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ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *JUGLANS REGIA*

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ABSTRACT

Juglansregia L. (walnut) leaves are considered a source of healthcare compounds, and have been widely used in traditional medicine. In this study antimicrobial effect of hydroalcoholic extract of *Juglansregia* was evaluated on four different bacteria, *Salmonella bongori, Bacillus subtilis, E. Coli* and *P. aeruginosa.* Antimicrobial effect of the hydroalcoholic extract, ciprofloxacin was evaluated using the well diffusion method by assessing the diameter of the growth inhibition zone of the extract were determined for the above-mentioned bacteria. Hydroalcoholic extract of *Juglansregia*had inhibitory effects on the proliferation of all four bacterial strains with maximum effect. The largest growth inhibition zone diameter belonged to *Bacillus subtilis* and the smallest to *P. aeruginosa*. Ciprofloxacin had the greatest inhibitory effect on *Salmonella bongori*. Hydroalcoholic extract of *Juglansregia* had a significant antibacterial effect on bacterial pathogens and appears to be a potent antimicrobial agents that could be considered as a medicinal plant.

Keywords: *Juglansregia*L., Hydroalcoholic extract, Phenolic compounds, Antioxidant activity, Antimicrobial activity

INTRODUCTION:

The emergence and spread of microorganisms that are resistant to the antimicrobials available in the market have been reported for decades, stimulating the search for new sources of substances with efficient antimicrobial activity, such as plants used in popular medicine. The treatment of diseases using plant extracts is the oldest method of natural medicine. The source of this knowledge is still remote, and it is believed that a large portion was acquired by observation of human and animal instincts. Thus, the man began to distinguish edible plants, or those that could treat diseases, from toxic plants. This knowledge has been passed down from generation to generation by communities that co-existed with herbs and depended on them to treat diseases [1]. Plants drugs are known to have protection systems beside pathogenic bacteria [2.] The genus juglans (family Juglandaceae) comprises several species and is widely dispersed throughout the world. Many parts of Green walnuts such as shells, kernel and seed, bark, and leaves are used in the pharmaceutical and beauty industry [3, 4.] *Juglansregia* L. bark is used in some countries as a toothbrush and as a dye for colouring the lips for makeup

purpose [5.] Walnut (*Juglansregia* L.) bark has been claimed to own anti-inflammatory, blood purify, anticancer, depurative, diuretic, and laxative activities. It contains several therapeutically active constituents, particularly polyphenols [6.] *Juglansregia* stem bark contains chemical constituents, namely, β-sitosterol, ascorbic acid5, juglone, folic acid, gallic acid, regiolone, and quercetin-3-α-L-arabinoside [7.] Antifungal, antibacterial, and antioxidant activities of this plant have been described [8-12.] The aim of the present study was to evaluate the antimicrobial activities of hydroalcoholic extract of *Juglansregia* against four species of bacteria.

EXPERIMENTAL

Chemical and reagents

All the chemicals and reagents used were of analytical grade.

Plant material

The plant *Juglansregia* was collected from local area of Bhopal (M.P.) in the month of January 2018.

Extraction procedure

Juglansregia was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Further defatted dried powdered of *Juglansregia*was extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 ºC.

Phytochemical screening

The extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of plant constituents [13, 14].

The total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [15].10 mg gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filtered. Two ml (1mg/ml) of this extract was for the estimation of phenols.2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vitro antimicrobial activity

The pathogenic bacteria used in the current study obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Table 1: Composition of nutrient agar media

Composition	Quantity
Agar	1.5 gms.
Beef extract	0.3 gms.
Peptone	0.5 gms.
Sodium chloride	0.55 gms.
Distilled water (pH- 7)	to make 100 ml

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes.

Preparation of plates

After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques, the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37^{o}C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar plates with loop full of microbes and further incubated for next 24 hours at 37^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antimicrobial sensitivity

The antimicrobial sensitivity test was employed on to the all the microbes used under present study with hydroalcoholic extract obtained from *Juglansregia.*For this experiment 6 mm diameter wells, stock of 100 mg/ml of extract separately applied on it. A nutrient agar plate was seeded with particular microbes with the help of spread plate technique prior and left for 5 minutes then incubated for 24 hours at 37^{o}C. After incubation, plates were observed to see the sensitivity of extracts towards test

bacterium at particular concentration in the form zone of inhibition.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37ºC for 24-48 hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antimicrobial activity of the extracts prepared from the plant material of *Juglansregia*using standard procedure [16]. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. Its essential feature was placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37ºC for 24 hour and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

The phytochemical tests of hydroalcoholic extract of *Juglansregia* showed presence of various phytoconstituents like carbohydrates, phenols and saponins. Further studies are required to separate the active constituents and to evaluate their antibacterial activity.

Table 2: Phytochemical screening of hydroalcoholic extract of *Juglansregia*

S. No.	Test	*Juglansregia*
1.	Detection of alkaloids	
	a) Hager's test	-ve
	b) Dragendroff's test	-ve
2.	Detection of carbohydrates	
	a) Fehling's test	+ve
3.	Detection of glycosides	
	a) Legal's test:	
		-ve
4.	Detection of saponins	
	a) Froth test	+ve
5.	Detection of phenols	
	a) Ferric chloride test	+ve
6.	Detection of flavonoids	
	a) Alkaline reagent test	-ve
	b) Lead acetate test	-ve
7.	Detection of proteins and aminoacids	
	a) Xanthoproteictest	-ve
8.	Detection of diterpenes	
	a) Copper acetate test	-ve

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X+0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 3: Preparation of calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

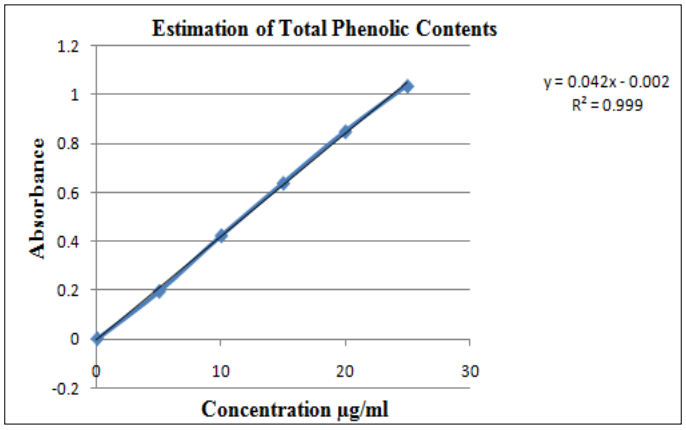

Figure 2: Estimation of total phenolic content

Table 4: Total phenolic content determination

S. No.	Extracts	Total phenol content (mg/100mg)
1.	*Hydroalcoholic extract*	0.716

Application of medicinal plants as food, preservatives, and drugs is mainly due their biological potentials such as antioxidant or antimicrobial activities [17]. The total phenolic content in hydroalcoholic extract was found to be 0.716 (GAE mg/100mg), as the increase in total phenolic content is related to the increase in *Juglans regia* antioxidant activity, while this relation is not between its antimicrobial activity and total phenolic content. It is reported that the antimicrobial activity of phenolic compound are due to iron deprivation or hydrogen bonding with vital protein such as microbial enzymes [18].

Table 5: Results of sensitivity of *Juglans regia*

S. No.	Microbes codes	Bacterial strains	*Juglans regia*
1.	Bact-1	*Salmonella bongori*	Yes
2.	Bact-2	*Bacillus subtilis*	Yes
3.	Bact-2	*E. coli*	Yes
4.	Bact-2	*P. aeruginosa*	Yes

Table 6: Antimicrobial activity of *standard drug* on selected microbes

S.N	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Salmonella bongori*	17±0.15	23±0.86	25±0.5
		Bacillus subtilis	12±0.5	17±0.74	20±0.15
		E. coli	9±0.5	12±0.57	14±0.74
		P. aeruginosa	15±0.57	18±0.74	19±0.5

Table 7: Antimicrobial activity of *Juglansregia* on selected microbes

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Salmonella bongori*	15±0.86	16±0.74	24±0.86
2.	*Bacillus subtilis*	15±0.74	23±0.5	27±0.57
3.	*E. coli*	18±0.86	22±0.5	26±0.86
4.	*P. aeruginosa*	15±0.5	19±0.5	20±0.74

The antibacterial activity of hydroalcoholic extract of *Juglan sregia* by agar-well diffusion method is shown in table 6 & 7. From the results of zone of inhibition it was revealed that the hydroalcoholic extract of possess an effective and equipotent antibacterial activity. This activity of the plant extract may be possibly due to phytoconstituents present in it. Bioactive compounds from the plant can therefore be used in the formulation of antimicrobial agents for the treatment of various bacterial infections. Thus the present experiment from the hydroalcoholic extract of *Juglan sregia* has been scientifically proven to be beneficial as an antibacterial agent against specific infections.

Conclusion

It can be concluded that antioxidant activity may account for antimicrobial activity of hydroalcoholic extract of *Juglan sregia*. The results of the present investigation is successful in identifying the antibacterial activity of selected medicinal plant which will help in further identifying the nature of the bioactive principle and its solubility, isolation and characterization of the active principle responsible for the activity. Based on the result of this study it can be said that *Juglan sregia* is an effective antimicrobial plant that can be used for finding new antimicrobial agents in order to treat and control infections.

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