). Antioxidants can scavenge the free radicals and inhibit lipid peroxidation. Antioxidants are added to a variety of foods to prevent or deter free radical-induced lipid oxidation of food (Angelo, 1996). Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer. Previous studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments (Wu et al., 2002). Therefore, many plants have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of action (Swamy and Tan, 2000).

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Keeping this front, the present study was planned to check the peroxyl radical scavenging activity of water extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del. employing lipid peroxidation assay.

MATERIALS AND METHODS

A. nilotica (L.) Willd. Ex Del., is a tree, with tough trunk. The bark is blackish gray or brown and rich in phenolics viz. condensed tannin and phlobatannin, gallic acid, protocatechuic acid pyrocatechol, (+)-catechin, (-) epigallocatechin-7-gallate, and (-) epigallocatechin-5,7-digallate. The bark is used extensively for colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma (Bhargava et al., 1998).

Preparation of extract

The dried and fine powdered bark material was extracted by adding solvents in increasing order of solvent polarity viz. hexane, chloroform, ethyl acetate, acetone, methanol, water and in reverse order. After filtering through folded filter paper (Whatman No.1), the solvent was recovered with vacuum rotary evaporator and this process was repeated thrice by adding solvent again in the residue three times. The crude water extract (C.E) was dried and partitioned by double distilled water and ethyl acetate to obtain water fraction (W.F) and ethyl acetate fraction (EA.F). For checking the antioxidant activity, each extract/fraction was dried and redissolved in methanol.

Chemical analysis

Nuclear magnetic resonance (NMR) spectrum:

The ^{1}H-NMR spectrum was recorded on Bruker AC-200 FTNMR spectrometer machine. The sample for recording NMR spectrum was prepared by dissolving the sample in methanol-d_6. Tetremethylsilane was use as internal standard.

UV spectra:

The UV-visible spectrum of extracts/fractions was recorded in acetonitrile, methanol and double distilled water on UV-1601 spectrophotometer from Shimadzu.

Lipid peroxidation by thiobarbituric acid assay (TBA)

TBA reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm (Halliwell and Guttridge, 1989). Normal male rats (250 g) were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared with homogenizer at 0-4°C with 0.15 M KCl. The homogenate was centrifuged at 8,000 x *g* for 15 min, and clear cell-free supernatant was used for the study with *in vitro* lipid peroxidation assay. Different concentrations (50-700 µg/ml) of extract/fractions dissolved in methanol and in test tubes. 1 ml of 0.15 M KCl and 0.5 mL of rat liver homogenates were added to the test tubes. Peroxidation was initiated by adding 100 µL of 0.2 mM ferric chloride. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2 mL of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.38% TBA, and 0.5% butylated hydroxytoluene. The reaction mixtures were heated at 80 °C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm. The percentage inhibition of lipid peroxidation is calculated by the formula:

Inhibition of lipid peroxidation (%) = 1 – (sample OD/blank OD) X 100

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± SE. The data obtained was statistically analyzed using SPSS for windows standard version (Release 7.5.1) for General Linear Model - General factorial. In order to estimate homogenous subsets for various treatments Post Hoc Test - Tukeys HSD at level of significance $\alpha = 0.05$ was applied.

RESULTS AND DISCUSSION

Chemical analysis

A. nilotica on extraction with water gives crude water extract as blackish brown colored. This powder is slightly soluble in DMSO-d_6 but is freely soluble in methanol. In ^{1}H-NMR spectrum, the integration of signals is directly proportional to their number. So, in discussing the ^{1}H-NMR, the integration of each signal has been given as percent, which represents relative number of protons. Its ^{1}H-NMR spectrum in methanol gives multiplet spread over δ 3.20-3.95 (65%), a region usually for carbohydrates. The ^{1}H-NMR spectrum exhibits broad signals at δ 4.80 (5%) and δ 6.40-6.90 (6%) in electron rich aromatic region, which may be due to phenolic compounds. In upfield aliphatic region also, ^{1}H-NMR spectrum gives peaks (~ 24%) spread over δ 0.80-2.40. These signals show the presence of saturated fatty esters in the extracts. Therefore, crude water extract of *A. nilotica* mainly has carbohydrates (~ 65%) and fatty esters (~ 30%) as well as small amounts of polyphenolics (~ 5%). The UV spectrum of this extract gives λ_{max} at 273 and 200 nm, and a small structureless absorption above 300 nm. This again points towards low concentration of polyphenolics in this extract. TLC of this extract showed two spots at Rf (retention factor) 0.48 and 0.64 when run in solvent system (toluene:ethyl acetate:formic acid :: 45:45:10). The spot at Rf 0.48 was comparable to the spot of authentic sample of gallic acid, which had the same Rf value.

Antioxidant activity

In LDL oxidation, the amount of TBARS, the breakdown product of LDL during lipid peroxidation, can be used as an index of lipid peroxidation. (Ernster, 1993). Our results showed that, the water extract/fractions of *A. nilotica* showed potent inhibition of lipid peroxidation. Table 1 exemplifies the inhibition of LDL oxidation potential of the water extract/fractions prepared in increasing and decreasing order of solvent polarity. The water fraction showed more peroxyl radical scavenging potential (52.14%) than the ethyl acetate fraction (49.55%) and crude water extract (31.42%) at 700 µg/ml concentration. On the contrary, in case of decreasing order of solvent polarity, the pattern of inhibition was little different. In this case ethyl acetate extract exhibited more peroxyl radical scavenging potential (56.68%) than water extract (50.95%) and crude water extract (35.14%) at same concentration i.e. 700 µg/ml concentration.

As phenolic antioxidants are suggested to act as inhibitors of LDL oxidation by means of free radical scavenging, so it is expected that the presence of these compounds in these extract/fractions might be responsible for inhibiting the LDL oxidation by donating the hydrogen atom (Frankel and Meyer, 2000). The main interest of the recent research suggests that LDL oxidation may play an important role in the pathogenesis of atherosclerotic complications, including coronary heart disease (CHD). The radical-mediated oxidative chain reaction is a possible mechanism involved in LDL oxidation. LDL oxidation is believed to be a complex and multistep process involving both lipid and protein fractions through different mechanisms (Marnett, 2000). Antioxidants, including vitamins C and E, flavonoids, and other plant phenolics, have been shown to suppress LDL oxidation and delay the development of heart diseases (Steer et al., 2002). The antioxidant and antiigenotoxic effects of *A. nilotica* have already been elucidated in our

Table 1. The effect of water extract/fractions of *Acacia nilotica* in lipid peroxidation assay.

Conc. of extract (µg/ml)	Increasing polarity			Decreasing polarity			BHT
	C.E	EA.F	W.F	C.E	EA.F	W.F	
50	06.46±0.71^{f}	11.15±1.48^{f}	12.53±0.80^{g}	08.24±0.80^{f}	11.63±0.40^{h}	15.22±0.88^{g}	57.24±0.70^{d}
100	13.27±1.22^{ef}	20.57±1.81^{e}	19.71±1.42^{fg}	14.19±0.79^{e}	19.70±0.85^{g}	23.52±0.83^{f}	89.17±1.29^{c}
200	19.20±0.65^{de}	31.52±0.71^{d}	27.97±1.65^{ef}	19.33±0.79^{d}	27.49±0.84^{f}	31.68±0.73^{e}	98.31±0.69^{ab}
300	23.20±1.77^{cd}	42.65±1.46^{c}	37.70±2.02^{de}	21.31±0.71^{d}	35.23±0.58^{e}	36.61±1.42^{d}	95.80±0.70^{ab}
400	28.72±2.05^{bc}	50.17±1.78^{c}	46.22±2.33^{cd}	28.64±1.06^{c}	39.65±1.24^{d}	39.15±1.09^{d}	97.76±0.92^{ab}
500	31.26±0.95^{abc}	59.91±3.04^{b}	55.23±1.19^{bc}	34.24±0.69^{b}	46.26±0.75^{c}	44.24±1.21^{c}	96.29±0.75^{ab}
600	36.72±2.95^{ab}	66.61±2.40^{ab}	61.76±2.67^{ab}	39.89±0.98^{a}	53.41±0.90^{b}	50.36±0.73^{b}	93.61±1.60^{bc}
700	39.69±2.57^{a}	71.21±1.70^{a}	68.17±3.48^{a}	42.24±0.41^{a}	59.48±0.77^{a}	56.31±0.91^{a}	98.45±0.74^{a}

Data shown are Mean ± SE of three experiments
Means followed by the different letter are significantly different from each other (General factorial - Tukeys HSD at α=0.05).
C.E: Crude water extract.
W.F: Water fraction.
EA.F: Ethyl acetate fraction.
BHT: Butylated hydroxytoluene.

laboratory (Singh et al., 2004; Arora et al., 2005). Further studies are in progress to isolate the antioxidant principle from the extract and fractions using different spectroscopic techniques.

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