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Antioxidant potential from stem bark of *Juglans regia* L.

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ABSTRACT

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. The role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may cause many diseases including cancer. Juglans regia L. is a valuable medicinal plant. Its root, stem, bark, leaves, fruits, seeds and seed oil are applied in traditional medicines to cure various health complaints. The solvent extracts of stem bark exhibit indicative to prominent antimicrobial activity. Antioxidant potential of various extracts is determined using spectrphotometric methods. Results of DPPH and Nitric Oxide assay confirm that extracts obtained from stem bark of J. regia L. possess significant antioxidant property.

Key words: *Juglans regia* L., Antioxidant potential, DPPH, Nitric Oxide, Spectrophotometer.

INTRODUCTION

Living cells produce different reactive oxygen species (ROS) such as superoxide, hydroxyl, peroxyl free radicals, singlet oxygen and hydrogen peroxide molecules. In living cells, ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanism [1-3]. Attack of ROS upon proteins produces carbonyls and other amino acid modifications that cause disorders of protein functions. ROS has also damage effects on the base and sugar units in DNA strand [4, 5]. Consequently accumulation of potentially harmful ROSs causes increase in stress, disease and aging periods and there is loss of haemostatic control and organ functions [6]. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT) have restricted use in food as they are under great consideration for toxicological reasons [7]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food. The

role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may cause diseases including cancer. Many plant phenols, flavonols etc. other than antioxidant vitamins such as: C, E and carotenoids exert powerful antioxidant effects [7]. In recent years, the search for natural antioxidants, especially plant origin has greatly increased [8].

Juglans regia L. is the medicinally useful species from family Juglandaceae. It is a frost tender deciduous tree growing up to 40-60m. It is found in the Himalayan regions in India. All parts of the plant; stem, bark, leaves, fruits, seeds, seed oil are used in folk medicines to treat variety of health disorders including cancer [9]. Decoction of the stem bark is useful in dental complaints [10]. It is reported that leaves of *J. regia* L. contain monoterpenes and sesquiterpenes whereas the bark shows presence of juglone, regiolone, sterols and flavonoids [11]. Quantification of phenolics and flavonoids from stem bark extracts (non-polar to polar solvents) using spectrophotometric method exhibited their higher percentage which is already mentioned [12]. Taking into consideration all these facts an attempt is made to evaluate the antioxidant activity of the extracts of *J. regia* L. stem bark using DPPH assay and Nitric Oxide method.

MATERIALS AND METHODS

1, 1-diphenyl-2-picrylhydrazyl (DPPH), sulphanilamide, naphthyl ethylenediamine dihydrochloride were obtained from Sigma Chemicals Co., USA. All other chemicals and reagents were of analytical grade.

UV Spectrophotometer (UV-VIS1700Pharma Spectrophotometer Schimadzu) was used to measure the absorbance at various concentrations of the extracts under study.

Plant material:
The plant material (stem bark) of the species was collected from local market. Its authentication was performed at Agharkar Research Institution (ARI), Pune, Maharashtra, India. The voucher specimen No. is 14319.

Preparation of extracts:
Air shade dried, finely pulverized and exactly weighed plant material was utilized to prepare extracts with measured volumes of solvents as ethyl acetate, acetone, ethanol, methanol and distilled water. Solvents were recovered under reduced pressure to obtain the crude extracts. Quath extract was prepared using standard conventional method. Exactly weighed amounts of dried extracts were dissolved in known volume of methanol and various aliquots of each extract were prepared and used for the DPPH and Nitric Oxide assays.

DPPH radical scavenging activity[13]:
1, 1-diphenyl -2-picryl-hydrazyl (DPPH) is converted to 1, 1-diphenyl -2-picryl hydrazine when it reacts with antioxidants. A change in color from purple to yellow is observed. Aliquots of extract solutions were taken and a total volume of 3ml was made using methanol. 0.15ml of freshly prepared DPPH solution (98µg/ml) was added, stirred and left to stand at room temperature (27^0C) for 30 minutes in dark. The control contains only DPPH solution in methanol while methanol served as the blank (negative control).The reduction capability of DPPH radicals

was determined by the decrease in its absorbance. Absorbance was noted at 517nm by using UV-VIS spectrophotometer.

Nitric Oxide scavenging activity[14]:
In this spectrophotometric method the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylenediamine dihydrochloride was measured. Sodium nitroprusside (SNP-5mM) in phosphate-buffer saline was mixed with an equivalent amount of methanol to get the control. Methanol served as blank. Methanol was added to test solutions at different concentrations to make up a volume of 3ml and incubated at room temperature (27^0C) for 90 minutes. This incubated solution (1.5 ml) was added to 1.5 ml of Greiss Reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyethylenediamine dihydrochloride). Absorbance at 546nm was noted using UV –VIS spectrophotometer.

In both methods the capacity of scavenging free radicals was calculated as follows:

Scavenging activity (%) = {(Control Abs.—Sample Abs.)/Control Abs} × 100

Ascorbic acid was used as the reference compound (positive control) with concentrations20 to 500µg/ml for both the above spectroscopic methods of evaluating the radical scavenging activity.

RESULTS AND DISCUSSION

Freshly prepared extracts of the dried plant material were subjected to screening for their possible antioxidant activities. For this purpose, DPPH free radical scavenging activity and Nitric Oxide scavenging methods using UV- VIS spectrophotometer were employed. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract [15].

Nitric Oxide (NO) is a diffusible free radical that plays many effective roles in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities [16]. Nitric oxide is generated from the decomposition of SNP and measured by Greiss Reagent.SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be measured by the use of Greiss reagent. A significant decrease in the NO radical is due to the scavenging activity of the extracts.

At the range of concentration under study, ascorbic acid exhibited 90.16% inhibition; acetone extract exhibited higher radical scavenging activity than all other extracts by DPPH assay and by Nitric oxide method but it is lower than ascorbic acid. IC_{50} values were calculated from plotted graphs of scavenging activity against the concentrations of samples. The values of IC_{50} for each extract are reported in Table-1.

Fig-1:

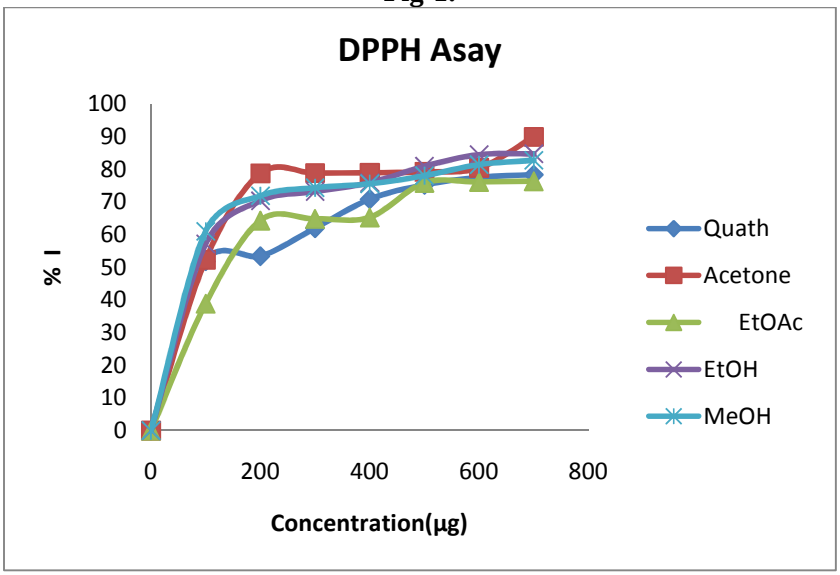

Fig-2:

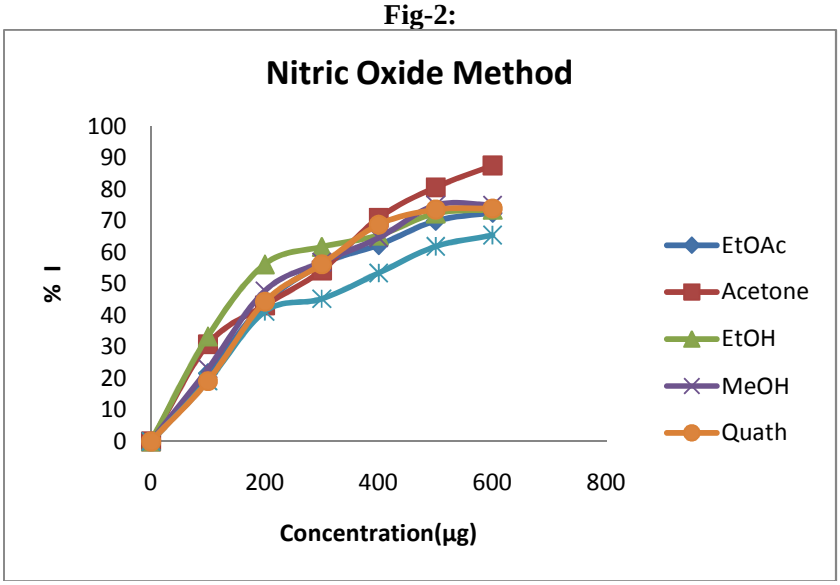

Table: 1- IC_{50} values of Extracts of *J. regia* L.

Sr. No.	Extract	DPPH assay	Nitric Oxide Method
1	EtOAc Extract	352.9	316.3
2	Acetone Extract	38.4	41.8
3	Ethanol Extract	186.5	179.6
4	Methanol Extract	187.7	190.4
5	Aqueous Extract	322.0	286.2
6	Quath Extract	227.1	206.2

Graphical presentation of the concentration against % I values of extracts for DPPH and Nitric Oxide methods is mentioned (Fig-1 and Fig-2).

CONCLUSION

The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc [17]. All extracts exhibit higher range of the radical scavenging activity. It means these extracts are rich in flavonoids as well as phenolic compounds which along with other polyphenolics in the plant material may be responsible for the antioxidant activities of these extracts. Above results strongly support the antioxidant potential of *J. regia L a*nd its importance as a rich source of natural antioxidants.

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REFERENCES

[1] B. Halliwerll, J. M. C. Gutteridge, Freeradicals in Biology and Medicine, Oxford University Press, Oxford, pp. 327-342**(1999)**.
[2] B. Halliwell, *Am. J.Med*. 30,145**(1991).**
[3] B. Halliwerll, J. M. C. Gutteridge, C. E. Cross, *J. Lab. Clin. Med*.,199, 598 **(1992).**
[4] M. Su. Y. Yang and G. Yang, *FEBS. Lett*., 580, 4136 **(2006)**.
[5] C. Chatgllialoglu and P. O'Neill, *Exp. Gerontol*., 36, 1459 **(2001)**.
[6] B. Halliwell, *Biochem. Soc. Symp*., 61, 73 **(1995)**.
[7] D. L. Madavi and D. K. Salunke, in eds.: S. S. Deshpande and D. K. Salunkhe, Toxicological Aspects of Food Antioxidants, Food Antioxidants, Marcel Dekker Inc. New York, pp.267 **(1995).**
[8] G. K. Jayaprakasha and L. J. Rao, *Zeitset Naturforsch*, 55C, 1018 **(2000)**.
[9] Bown D. Encyclopedia of Herbs and their uses. Darling Kinderley, London **1995** ISBN O-7513-020-3.
[10] Duke J. A. Handbook of Energy Crops, (269), **1983).**
[11] Inbaraj and Chignell, *Toxicology and Applied Pharmacology*, Vol.209, Issue1, pp-1-9, 15 NOV **2005**.
[12] Kale A., Gaikwad S., Mundhe K, Deshpande N R, Salvekar J P , *Int. J. Pharma and Biosci*., Vol. 1, Issue3, Jul-Sep**2010**.
[13] Stanojevic L., Sanojevic M., Nikolic V., Nikolic L., Ristic D, Canadanovic-Brunet J, Tumbas V, *Sensors*, 9, pp 5702-5714 **(2009)**.
[14] Madan M. P., G. Raghavan, A. K. Singh and P. Palpu, *Acta. Pharm*., 55, pp 297-304 **(2005).**
[15] Ghafar MFA, Nagendra Prasad K, Weng kk, Ismail A, *African J. Biotechnology*, 9, pp 326-330, **2010.**
[16] Haggerman AE, Riedl KM, Jones GA, Sovik RN,Ritchard NT and Hartzfeld PW, *J. Agric. And Food Chem*., 46, pp.1887-1892 **1998**.
[17] Miller A. L., *Alt. Med. Rev*., 1:103-111.