

# Antimicrobial Activities of Different Organic Extracts of Nut Shells of *Juglans Regia* (walnut)

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## Abstract

*Juglans regia* L. (walnut) is widely distributed all over the world. This work compares the antimicrobial activities of hexane, chloroform and methanol extracts of nut shells of *Juglans regia* against three Gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*), three Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) and one fungi (*Candida albicans*) by disc diffusion method. The zone of inhibition of the extracts was compared with ciprofloxacin (antibacterial) and ketoconazole (antifungal).

Key words: *Juglans regia*, medicinal plant, antimicrobial activity, disc diffusion

## Introduction

Over the past few decades, there has been much interest in natural products as sources of new antimicrobial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms. As a result, plants have become one of the bases of modern medicine (Evans *et al.*, 2002). Plant-derived products with phenolics and polyphenolic having major interest and used as antimicrobial agents, (Cowan, 1999). The increased resistance to antibiotics and the problems presented by antimicrobial agents added in food (resistance, mutagenesis and carcinogenesis effects, for example) and public's pressure on the food industry to avoid chemical preservatives are the main factors justifying the search and development of new antimicrobial agents, especially those of natural origin (Rauha *et al.*, 2000; Proestos *et al.*, 2005). The antioxidant activity and antimicrobial potential of walnut leaves were also evaluated (Pereira *et al.*, 2007). Recently, the chemical composition, antioxidant potential and antimicrobial activity have been studied in six walnuts (*Juglans regia* L.) cultivars (cv. Franquette, Lara, Marbot, Mayette, Mellanaise and Parisienne) produced in Portugal (Pereira *et al.*, 2008).

In the present work, powder of nut shells of *Juglans regia* was extracted successively with hexane, chloroform and methanol and the extracts were subjected to antimicrobial screening

against three Gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*) and three Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) and one fungi (*Candida albicans*) by disc diffusion method. The zone of inhibition of the extracts was compared with ciprofloxacin (antibiotic) and ketoconazole (antifungal).

## 2. Materials and methods

### 2.1. Sample preparation

*Juglans regia* (walnut) seeds were broken and kernels were removed. After the removal of kernels, the nut shells were ground to coarse powder and the powdered shell was extracted with hexane for 12 h at room temperature. This process was repeated successively with chloroform and methanol until the color of the extract become colorless. The hexane, chloroform and methanol extracts obtained were filtered separately using Whatman filter paper No.1 and concentrated under reduced pressure in a rotary evaporator. The crude extracts (from hexane, chloroform and methanol extracts) obtained were stored in refrigerator at 4°C.

### 2.2. Antimicrobial activity

Antibacterial and antifungal activities of the extracts were tested by the disc diffusion method using Muller Hinton agar and

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Sabouraud dextrose agar (Hi Media Labs Ltd). The zone of inhibition was recorded on the completion of the incubation and the mean diameter for each extracts at 100, 200 and 300 µg concentrations was recorded. The zone of inhibition was recorded in millimeters (mm). Stock solutions of tested compounds were prepared in relevant solvents. The diameters of the zone of inhibition produced by the extracts were compared with the standard antibiotics ciprofloxacin (10 mcg per disc) and ketoconazole (10 mcg per disc) for *Candida albicans*. Each experiment was repeated three times to minimize the error.

### 2.3. Inoculum preparation

Fresh bacterial/*Candida* cultures were used for the antimicrobial activity. Seven ATCC colonies of the strains were inoculated to Brain Heart Infusion broth and Sabouraud dextrose agar and incubated at 37°C for 22–24 h. The turbidity was adjusted with sterile broth to correspond to the 0.5 McFarland standard; standard inoculum of the microorganism of  $1.5 \times 10^6$  colony forming units (CFU mL<sup>-1</sup>) diluted to 1 : 100 gives suspension of turbidity equal to a Mc Farland standard 0.5. The turbidity was adjusted to match a McFarland 0.5 barium sulfate, prepared by adding 0.5 mL of 1.175% w/v (0.048 m) hydrate (BaCl<sub>2</sub> · 2H<sub>2</sub>O) to 99.5 mL of 1% w/v (0.36) sulfuric acid.

### 2.4. Antimicrobial activity

The different organic extracts were tested for antibacterial activity against *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus epidermidis* (ATCC 155), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 29665), *Pseudomonas aeruginosa* (ATCC 25619) and antifungal activity against *Candida albicans* (ATCC 10231). The screening results are shown in Table 1 and 2.

### 2.5. Disc diffusion Method

The disc diffusion test (Bauer *et al.*, 1966) was performed using Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. Once the medium had solidified, then the culture was inoculated on the medium. Within 15 min of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacteria/*Candida* suspension and inoculated with 1 mL of the organism suspension. The sterile swab was used to streak on the surface of the MHA medium to ensure even distribution of the inoculum. The plates were allowed undisturbed for 3 to 5 min for absorption of excess moisture. The extract loaded discs were placed on the inoculated plates and pressed firmly onto the agar with the sterile forceps to ensure the complete contact with the agar. The plates were incubated at 35–37°C for 24 h. The extracts were used in the concentrations of 100, 200 and 300 µg/disc in relevant solvents. Ciprofloxacin and Ketoconazole were used as standard drugs in the concentration of 10 mcg/disc for antibacterial and antifungal, respectively.

## 3. Results and Discussion

In the present study, antibacterial and antifungal activities (zone of inhibition in mm) of hexane, chloroform and methanol extracts of nut shells of *Juglans regia* were studied against three gram positive and three gram negative and one fungal microorganism and the results are given in Table 1 and Table 2, respectively.

Antimicrobial activities of various other parts of *Juglans regia* have already been studied with different microorganisms. Alkhawajah (1997) reported the growth inhibition effect of *Juglans regia* bark extract against gram positive (*S. aureus* and *S. mutans*), gram negative (*E. coli* and *Paeruginosa*) and pathogenic yeast (*C. albicans*). Mehrabian *et al.* (2000) reported the presence of least microbicidal activity in chloroform extract. Fadi Qa'dan *et al.* (2005) studied the antimicrobial activity of *Juglans regia* leaf extracts, in which they reported the zone of inhibition ranged from 15.8–17.6 mm against *P. acnes*, 11.3–15.7 mm against *S. aureus* and 12.9–15.5 mm against *S. epidermidis* by disc diffusion method. Oliviera *et al.* (2008) reported the antimicrobial activity of the green husk extracts of *Juglans regia*. The chemical composition, antioxidant potential and antimicrobial activity were studied by Pereira *et al.* (2008) in the fruits of six walnuts (*Juglans regia*) cultivars (cv. Franquette, Lara, Marbot, Mayette, Mellanaise and Parisienne) produced in Portugal. Their antimicrobial activities were checked against gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*), revealing activity against the different tested microorganisms.

Coban and Bivik (2010) studied the antimicrobial activity of ethanol extracts of *Juglans regia* (walnut) leaves against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Bacillus thuringiensis*, *Serratiamarcescens*, *Pseudomonas extorquens*, *Proteus sp.* and *Saccharomyces cerevisiae*, *Candida albicans*, *Candida glabrata*, *Candida utilis*, *Candida tropicalis*. The results showed that the ethanol extracts of *Juglans regia* inhibited the growth of nine bacteria and the inhibition zones ranged between 12–25 mm. In addition, the ethanolic extracts of this plant inhibited the growth of all used yeasts and the inhibition zones ranged between 8–16 mm. However, ethanol extract of *Juglans regia* did not show any antimicrobial effects against used three gram negative bacteria such as *E. coli*, *S. pneumoniae* and *S. marcescens*.

Upadhyay *et al.* (2010) reported the in vitro antifungal activity of petroleum ether, benzene, chloroform, acetone and methanol extracts of stem bark of *Juglans regia*. The maximum zone of inhibition (9.33 mm) was shown by methanol and chloroform

extracts (300 µg/ml) against *Aspergillus niger* and *Trichoderma virens*. At the same concentration (300 µg/ml), benzene, acetone and petroleum ether extracts also inhibited the growth of *Fusarium solani*, *Alternaria alternata* and *Aspergillus niger* (9 mm, 8.33 mm, 7.33 mm), respectively, but the petroleum ether, chloroform and methanolic extracts did not show any activity against *Fusarium solani*, *Alternaria alternata* and *Aspergillus niger* at all. These results show that these fungal species are known to be resistant to the action of tested extracts. The methanolic extract was found to be more effective than other extracts which indicates the potency of the bioactive components of the plant against all the test species. The minimum zone of inhibition was found to be of acetone extract against *Fusarium solani*.

The results obtained in the present study, clearly showed that the anti-bacterial activity increases with increasing concentration of the extract in all the tested organisms. All the extracts exhibited more activity against gram negative bacteria particularly with *E. coli* and *P. aeruginosa*. The rest of the gram negative bacteria (*K. pneumonia*) and all the tested gram positive bacteria (*S. aureus*, *S. epidermidis* and *B. cereus*) showed comparatively less activity. Amongst the extracts tested for antibacterial activity with low concentration (100 µg), moderate activity was observed against *E. coli* and *P. aeruginosa*. All the extracts exhibited promising activities against all the six organisms at high concentration (200 to 300 µg). In the case of antifungal activity, methanol extract showed better activity than the hexane and chloroform extracts almost in all the concentrations.

### 5. Conclusion

Among the three different organic solvent extracts hexane showed better activity against antibacterial and methanol extract showed better activity against *Candida*. This may be attributed to single or the combined effect of the phytoconstituents present in the extracts

Test organism	Hexane extract (in µg)			Chloroform extract (in µg)			Methanol extract (in µg)			Ciprofloxacin (10 mcg/disc)
	100	200	300	100	200	300	100	200	300	
<i>S. aureus</i>	10	14	20	09	12	15	11	13	16	33
<i>S. epidermidis</i>	12	15	23	13	18	24	14	17	22	33
<i>B. cereus</i>	13	16	23	14	19	22	13	16	19	33
<i>E. coli</i>	16	22	27	17	24	29	18	22	28	33
<i>K. pneumonia</i>	13	18	22	12	15	20	11	16	21	32
<i>P. Aeruginosa</i>	19	26	29	18	27	28	17	26	31	33

Table1. Antibacterial activity of different organic extracts of nut shells of *Juglans regia*. (Zone of inhibition in mm).

Test organism	Hexane extract (in µg)			Chloroform extract (in µg)			Methanol extract (in µg)			Ketoconazole (10 mcg/disc)
	100	200	300	100	200	300	100	200	300	
<i>C. Albicans</i>	12	15	22	16	18	24	18	20	25	33

Table2. Antifungal activity of different organic extracts of nut shells of *Juglans regia*. (Zone of inhibition in mm).

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