International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue: 7 2014 Pages:51-55

(pISSN: 2348-5213; eISSN: 2348-5221)

RESEARCH ARTICLE

STUDY ON ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF WALNUT (*Juglans nigra*) OIL

PAYAL RATHI[1*], MAMSHAD AHMAD[1], ARTI TOMAR[2]

[1]Department of Chemistry, Uttaranchal College of Science and Technology, Dehradun, Uttarakhand, India
[2]Department of Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India

Corresponding Author: dr.payal.chem@gmail.com

Abstract

Walnut is common fruit which is used as food supplement all over the world. All the plant parts of walnut are used as medicine in the treatment of various diseases. The present study is based on the medicinal properties of the walnut oil extracted from the kernel of the fruit. It is found that walnut oil shows antimicrobial activity against microbes *Staphylococcus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris*. Walnut oil can be used to fight against bacterial infections. The authors also found that walnut oil has a great number of phytochemicals fully armed with the potential to reduce Fe^{3+} of FRAP reagent to Fe^{2+}. The extent of reduction was taken as the power of neutralizing free radicals in the body. Thus the walnut oil has great anti-microbial activity and high antioxidant potential.
.

Keywords: Antimicrobial activity, Antioxidant property, *Juglans nigra*, walnut oil, FRAP method.

Introduction

In recent times, there has been much interest in the Walnut is a very old fruit used by people as dry food in their special diet. The fruit is known by people from 7000 BC. This fruit is known by different names in the various parts of the world. The Romans called walnut (*Juglans regia*), a species of walnut, “Jupiter’s royal acorn”. The historical evidences show the modern walnut came from ancient Persia. So the walnut is called “Persian walnut”. Walnut is found mostly in mountain area. Green walnuts, shells, kernels and seeds, bark and leaves are used in the pharmaceutical and cosmetics industries (Pereira *et al.*, 2007; Stampar *et al.*, 2006). Besides this walnut species are important sources of nuts and timbers in the temperate zones across the world. In China, *Juglans* (Juglandaceae) is not only an agricultural commodity, but its leaves, barks, stems, pericarps, fruits, flowers and ligneous membranes are all applied for different medicinal uses. In fact, walnut leaves are considered to be a source of healthcare compounds and have been intensively used in traditional medicine for the treatment of venous insufficiency, hemorrhoids, hypoglycemia, diarrhea, and fungal or microbial infections (Wichtl *et al.*, 1999). Phenolic compounds, natural antioxidants, are found in walnut trees. These compounds are of much importance due to their benefits in improving health and decreasing the risk of degenerative diseases (Oliveira *et al.*, 2008). A lot of work has been done to determine antifungal, antibacterial and oxidative stability of food components of this plant species (Isanga *et al.*, 2007; Miraliakbari *et al.*, 2008). Linoleic acid is the major fatty acid, followed by oleic, linolenic, palmitic and stearic (Amaral *et al.*, 2003). In addition, walnuts have other components that may be beneficial for health including plant protein, dietary fiber, melatonin

(Reiter *et al.*, 2005), and plant sterols (Amaral *et al.*, 2005). Vitamin E family compound found in the plant, which has antioxidant activity, mainly in the prevention of lipid oxidation process (Koksak *et al.*, 2006). Plant-derived products can also be used as antimicrobial agents, with phenolics and polyphenolic having major interest.

This study is based on *Juglans nigra* called as eastern black walnut. Black walnut (*Juglans nigra*) husk was studied for its composition and applications as dye on Pashmina fabric (Lal *et al.*, 2011). Medicinal value of black walnut was explored as it creates toxicity on rates and can be used to control the rate population (Owumi *et al.*, 2013). Antimicrobial property of black walnut juice of unripe hull was studied and found that the presence of a naphthaquinone (juglone) had a great antimicrobial activity. Black walnut has a great antioxidant property. Due to this, it is used as food to reduce the cardiovascular diseases (Rorabaugh *et al.*, 2011). In this paper authors presents the study on anti-microbial and anti-oxidant properties of black walnut oil extracted from the kernel by cold pressing method.

Materials and Methods

Chemicals and Reagents

All the chemicals used in this study were AR grade and procured from Sigma, Merck, Acros organics and Qualigens etc. Along with this, deionised water form Himedia is used throughout the study. The most important chemical TPTZ (2,4,6-tripyridyl-s-triazine) is procured from Sigma. HCl (LR grade), sodium acetate and acetic acid were taken from Qualigens. $FeSO_4.7H_2O$ (Ferrous sulfate heptahydrate) was from Acros Organics and $FeCl_3.6H_2O$ (Ferric chloride hexahydrate) from Merck. Other supporting chemicals including oxalic acid (Himedia), NaOH (Himedia) etc were also procured.

Test Microorganisms

List of the range of bacterial microorganisms used in this study are given in the Table 1. These microorganisms were propagated in the conditions as nutrient agar (NA, 28.0 g, pH 7.4±0.2), nutrient broth (NB, 13.0 g, pH 7.4±0.2) and Muller Hinton agar (MHA, 38.0 g, pH 7.3±0.1). Sterilization by autoclaving was at 121^{o}C for 15 min as per the manufacturer specification.

Plant Material (Black Walnut Oil)

Black walnut used in the study was collected from northern part of India. The kernels were separated from the rip and dried fruit of nut. The oil was extracted from the kernel in a special designed apparatus by cold pressing method. In this method kernel are filled in a hollow thick walled iron tube. A piston is installed in the tube and put pressure on the kernel. Firstly the kernels are broken and then became solid dense material. At this stage oil comes out from the kernel material which was collected from the bottom of the tube, drop by drop. This oil was filtered and stored in air tight container.

Evaluation of Antimicrobial Activity

The Agar well diffusion method was used to evaluate the antimicrobial activity. The bacterial culture were grown in nutrient broth and incubated at 37^{o}C for 24 hrs. The absorbance of the culture was adjusted to 0.5 according to McFarland turbidity standard with sterile nutrient broth. The 0.02 mL of each culture was seeded on the sterile petre plates containing sterile Muller Hinton Agar media. The well was bored with 9 mm borer in seeded agar. An amount of 100 μL of the walnut oil was added in each well. Plates were incubated at 37^{o}C for 24 hrs. After incubation period the zone of inhibition was measured.

Measurement of Antioxidant activity

The antioxidant activity was measured by FRAP (Ferric Reducing Ability of Plasma) method (Benzie *et al.*, 1999). For this study solutions including acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM), HCl (40 mM), ferric chloride solution (20 mM) and ferrous sulphate solution (1.8 mM) were prepared. FRAP working solution (Shahwar *et al.*, 2012) was prepared freshly each time. 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ were mixed in 10:1:1 (v/v/v) and kept away from light. 5.0 mg/mL of walnut oil (final concentration 50 to 5000 µg/mL) or $FeSO_4.7H_2O$ (final concentration 0 to 1.8 µmol Fe^{2+}/mL) solution were added to 2.25 mL FRAP working solution and 0.225 mL of deionized water. The mixture was incubated at 37°C for 30 min away from light. Absorbance was measured at 593 nm using the spectrophotometer. FRAP working solution with deionized water instead of a sample was used as a blank.

Result and Discussion

Six bacterial strains (pathogens) were used (Table 1) for the study. Out of these two were gram +ve and rest four were gram –ve. Black walnut oil

showed antimicrobial activity against all the pathogens tested (Fig. 1).

From the above graph it is clear that *Staphylococcus* showed 31 mm as the zone of inhibition at 0.1 μL oil concentration. *Bacillus subtilis* showed least antimicrobial activity i.e. 11 mm. *Klebsiella pneumoniae* showed good activity of 24 mm inhibition zone, followed by *Pseudomonas aeruginosa* (22 mm), *Escherichia coli* (14 mm) and *Proteus vulgaris* (12 mm). The results clearly indicate that black walnut oil has a capacity to fight bacterial infection caused by these pathogens. The antimicrobial activity of oil is due to the presence of juglone (5-hydroxy-1,4-naphthaquinone). Juglone has a capacity to destroy the activity of bacteria due to hydrogen bonding of >c=o group to the corresponding molecular site i.e. proteins or enzymes.

The amount of black walnut oil was extracted by cold press method from the kernel of the fruit. The reducing potential of the oil is summarized in the Table 2 and Fig. 2.

The walnut oil has been said to contain a great numbers of phytochemicals fully armed with the potential to reduce Fe^{3+} to FRAP reagent Fe^{2+}. The different concentrations (from 50 g to 5000 g) of oil in benzene were used. It is seen that as the concentration of oil is increased the FRAP values are increased gradually up to a concentration of 750 μg. The limiting value was recorded at 2500 μg, beyond this FRAP value became constant. Thus it can be said that walnut oil is a good antioxidant agent for the body. Antioxidant properties arise due to the tendency of generation of free radicals. Juglone and other compounds present in the walnut oil has a tendency to generate the free radicals. These free radicals capture the free radicals present in the body or formed for a moment in the body. The quinonic group is responsible for the antioxidant properties. quinone form easily diracial of quinol whick capture the other free radicals. Thus walnut oil has antioxidant property.

Table 1: List of Bacterial Organisms Used in The Study

S.No.	Bacterial species	Liquid Medium	Solid Medium	Temp. ^{o}C	Strain No.
1	*Bacillus subtilis*	NB	NA	37^{o}C	MTCC-121
2	*Pseudomonas aeruginosa*	NB	NA	37^{o}C	MTCC-1036
3	*Staphylococcus*	NB	NA	37^{o}C	Local
4	*Klebsiella pneumoniae*	NB	NA	37^{o}C	MTCC-432
5	*Proteus vulgaris*	NB	NA	37^{o}C	MTCC-1771
6	*Escherichia coli*	NB	NA	37^{o}C	Local

Fig. 1: Zone of inhibition of pathogens studied using black walnut oil

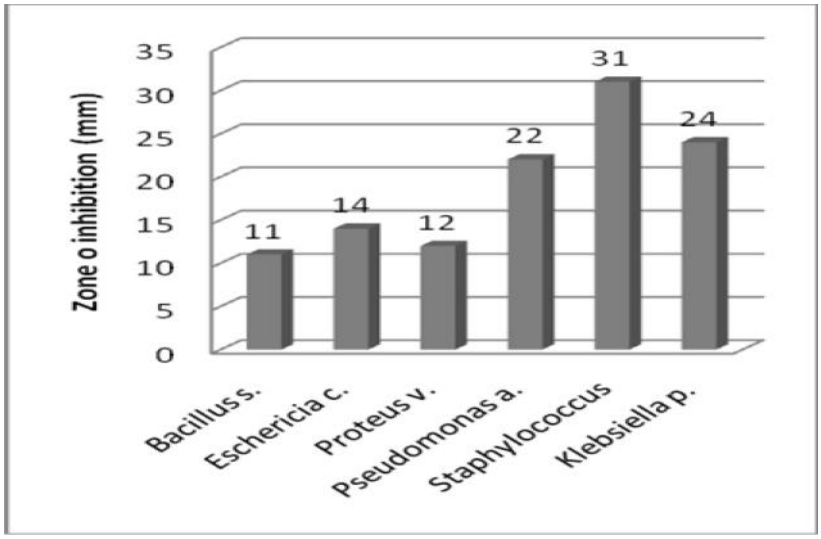
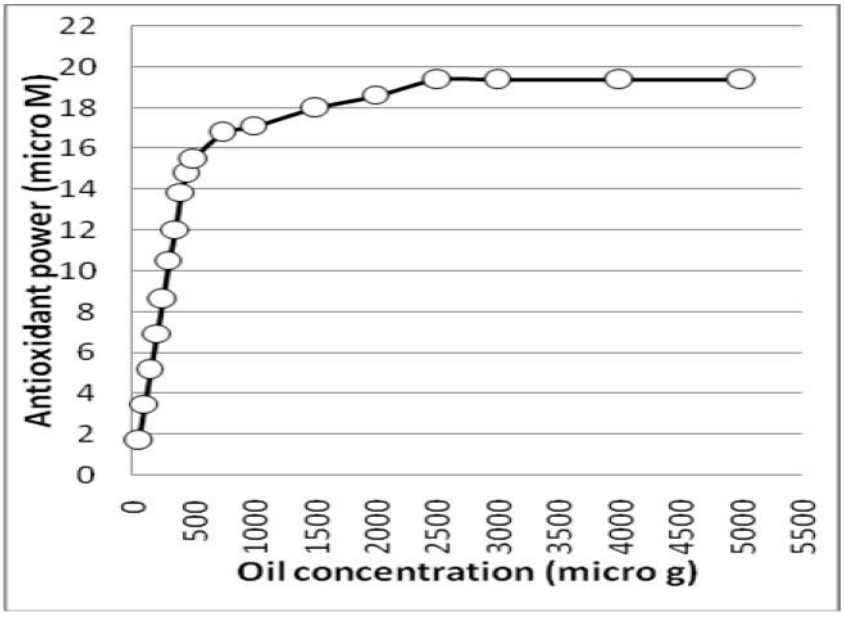


Table 2: Antioxidant FRAP value of black walnut oil at different concentrations

S. No.	Oil concentration (g)	Antioxidant power ( )	S. No.	Oil concentration (g)	Antioxidant power ( )
1	50	1.73	10	500	15.52
2	100	3.46	11	750	16.83
3	150	5.19	12	1000	17.12
4	200	6.92	13	1500	18.04
5	250	8.65	14	2000	18.61
6	300	10.51	15	2500	19.41
7	350	12.00	16	3000	19.42
8	400	13.84	17	4000	19.42
9	450	14.81	18	5000	19.43

Fig. 2: Antioxidant power of black walnut oil



Conclusion

The result on antimicrobial and antioxidant activities of the walnut oil indicate that, walnut oil has a great curing effect of bacterial infections and make the conditions in the body to fight against free radicals in the body. The walnut oil is a good for health purpose due to its medicinal effect and needs to be consumed daily.

References

Amaral J. S., Casal S., Pereira J., Seabra R. and Oliveira B., Determination of Sterol and Fatty Acid Compositions, Oxidative Stability and Nutritional Value of Six Walnut (*Juglans regia L.*) Cultivars Grown in Portugal, *J. Agric. Food.Chem.*, 51, 7698-7702, (2003).

Amaral J. S., Alves M., Seabra R. and Oliveira B., Vitamin-E Composition of Walnuts (*Juglans regia L.*): A 3-Year Comparative Study of Different Cultivars, *J. Agric. Food. Chem.*, 53, 5467-5472, (2005).

Benzie I. F. and Strain J. J., Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration, *Methods Enzymol*., 299, 15-27, (1999).

Clark A. M., Jurgens T. M. and Hufford C. D., Antimicrobial Activity of Juglone, *Phytother. Res.*, 4, 11-14, (1990).
Koksak A., Artik N., Simsek A. and Gunes N., Nutrient Composition of Hazelnut (*Corylus avellana L.*) Varieties Cultivated in Turkey, *Food Chem.*, 99, 509-515, (2006).
Lal C., Raja A. S. M., Pareek P. K., Shakyawar D. B., Sharma K. K. and Sharma M. C., *Juglans nigra*: Chemical Constitution and Its Aapplication on Pashmina (Cashmere) Fabric as A Dye, *J. Nat. Prod. Plant Resour.*, 1(4), 13-19, (2011).
Isanga J., and Zhang G. N., Biological Active Components and Nutraceuticals in Peanuts and Related Products: Review, *Food Rev. Intl.*, 23, 123-140, (2007).
Miraliakbari H. and Shahidi F., Oxidative Stability of Tree Nut Oils, *J. Agric. Food. Chem.*, 56, 4751-4759, (2008).
Oliveira I., Sousa A. and Ferreira I. C., Total Phenols, Antioxidant Potential and Antimicrobial Activity of Walnut (*Juglans regia L.*) Green Husks. *Food Chem Toxicol*, 46, 2326-2331, (2008).
Owumi S. E., Odunola O. A. Gbadegesin M. A. and Nulah K. L., Protective Effect of *Juglans nigra* on Sodium Arsenite-Induced Toxicity in Rats, *Pharmacognosy Res*., 5(3), 183–188, (2013)
Pereira J. A., Oliveira I. and Sousa A., Walnut (*Juglans regia L.*) Leaves: Phenolic Compounds, Antibacterial Activity and Antioxidant Potential of Different Cultivars. *Food Chem Toxicol*., 45, 2287-2295, (2007).
Reiter R. J., Manchester L. C., Dun-Xian Tan M. D., Melatonin in Walnuts: Influence on Levels of Melatonin and Total Antioxidant Capacity of Blood, *Nutrition*, 21, 920-924, (2005).
Robrabaugh J. M., Singh A. P., Sherrell I. M., Freeman M. R., Vorsa N., Fitschen P., Malone C., Maher M. A. and Wilson T., English and Black Walnut Phenolic Antioxidant Activity in Vitro and Following Human Nut Consumption, *Food and Nutrition Science*, 2, 193-200, (2011).
Shahwar D., Raza M. A., Bukhari S. and Bukhari G., Ferric Reducing Antioxidant Power of Essential Oils Extracted from *Eucalyptus* and *Curcuma* Speceis, *Asian Pacific Journal of Tropical Biomedicine*, S1633-S1636, (2012).
Stampar F., Solar A. and Hudina M., Traditional Walnut Liqueur - Cocktail of Phenolics, *Food Chemistry*, 95, 627-631, (2006).
Wichtl M., and Anton R., Plantes Thérapeutiques. *Technique et Documentation. Paris*, (1999).

