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QUANTIFICATION OF PHENOLICS AND FLAVONOIDS BY SPECTROPHOTOMETER FROM -*JUGLANS regia*

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ABSTRACT

Natural antioxidants such as dietary plant phenolics and flavonoids are increasingly attracting attention. They are natural disease-preventing, health-promoting, and anti-ageing substances. Medicinally useful royal Juglans species is worth studying for its traditional therapeutic applications as anticancer, diuretic, anti-inflammatory and laxative effects. It is a rich source of antioxidants i.e. phenolics and flavonoids. Present study involves spectrophotometric determination of phenolics and flavonoids content from chloroform, ethyl acetate, ethanolic and methanolic extracts of walnut bark using Folin-Ciocalteau reagent and Quercetin as standard. The results show high contents of phenolics and flavonoids supporting the antioxidant activity of the extracts and suggest need for further investigations to isolate secondary metabolites.

KEY-WORDS: Phenolics, Flavonoids, Folin-Ciocalteau, Quercetin, *Juglans regia*.

INTRODUCTION:

Antioxidants are vital in combating the free radicals which damage human cells under 'oxidative stress' conditions and an imbalance of free radicals may cause grave disturbances in cell metabolism. Oxidative stress conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing, and inflammatory activities [1, 2] . Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom occurring in all parts of the plants. They offer resistance to diseases, Phenolics effect to reduce risk of cancer. Higher the phenolics content stronger is the antioxidant activity. Total phenolics content of the plant material is determined by conventional chemical method as well as spectrophotometer using Folin-Ciocalteau reagent. [3, 4].

Natural antioxidants such as dietary plant flavonoids have an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant and anti-cancer agents [5]. Flavonoids are plant secondary metabolites widely

distributed in the plant kingdom. More than 6000 flavonoids have been identified in plants[6].
The royal Juglans species of Junglandaceae family is well known for its use in traditional medicines all over the world. All parts of the plant are medicinally useful for curing various health disorders. Some extracts of leaves are reported to show anticancer activity[7]. Literature survey reveals that amounts of phenolic compounds from fruit kernel and green husk of Juglans species are determined. The Antioxidant potential of aqueous extract of walnut bark along with phenolics and flavonoids contents and its modulatory effect on cyclophosphamide-induced urotoxicity in Swiss albino male mice has been studied[8]. Walnut (*Juglans regia* L.) bark has been claimed to possess anti-inflammatory, blood purifying, anticancer, diuretic and laxative activities. It contains several therapeutically active constituents. [5]. According to Hartwell (1967-1971), the English walnuts are used in folk medicines especially cancerous conditions of breast, epithelium, gullet, intestine, kidneys, liver, mouth, stomach, throat and uterus. Preliminary phytochemical analysis of the plant revealed the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids.

Present study involves spectrophotometric estimation of phenolics and flavonoids content from chloroform, ethyl acetate, ethanolic and methanolic extracts of walnut stem bark. From the standard curve their concentration in the test samples were calculated.

MATERIALS AND METHOD:

Finely powdered and air shade-dried plant material was taken for experiments. Folin-Ciocalteau reagent, Catechol, Quercetin and all other chemicals used were from Merck.

The UV spectrophotometer (UV-VIsS1700Pharma Spectrophotometer Schimadzu) was used for the measurement of absorbance at various concentrations of the extracts under study. Exactly weighed sample powder was ground with a pestle and mortar in the measured volume of solvents chloroform, ethyl acetate, ethanol and methanol to prepare respective extracts. The extract was filtered through a Whatman Filter paper. Each extract was prepared just before the experiment so as to prevent any further degradation.

Total Phenols determination:

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue).According to this principle various concentrations of the prepared extracts when react with the Folin-Ciocalteau reagent and 20% Na_2CO_3 solution give shades of blue colour. The absorbance measured at 650nm.is represented A standard curve using different concentrations of catechol was drawn from which the concentration of phenols in the test samples was calculated and expressed as mg phenols/g material.

Total flavonoids determination:[9]
Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Each plant extracts (0.5 ml of1:10 g ml-1) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm The calibration curve was obtained by preparing Quercetin solutions at concentrations 12.5 to 100 g/ml

RESULTS AND DISCUSSION:

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler etal., 2003, Cook and Samman, 1996). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992).

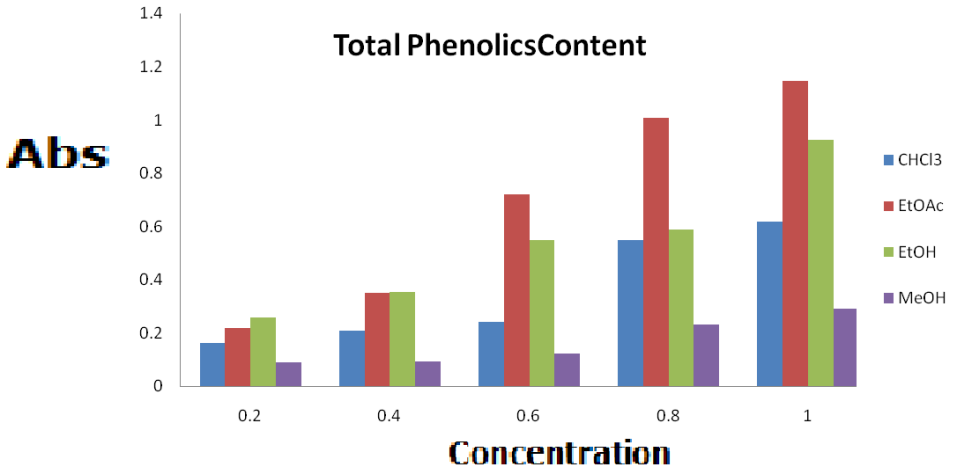
The result of the present study showed that the flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y =0.0307x + 0.0035) were between 3.50 to 32.81.Total phenols that were measured by Folin Ciocalteu reagent equivalent (standard curve equation: y = 0.0275x +0.0278,). The total phenol varied from 20.32 to 44.87mg/g in the extract powder.The ethyl acetate extract of the plant material shows higher phenolic and flavonoid content than chloroform, ethanol and methanol extracts. Hence bioactive antioxidant components can be isolated by further separation of ethyl acetate extract. The high scavenging property of *Juglans regia* may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. The results indicate that the plant material may become an important source of compounds with health protective potential.

Table 1

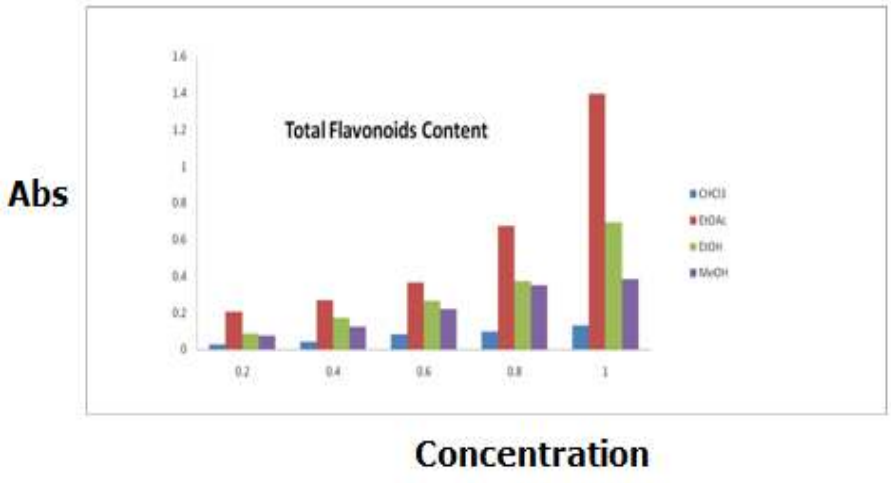
Flavonoid and phenol contents in the plant extracts.

Extract	Flavonoid(mg/g)	Phenol (mg/g)
Chloroform	3.50	34.50
Ethyl acetate	32.81	44.87
Ethanol	13.59.	36.78
Methanol	.11.48	20.32

Each value in the table was obtained by calculating the average ofthree experiments ± standard deviation



GRAPH-I



GRAPH-II

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