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Research Paper

Antioxidant and DNA protection activities of the hill toon, *Cedrela serrata* (Royle) leaves extract and its fractions

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ABSTRACT
Antioxidant activity of methanolic leaves extract of the hill toon, *Cedrela serrata* (Royle) was determined *in vitro* by DPPH free radical scavenging assay (using ascorbic acid as standard) and DNA protection assay. All the analysis was made by using of UV-visible spectrophotometer (DAD 8453, agilent). The antioxidant activity of *C. serrata* leaves extracts was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay in which all of the leaf extracts showed significant activities compared with standard. Inhibitory concentration (IC_{50}) of ascorbic acid was 5.54ppm, whereas, IC_{50} for methanolic extract was 4.39ppm. For n-butanol fraction IC_{50} was <1ppm and for ethyl acetate faction and aqueous fraction IC_{50} was 4.70 and 5.60ppm, respectively. DNA protection against hydroxyl radical from hydrogen peroxide was assayed by agarose gel electrophoresis. Plant has no damaging effects on DNA and was able to reduce the hydroxyl radical-induced DNA damage. It is concluded that extracts have a potential source of antioxidants of natural origin and may act as a chemo-preventative agent, providing antioxidant properties and offering effective protection from free radicals.

Keywords: Antioxidant activity; *C. serrata*; DNA protection; Inhibitory conc.; Free radicals.

INTRODUCTION
Aerobic life on earth depends upon oxygen but it is also involved in a number of toxic chemical reactions. Auto-oxidation occurs when any organic molecule reacts with atmospheric oxygen. Oxidation of lipids of food stuff results rancidity of food. Human physiology also involves a number of oxidation reactions. The continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Tseng, et al., 1997). Oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury, stroke, arteriosclerosis, neurodegenerative and cardiovascular disease as well as for aging process (Willcox, et al., 2004). A potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated diseases (Ames, et al., 1995).

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Recent studies showed that a number of plant products including polyphenolic substances (e.g., flavonoids and tannins) and various plant or herb extracts (Naqvi and Perveen, 1991; 1993) exert potent antioxidant actions (Sheng, et al., 2008; Iqbal, et al., 2009). It is postulated that free radical induced DNA damage is involved in development of renal and hepatic cancer due to Fe overload (Toyokuni, et al., 1994). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super-oxide, peroxyl radicals and hydroxyl radicals and the formation of hydrogen peroxide is involved in the mechanism for free radicals to damage genetic material. These are responsible for balancing oxidation processes in our body and these compounds can be extracted from natural sources or by synthetic processes (Florence, 1995).

The objective of the present study is to evaluate antioxidant activities of hill toon, *Cedrela serrata* (Royle) leaves extracts and fractions (*in vitro*) by the DPPH (1, 1-diphenyl, 2-picrylhydrazyl) and DNA protection assay, as no similar work on antioxidant activity of *C. serrata* has been reported so far.

MATERIALS AND METHODS

Plant material: Leaves of *Cedrela serrata* (Royle) was collected in the month of July, 2010 from Balakot road, Mansehra, Pakistan and was authenticated by Dr. Habib Ahmed, Chairman and Incharge of the herbarium of the Hazara University, Garden Campus, Mansehra-21300, Pakistan, where voucher specimen was deposited with number 6998. They were air-dried at laboratory temperature 25-27ºC and reduced to powdery form using an electric blender. Then concentrated in 80% methanol and dark green gummy residue (30g) was obtained (Perveen, et al., 2010). Fractionations were made according to Rashid, et al., (2009) with some modification that the residue was dissolved in distilled water on the basis of increasing polarity to obtain methanolic extract (ME), n-butanol fraction (NBF), ethyl acetate fraction (EAF), and aqueous fraction (AQF). They were investigated for different biological activities.

Antioxidant activity: For antioxidant activity, the free radical scavenging activity was measured using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) assay according to the procedure described by Kulisic, et al., (2004) and modified by Obeid, et al., (2005). Then 3.2mg DPPH dissolved in 100ml of 82% methanol. When 2800µl of DPPH solution was added to glass vial followed by 200µl of test sample solution, then stock solution was obtained. Further 3 concentrations of 100ppm of stock solution, 10ppm of stock solution and methanol (1:1) and 1ppm of the same solutions (1:3) was obtained in 2 ml final volume. Mixture was shaken well and kept in dark at controlled temperature (25±3ºC) for 1h. Absorbance was measured at 517 nm using spectrophotometer (DAD 8453, Agilent). Mixture of 82% methanol and 100% methanol (14:1) was used as blank (transparent) while DPPH solution and methanol (14:1) were taken as control (dark purple color). The test was performed in triplicate. Inhibition% was measured according to following formula:

$$\% \text{ scavenging effect} = [(Ac-As)/Ac] \times 100$$

- Ac: absorbance of control; As: absorbance of test sample; IC_{50} value was calculated by graphical method.

DNA protection assay: This assay was performed according to Tian and Hua (2005), with little modifications. Three µl (0.5µg/3µl in 50mM phosphate buffer having pH 7.4) of pBR322 plasmid DNA, 4µl of 30% H_2O_2 and 3µl of tested samples were added to make stock solution. Then various concentrations of 1ppm of stock solution and methanol (1:9), 10ppm of the same solutions (1:1) and 100ppm of stock solution were obtained in 1ml final volume. The mixture was incubated at 37ºC for 1h. The control, untreated-DNA, and treated-DNA with 30% H_2O_2 (final volume made up 10µl with 50

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mM phosphate buffer having pH 7.4) were made simultaneously. The mixture was incubated at 37ºC for 1h and then run on 1% agarose gel electrophoresis. DNA bands (super coiled, linear, and open circular) were stained with ethidium bromide and were observed for qualitative analysis by scanning with Doc-IT, VWR. Antioxidant or pro-oxidant effects on DNA were evaluated in the dark on the basis of the increase or loss percentage of super coiled monomer, compared with the control value.

RESULTS

Antioxidant assay rocking: The pro-oxidant and antioxidant effect in ME and fractions of *C. serrata* were checked by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging antioxidant assay. All the extracts showed significant antioxidant activity at all concentrations when ascorbic acid was used as positive control (Table 1). While it's IC_{50} was 5.60ppm (Figure 1).

Table-1: Antioxidant assay by DPPH scavenging activity of different concentrations of all fractions of *Cedrela serrata* as compared to ascorbic acid.

Plant name	Extract*	Scavenging effect (%) at conc. (ppm)		
		100	10	1
Ascorbic acid	standard	95.04	86.45	20.34
C. serrata	ME[1]	70.58	69.57	41.96
	NBF[2]	70.75	58.53	52.06
	EAF[3]	71.94	64.37	27.37
	AQF[4]	67.96	65.04	10.70

- *ME[1]: methanolic extract; NBF[2]: n-butanol fraction; EAF[3]: ethyl acetate fraction; AQF[4]: aqueous fraction.

DNA protection assay: The results of DNA protection assay of *C. serrata* (Figure 2a; b). When plasmid DNA was treated with H_2O_2, supercoiled form completely disappear indicating complete damage to plasmid DNA. All extracts were tested without H_2O_2 for any positive damaging effect on DNA. Total 4 extracts were tested for DNA protection effect with conc. 100, 10 and 1ppm. All 4 extracts showed protection against all dilutions tested especially at lowest concentration. The results indicate that none of the extracts had any damaging effect on plasmid DNA (Table 2).

Table-2: DNA Protection effect of methanolic extract and fractions of *Cedrela serrata*.

Concentration (ppm)	Extract/Fractions of *Cedrela serrata*			
	ME[1]	NBF[1]	EAF[1]	AQF[1]
100	+[2]	++	+	+
10	++[2]	++	++	++
1	+++[2]	+++	+++	+++

- [1]ME: methanolic extract; NBF: n-butanol fraction; EAF: ethyl acetate fraction; AQF: aqueous fraction.
- [2]+: Weak; ++: Moderate; +++: Significant Protection.

DISCUSSION

The leaf extracts *Cedrela serrata* exhibited potent *in vitro* antioxidant activity in DPPH-radical scavenging assay, in comparison to the known antioxidants, such as ascorbic acid. The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, 2, 2-Diphenyl-Picryl Hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). The efficacies of anti-oxidants are often associated with their ability to scavenge stable free radicals (Wang, et al., 1999). The degree of discoloration of DPPH indicates the scavenging potential of the antioxidant compound (Benabadji, et al., 2004).

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Saha et al., (2008a) reported antioxidant potential of the methanol extract of the leaves of *Mimusops elengi* Linn. and found the IC_{50} value of the extract was 43.26 µg/ml, as opposed to that of ascorbic acid (IC_{50}: 5.89µg/ml), which is a well known antioxidant. The present result showed that the IC_{50} value of the methanol extract of the leaves of *C. serrata* was 4.39µg/ml, as compared to that of ascorbic acid (IC_{50}: 5.6 µg/ml). The difference may be due to difference in plant specie or due to different environmental conditions.

Ara and Nur, (2009) showed that methanol leaves extract of *Lippia alba* exhibited a significant dose dependent inhibition of DPPH activity. The IC_{50} value of methanol leaves extract of *L. alba* and ascorbic acid was found to be 34.4µg/ml and 40.8µg/ml, respectively. At the present, it is reported that methanol leaves extract of *C. serrata* exhibited a significant dose dependent inhibition of DPPH activity. The IC_{50} value of methanol leaves extract of *C. serrata* and ascorbic acid were found to be 4.39µg/ml and 5.6µg/ml, respectively. The difference may be due to use of different plant species or different parts of plants used.

Krishnaraju et al., (2009) showed methanol and aqueous extracts exhibited comparable DPPH radical scavenging activity with IC_{50} values 5.25 and 5.33µg/ml, respectively compared to vitamin C (IC_{50}: 4.5µg/ml). At the present, it is reported that methanol and aqueous extracts of *C. serrata* exhibited IC_{50} values 4.39 and 5.6µg/ml, respectively as compared to ascorbic acid (IC_{50}: 5.54µg/ml). Two studies are about similar to each other as far as IC_{50} values are concerned. This may be due to similar method used.

Saha et al., (2008b) shown that methanol extract of *Ixora coccinea* L. exhibited a significant dose dependent inhibition of DPPH activity, with IC_{50} at a concentration of 100.53µg/ml whereas IC_{50} of standard ascorbic acid is 58.92µg/ml. In the present studies, it is reported that IC_{50} of methanolic extract of *C. serrata* is 4.39µg/ml compared to ascorbic acid (5.54µg/ml). The difference may be due to use of different plant species or different environmental conditions.

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS) that are continuously produced in vivo, result in cell death and tissue damage. Hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damage (Halliwell, 1991). Pro-oxidants may represent a threat to health, whereas antioxidants may counteracts these effects by scavenging pro-oxidants (Kool, et al., 2007).

The oxidative damage of DNA is an important mechanism in the initiation of cancer. The damage is usually caused by hydroxyl radicals (Athukorala, et al., 2006). Activity of these radicals can be reduced by natural antioxidants found in plants including herbs (Reddy, et al., 2003). The fenton reaction involves the reaction between hydrogen peroxide and Fe^{2+} to form hydroxyl radicals. Scavengers of hydroxyl radicals inhibit this reaction through the reduction of Fe^{2+} (Halliwell, et al., 1987).

Antioxidant and pro-oxidant effects of crude extract and fractions of selected *C. serrata* was investigated in vitro by using free radical-induced oxidative plasmid pBR322 DNA break system. Results of H_2O_2 induced plasmid pBR322 DNA damage assay showed that DNA protection effect of ME of *C. serrata* showed high DNA protection activity which increases with decrease in conc. of the extracts in the reaction mixture. The ME of *C. serrata* showed maximum protection at 1 ppm while weak protection at 100ppm. In case of fractions, all of the fractions showed DNA protection up to some extent with highest protection at 1 ppm while AQF also showing

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maximum protection at 100ppm as well. It indicates that *C. serrata* showed significant DNA protection ability.

Lin, (2008) found that water extract from the residue of jelly fig achenes (WERJFA) at 200µg total solids/ml concentration was able to reduce the hydroxyl radical-induced damage in calf thymus DNA by approximately 30%. These results suggest that WERJFA protects DNA through the antioxidant activity. In the present result, it is reported that methanol and aqueous fractions of *C. serrata* showed high DNA protection activity which increases with decrease in concentration of the extracts in the reaction mixture. Both the results are similar up to some extent.

Cedrela serrata contains significant amount of flavonoids which is associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals as was found by Rice-Evans et al., (1997) and Jorgensen et al., (1999). It is concluded that *C. serrata* can be used against aging, cancer as well as inflammatory diseases, so further work should be carried out on this plant.

CONCLUSION

Cedrela serrata extracts showed significant antioxidant activity at all concentrations when ascorbic acid was used as positive control. All extracts also showed DNA protection effect against all dilutions tested especially at the lowest concentration. The results indicate that none of the extracts had any damaging effect on plasmid DNA.

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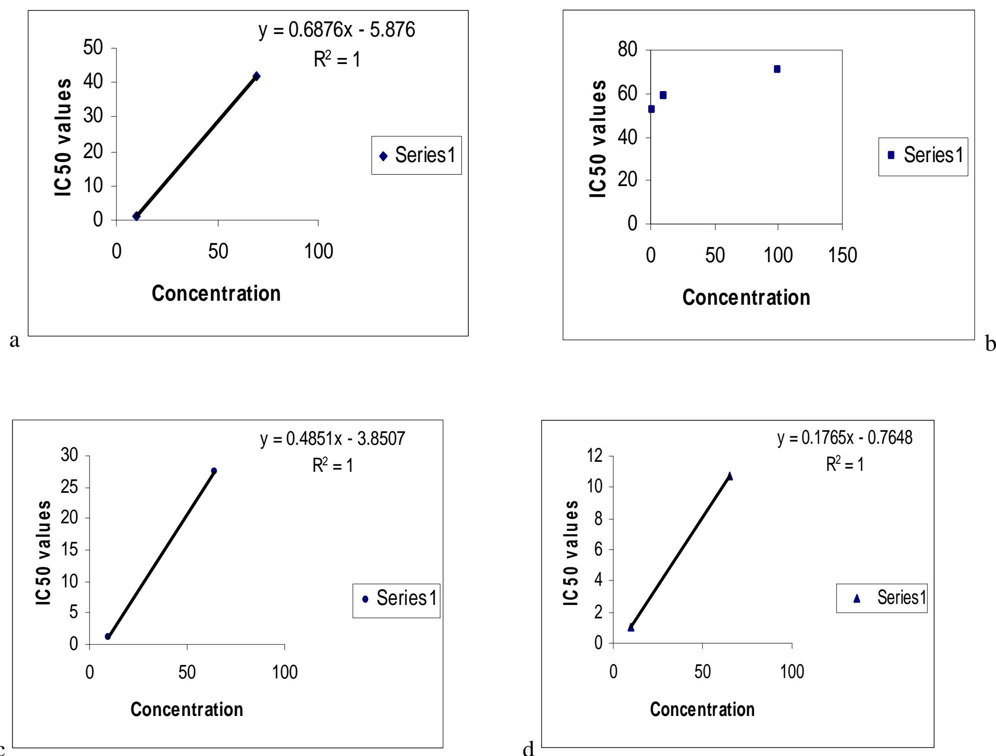
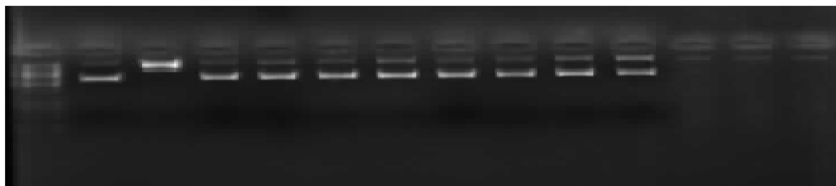


Figure-1: Antioxidant assay of *C. serrata* and inhibitory concentration at 50 ppm.

- a: methanolic extract; b: n-butanol fraction; c: ethyl acetate fraction; and d: aqueous fraction.

a) 1 2 3 4 4a 4b 4c 5 5a 5b 5c



b) 1 2 3 6 6a 6b 6c 7 7a 7b 7c

Figure-2: Agarose-gel electrophoretic patterns of plasmid DNA breaks by OH generated from fenton reaction in the presence of *Cedrela serrata*.

- Half µg of pBR322 DNA was incubated at 37°C for 1 h with 3µl 30% H_2O_2 and the following additives: Lane 1: 1kb DNA Ladder; 2: Plasmid DNA (pBR322); 3: Plasmid DNA treated with H_20_2; a: 4: n-butanol fraction (NBF) + plasmid; 4a: NBF100; 4b: NBF10; 4c: NBF1; 5: methanolic extract (ME) + plasmid; 5a: ME100; 5b: ME10, and 5c: ME1. b: 6: ethyl acetate fraction (EAF) + plasmid; 6a: EAF100; 6b: EAF10; 6c: EAF1; 7: aqueous fraction (AQF) + plasmid; 7a: AQF100; 7b: AQF10, and 7c: AQF1.

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