Research Article

Preliminary Phytochemical Evaluation and Antibacterial Potential of Different Leaf Extracts of *Juglana Regia*: A Ubiquitous Dry Fruit from Kashmir-India

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Accepted on: 18-02-2013; **Finalized on:** 31-03-2013.

ABSTRACT

Juglans regia, the royal species from *Junglandaceae* family, well-known for its valuable medicinal uses and is grown in the forests of Himalayas in India. It is a woody, deciduous and frost-tender tree. The root, stem bark, leaves, seeds, cotyledons and seed oil are useful to treat various health complaints including cancer in the folk medicines. The fresh leaves were collected in the month of May 2012 from orchards of Bandipora (J&K). Leaves were shade dried, powdered and extracted using different solvents viz., acetone, methanol, ethanol and distilled water in ascending order of polarity. Preliminary phyto-chemical screening of the crude extracts revealed the presence of carbohydrates, cardiac glycosides, flavonoids, steroids and tannins. Crude extract of the leaves of *Juglans regia* Linn. were tested for antimicrobial activity by observing the zone of inhibition against four species of Gram +ve and Gram –ve bacteria. Antifmicrobial activity was done by disc diffusion method at concentrations of 50 and 100µg/ml/disc of the extracts. The extracts showed selective bacteriostatic action against some species. All extracts showed varying degrees of inhibitory activity against all bacterial species. Ethanolic and acetone extract showed significant activity against *E.coli and Klebsella* respectively.

Keywords: *Juglans regia* L., *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsella pneumoniae.*

INTRODUCTION

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin. Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents[1]. *Juglans regia*, known as Akhrot in India, a native of Eastern Europe to North Asia i.e. China, Iraq, Mexico, Spain, Turkey, Nepal, India (forests in Himalayas) is a member of *Juglandaceae* family. It is a woody, deciduous and frost-tender tree growing to 20m. height[2]. The wood is heavy, durable and polishes well. The bark is resinous and scented. The tree is in flower in June and a seed ripe in October. This valuable tree has a long history of medicinal use to treat a wide range of health complaints. Almost all parts of the plant are medicinally important. The dried green husks contain 2.5-5% ascorbic acid (vitamin C) which can be extracted and used as a vitamin supplement. The root and stem bark are antihelmentic, astringent[3], antibacterial[4] and detergent. The stem bark is dried and used as a tooth cleaner. The decoction of leaves and bark is used with alum for staining wool brown. The cotyledons are used in the treatment of cancer since a long time. Some extracts of the plant have shown anticancer activity. *Juglans regia* Linn. stem bark contains chemical constituent's viz. β-sitosterol, ascorbic acid[5], juglone, folic acid, gallic acid, regiolone, and quercitin-3-α-L-arabinoside[6]. Juglone found in the leaves and its derivatives show a wide spectrum of applications in the field of cosmetics, pharmacology and ecology. This tree is reputed to possess varied medicinal properties. Considering the vast medicinal applications of the royal species, an attempt is made to technically analyze the leaves for *in vitro* antibacterial activity as there is negligible report on antibacterial activity of this plant. The present work therefore, attempts to evaluate the antimicrobial activity of the leaves of *Juglans regia* Linn.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *Juglans regia* Linn. was collected in the month of May, 2012 from Orchards of bandipora (J&K) and was authenticated.

Processing of plant material

The leaves were shade dried over a period of two weeks. The dried samples were milled into fine power by pounding manually with a clean and sterile mortar, stored in sterile cellophane bags in a cool dry place till further use.

Preparation of extracts

The dried coarse powder (172gm) was extracted in separating funnel sequentially in 400ml of various solvents viz., acetone, methanol, ethanol and distilled water in the ascending order of polarity. The process was run till the decolourisation of the solvent, after which the sample was concentrated in water bath at the temperature of 45°C and further condensed to powdered form. The dried extracts were weighed and kept in labeled sterile specimen bottles.

Solubility Test

To check the solubility, extract was dissolved in different solvents e.g., DMSO, water, methanol, ethanol, chloroform and acetone to prepare different concentrations ranging from 50µg/ml to 100µg/ml and used for screening the antibacterial activity. Methanolic extract was soluble in distilled water and methanol, benzene extract was soluble in benzene and acetone, ethanolic extract was soluble in methanol and ethanol, acetone extract was soluble in acetone, benzene and ethanol and water extract was soluble in water and partially in methanol.

Preliminary Phytochemical screening

The secondary metabolites classes such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids and tannins were screened according to the standard phytochemical methods[7]

Screening for antibacterial activities

Bacterial strains

On the basis of pathogenic importance and literature surveys following test micro-organisms were selected for antibacterial activity assay. The bacterial isolates were cultured on Nutrient agar media incubated at 37˚C for 24 hrs and the microorganisms were repeatedly sub-cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification.

Preparation of media

The medium was prepared by dissolving Nutrient agar media (HiMedia Laboratories Pvt. Ltd) in distilled water and autoclaved at 121ºC for 15 minutes. It was used for antimicrobial study.

Preparation of inoculums

Stock cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsella pneumonia* were maintained at 4^{o} C on slopes of Nutrient agar media. Active cultures for experiment were prepared by transferring a loopful of bacterial organisms from stock cultures to test tubes of Nutrient agar media slants and incubated for 24 hours at 37^{o} C.

Antibacterial susceptibility test /Agar disc diffusion assay

The Nutrient agar media in sterile Petri plates was used for the test cultures. The NA (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were prepared by pouring molten media in to sterile petriplates. NA plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. The plates were allowed to solidify and inoculum suspension was spreaded uniformly with glass spreader. Sterile discs (6.0mm in diameter) were dipped in solution of the different concentration (50µg/ml and100µg/ml) of various extracts serially dissolved in their respective solvents till saturation and were dried. The methanol was used as control for methanolic extract. The plates were incubated to 37°C for 24 hrs. The diameter of the Zone of inhibition was measured in mm and the antibacterial experiments were performed in triplicates.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Phytochemical screening of the crude leaf extracts of *Juglans regia* Linn. revealed the presence of carbohydrates, cardiac glycosides, flavonoids, alkaloids, proteins, steroids and tannins. Results of preliminary phytochemical screening are tabulated in (Table 1). The increasing reliance on the use of medicinal plants worldwide has been traced to the extraction and development of several drugs from these plants as well as from traditionally used rural herbal remedies. Further, detailed investigation needs to be underway to determine the exact phytoconstituents and isolate the active principles which are responsible for the antibacterial activity of the leaves of *Juglans regia* Linn.

Agar disc diffusion assay

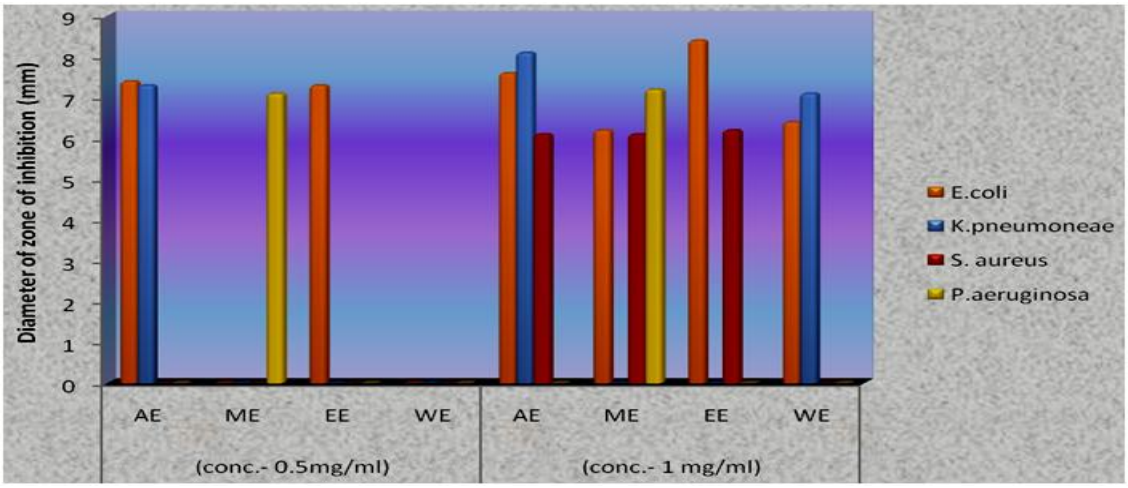
In vitro antibacterial activity of acetone, methanol, ethanol and water extracts of leaves of *Juglans regia* Linn. were evaluated by measuring the diameters of zones of growth inhibition of the bacterial colonies and the results are tabulated in table 2. The highest zone of growth inhibition was shown by acetone and ethanolic extracts (1mg/ml) against *Kelebsella pneumonia* and *E.coli* respectively (8.1mm and 8.6mm).

Table 1: Preliminary Phytochemical Evaluation of the Leaves of *Juglana regia* L.

Phytoconstituents	Benzene extract	Acetone extract	Methanolic extract	Ethanolic extract	Water extract
Alkaloids	-	+	+	-	+
Flavonoids	-	-	+	-	+
Glycosides	+	-	+	+	+
Proteins	-	-	-	-	+
Steroids	+	+	-	-	-
Tannins	-	-	+	+	-
Carbohydrates	-	-	-	-	+

Table 2: Antibacterial Activity of the Different Extracts of the leaves of *Juglans regia* linn.

Test organism	Diameter of zone of inhibition (mm) With SD				Diameter of zone of inhibition (mm) With SD			
	Extract concentration (0.5mg/ml)				Extract concentration (1mg/ml)			
	Acetone extract	Methanol extract	Ethanol extract	Aqueous extract	Acetone extract	Methanol extract	Ethanol extract	Aqueous extract
E.coli	7.4±0.1	0.00±00	7.3±0.1	0.00±00	7.6±0.1	6.2±0.1	8.6±0.06	6.4±0.1
Klebsella pneumoneae	7.3±0.1	0.00±00	0.00±00	0.00±00	8.1±0.06	0.00±00	0.00±00	7.1±0.06
Staphylococcus aureus	0.00±00	0.00±00	0.00±00	0.00±00	6.1±0.06	6.1±0.06	6.2±0.1	0.00±00
Pseudomonas aeruginosa	0.00±00	7.1±00	0.00±00	0.00±00	0.00±00	7.2±0.1	0.00±00	0.00±00



Graph 1: Graphical Representation of Antibacterial Activity of extracts of *regia* Linn leaves.

At same concentration (1mg/ml) water and methanolic extracts also inhibited the growth of *Kelebsella pneumonia* and *Pseudomonas aeruginosa* (7.1mm, 7.2mm,) respectively, but all types of the extracts (Acetone, Methanol, Ethanol, Water) at 0.5mg/ml conc depicted negligible activity against *staphylococcus*. The ethanolic extract was found to be more effective than other extracts which indicates the potency of the bioactive components of the plant against the test species. The lowest zone of growth inhibition was found to be of acetone extract and of methanolic extract against. *staphylococcus* (6.1mm) at conc. of 1mg/ml. The antibacterial potency of the leaves of *Juglans regia* Linn. may be attributed to single or the combined effect of the phytoconstituents present in the leaves. Our results are in agreement with the findings reported by Upadhyay *et. al.* 2010[8] and to some extent similar to the findings exhibited by kale *et. al.* 2011[9].

CONCLUSION

The phytochemical assay of the leaf extracts of *Juglans regia* Linn. revealed the presence of carbohydrates, cardiac glycosides, flavonoids, steroids and tannins. Thus, antibacterial activity of *Juglans regia* Linn. is evident due to the active compounds present in the crude extracts. The findings in the present study offers a scientific support to the use of stem bark of *Juglans regia* Linn. as an antibacterial in new drugs for therapy as it showed good antibacterial activity. Further pursuit on the isolation of bioactive compounds would enable more potential and natural anti-bacterials against several strains.

In conclusion, this study supports the use of natural products as medicines because active biomolecules in plant extracts exhibit antibacterial activity to a considerable extent. It provides the basis for the present rapidly increasing interest for the use of natural antioxidants and antimicrobials. The results strongly support the reported traditional use of *j. regia* plant.

Conflict of Interest

Authors have no competing conflict of interest.

Acknowledgement: The authors are thankful to the Head, Department of Research Jawaher Lal Nehru Cancer Hospital and Research Center for providing laboratory facilities to perform the experiments.

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Source of Support: Nil, **Conflict of Interest:** None.