

Research Article

Pharmacognostic study of Walnut (*Juglans regia* L.) endocarp from Azad Jammu Kashmir (AJK)

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Citation

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Abstract

The present study explains antimicrobial activity and acute toxicity of walnut (*Juglans regia* L.) endocarp from Azad Jammu Kashmir (AJK). The walnut endocarp extract tested against fungal species i.e. *Aspergillus niger* and *Penicillium notatum* and bacterial species i.e. *Staphylococcus aureus* and *Escherichia coli*. *Aspergillus niger* and *Penicillium notatum*. at 10, 100 and 1000 µg/ml concentrations showed Diameter Inhibition Zone (DIZ). Diameter Inhibition Zone (DIZ) was maximum against *Aspergillus niger* in all concentrations as compared to *Penicillium notatum*. Similarly, *Staphylococcus aureus* showed higher Diameter Inhibition Zone (DIZ) at 10, 100 and 1000 µg/ml concentration respectively as compared to *Escherichia coli* Diameter Inhibition Zone (DIZ) at the same concentrations. Acute toxicity of walnut endocarp extract showed the significant result at 100 mg/kg, 200 mg/kg and 400 mg/kg concentrations with 0% mortality. It was concluded that walnut endocarp may be important source of antimicrobial activity and may be used in pharmacognosy.

Keywords: Acute toxicity; AJK; Antimicrobial activity; Pharmacognosy; Walnut

Introduction

Pharmacognosy is the systematic study of properties that is structural, chemical and biological effects of crude drugs are studied, method of cultivation, drying, collection, preservation and preparation. Mostly the crude drugs are obtained from different parts of plant or from a whole plant [1]. Therapeutic agents are isolated from different medicinal plant by different way that is modern, Unani, Chinese and traditional system of medicine. On the basis

of chemical analysis, modern systems of medicine have negative reaction and traditional system of medicine have no side effect and produce good actions [2]. Medicinal plants comprise some bioactive organic complexes that is carbohydrates, tannins, flavonoids, alkaloids, steroids and terpenoids which offer precise biological action on the human body. The chemical constituents of plant are helpful because such information was important for the manufacture of complex chemical

constituents [3]. Different parts of the plants such as flowers, fruits, leaves, seeds, roots, endocarp and bark are bases for the origin of secondary metabolites. The plants that contains phytochemicals have constantly played a vital role in medicine [4]. Medicinal plants are used by hakims as 80% of the population living in the villages that are totally dependent on the traditional system of medicines [5].

Juglans regia L. commonly known as “walnut plant” is the wide spread nut tree in the biosphere belongs to family Juglandaceae. It is a distinctive plant having medicinal uses. All parts of walnut can be useful for humans. Previously the Ancient, Greeks and Romans describe and studied the walnut and Theophrastus, “the father of botany” was one of the first to designate walnut [6]. The production of walnut is increase worldwide. The pairs of walnut have been rotated and played with in the palm of the hand to stimulate the circulation blood and as a status symbol in Chinese culture [7]. *J. regia* L. are used for the preparation of Batch flower remedies, a kind of alternative medicine promoted for its effect on health [8]. Juglone is a chemical release from *J. regia* L. has toxic properties and it is toxic to different plant species at diverse levels. Juglone is occur in significant

quantities in all parts of walnut and in endocarp is very little or absent [9]. They recognized the juglone had cytotoxic potential against tumor cells in human [10]. All parts of *J. regia* L. have vitamins i.e. C, A, E, and group B, organic acids, minerals, and tannins are also present. When fruit is unripe it consist of vitamin C about 3 to 5%. The commencement of endocarp solidification vitamin C was found in large quantity [11]. *J. regia* L. has nutrient properties carbohydrates, vitamins, minerals, fats and proteins. It is an important basis of flavonoids, steroids, pectic compounds and phenolic acids [12].

Materials and Methods

Plant collection and powder preparation

The walnut fruits were collected from Azad Jammu Kashmir (AJK) in clean polythene bags in March 2016. The collected sample was brought in to the laboratory, Department of Botany Islamia College Peshawar. The fruits were broken and the endocarp was separated. The endocarps were grinded with the help of electric grinder in the form of powder. The powder was closed in the air tight bottle and protected from the dust and moisture. This powder was further used for pharmacognostic studies (Fig. 1).



Figure 1. Showed walnut endocarp and its powder. It showed the *J. regia* L. endocarp and its powder form after grinding, it indicate the p physical character (brown in colour, rough texture, no smell and tasteless).

Preparation of crude extract

200g powder of walnut endocarp was taken in the clean beaker and add 500ml ethanol. The beaker was covered with aluminum foil and protect from moisture, dust particle and other materials. Left it for 15 days, every day shaking and stirring would be done for few hours. After 15 days solution was filtered by using cotton material, then it was again filtered by Whatman filter paper. The mixture was transferred in to rotary evaporator to evaporate the ethanol from extract. At the end, concentration was obtained that is crude ethanolic extract of walnut endocarp. The crude extract was collected in a close voile, and would be used for performing further activities.

Antifungal activity

The agar well diffusion assay method [13] was used to determine the activity of walnut endocarp extract. The Saboured Dextrose (SD) media was cast off for antifungal activity. *Aspergillus niger* and *Penicillium notatum* was used to studied antifungal activity at various concentrations.

Media preparation

For preparation of Saboured Dextrose Medium (SDM), 40g of Dextrose and 14-15g of Agar was dissolved in 1000 ml distilled water and 10g of peptones was included in it and autoclaved at 121°C temperature for 20 minutes at 15 psi pressure.

Inoculation and antifungal activity

Fungal lawns were prepared with the help of cotton swabs by dextrose broth. Well was formed in petri dishes by metallic borer. Various concentrations 10, 100 and 1000µg/ml of walnut endocarp extract was passed by micropipette in each well of petri dish. Fungicide (Amoxicillin) was taken and added in the well, served as a positive control and DMSO (Dimethyl sulfoxide) was castoff as negative control. Petri dishes were transferred in to incubator at 28°C for 5 to 6 days. Diameter of zone of inhibition

(DIZ) was forced round the well in each petri dish after incubation period. This zone of inhibition showed that the extract have the potential of antifungal activity [14]. After 6 to 7 days, Diameter of zone of inhibition (DIZ) was measure using scale. Percentage inhibition was designed by formula;

$$\% \text{ inhibition} = \frac{\text{(Zone of inhibition of sample)}}{\text{(Zone of inhibition of standard)}} \times 100$$

Antibacterial activity

The agar well diffusion method was used for purpose of antibacterial activity [15]. Two bacterial strains i.e. *Staphylococcus aureus* and *Escherichia coli* were used for antimicrobial activity.

Media preparation, inoculation and autoclaving

For this 2g of agar, 0.5g of Peptone and 0.3g Yeast extract was dissolved in 100ml distilled water and then autoclaved. The Nutrient broth medium (NBM) was made as the Nutrient agar medium (NAM) except agar. Nutrient broth medium was poured in to each test tube (10ml). Put the test tubes in a beaker and Nutrient agar medium in a flask which was covered with cotton and aluminum foil. Petri dishes was covered with newspaper and placed in autoclave along with nutrient agar and nutrient broth media at 121° C temperature for 20 minutes at 15 psi pressure for pasteurization. After autoclaving Nutrient agar medium (NAM) and Nutrient broth medium (NBM) was placed in Laminar Flow Hood (LFH) while petri dishes were transferred in oven at 189° C for oven at aridness. Petri dishes was also transferred to Laminar Flow Hood (LFH) after dryness. Nutrient agar medium was poured into petri dishes and leave these petri dishes for few minutes to solidify. Petri dishes was wrapped with sticky tape. The petri dishes were labeled and reserved in incubator for 24 hours.

Inoculation and antibacterial activity

For antibacterial activity small slice of bacterial strain was transferred by the loop

that was sterilized by heating into the test tubes having nutrient broth medium was present in it. Test tubes were covered with cotton and aluminum foil. The test tubes were given names. Then the test tubes were placed in a beaker with great care and were transferred to incubator at 37° C for 24 hours. Next day test tubes that contained distilled water and cotton swabs were covered with the foil were put in autoclaved for sterilization. After this test tubes, petri dishes having culture of bacteria and cotton swabs were taken and placed in Laminar Flow Hood (LFH). Now test tubes were taken that contained bacteria and nutrient broth. By the help of micropipette a portion of it was relocated in to the test tube have distilled water. Uniform lawn of the bacteria was formed by the cotton swabs on the nutrient agar petri dishes and permitted for 5 minutes. Using cork borer wells were made on the petri dishes that contained nutrient agar media.

Stock solution was formed by 5mg of the walnut endocarp extract that was dissolved in the 3 mL DMSO (Dimethyl sulfoxide). Prepared 10, 100 and 1000µg/ml concentrations. They were added to every well and was labeled by the help of marker. Amoxicillin was used as positive control and DMSO (Dimethyl sulfoxide) was for the negative control. The petri dishes were covered with sticky tape and were placed in the incubator for one day at 37 °C for the purpose of zone of diameter of inhibition. Zone of inhibition was measured on next day by the use of the ruler. % inhibition was calculated by the formula;

$$\% \text{ inhibition} = \frac{(\text{Zone of inhibition of sample})}{(\text{Zone of inhibition of standard})} \times 100$$

Acute toxicity

The study of acute toxicity was performed on ethanolic extract of *J. regia* L. by using albino mice having weight of 25kg to 30kg. The mice were arbitrarily dispersed in to 4 groups each with 6 mice. The mice were used to research laboratory environment

before the beginning of test. The mice were fasted from food full night, control group was treated with normal saline and the residual 2 to 4 groups were treated with 100mg/kg, 200mg/kg and 400mg/kg body weight respectively of the walnut endocarp extract. The mice were perceived constantly for the first 4 hours and then for the succeeding 24 hours [16]. Mice were kept fast for 24 hours before the experiment was performed and divided into 6 groups. Group one was inoculated with normal saline (10ml/kg) as a negative control, group two with standard (10mg/kg) as positive control, group three with 1% acetic acid for stimulate pain (writhing) while group 4 to 6 were injected with 100, 200 and 400 mg/kg of Walnut endocarp. After 20 minutes, 1% acetic acid was inserted to every mice. After 5 minutes, mice were separately observed to count the number of writhing (painful muscle contraction) or elongating reaction for 20 minutes. The mean number of writhing and the % inhibition were designed and the groups were related with the control group;

$$\text{Percent writhes inhibition} = \frac{100 - A - B}{A} \times 100$$

Where;

A= Average number of writhes of the positive control

B= Average number of writhes of the test group

Results and Discussion

Following biological activities were performed for the determination of pharmacological potential on the ethanolic extract of *J. regia* L. endocarp.

Antifungal activity

In antifungal activity the ethanolic extract of *J. regia* L. was tested on different fungi *Aspergillus niger* and *Penicillium notatum*. Dissimilar dose of concentrations that is 10, 100 and 1000 µg/ml inhibit the growth with different zone of inhibition. In Walnut endocarp dose of 10, 100 and 1000 µg/ml concentrations displayed the zone of

inhibition i.e, 7.00 ± 0.75 , 11.83 ± 0.76 and 15.67 ± 1.04 against *Aspergillus niger*. Amongst this walnut endocarp has higher Diameter of zone of inhibition (DIZ) at 1000 $\mu\text{g/ml}$ concentration (Table 1; Fig. 2). Results were fairly related with the results detected by [17], who worked on antifungal activity of *Punica granatum* shell and determined maximum activity against *Aspergillus niger* i.e. 22mm. The outcomes are also similar with [18], that showed highest antifungal activity of *Aspergillus niger* against shell of pomegranate i.e. 16, 12 and 10 mm respectively. Dose of 10, 100 and 1000 $\mu\text{g/ml}$ concentration of Walnut endocarp extract have exhibited the zone of inhibition 5.90 ± 0.79 , 9.83 ± 1.04 and 12.67 ± 0.76 respectively against *Penicillium notatum*. Highest Diameter of zone of inhibition (DIZ) has found at 1000 $\mu\text{g/ml}$ concentration. Our results also showed overall higher diameter of inhibition zone at 10, 100 and 1000 $\mu\text{g/ml}$ concentration against *Aspergillus niger* as compare to *Penicillium notatum* at each concentration.

Antibacterial activity

The antibacterial activity of walnut endocarp extract against different bacteria *Escherichia coli* and *Staphylococcus aureus*. The results confirmed that *Staphylococcus aureus* inhibits highest Diameter of zone of inhibition (DIZ) 12.66 ± 0.76 against walnut endocarp at 1000 $\mu\text{g/ml}$ concentration and

lower diameter of inhibition zone 9.16 ± 0.76 , 6.00 ± 1.0 at 100 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ concentration respectively. *Escherichia coli* prevent minimum Diameter of zone of inhibition (DIZ) as 4.83 ± 0.38 at 10 concentrations, 7.0 ± 0.50 at 100 and maximum Diameter of zone of inhibition (DIZ) 9.5 ± 0.50 at 1000 $\mu\text{g/ml}$ concentration. Our results also showed overall higher diameter of inhibition zone at 10, 100 and 1000 $\mu\text{g/ml}$ concentration against *Staphylococcus aureus* as compare to *Escherichia coli* at each concentration (Table 2; Fig. 3).

Staphylococcus aureus showed the maximum diameter of inhibition zone $34.0 \pm 1.7\text{mm}$ in the shell of pomegranate [19]. Present findings are similar with [20] who described that maximum antibacterial influence was noted on *Staphylococcus aureus* and showed higher diameter of inhibition zone that is 25mm. The results given in the table were also related to the consequences detected higher Diameter of zone of inhibition (DIZ) 19, 14 and 10mm against *Staphylococcus aureus* respectively at dissimilar absorptions of shell of pomegranate [18]. Extract of barks, fruits and green rinds of walnut from various area showed antibacterial activity against bacterial strains i.e. *Staphylococcus aureus*, *Pseudomonas spp.* and *Escherichia coli*, using agar streak method [21, 22].

Table 1. Diameter of inhibition zone (DIZ) of pathogenic fungi against *J. regia* L. endocarp at three different concentrations.

Fungi	10 Concentration ($\mu\text{g/ml}$)	100 Concentration ($\mu\text{g/ml}$)	1000 Concentration ($\mu\text{g/ml}$)
<i>Penicillium spp.</i>	5.90 ± 0.79	9.83 ± 1.04	12.67 ± 0.76
<i>Aspergillus niger</i>	7.00 ± 0.75	11.83 ± 0.76	15.67 ± 1.04

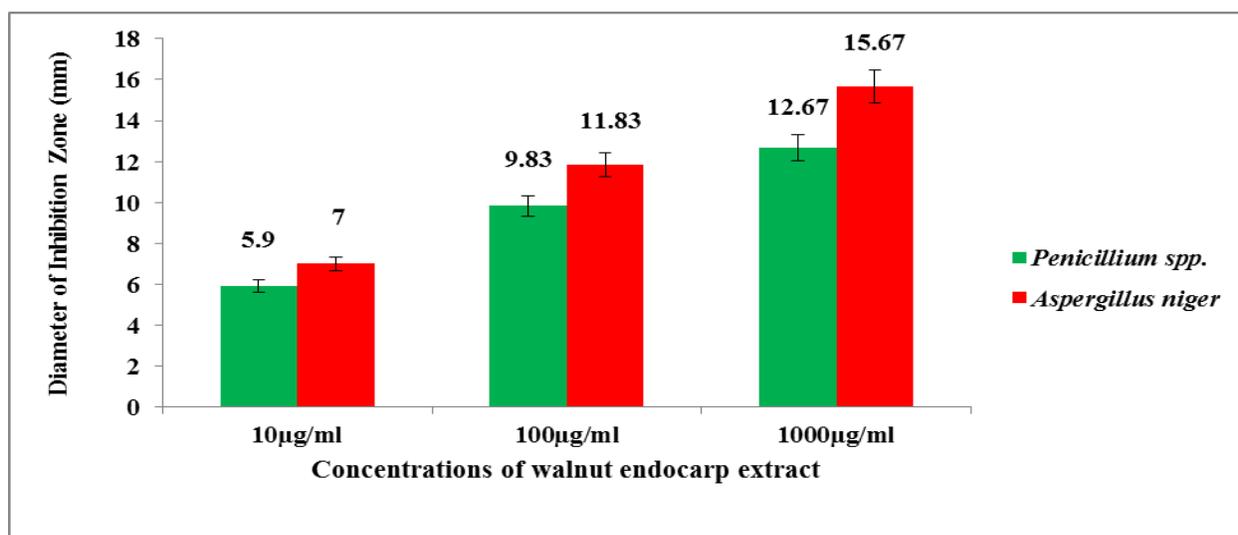


Figure 2. Antifungal activity of ethanolic extract of walnut endocarp. It shows the DIZ is greater in *Aspergillus niger* as compare to *Penicillium notatum*. at 10,100 and1000 µg/ml concentration

Table 2. Diameter of inhibition zone (DIZ) of Bacteria against *J regia* L. endocarp at three different concentrations.

Bacteria	10 Concentration (µg/ml)	100 Concentration (µg/ml)	1000 Concentration (µg/ml)
<i>Staphylococcus aureus</i>	6.00±1.0	9.16±0.76	12.66±0.76
<i>E. coli</i>	4.83±0.38	7.0±0.50	9.5±0.50

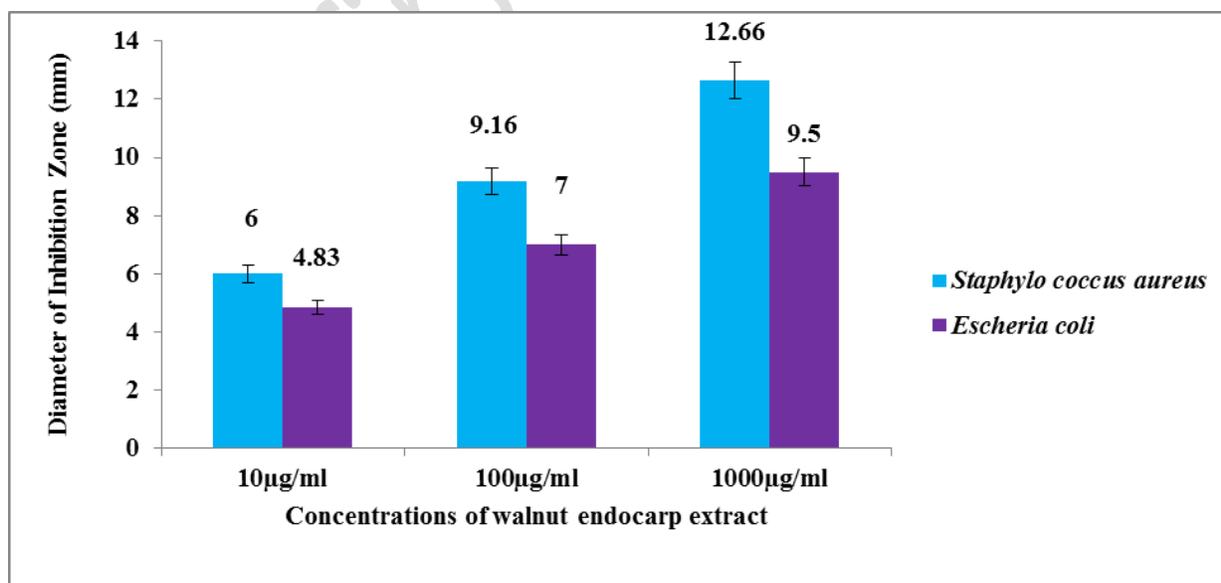


Figure 3. Antibacterial activity of ethanolic extract of walnut endocarp. It shows that the *Staphylococcus aureus* have high DIZ at 10,100 and 1000 µg/ml as compare to the *E coli*

Acute toxicity

The present study was conducted using mice for acute toxicity of *J. regia* L. endocarp ethanolic extract following the method [16]. The evaluation of toxicity is essential, as plant extract was used for the beneficial of human beings for effective and safe dose management [23]. Therefore, our result showed that doses of the *J. regia* L. endocarp extract is safe for living organisms. Endocarp is not use as a crope residue but have a large amount of lignin content and energy which is used for the bio-products as compare to other biomass resources [24]. The methanolic extract of walnut endocarp use as a natural additive constituent in food and pharmacological industries [25].

Conclusion

The aim of this research was to investigate the medicinal value of endocarp of *J. regia* L. and its possible uses, as endocarp of walnut is wasted mostly by the human being. So this study was conducted to determine the medicinal importance in endocarp of *J. regia* L. Pharmacognostic studies help us in the detection of adulteration and identification of crude extract which is used against bacterial, fungal diseases and acute toxicity in *J. regia* L were analyzed. We tried to explore the endocarp for pharmacological value and to commercialize on industrial scale.

Authors' contributions

Conceived and designed the experiments: N Azam, Performed the experiments: S Qadar, Analyzed the data: S Qadar, Contributed materials/analysis/tools: I Ahmad & S Khalid, Wrote the paper: M Anwar.

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