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In Vitro Antioxidant Activity of Different Extract of Bark of *Juglans Regia*

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ABSTRACT

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing. etc. In the treatment of these diseases, antioxidant therapy has gained a great importance. Different extract (Hot water, Hydro alcoholic, Chloroform and Petroleum Ether) of *Juglans regia* bark was studied for its in vitro antioxidant activity using different models viz. DPPH radical scavenging, ABTS radical scavenging, FRAP assay and Superoxide Radical Scavenging Assay. The results were analyzed statistically by the regression method. Its antioxidant activity was estimated by IC50 value. The results showed that the Hot water and Hydro alcoholic extracts of *Juglans regia* showed potent antioxidant activity than the Chloroform and Petroleum Ether extracts. The antioxidant potency of different extracts has been expressed as in order of RGHW>RGHA>RGCH>RGPEt In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. These results clearly indicate that *Juglans regia* is effective against free radical mediated disease.

Key words: DPPH, FRAP, *Juglans regia*, Superoxide.

INTRODUCTION

Free radicals [reactive oxygen species (ROS)] are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, these radicals can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer (Ames B, 1998; Cox DA *et al*., 1996; Finkel T *et al*., 2000). Although many anti-oxidant defense systems consisting of enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and nonenzymatic (ascorbic acid, glutathione and α-tocopherol) compounds can maintain the balance between free radical generation and protection from damage by these redicals but these anti-oxidant systems do not provide complete protection from against ROS under conditions of severe oxidative stress (Cesaratto L *et al*., 2004). Walnut (*Juglans regia*, Fam. Juglandaceae) is a large, deciduous tree attaining heights of 25–35 m, and a trunk up to 2 m diameter, commonly with a short trunk and broad crown, though taller and narrower in dense forest competition (Asgary S *et al*., 2008). It is a light-demanding plant requiring full sun to grow well. The bark is smooth, olive-brown when young and silvery-grey on older branches, and features scattered broad fissures with a rougher texture. In traditional system of medicine Walnut is used for its antioxidant, antiproliferative (Wang YN *et al*., 2009), antiatherogenic, osteoblastic, cardioprotection, lipid metabolism, suppresses functional insufficiency of liver (Dzhafarova RE *et al*., 2009; Negi AS *et al*., 2011), links synthesizing enzymes, increases the antitoxic action of hepatocytes and improves the functional insufficiency of kidneys (Cruz-Vega DE *et al*., 2008). Walnut also effective against Mycobacterium tuberculosis. The aim of this study is to investigate antioxidant activity of different extract of bark of *Juglans regia*.

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MATERIAL & METHODS

Preparation of plant extracts:

Samples of *Juglans regia* (JR) were collected from the district. Almora, Dehradun, UK & identified by Department of Botany, FRI, Dehradun. The freshly cut bark were dried in the drying room with active ventilation

at ambient temperature. 500g of powdered form bark of Juglans regia.(JR) has taken. Fractionation has been done according to increase polarity as Petroleum ether, Chloroform, Hydro alcoholic and Hot water. The obtained extract was filtered and evaporated by using vacuum evaporator under 40C to give different %yield [JRPEt: 13.92gm, JRCH: 19.68gm, JRHA: 42.6gm, JRHW: 163.32gm. of dried crude extract.

DETERMINATION OF ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF THE JUGLANS REGIA *IN VITRO*:

DPPH Radical Scavenging Assay:

To the ethanolic solution of DPPH (100µM) an equal volume of test drug was added, dissolved in distilled water at various concentrations (10µg to 50µg/ml). After incubation for 20 minutes at room temperature absorbance was recorded at 517nm. Blank was carried out in the same manner without the drug. For rate kinetic study absorbance was taken immediate after addition of DPPH up to 500 seconds. The experiment was conducted in triplicate.

$$\text{DPPH Scavenged (\%)} = \frac{A_{CONTROL} - A_{TEST}}{A_{CONTROL}} \times 100$$

Where Acontrol is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the *Juglans regia* extracts were expressed comparing with standard (Akhtar N *et al*., 2009).

ABTS Radical Cation decolonization Assay

To the reaction mixture containing 0.3 ml of ABTS radical, test compound was added at various concentrations from 10µg to 500 µg/ml in suitable solvent in a final volume of 2.5 ml make up with phosphate buffer, pH 7.4 (20mM). Blank was carried out without drug. Absorbance was recorded at 734nm immediate after the addition of ABTS radical. The experiment was repeated thrice in triplicate.

Ferric Reducing Antioxidant Potential (FRAP) Assay

Various concentrations of the bark extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

$$\text{\% increase in Reducing Power} = \frac{A_{TEST}}{A_{BLANK}} - 1 \times 100$$

Where A_{Test} is absorbance of test solution; A_{Blank} is absorbance of blank. The antioxidant activity of the *Juglans regia* extracts were compared with the standard.

Superoxide Radical Scavenging Assay

To the reaction mixture containing 0.5 ml of various concentrations of drug (10µg to 500µg /ml), 0.2 ml of NBT and 1ml of alkaline DMSO were added. Blank was carried without drug, only DMSO in was added. Absorbance was recorded at 560 nm. The experiment was repeated thrice in triplicate.

RESULTS AND DISCUSSION

Several concentrations ranging from 10–600 µg/ml of all four extract of *Juglans regia* were tested for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC50 values were calculated for all models given in table.1.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing (Velioglu YS *et al*., 1998). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm (Ganapaty S *et al*., 2007) (Fig.1).

The antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies (Evans C *et al*., 1997) (Fig.2).

The potency of ferric ion reducing antioxidant potency has been observed. The result indicated free radical scavenging activity of the extracts in dose dependent manners, which are shown in (Fig.3) (Papoutsi Z *et al*., 2008).

Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse the breakdown of superoxide radical (Shirwaiar A *et al*., 2007). In our study, alkaline DMSO used for superoxide generation indicates that J.regia is a potent superoxide scavenger (Fig.4).

Figure.1 Dose response curve of DPPH radical scavenging assay of various extracts isolated from Juglans regia

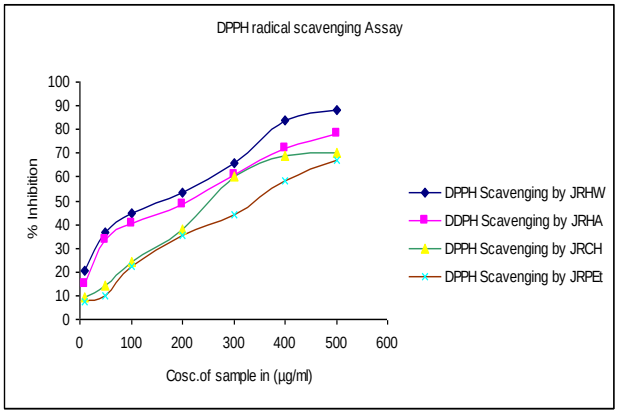


Table.1 Comparison of IC_{50} values of different extracts of Bark of *Juglans regia*

SNo.	Model	IC_{50} value of Hot Water extract (µg/ml)	IC_{50} value of Hydro alcoholic extract (µg/ml)	IC_{50} value of Chloroform extract (µg/ml)	IC_{50} value of Peroleum Ether extract (µg/ml)
1	DPPH Radical Scavenging Assay	182.6	238.6	274.3	347.4
2	ABTS Radical Cation decolonization Assay	148.8	224.6	288.4	327.2
3	Ferric Reducing Antioxidant Potential Assay	98.8	204.3	288.6	336.3
4	Superoxide Radical Scavenging Assay	201.8	254.4	301.6	499.3

Figure.2 Dose Response Curves of ABTS cation scavenging activity of various extract fractions isolated from *Juglans regia*

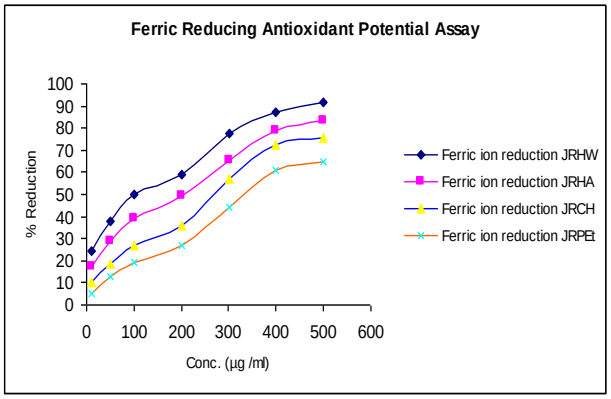


Figure.3 Dose Response Curves of reducing power of various extract fractions isolated from *Juglans regia*

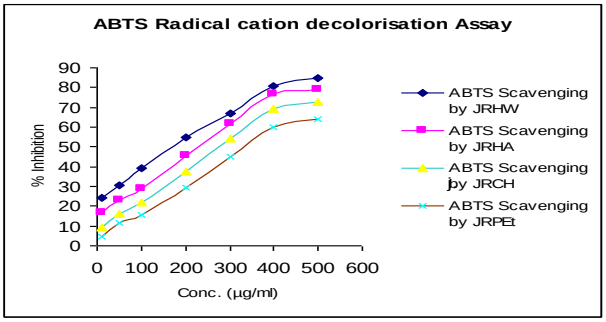
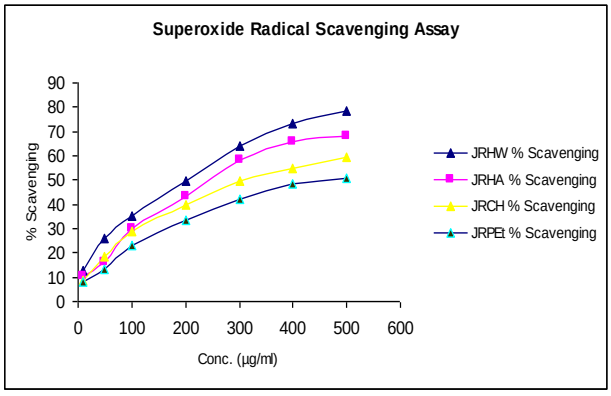


Figure.4 Dose Response Curve of Superoxide Radical Scavenging Assay of various extracts isolated from *Juglans regia*



CONCLUSION

The results of the present study show that the extract of *Juglans regia* exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. This observation also shows presence of a great amount of polyphenols.

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