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Phytochemical Analysis and Antioxidant Activity of *Juglans Regia L.*

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Abstract

Free radicals are the primary cause of the majority of degenerative diseases. The substances that collect free radicals are antioxidants. The main intent of current research was to make an inquiry into the phyto-chemical & in-vitro anti-oxidant profile of fruit husk of Juglans regia extract. The technique used to put to the test the antioxidant abilities of extracts included the DPPH assay, superoxide radical scavenging, ferric thiocyanate (FTC], and hydroxyl radical scavenging assay. Preliminary phytochemical analysis was screened using standard methods. Phytochemical investigation found that it contains carbohydrates, alkaloids, terpenoids, tannins, flavonoids, glycosides and saponins. Methanolic extract of Juglans regia showed better antioxidant capability than ethyl acetate extract. According to the results of this study, Juglans regia fruit husks may be a reliable source of natural antioxidants.

Keywords

Ethyl acetate extract, flavonoids, Juglans regia, Scavenging, terpenoids.

INTRODUCTION

The *Juglandaceae* family comprise multiple genera, among them the most notable is Juglans. The *Juglans regia* is a premium tree that was extensively cultivated, because of its excellent fruits & timber. The nut is of considerable commercial relevance to food sector, and it is valued and treasured around the world for its beneficial nutritional, medicinal, and sensory properties [1]. This royal species, which grows up to 25–35 meters in the Kashmir region, is a product of the Himalaya's rich biodiversity.

Juglans regia has traditionally been used to manage wide range of medical issues, such as tumors, inflammation, type 2 diabetes, antiradicalar, hyperhidrosis, antidiarrhea, prostate, and heart disease [2] [3] [4]. On the other hand, researchers discovered that practically all components of the plant are beneficial in fighting various diseases as well as preserving food grains. *Juglans regia* nut extracts prevented oxidative damage, [5] [6] [7] [8] [9] tumour growth, inflammation, anti-wrinkle, and pre-mature ageing. Nuts as a dietary meal, against some skin illnesses, hypoxia, diabetes, and inflammation [10] [11]; leaves as an antidiarrheal, anthelmintic, depurative [12] and pesticide and fungicide [12] [13] when mixed with stored grains. Astringent, depurative, digestive, anthelmintic, bactericide, laxative, diuretic, detergent, stimulant, and insecticidal [14] properties of stem bark. *Juglans regia* L. shell has been used to clean gun barrels, jewels, and metal stuff, as well as crude oil segregation & water [15].

Juglans regia L. contains cardiac glycosides, steroids, polyphenols, tannins, flavonoids, carbohydrates, dietary fibre, flavonols, fatty acids, plant sterols, minerals, melatonin, folate, protein, tocopherol, tannins, vitamin C, vitamin A, and vitamin E compound [16] [17]. Numerous investigations have shown that phenolic extracts have antibacterial activity, making them an excellent alternative to medications and food preservative agents. *Juglans regia* is a plant-based item that has an important effect on the food industry. It is appreciated for its nutritive value and sensory qualities and is frequently consumed as royal food throughout the world. Because of antibiotic resistance, there is a growing interest in employing natural antibacterial substances. There hasn't been any published research on the phytochemical analysis, anti-oxidant, or antibacterial properties of the fruit husk of *Juglans regia* from the Himalayan region, despite the fact that many parts of this plant have been researched for their biological qualities. The goal of this research is to inspect the phytochemical analyses and antioxidant activities of *Juglans regia* L. fruit husk and its potential application in food items.

MATERIAL AND METHOD

Reagents and Chemicals

Petroleum ether and methanol were brought from Avantor Performance Materials (RANKEM) Pvt. Ltd., Gurgaon, India. All other chemicals, solvents, and reagents utilised were of the laboratory reagent calibre.

Plant Material Collection

The fruit husk of *Juglans regia* was collected from Shangus, Anantnag, Kashmir in the month of August-2021 and were identified at the Centre for Biodiversity, University of Kashmir and endorsed by Akther H. Malik (Jr. Scientist and curator) for future use. The voucher specimen has been

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maintained in the KASH Herbarium at the University of Kashmir under voucher specimen number 4312-KASH Herbarium, Centre for Biodiversity and Taxonomy.

Extraction

Cold Maceration

The essential specimens of plants were gathered, cleaned, and dried. Cold maceration was used to remove the plant material. Various organic solvents with different polarity, such as Pet. Ether, ethyl-acetate (EA) and methanol were used to extract dried plant powder and each was allowed for four to five days. The extract was cleaned of all non-extractable material. After removing excess moisture, the extract was transferred to a beaker and evaporated before being collected in an airtight vessel. All of the extracts' yields from extraction were calculated.

Phytochemical Screening

Introductory investigation tests for alkaloids, flavonoids, sterols, tannins, and other natural compounds were executed out as a result of those earlier reported methods in order to analyse the distinct classes of natural compounds in the methanol and ethyl acetate extracts [18].

***In-vitro* anti-oxidant activities**

DPPH radical scavenging activity

The antioxidant ability of plant extracts was measured *in vitro* using the DPPH free radical scavenging test. A 0.1 mM concentration of DPPH in the methanol was first produced, 2 ml of the resulting solution was then mixed with various doses of 1 mg/ml methanolic raw extract. The resulting mixture were then infused and allowed to stand at room temperature for almost 30 minutes. Then, using a spectrophotometric determination of absorbance at 517 nm, the percentage of inhibition was determined using the below given equation;

% DPPH-free radical scavenging activity = $([A_0–A_1]/A_0) \times 100$.

A0 and A1 denote the absorption rate of a control substance or a blank and the absorption of a plant extract or a positive control, respectively. The chart for the inhibitory level 50% (IC50) was then constructed by plotting the percentage of activity for scavenging against log concentration [19].

Super-oxide anion radical scavenging assay

The method developed by M. Nishikimi *et al.* was used to calculate the superoxide anion scavenging activity [20]. One millilitre of nitro-blue tetrazolium (NBT) (100 µl of nitro-blue tetrazolium in 100 mM of phosphate buffer having pH 7.4), one millilitre of NADH (468 µl in 100 mM of the phosphate buffer with pH 7.4), and various proportions of extracts (20, 40, 60, 80, and 100 g/ml) make up the resulting composition. The resulting mixture were incubated at 30°C for 15 min. Absorbance of the samples were recorded at 560 nm against blank samples in the spectrophotometer [20]. The following formula was used to determine the percentage of scavenging:

% Inhibition = [Absorbance of control- Absorbance of sample/ Absorbance of control × 100]

Ferric thiocyanate (FTC) method

A mixture composition of 2 mL sample [or methanol (as blank) or butylated hydroxyanisole (as reference)], 2.05 mL of linoleic acid (2.51%) in ethanol (99.8%), 4 mL (0.05 mol/L phosphate buffer) having pH 7.0 and distilled water of 1.95 mL concentration was incubated in an Erlenmeyer flask in a rotary incubator having 150 r/min at 40 C) in the dark place. A test tube was filled with the 0.1 mL of the mixture used for the reaction to measure the antioxidant activity. It was then mixed with 9.7 mL concentration of ethanol (75%), 0.10 mL of 30% ammonium thiocyanate, and 0.02 mol/L of ferrous chloride in 3.5% hydrochloric acid. The reaction composition's absorbance had been determined at 532 nm three minutes after ferrous chloride was added. As an adverse control, this mixture was developed as well without linoleic acid [21]. Positive tests were shown by the vitamin E and BHA. The following equation was applied to calculate antioxidant activity.:

% inhibition = $(A_{control} – A_{sample} / A_{control}) \times 100$

Where Asample denotes the absorbing capacity of tested extract samples and Acontrol denotes absorbance of control sample media.

Hydroxyl radical scavenging activity

100 µM of $FeCl_3$, 104 µM EDTA, 1 mM H_2O_2, and 2.8 mM 2-deoxy-D-ribose were combined to the reaction composition together with extract at various concentrations (20-100 µg) in a final reaction volume of 1 ml formulated with 20 mM potassium phosphate buffer having pH 7.4. The reaction mixture was then nurtured for one hour at 37ºC. 2.8% of TCA and 0.5% of TBA in 0.025 M NaOH including 0.02% of BHA were added to the combination after it had been warmed in water bath at 95 ºC for 15 min. The reaction combination was then centrifuged for 15 minutes at 5000 rpm after being cooled on ice. At 532 nm the absorbance of the supernatant was determined. By providing appropriate controls, all readings were adjusted for any interference from the antioxidant's or extracts brown colour. 100% deoxyribose oxidation was assumed to have occurred in the adverse control without any antioxidants or phytochemicals. The test sample's percentage of hydroxyl radical scavenging activity was determined in contrast to the adverse control. We used ascorbic acid as the positive control. [7].

% inhibition = $(A_{control} – A_{sample} / A_{control}) \times 100$

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RESULTS AND DISCUSSION

Quantitative determination of the chemical constituency

Extraction yield

Table-1 demonstrates the crude sequential extracts' % yields (in petroleum ether, ethyl acetate, and methanol) of fruit husk of the *Juglans regia* (walnut). Methanolic extracts exhibited higher yield (4.33%) followed by ethyl acetate (0.253%) and pet ether. The lowest yield was found in ether extract (0.137%).

Table 1. Percentage yield of *Juglans regia* extract

S. No	Solvent	Colour of extract	Theoretical Yield (gms)	Actual Yield (gms)	% Yield
1	Pet. ether	Brown	137.97	0.19	0.137
2	Ethyl acetate	Brown	133.55	0.35	0.253
3	Methanol	Brown	130.99	5.98	4.33

Preliminary qualitative phyto-chemical investigation

The current investigation found that the extracts of *Juglans regia* contained carbohydrates, alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and proteins and amino acid. Methanolic extracts included more metabolites that were secondary than any other solvent extracts, whereas pet ether extract only included saponins..

Table 2. Results of Qualitative Phytochemical Analysis

Chemical Constituents	Pet. ether extract	Ethyl acetate extract	Methanol extract
Carbohydrates	-	-	+
Alkaloids	-	-	+
Terpenoids	-	+	+
Flavonoids	-	+	+
Tannins and Phenolic Compounds	-	+	+
Saponins	+	-	-
Protein and Amino acids	-	-	-
Glycosides	-	-	+

In-vitro anti-oxidant activity

DPPH radical scavenging activity

Quantitative anti-oxidant potential was gauged by DPPH-free radical scavenging assay where IC_{50} values of ethyl acetate (EA) & methanol crude extract of *Juglans regia* were found to be 62.94µg/ml and 52.98µg/ml, while the reference (ascorbic acid) revealed the value as 25.82 µg/ml.

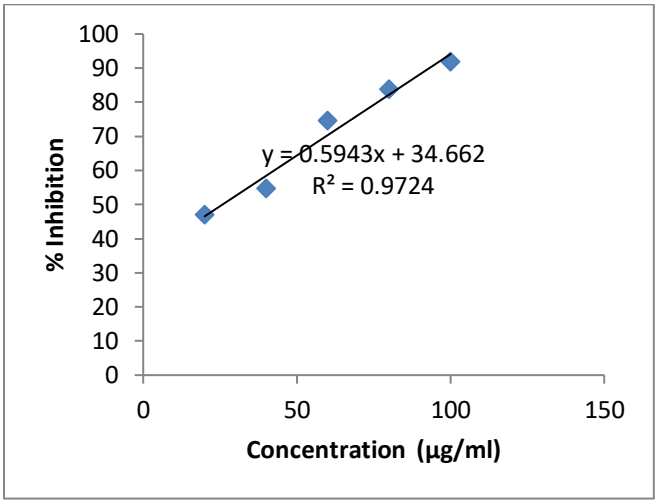

Figure 1. DPPH radical scavenging assay of Std. Ascorbic acid

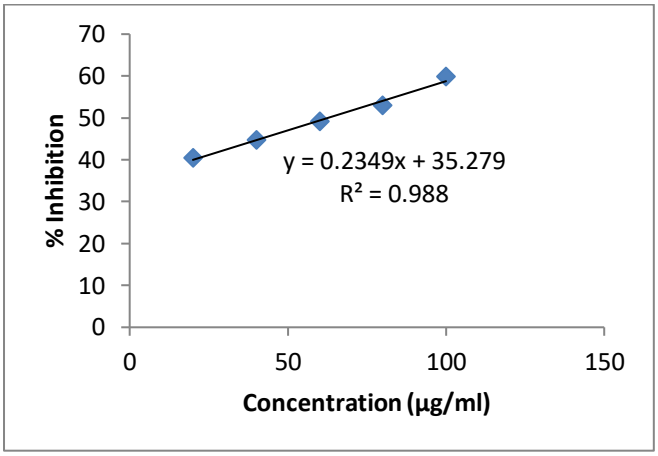

Figure 2. Represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract of *Juglans regia*

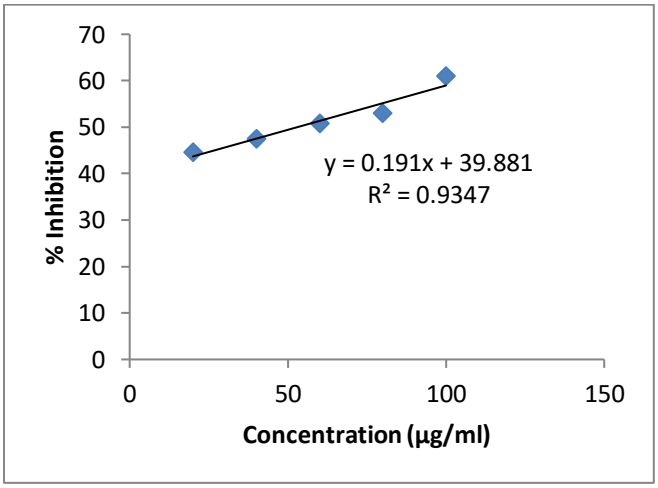

Figure 3. Represents the Percentage Inhibition *Vs* Concentration of Methanolic extract of *Juglans regia*

Scavenging activity of superoxide anion

Among the most potent reactive oxygen species that have been generated is the superoxide anion radical. The ethyl acetate & methanol extract of *Juglans regia* superoxide

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radical scavenging activity were 70.37µg/ml and 53.03µg/ml each. However the IC_{50} of standard ascorbic acid was 12.01µg/ml.

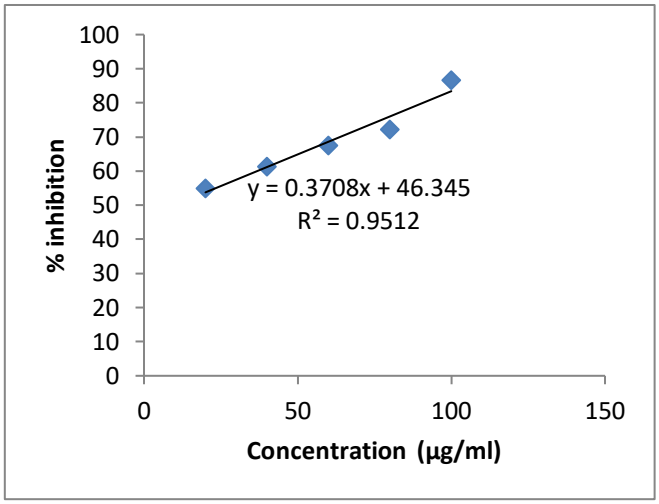

Figure 4. Superoxide radical scavenging activity of Std. Ascorbic acid

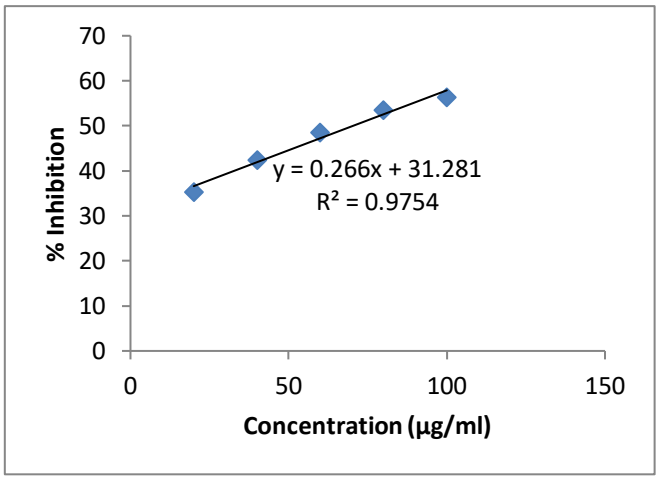

Figure 5. Represents the Percentage Inhibition Vs Concentration of Ethyl acetate extract of Juglans regia

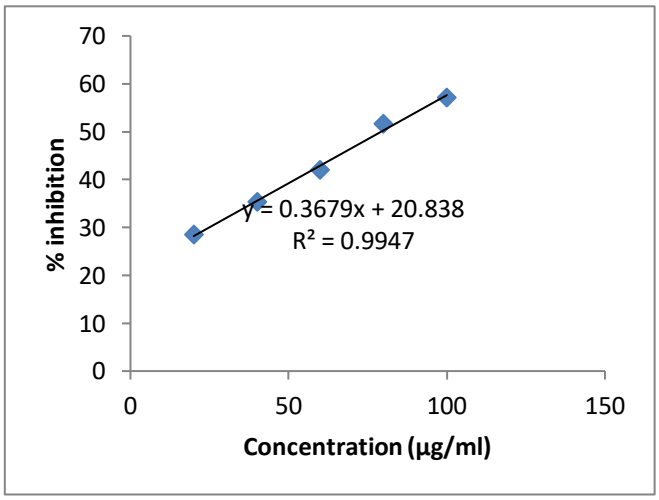

Figure 6. Represents the Percentage Inhibition Vs Concentration of Methanolic extract of Juglans regia

Ferric thiocyanate (FTC) method

The ferric thiocyanate procedure was developed to evaluate lipid peroxide concentration, with the quantity of Fe2+ converted by lipid peroxides to Fe3+ as the end point measurement. At 500 nm, the Fe3+ -thiocyanate combination exhibits a vibrant red colour. Ammonium thiocyanate has an advantage over other colouring agents in that it binds iron selectively to Fe^{3+} ions only, and the Fe^{3+} thiocyanate complex creates a single exclusive absorbance peak at 500 nm. The FTC assay results (Figure 8 & 9) demonstrated that ethyl acetate and methanol extract of *Juglans regia* has the antioxidative ability for chain-breaking suppression of lipid peroxidation, with 65.87 µg/ml and 44.26 µg/ml inhibition when compared to standard ascorbic acid (16.85 µg/ml).

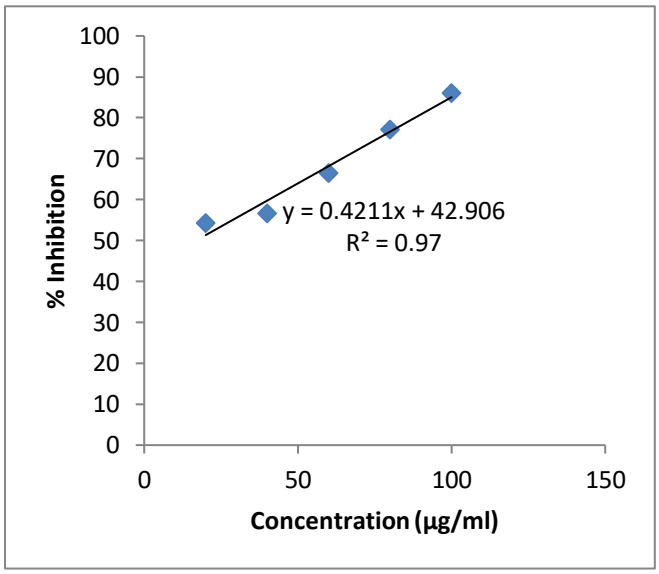

Figure 7. Ferric thiocyanate (FTC) activity of Std. Ascorbic acid

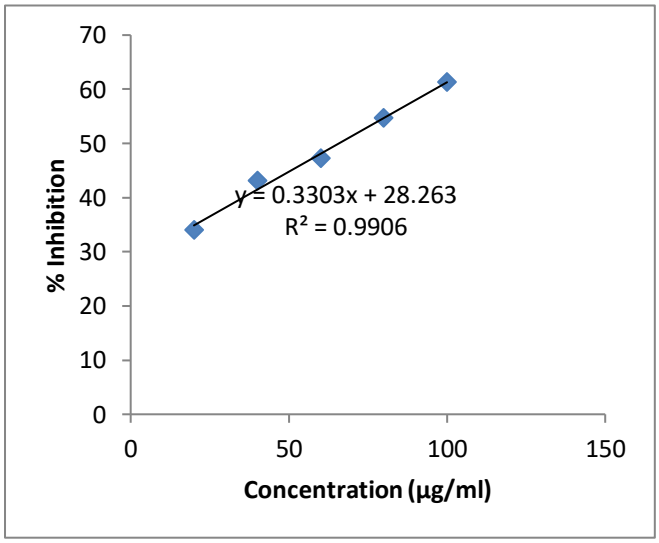

Figure 8. Represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract of *Juglans regia*

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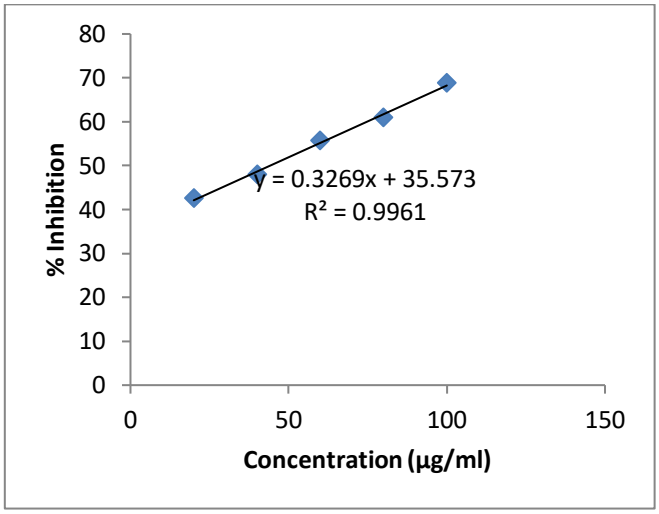

Figure 9. Represents the Percentage Inhibition *Vs* Concentration of Methanolic extract of *Juglans regia*

Hydroxyl radical scavenging activity

This test demonstrates that the extract and standard can impede the hydroxyl radical-mediated deoxyribose degeneracy in Fe^{3+} EDTA-ascorbic acid and H2O2 reaction combination. The results are shown in Figure 11 & 12. In this test, the IC_{50} values for both the extract and standard were 67.34, 47.5 and 9.33 µg/ml respectively. At 100µg/ml, the percentage inhibition values for ethyl acetate & methanol extract were 62.48 % and 66.03 % respectively.

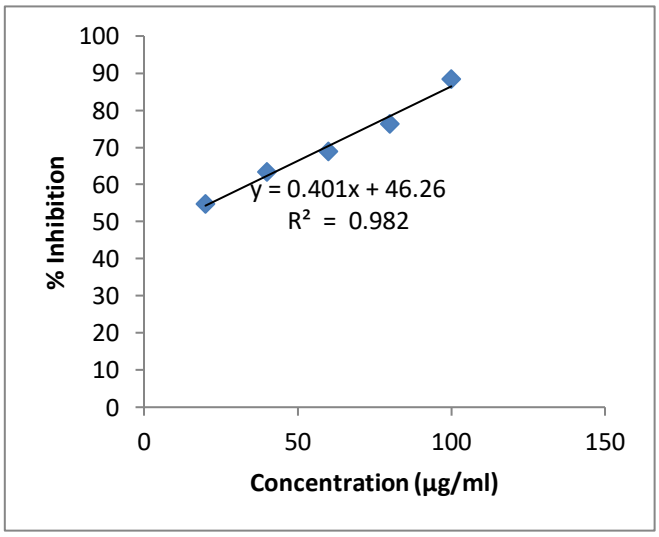

Figure10. Hydroxyl-radical scavenging assay of Std. Ascorbic acid

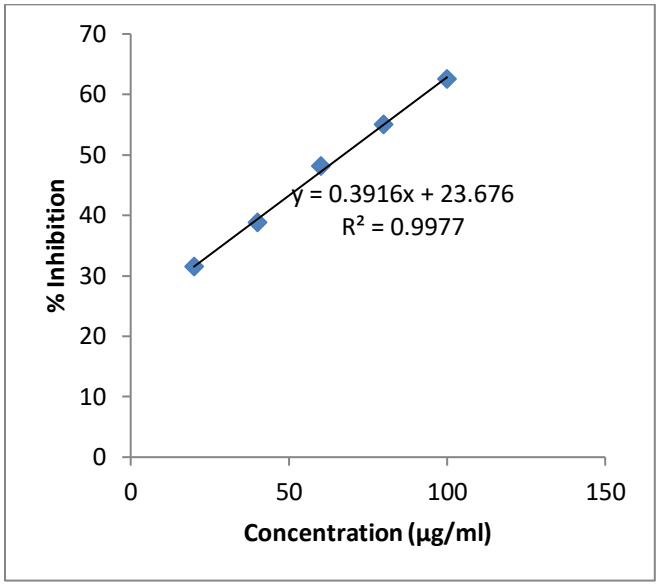

Figure 11. Represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract of *Juglans regia*

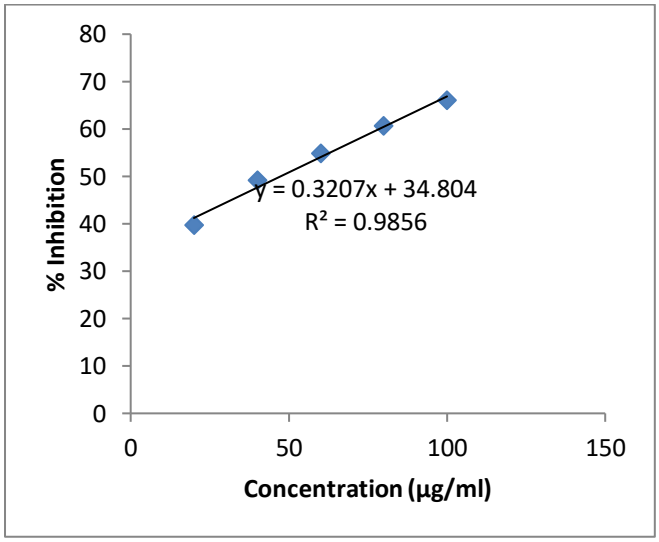

Figure12. Represents the Percentage Inhibition *Vs* Concentration of Methanolic extract of *Juglans regia*

CONCLUSIONS

The results of several studies have shown that plants belonging to the *Juglandaceae* family contain alkaloids, flavonoids, and terpenoids. Walnut is a medicinal plant belonging to this family that has been used in traditional medicine for the treatment of a lot of diseases. Due to having monoterpenes, coumarin, flavonoids, tannins, saponins, alkaloids, and other components, it has many medicinal properties. This component has been suggested to reduce the risk of hypertension, diabetes mellitus, cancer, and microbial activity. The data reported in the previous studies confirmed that walnuts are a rich source of important nutrients that can be beneficial to human health as well.

The antioxidant potential and phytochemical screening inquiry of *Juglans regia* extracts were evaluated. Different methods of antioxidant activity were effectively applied to

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evaluate the assays of *Juglans regia* extracts. Fruit husk of *Juglans regia* plant showed a potential antioxidant activity and are capable of scavenging ROS. This study verified that *Juglans regia* fruit husk extract had antioxidant properties in vitro. The ability of this plant to cure oxidative stress-related human illnesses in vivo requires more study. Because of its multiple compounds and pharmacological properties, it is necessary to conduct further studies on other unknown useful properties of this plant; so that it could be used as a drug to treat human diseases. It is also recommended that more research and clinical trials to be conducted to identify molecules, information pathways, and related genes. A key issue that can be used in these studies is to evaluate the therapeutic effects of walnuts on diseases such as diabetes, hypertension, infectious, and liver diseases which should be investigated in clinical trials.

REFERENCE

[1] Martínez ML, Labuckas DO, Lamarque AL, Maestri DM. Walnut (Juglans regia L.): genetic resources, chemistry, by-products. Journal of the Science of Food and Agriculture. 2010 Sep;90(12):1959-67.
[2] Girzu M, Carnat A, Privat AM, Fialip J, Carnat AP, Lamaison JL. Sedative effect of walnut leaf extract and juglone, an isolated constituent. Pharmaceutical biology. 1998 Jan 1;36(4):280-6.
[3] Mouhajir F, Hudson JB, Rejdali M, Towers GH. Multiple antiviral activities of endemic medicinal plants used by Berber peoples of Morocco. Pharmaceutical biology. 2001 Jan 1;39(5):364-74.
[4] Baharvand-Ahmadi B, Bahmani M, Tajeddini P, Naghdi N, Rafieian-Kopaei M. An ethno-medicinal study of medicinal plants used for the treatment of diabetes. Journal of nephropathology. 2016 Jan;5(1):44.
[5] Almeida IF, Fernandes E, Lima JL, Costa PC, Bahia MF. Walnut (Juglans regia) leaf extracts are strong scavengers of pro-oxidant reactive species. Food Chemistry. 2008 Feb 1;106(3):1014-20.
[6] Varella SD, Pozetti GL, Vilegas W, Varanda EA. Mutagenic activity of sweepings and pigments from a household-wax factory assayed with Salmonella typhimurium. Food and Chemical Toxicology. 2004 Dec 1;42(12):2029-35.
[7] Sharma P, Ravikumar G, Kalaiselvi M, Gomathi D, Uma C. In vitro antibacterial and free radical scavenging activity of green hull of Juglans regia. Journal of pharmaceutical analysis. 2013 Aug 1;3(4):298-302.
[8] Zhang H, Wang J, Zhao HY, Yang XY, Lei H, Xin M, Cao YX, Zhang SQ. Synthesis and biological evaluation of irreversible EGFR tyrosine kinase inhibitors containing pyrido [3, 4-d] pyrimidine scaffold. Bioorganic & Medicinal Chemistry. 2018 Jul 23;26(12):3619-33.
[9] Zhao MH, Jiang ZT, Liu T, Li R. Flavonoids in Juglans regia L. leaves and evaluation of in vitro antioxidant activity via intracellular and chemical methods. The Scientific World Journal. 2014 Jan 1;2014.
[10] Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010 Dec 10;2(12):1231-46.
[11] Verma RS, Padalia RC, Chauhan A, Thul ST. Phytochemical analysis of the leaf volatile oil of walnut tree (Juglans regia L.) from western Himalaya. Industrial crops and products. 2013 Mar 1;42:195-201.
[12] Cosmulescu S, Trandafir I. Seasonal variation of total phenols in leaves of walnut (Juglans regia L.). Journal of Medicinal Plants Research. 2011 Sep 23;5(19):4938-42.
[13] Negi KS, Kanwal KS. Plants used as fish toxins in Garhwal region of Uttarakhand Himalaya.
[14] Stehle S, Kirchheiner J, Lazar A, Fuhr U. Pharmacogenetics of oral anticoagulants: a basis for dose individualization. Clinical pharmacokinetics. 2008 Sep;47:565-94.
[15] Srinivasan A, Viraraghavan T. Removal of oil by walnut shell media. Bioresource technology. 2008 Nov 1;99(17):8217-20.
[16] Çağlarırmak N. Biochemical and physical properties of some walnut genotypes (Juglans regia, L.). Food/Nahrung. 2003 Jan 1;47(1):28-32.
[17] Crews C, Hough P, Godward J, Brereton P, Lees M, Guiet S, Winkelmann W. Study of the main constituents of some authentic hazelnut oils. Journal of agricultural and food chemistry. 2005 Jun 15;53(12):4843-52.
[18] Kokate CK. Practical pharmacognosy, 4thedn. Vallabh Prakasan, New Delhi. 1994:179-81.
[19] Athavale A, Jirankalgikar N, Nariya P, De S. Evaluation of in-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. International journal of pharmaceutical sciences and research. 2012 Aug 1;3(8):2543.
[20] Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972 Jan;46(2):849–54.
[21] Ghaima KK, Hashim NM, Ali SA. Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale). Journal of Applied Pharmaceutical Science. 2013 May 30;3(5):096-9.