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In vitro Phytochemical and antimicrobial Activity of Walnut (*Juglansregia L.*)Husk

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Abstract: The aqueous extracts of different concentration of walnut's husk were investigated for their phytochemical & antibacterial properties. The husk extracts were made using the simple infusion, decoction infusion and microwave infusion. The different concentration of extracted extracts showed antibacterial and antifungal activity, which were shown in the results of our study. Phytochemical analyses revealed the presence of Alkaloid, Phenols, Flavanoids, Protiens and Amino acid, Saponins, Quinones, Terpenoids. Antibacterial potentials were evaluated using the agar disk diffusion method. The extracts were inactive against all the gram negative bacteria which were used in current study but on other hand it inhibits the gram positive bacteria effectively on different concentration. The walnut, leaves , bark and its stem's extracts have unique quality to inhibit the fungal and bacterial growth but on the other hand we observed that walnut husk has different property as compare to its inner seed, husk extract were enhancing the growth of some fungal strains which we tested during our study.

Keywords Walnut, protein, husk, fungal

INTRODUCTION

Walnut (*Juglansregia L.*) belongs to the Juglandaceae family and delivers amazing antioxidant components help in disease prevention and control [1-5]. It is native of western Himalayan chain and was highly grow in Europe as early as 1000 BC. Today, walnut is cultivated commercially every part of earth[6].

There is number of medical advantages of this plant. It has been use in the treatment of piles, nose troubles, scabies, cough, scurvy, gonorrhea, toothache and boils to treat hook worm, venereal diseases, for teeth cleaning, to lower cholesterol plasma levels in rheumatism, and asthma, reestablishment of the components of

gastric mucosa, and as a laxative[7,8]. Various studies support that systematic consumption of walnuts may have beneficial properties against and oxidative stress diseases such as cardiovascular disease and cancer [9,10]. In spite of this dry fruit leaves have been used in both cosmetic and pharmaceutical manufacturing [11]. Ingestion of assured phenolics in the food is considered supportive for human nutrition[12].

Regular antioxidants, such as phenolic mixtures, have significance value, due to decreasing the risk of declining diseases burden by lowing oxidative tension and reserve of macromolecular oxidation [13-15]. Numerous Studies have also exposed the antimicrobial potential of walnut products, specially leaves, bark and fruits [16-19]. Halvorsen *et al.*[20] found that walnuts have one of the ultimate ingredients of antioxidants among all scrutinizes nuts and seeds. Juglone, is widely vital phenolic complex of walnut and famous for its antimicrobial effectively. It was also published to reduction the events of tumors of the small intestine of rats [21]. Kris-Etherton *et al.*[22] research that flavonoids in walnuts have great serum cholesterol-modulating effects, and one cluster of flavonoids has cardio protective effects. The objective of our study is to evaluate the phytochemical and antibacterial and antifungal activity of walnut husk. This green walnut husk is used as a green dye it contains high amount of phenolic compounds. The analysis were perform in the duration of September to December we make the husk extract by infusion, decoction infusion, and microwave infusion with different concentrations.

MATERIAL AND METHOD

The Study of Antimicrobial activity and phytochemical test of Walnut Husk has been done in the Laboratory of Institute of Environmental Studies, University of Karachi. In this Study Seven different microbes and two fungal strains were used.

Preparation of Extract of Walnut Husk: Pakistan is one of the biggest producers of walnut. The walnut husk used in the production of biofuel in Northern areas of Pakistan. We collected the Raw Walnut and separate out the husk and crushed it in motor and piston and the paste was used for making of different concentration and different category of walnut husk extract.

Preparation of Decoction: We made different concentration of aqueous decoction by boiling. 5% Concentration was prepared by adding 5gm of crushed walnut husk in 100ml of distilled water in a flask and give heat for 15 to 20 minutes and then remove the flask from heat and let it cool then filter through what man filter paper of 0.45µm and store it in small vials [23]. (We made 3 concentrations 5%, 10% and, 15%).

Preparation of Infusion: We made different concentration of aqueous infusion. 5% Concentration was prepared by adding 5gm of crushed walnut husk in 100ml of distilled water and left for 48 hours with occasional shaking at normal temperature/ room temperature and then filter through whatman filter paper of 0.45µm and store it in small vials.[24] (We made 3 concentrations 5%, 10% and, 15%).

Extract Antibacterial Test: First we swab the isolated cultures on the nutrient agar plates, than we put the extract disc in the cultured plates and incubate the plate for 24hours at 37◦C next day we observed the results.

Extract Antifungal Test: First we swab the isolated cultures on the Sabouraud dextrose agar plates, than we put the extract disc in the cultured plates and incubate the plate for 24 hours at 20◦C next day we observed the results.

Standard disc Antibacterial Test: First we swab the isolated cultures on the nutrient agar plates, than we put the standard disc in the cultured plates and incubate the plate for 24hours at 37◦C next day we observed the results flow chart no.3 illustrate the idea.

Standard disc Antifungal Test: First we swab the isolated cultures on the Sabouraud dextrose agar plates, than we put the standard disc in the cultured plates and incubate the plate for 24hours at 20◦C next day we observed the results flow chart no.4 illustrate the idea.

Phytochemical Test: Qualitative phytochemical analysis of the Walnut husk extract carried out by using standard procedures to assess the different types of phytochemical constituents present in the Walnut husk it detect by using the different chemical tests. Screenings will be done for alkaloids, saponins, phytosterols, amino acids, flavonoids, quinines, terpenoids and, phenols [31].

Detection of Alkaloids: Wagner`s Test: Extracts were dissolved in 1 ml of hydrochloric acid and filter them individually. Filtrated were treated with few drops of Wagner`s reagent (Iodine in potassium Iodide).Appearance of Brown/reddish precipitate indicates the presence of alkaloids[25.]

Detection of Saponins: Foam Test: To 2ml of extract was added 6ml of water into test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins [26].

Detection of Phytosterols: Salkowski's Test: Extracts were treated with chloroform and later filtered. The filtrates were treated with few drops of Concentrated Sulphuric acid, shaken for some times and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes [25].

Detection of Tannins: Gelatin Test: 1% gelatin solution containing sodium chloride was added.Apperance of white precipitate indicates the presence of tannins [25].

Detection of Flavonoids: Ferric chloride Test: few drops of ferric chloride solution treated with extract that result in the formation of blackish red colour indicating the presence of flavonoids [27].

Detection of Proteins and Aminoacids: Xanthoproteic Test: Extracts were treated with few drops of conc. Nitric acid.Yellow colour appearance indicates the presence of proteins [25].

Detection of Quinones: Few drops of extract was treated with concentrated Hydrochloric acid and observed for the appearance of yellow precipitation (or colouration) [26].

Detection of Terpenoids: 1ml of chloroform was reacted with 2ml of extract then added few drops of Concentrated Sulphuric acid.A reddish brown precipitation produced immediately indicated the presence of terpenoids [26].

Detection of Phenol: Ferric Chloride Test: 3-4 drops of ferric chloride solution were added in the extract solution. Appearance of bluish black colour indicates the presence of phenols[25].

RESULT

Phytochemical Test: The result of phytochemical test is shown below in table no. 2 in which 9 constituent were tested, bioactive constituents such as alkaloids, saponins, phytosterols, amino acids, flavonoids, quinines, terpenoids and, phenols were tested for their qualitative analysis. Extract give positive result for alkaloid, phenols, flavanoids, saponins, quinines, aminoacids and, Terpenoids while Physterols and tannins were not detected in walnut husk. Results of the present study indicate that the identified phytochemicals are possibly the bioactive constituents in walnut husk.

Table 1: Phytochemical Test

S.no	Phytochemical	Husk Extract
1	Alkaloid	+
2	Phytosterols	-
3	Phenols	+
4	Tannins	-
5	Flavanoids	+
6	Protiens and Amino acid	+
7	Saponins	+
8	Quinones	+
9	Terpenoids	+

Key: +sign indicate presence and – sign indicate absence of constituent.

Antibacterial Activity: The antibacterial activity of walnut husk shows that it is active against some gram +ve bacteria because it shows zone of inhibition against *bacillus subtilis* and *Staphylococcus aureous* and it donot work against the gram –ve bacteria the result shown below in table number 2.

Table 2: Walnut Husk Extraction Antibacterial Activity Chart

S.no.	**Extract type**	**Conc. In %**	**Micro-organism used (Zone of Inhibition in mm)**					
			PV	**MC**	**EC**	**BS**	**SA**	**PA**
1	Infusion	5	-	-	-	11.3	7.8	-
		10	-	-	-	5	17.6	-
		15	-	-	-	-	-	-
2	Decoction	5	-	-	-	11.3	-	-
		10	-	-	-	9.5	-	-
		15	-	-	-	5	-	-
3	Microwave extract	5	-	-	-	5	-	-
		10	-	-	-	17.6	6.3	-
		15	-	-	-	-	20.1	-

Key: -ve signs show no zone of inhibition microbe codes full form given below in table no.7

Antifungal Activity: Antifungal test shows that the walnut husk is a promoter for most of the fungus strains because it enhance the growth of the fungus. The result shows below in table number 3.

Table 3: Walnut Husk Extraction Antifungal Activity Chart

S.no.	Extract type	Conc. In %	Fungus used (Zone of inhibition in mm)		
			P	AN	CA
1	Infusion	5	-	-	-
		10	-	-	-
		15	-	-	-
2	Decoction	5	-	-	-
		10	-	-	-
		15	-	-	-
3	Microwave extract	5	-	-	-
		10	-	-	-
		15	-	-	-

Key: -ve signs show no zone of inhibition microbe codes full form given below in table no. 7

Antibacterial Standard Test: Standard disc of different antibiotics used as a control. Table number 4 illustrate the result.

Table 4: Standard Disc Antibacterial Test

S.no.	Antibiotic discs	Culture Used (zone of inhibition in mm)						
		PV	MC	CA	EC	BS	SA	PA
1	CIP	42.41	10.50	87.97	28.60	63.23	122.70	82.70
2	AMP	12.57	-	-	31.82	28.6	-	-
3	AMC	1.02	11.82	-	10.28	-	-	-
4	CFM	-	-	-	-	2.20	-	-
5	NN	-	-	-	-	-	-	5.03
6	ATM	-	-	28.59	-	-	28.50	-
7	CRO	36.44	-	50.27	14.85	28.50	-	-

Key: -ve sign shows no growth and antibiotics codes microbe full form given below in table no. 6 and table no.7 respectively.

Standard Antifungal test: Standard disc of different antibiotics used as a control. Table number 5 illustrate the result.

Table 5: Standard disc antifungal Test

S.no.	Antibiotic Disc	Fungus used (zone of inhibition in mm)	
		P	AN
1	VOR	-	8.48
2	FLV	-	-
3	NYS	129.18	38.01

Key:-ve sign shows no growth and antibiotics codes microbe full form given below in table no.6 and table no.7 respectively.

Table 6: Antibiotics Codes

S.no.	Antibiotics disc	Codes
1	Ciprofloxacin	CIP
2	Ampicillin	AMP
3	Amoxycillin	AMC
4	Cefixime	CFM
5	Tobramycine	NN
6	Aztreonam	ATM
7	Ceftriaxone	CRO
8	Voriconazole	VOR
9	Flvconazole	FLV
10	Nystatin	NYS

Table 7: Microbes Codes

S.no.	Micro-organism	Codes
1	*Proteus Vulgaris*	PV
2	*Micro-cocci*	MC
3	*Candida Albikan*	CA
4	*Eschericia coli*	EC
5	*Bacillus Subtilis*	BS
6	*Staphylococcus aureus*	SA
7	*Pseudomonas aeroginosa*	PA
8	*Penecillium* (fungus)	P
9	*Aspergillus niger*	AN

DISCUSSION

In recent time large number of medicinal plants and their compound has shown helpful therapeutic activity [28]. In different countries plants are used medicinally for the extraction of potential active and powerful drugs [29]. Juglone has been reported to inhibit intestinal carcinogenesis induced by azoxymethane in rats and might be a promising chemo preventive agent in human intestinal neoplasia [21]. In our study the extract of walnut husk was tested for its antibacterial and phytochemical test. As we know that walnut has its medical significance it is good for health. Interesting fact that walnut tree leaves, stem and its seed has very good antibacterial and antifungal effects. Walnut seeds, leaves and bark aqueous and solvents extract inhibited antifungal activity against wide range of fungi using disc diffusion method, agar dilution method, agar streak dilution and Raddish method[19]. Extract of our sample do not inhibit the gram –ve microbes like *E.coli, P.vulgaris, Micrococcus, P.aerogenosa and* we also observed that walnut husk extract effect on fungus it enhanced the growth of *A.niger* and *pennicillium* it is a unique feature that inner part inhibit the growth and outer covering promote the growth.

Phytochemical test shows that it possess alkaloid, phenols, Flavanoids, Aminoacids, saponins, quinines and,terpenoids. Only tannins and physterols were absent in husk.A few studies were developed concerning the phenolic composition of green walnut husks. Juglone (5-hydroxy-1,4-naphthoquinone) is known as a characteristic compound of Juglans spp. Being reported its existence in green walnut husks [30]. According to Stampar *et al.*[11] identified thirteen phenolic compounds in walnut husks: chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, gallic acid, ellagic acid, protocatechuic acid, syringic acid, vanillic acid, catechin, epicatechin, myricetin, and juglone.

CONCLUSION

In our study walnut husk found as fungus growth promoter because it's promoted the growth of some fungus species like *Aspergillusniger* and *Penicillium notatum* as increase in the concentration of the extract which we tested in our project. On other hand extract inhibit some gram +ve microbes like *Staphylococcus aureous* and *Bacillus subtillis*. Juglianregia leaves and its stem extract are very good inhibitor against both positive and negative microbes but with the walnut husk has different property towards gram –ve microbes it do not inhibit the gram negative micro-organisms like *E.coli*, *P.vulgaris*, *Micrococcus* and, *Pseudomonas.aerogenosa* and fungus *C.albicans*. Juglian regia is a natural source as antibacterial and antioxidant agent. It has good property of inhibition which used in many medicines and cosmetics because of its dye pigment quality.

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