ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antibacterial Potentials of Bark and Leaves Extracts of Juglans *Regia* against Antibiotic Resistant Bacteria

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Manuscript Info

Manuscript History:

Received: 15 February 2013
Final Accepted: 10 March 2013
Published Online: March 2013

Key words:
antibacterial activity,
antibiotics,
gram positive bacteria,
skin diseases.,

Abstract

Plant parts were collected from Abbottabad District, Khyber Pakhtunkhwa. Then crude methanolic extract of bark and leaves of *Juglans regia* were taken. Each methanolic crude extract was diluted in Dimethyle sulphoxide (DMSO) up to the concentration of 60 mg/ml, 90 mg/ml and 120 mg/ml. The plant parts were examined for antibacterial activity against the gram positive bacteria (*Staphylococcus aureus)* and gram negative bacteria (*Agrobacterium tumefaciens)*. *Juglans regia* bark and leaves show remarkable antibacterial activity against gram positive and gram negative bacteria. By comparing the results with the standard antibiotics lincomycine and ceftrixone, both standards have greater zone of inhibition against the gram positive and gram negative bacteria. From both the plant extracts as well as antibiotics, it was found that the gram positive bacteria (*Staphylococcus aureus*) was more sensitive than gram negative bacteria (*Agrobacterium tumefaciens*). Thus, the plant extract of *Juglans regia* bark was found to have more potent antimicrobial activity against gram positive bacteria, *Staphylococcus aureus*, which can be used to treat skin diseases.

Introduction

The use of drugs derived from plants has been in practice for a very long time because of the high rate of mortality caused by bacterial infections and diseases in human population and its significance cannot be over emphasized with the recent trend of high percentage of multidrug resistance to the present day antibiotics (Adegoke et al., 2010).

There has been a very strong traditionally dependency on the medicinal plant, for the treatment of illness. In Jamaica tropical research with seventy one percent of the patients have been treated with herbal medicines, before reported to the medical services. In Jamaica the most folk uses to control the cold, fever, coughs, is also through use of the medicinal plants. The medicinal plant is also used as pesticides in the past time and thus in 1960 the trend has been changed that how this services has been incorporated in to the modern medical practices of the country in that time (Mitchell & Ahmad, 2006).

Diarrhea is one of the most dangerous diseases of the world and is also estimated that it is responsible for the death of 3-4 million individuals per each year. The major facts of infection is due to food borne by *Salmonella*, and the *Escherichia coli* also causes the water borne infection due to the use of domestic water. In 1998 in South Africa essentials drug programme was recommended for the diarrhea infection control (that was also adopted by world health organization). But in this case the people of that place take help from the traditional healers. They also provide to the people alternative of the drug which is also derived from the plants, and is also easily available and so helpful from the modern medicines (Mathabe *et al.*, 2006). In South Africa in the Limpopo province plants were used by vhenda speaking traditional healers from different places and different localities, also use the medicinal plants for the treatment of diarrhea (Ngobeli, 2002).

In Africa medicinal plants are used for the treatment of diarrhea (Mathabe *et al.*, 2006). Typhoid fever is also the most danger and a major scourge most affective besides malaria. This disease causes the

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death of 16.6 million people globally each year (WHO, 2003). The same work was carried out in the Ebonyi state university, Department of food technology, and used the plants material against the causative agent of typhoid fever, and the extract of *Allium sativum* and *Moringa oleifera* against the Sal*monella typhi* to determine the antibacterial effect against the typhoid fever (Ayogu & Amadi, 2009).

The herbal medicines are used from primitive to the present time in very strong position. Ethno botanical pharmacology is older as the man in the world. In Indo-Pak the plant medicine were used in Rig Veda between 4500 -1600 BC and Ayurveda between 2500 -600 BC. Its origin is also traces with Greek medicines; the Greek medicine was first adopted by the Arabs and transferred to Indo-Pak and Europe. Globally, about 80% population depends upon traditional system of the treatment (Ahamad, 1999). The plant medicines have no side effect, and human being getting it easily from the nature. In Pakistan Unani medicine is dominant but in the remote areas the ethno medicinal plants are also used (William, 2001). The people used about 90% of the medicinal plants species, the area where most of the medicinal plants are present. This also indicates of the vast repository to knowledge of plant medicine that is also available for global uses. Traditional and indigenous medical uses of plants, oral and codified, are undoubtedly eroding. (Shah & Khan, 2008).

In Pakistan the very large variety of the medicinal plants is due to the climatic effects and is also the great amount of the valuable medicinal plants are present in the forests of the Pakistan. But very little work is done on these plants. The *Colchicum lutem* Baker is one of the Pakistani medicinal plants from the Pakistani forests. The whole plant was collected from upper Dir N.W.F.P and also used as anti bacterial and antifungal and have a good results (Ahmad *et al.,* 2006). The present study focused on investigation of antibacterial potentials of the *Juglans regia* plant, collected from District Abbottabad, Khyber Pakhtunkhwa for first time in Pakistan as there is no such available research work.

Material and Methods

Plant material collection, Identification and preparation of extract

Bark and leaves of *Juglans regia* were collected from Thandiani (Abbottabad) and identified by a taxonomist. The research work was conducted in the Microbiology Laboratory, department of Biological sciences, Gomal University Dera Ismail Khan NWFP, Pakistan. After cleaning of adulterant material, the leaves rinsed with distilled water and kept under shade till drying and then weighed. Extraction from bark and leaves was taken by simple maceration process. The bark and leaves were taken and grinded up to powder form by kitchen blender, and then deepen in the methanol. This poorly homogenized mixture of leaves was kept for 4 weeks and of bark for 5 weeks under the shade at room temperature (25C˚±2C˚) in extraction bottle. After 4 weeks (for leaves) and after 5 weeks (for bark) maximum amount of methanol was separated from the mixture, Filtrate was filtered twice, first using the ordinary filter paper and then Watt's man No. 4 filter paper. The remaining methanol was then completely evaporated by vaparotator (rotary) at a temperature of 60C˚ and at a rotation of 80 rpm to obtain the *Juglans regia* crude extract.

Preparation of samples

The 120 mg of *Juglans regia* methanolic extract was dissolved in 1 ml of dimethyle sulphoxide (DMSO). This stock solution was used for further dilution with DMSO i.e. 120 mg/ml, 90 mg/ml and 60 mg/ml. Lincomycine 400 mg and ceftrixone 500 mg was also diluted as i.e. 2 mg/ml in the DMSO for comparative study.

Isolation of *Staphylococcus aureus*

Staphylococcus is gram positive bacteria and is normal flora of skin. Sterilized cotton swab was rubbed with neck and then spread on the nutrient agar media. The plates were incubated at 35C˚ for 24hrs. All colonies were screened for gram positive bacteria, *Staphylococcus aureus.*

Isolation of *Agrobacterium tumefaciens*

Agrobacterium tumefaciens is gram negative bacteria which causes crown gall diseases in the plants (plant cancer) was also isolated from the infected parts of *Rosa indica* family *Rosacea*, number of infected plants parts were cut and reins with distilled water and then dropped into the sterilized water for 3 hrs, then transferred the culture from water to the carrot discs in a sterile dishes in filter paper and incubated at a temperature of 25C˚ for 3 weeks. Only those samples were taken which cause gall formation. The culture from gall was taken by wire loop and streaked on the yeep agar media and incubated at 25C˚ for 24hrs. The culture from the yeep agar media was transferred to yeep broth media and incubated at 25C˚ for 24hrs. Then by the spread plate technique 0.01 ml of culture broth was spread on Yeep agar media. All the colonies were screened for *Agrobacterium tumefaciens*.

Inoculums preparation for *Staphylococcus aureus*

The bacterial strain from 24 hrs old culture were streaked on the same fresh culture media and incubated for 24 hrs. Then a loop bacterial culture

was formed. The streak plate was transferred to nutrient broth and incubated for 24 hrs at 35C˚ at 120 rpm and then 0.01 ml of the broth culture was spread on the nutrient agar and observed the number of colony forming unit (CFU).

Inoculums preparation for *Agrobacterium tumefaciens*

The bacterial strain from 24 hours old culture was streaked on the same fresh culture media and incubated for 24 hrs. Then a loop bacterial culture was formed. The streak plate was transferred to nutrient broth and incubated in shaking incubator for 24 hrs, 25C˚ at 210 rpm and then 0.01 ml of broth culture was spread on the yeep extract medium and observed the CFU.

Disc diffusion

The antimicrobial assay was performed by using the disc diffusion method. Actively growing cell of both the strains were spread by spread plate technique with the help of micropipette. Culture of 0.01 ml was spread on the nutrient agar medium plates. Subsequently, filter paper discs (6 mm diameter) saturated with plant crude extract/antibiotic was placed on the surface of each inoculated nutrient agar medium. After placing the discs the plates were incubated at 35C˚ for 24 hrs. Then the zone of inhibition around the discs was measured (Rodrigues et al., 2008).

Agar well diffusion

The antibacterial activity was also performed by using agar well diffusion method. The agar plates were prepared by using nutrient agar (Merck). The microorganism culture was evenly spread on the surface of agar plates by sterile swab sticks. Four wells (5 mm in diameter) were made in each plate with the help of sterile cork borer. The plant extracts/antibiotic were added in each well and incubated at 35C˚ for 24 hrs. After incubation the plates were observed for the presence of bacterial growth and for the measurement of zone of inhibition around the well. The size of zone of inhibition was measured in millimeters for antibacterial activity (Mathab et al., 2006).

Pouring of test solution, incubation and measurement the zone of inhibition

The test solution was poured by micropipette in the respective wells, and in case of disc diffusion, four concentrations of the extracts (150 mg/ml, 120 mg/ml, 90 mg/ml and 60 mg/ml) for positive control (lincomycine and ceftrixone) were applied to plates. The plates were incubated at 35C˚ for *Staphylococcus* and 25C˚ for *Agrobacterium*. After 24 hrs of incubation the diameter of zone of inhibition around the well and disc was measured. Antibacterial activities of all dilution of extracts were determined against the two strains of the bacteria.

Result and Discussion

The leaves and bark extract of *Juglans regia* shows the highest activity against the gram positive bacteria and gram negative bacteria as compared to the standard antibiotic; lincomycine and ceftrixone.

Juglans regia bark extract have the zone of inhibition 15 mm, 16 mm and 40 mm against the gram positive bacteria, *Staphylococcus aureus* while it is 13 mm, 15 mm and 20 mm against the gram negative bacteria, *Agrobacterium tumefaciens* (Table 1). The antibiotic, lincomycine have 30 mm zone of inhibition against the gram positive bacteria, *Staphylococcus aureus* and 20 mm zone of inhibition against the gram negative bacteria, *Agrobacterium tumefaciens*. Ceftrixone have the zone of inhibition, 32 mm against the gram positive bacteria, *Staphylococcus aureus* and 25 mm zone of inhibition against the gram negative bacteria, *Agrobacterium tumefaciens* (Fig. 1).

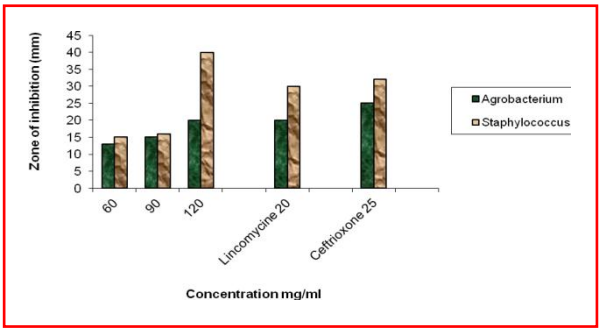

Figure 1. Antibacterial activity of *Juglans regia* Bark and comparison with antibiotics.

The *Juglans regia* leaves extract have the zone of inhibition, 9 mm, 12 mm and 20 mm against the gram positive bacteria and no zone of inhibition at 60 mg/ml concentration, while it is 7 mm and 11 mm against the gram negative bacteria (Table 2). The antibiotic lincomycine, have 30 mm zone of inhibition against the gram positive bacteria and 20 mm zone of inhibition against the gram negative bacteria while ceftrixone have the zone of inhibition, 32 mm against the gram positive bacteria and 25 mm against the gram negative bacteria (Fig. 2).

From these results it becomes clear that the *Juglans regia* bark and leaves extract show highest antibacterial activity against the gram positive bacteria and low activity against the gram negative bacteria. Similarly, the bark and leaves extract have the highest activity as compared to the standard antibiotic.

Medicinal plants are present in Pakistan at a very high level and thus Pakistan is a rich source of medicinal plants, the peoples are willing to use these plants as a traditional medicine. Since Unani system of medicine is now revived in recognition of unparallel contribution of Arabs and Greeks, closely associated with Muslims. This system of medicine is gaining revival in Pakistan and many Muslims countries in the name of Tibe-Islami, thus now a day Pakistan is the reach source of the traditional medicines.

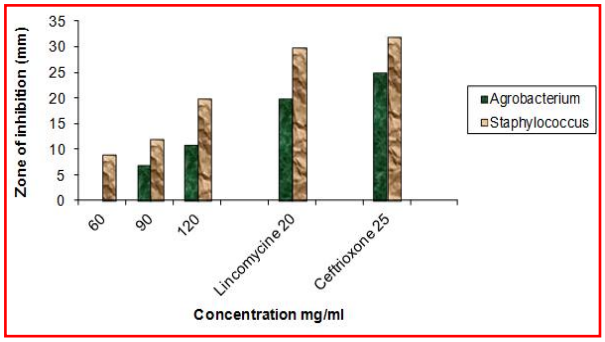

Figure 2. Antibacterial activity of *Juglans regia* Leaves and comparison with antibiotics.

The agar well diffusion methods are very commonly used for the screening of antimicrobial activities. In this study we have used the agar well diffusion method for the determination of antimicrobial activity of methanolic extracts of medicinal plants. The same technique was also used by some other researcher for their work as Doughari & Manzara (2008) Mathab *et al.* (2006) & share *et al.* (2008).

Disc diffusion methods are also used very commonly for the antibacterial activity of bacteria and for the antibiotic comparison with extracts. In this study the disc diffusion method for determining the antimicrobial activity of the methanolic extracts of medicinal plants was observed. Similar technique of the disc diffusion method has been used by some other research workers as (Jonathan *et al.,* 2000, Maksimovic *et al.,* 2008, Doughari & Okafor, 2008).

The culture medium used in the study was the nutrient agar which is also used for culturing routine pathogens. This medium is also suitable for the growth of the pathogens used in this study i.e. *Staphylococcus aureus.*

The yeep medium was also used in study was the culturing medium for the *Agrobacterium tumefaciens.* The same medium was also used by Huang *et al*. (2001) in his work.

Methanol was used as a solvent for extraction of the crude extract from the plants parts. The methanol is better solvent, and in other hand all the chemical compounds which have the best antimicrobial activity are soluble in methanol (Chanrasekaran & Venkatesalu, 2004). Some research workers also used the solvent methanol in their research works as (Polat *et al.* 2008, Alzoreky & Nakahara, 2003).

The plants in the present study shown antibacterial activity against gram positive and do not show the remarkable activity against gram negative like *Juglans regia* leaves. This plant show the best activity against gram positive *(Staphylococcus aureus),* the same results was also reported by Kaushik & Goyal (2007) that the gram positive bacteria are more sensitive than the gram negative bacteria and both are not sensitive to the plant extracts or antibiotic.

Table. 1- *Juglans regia* (Bark), Zone of inhibition (mm) after 24hrs

S. No.	Conc. mg/ml	*Staphylococcus aureus*	*Agrobacterium tumefaciens*
1	60mg/ml	15mm	13mm
2	90mg/ml	16mm	15mm
3	120mg/ml	40mm	20mm

The zone of inhibition of the activity on plant extracts was also different among the gram negative and gram positive as well as the zone of inhibition of *Juglans regia* bark was 40mm against the gram positive bacteria (*Staphylococcus aureus*). On the other side, gram negative bacteria (*Agrobacterium tumefaciens*) the zone of inhibition of *Juglans regia* bark was 20mm. Therefore, it is clear that the gram positive bacteria are more sensitive than the gram negative bacteria.

Table. 2- *Juglans regia* (Leaves), Zone of inhibition (mm) after 24hrs

S. No.	Conc. mg/ml	*Staphylococcus aureus*	*Agrobacterium tumefaciens*
1	60mg/ml	09mm	No inhibition
2	90mg/ml	12mm	07mm
3	120mg/ml	20mm	11mm

The antibiotic zone of inhibition was also differ among the gram positive and gram negative bacteria i.e. the zone of inhibition of lincomycine (400mg) against the gram positive bacteria was 30mm and of the ceftrixone (500mg) was 32mm, and against the gram negative bacteria lincomycine 20mm and ceftrixone 25mm zone of inhibition. From this comparison it becomes clear that the gram positive bacteria *Staphylococcus aureus* is more sensitive than the gram negative bacteria, *Agrobacterium tumefaciens*. The same work of comparison of plant extracts with antibiotic was reported by Doughari & Okafor (2008) & Mathab *et al.* (2006).

Conclusions

The ordinary belief of the people in area regarding the specific plants against the diseases is highly valuable. Plants can be used safely as compeered to antibiotic have low cost low side effects. The results of plant extracts against the bacteria, the antimicrobial essay confirmed the great potential of medicinal plants for the production of bioactive compounds and are useful for the use of medicinal plants in primary healthcare, and also to improve the production for their traditional use of the medicinal plants. The phytochemical study of the characterization of the plant extracts, and to investigate the responsible bioactive compounds, quality standard, chemistry and structure of the compound are also necessary.

References

Adegoke Iberi PA., D.A. Akinpelu, O.A. Aiyegoro and C.I. Mboto. (2010). Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. *Int. J. Appl. Res. Nat. Prod.,* 3 (3): 6-12.

Ahmad, B., H. Khan, S. Bashir and M. Ali. (2006). Antimicrobial bio assay of *Colchicum luteum* baker. *J. Enzy. Inhib. medicinalchem.,* **21(6):** 765-769.

Ahmad, H. (1999). Issues Regarding Medicinal Plant of Pakistan *Udyana Today*., **6(3):** 6-7.

Alzorky, N.S. and Nakahara. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. food MicrobioL.,* **80**:223-230.

Ayogu, T.E. and E.S. Amadi. (2009). Antibacterial Activities of Medicinal Plants on Typhoid Fever organism. *The int. J. Third World Med*., **7 (2).**

Chanrasekaran, M. and V. Venkatesalu. 2004. Antibacterial and Antifungal activity of *Syzgiwn jambulanum* seed **91**:105-108.

Doughari, J.H. and N.B. Okafor. 2008. Antibacterial activity of *Senna siamae* leaf extracts on *Salmonella typhi*. *Afr. J. Microbiol. Res*., **2:** 42-46.

Doughari, J.H.H. and S. Manzara. 2008. In-Vitro antibacterial activity of crud leaf extracts of *Mangifera indica* Linn. *Afr. J. Microbiol. Res*., **2:** 67-72.

Huang J.Q., W.E.I. Zhi Ming and H.Z.A.Y. Zhu. 2001. *Agrobacterium tumefaciens* mediated transformation of rice with the spider insecticidal gene conferring resistance to leaf folder and striped stem borer. *Cell Res*., **11**:149-155.

Jonanthan, E., A. Kelmanson, , J. Jagar Q and V. Staden. 2000. Zulu medicinal plants with antibacterial activity. *J. Ethnopharmacol*.,**69:** 241-246.

Kaushik, P. and P. Goyal. 2007. In vitro evaluation of *Datura innoxia* (thorn-apple) for potential antibacterial activity. *Indian J. Microbial*., Springer.

Maksimovic, Z., M. Milenkovic, D. Vucicevic and M. Ristic. 2008. Chemical Composition and antimicrobial activity of *Thymus pannonicus* All (*Lamiacea*) essential oil cent. *Eur.J.Biol*., **3(2):**149-154.

Mathab, M.C., R.V. Nikolovia, N. Lall and N.Z. Nyazema. 2006. Antibacterial activities of Medicinal Plants used for the treatment of diarrhoea in LimPopo Province, South Africa. *J. Ethnopharmacol*., **105:** 286-293.

Mitchell, S.A. and M.H. Ahmad. 2006. A review of Medicinal Plant Research at University of the West Indies, Jamaica; 1948-2001. *West Ind. Med* J., **55** (4): 243.

Ngobeli, A. M., 2002. Biological and chemical screening of plants used traditionally to treat diarrhea in children. Department of Botany, University of Venda for science and Technology, Master thesis.

Polat, Z.A., A. Vural, T.B. Fathozan, S. Ozceik and A. Cetine. 2008. In vitro Evaluation of the Amoebicidal activity of Garlic *(Allium sativum*) on *Acanthamoeba castellanii* and its Cytotoxic Potential on Corneal Cells. *J. Ocular Pharmacol. Therapeut.,* **24:** (1). Doi: 10.1089/jop.2007.0035.

Rodrigues, J., D.C. Michelin, D. Rinaldo, G. Zoloco, L.C.D. Santos and WVHRN. Salgado. 2008. Antimicrobial Activity of *Miconia* Species (Melastomataceae). *J. Med. Food,* **11(1)**: 120-126.

Shah, G.M. and M.A. Khan. (2008). Check list of Medical Plants of Siran Valley Mansehra Pakistan.

Share, K.N., V. Anandhraman, V.G. Meshram, S.U. Meshram, M.V.R. Reddy, P.M. Tumane and K.. Goswami. 2008. Anti-microfilarial activity of methanolic extract of *Vitex negundo* and *Aegle*

marmelos and their phytochemical analysis. *Ind. J. Exp. Biol.,* **46**:128-131.

Williams, L.A.D., H. Rosner, E.A. Vasquez, B. Vogler, J. Conrod and I. Klaiber. 2001. Biological activities of selected Caribbean plants. *Proc. Int. Sympos.,* "Utilization of natural products in developing countries: trends and needs", Natural Products Institute, UWI, Mona, Kingston, July 9–14: 321.

World Health Organization (WHO) Conference, News paper, "Daily Mashriq" 2003.