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Antibacterial activity of hexane extract of *Juglans regia* against some clinical isolates of urinary tract infection

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Abstract

Background: To investigate the antibacterial activity of crude hexane extract of *Juglans regia* against five clinical isolates of urinary tract infection. Urinary tract infections (UTI) caused by bacterial pathogens have become a serious global health concern. Clinical isolated bacterial agents responsible for UTI are *Escherichia coli, Klebsiella pneumonia, Staphylococcus saprophyticus, Proteus mirabilis* and *Pseudomonas aeruginosa.* Recently, medicinal plants have discovered extraordinary fame in therapeutic treatment for various types of diseases including urinary tract infection. Medicinal plants may be considered as new sources for producing antibacterial. Anti-bacterial study was carried out by disc diffusion method against the pathogens viz., *Escherichia coli, Klebsiella pneumonia, Staphylococcus saprophyticus, Proteus mirabilis* and *Pseudomonas aeruginosa.* maximum degree of antibacterial activity was seen against *E. coli* (22 mm) followed by *Proteus mirabilis* (17 mm) and *Klebsiella pneumonia* (16 mm) moderate activity shown by *Pseudomonas aeruginosa* (12 mm) and least activity shown by *Staphylococcus saprophyticus* (07 mm). In this way, the utilization of *J. regia* for treatment of urinary tract infection might be fulfilled. Additionally work will emphasize the isolation and characterization of dynamic standards in charge of bio-efficacy.

Keywords: UTI, juglans regia, hexane

Introduction

Urinary tract infections are serious health problem affecting millions of people throughout world. (Stauffer *et al*., 2004) [17]. UTI is an important cause of morbidity and mortality in Indian subjects affecting all age groups across the life span. Urinary tract infection can be defined by the presence of significant quality of bacteria in the urine along with signs and symptoms of infection (Zelikovic *et al*., 1992) [18]. Millions of people are diagnosed with urinary tract infections (UTI) every year all over the world (Shaikh *et al*., 2005) [16]. Urinary tract infections caused by pathogens have become a severe health problem worldwide. The main infective driving force that causes such kind of infections are *Escherichia coli*, *Candida albicans, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus faecalis* (Diab *et al*., 2002) [7]. (Shaikh *et al*., 2005; Raz, 2001) [16, 14]. The antibacterial activities of some medicinal plants used against UTI causing pathogens were studied in recent time. Traditional medical methods, especially the use of medicinal plants still plays a major role in the developing countries. Thus the use of plants as medicine is an ancient practice common to all societies, especially in Indian and African society. Several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs (Akenga *et al*., 2005) [1]. The plant has a long history of medicinal usage. According to the reports of world health organization (WHO), about 80% of the third world populations rely on traditional herbal therapies (Dubey *et al*., 2012) [9]. There are several studies that have analyzed the therapeutic potential of the plants (Djeussi *et al*., 2013; Habiba *et al*., 2016; Mustafa *et al*., 2016) [8, 11, 13]. Several plants have been investigated to treat the UTI, and other types of infection caused due to the pathogenic organisms (Eisenberg *et al*., 1993) [10]. Their extracts are used as raw medicine and believed to be the important source of new chemical substances with potential therapeutic properties (Chandra, 2013) [4]. *Juglans regia*, is a member of Juglandaceae family. This valuable tree has a long history of medicinal use to treat a wide range of health complaints. It is 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). Almost all parts of the plant are medicinally important. The root and stem bark are anthelmentic, astringent, antibacterial (Citolgu and Atlantar, 2003) [6].

Material and Methods

Plant material and extraction

Juglans regia was collected from the Kashmir Valley, India in the month of July, 2016. After collection, the roots were sun dried for 7 days and pounded using pestle and mortar and stored at 35 - 37°C until required. Fifty grams powdered roots were taken with 250ml of hexane solvents for 12h at 30 °C temp. in soxhlet apparatus and then filtered using Whatman No.1 filter paper. The filtrate evaporated to dryness using

rotary evaporator and the resultant extract stored in a reagent bottle at 4 –8°C.

Isolation and Identification of UTI bacteria

The bacteria used were *E. coli*, *S. saprophyticus K. pneumoniae, P. mirabilis* and *P. aeruginosa* isolated from clinical specimens obtained from patients diagnosed with urinary tract infections at the Bombay Hospital & Research Centre Jabalpur, M.P. Isolation and identification of the organisms were done following standard procedures in handling clinical specimens (Cheesbrough, 2000). Bacterial agents were grown on Blood agar or MacConkey agar plates at 37°C and maintained on nutrient agar slants and were differentiated using the gram staining procedure into gram positive and gram negative organisms. The organisms were transferred to cystine lactose electrolyte deficient (CLED) agar medium for further differentiation of urinary organisms. Urine samples were shake well in their containers for even distribution of bacteria. A calibrated wire loop with internal diameter 3.26mm that hold 0.004 ml of urine was inoculate into the above media. The inoculums were spread with the wire loop on the media plate. Plates were incubated aerobically at 37°C for 24 hours.

Antibacterial Activity

The antibacterial potential of root extract of *Juglans regia* was tested by disc diffusion method (Bauer *et al*., 1966) [3]. Mueller-Hinton agar plates were used for determining the antibacterial activity. The colonies were inoculated in normal saline solution. The turbidity was then adjusted to equal the turbidity of 0.5 McFarland standard giving a final inoculum of 1.5 × 108 CFU/mL. Solution of known concentration of the test samples in calculated volume of solvents dried and sterilized filter paper discs (6mm diameter) were then impregnated with known amount of the test substances using micropipette. Standard antibiotic (Ciprofloxacin) disc was used as a positive control. These plates were then kept at low temperature (4 °C) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37 °C for 12 h to allow maximum growth of the microorganisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Results and Discussion

The activity showed by root extract of selected plant was promising against bacteria responsible for urinary tract infection.

A total of 300 patients of all age group clinically diagnosed as urinary tract infection (UTI) were studied to isolate bacteria from urine. Out of 300 UTI 160 (44%) were culture positive.

Table 1: Distribution of culture by UTI

Type of UTI	Urine cultured	Culture positive cases	Percentage
UTI	300	160	47.33%

Among the samples analyzed and data obtained by the patients of UTI it could be assessed that female is more prone (58.12%) than male (41.87 %) for the above infection

Table 2: Distribution of infected patient male and female.

Male UTI Patients	Female UTI Patients
67 (41.87 %)	93 (58.12 %)

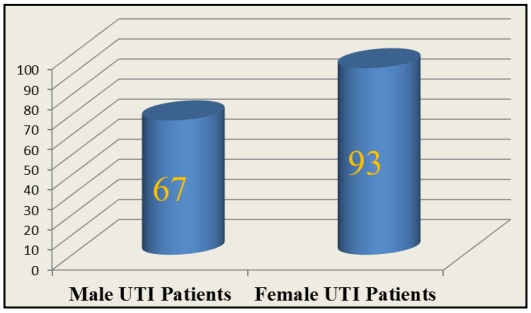

Fig 1: Distribution of infected patient male and female.

From table iii it is found that Paralysis (61.53%) was identified as risk factor for maximum patients. Diabetic (57.73%) was the next to it.

Table 3: Prevalence of risk factor among suspected UTI patients

Risk Factor UTI Patient	Total No. of Patients	No. of Infected Patients	Percentage
Diabetic	97	56	57.73%
Surgical	37	17	45.94%
Cardiac	25	09	36%
Paralysis	78	48	61.53%
Other	63	30	47.61%
Total	300	160	53.33%

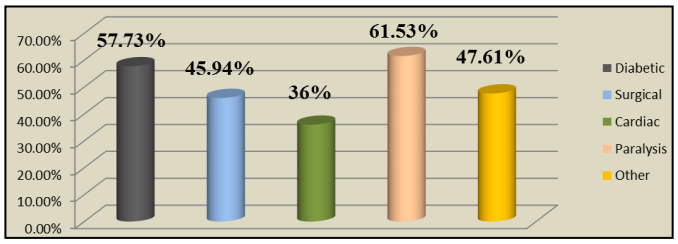

Fig 2: Prevalence of risk factor of bacteria among suspected UTI patients

The most common organisms isolated were *Escherichia coli* (85), *Proteus mirabilis* (48), *Pseudomonas aeruginosa* (29), *Klebsiella pneumonia* (33) *Staphylococcus aureus* (18) and *Staphylococcus saprophyticus* (07) form diseased patients.

Table 4: Distribution of isolated bacteria responsible for urinary tract infection

Isolated bacterial species	
E. coli	43
P. aeruginosa	16
Proteus mirabilis	38
Klebsiella pneumonia	22
Staphylococcus saprophyticus	29
Total	160

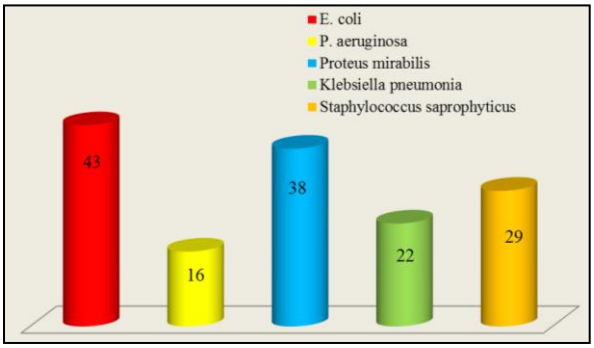

Fig 3: Distribution of bacteria responsible for urinary tract infection

According to the results of this study, the highest isolate bacteria was *E. coli* (26.87%) followed by *Proteus mirabilis* (23.75%), *Klebsiella pneumonia* (13.75%) *P. aeruginosa* (10%), and *Staphylococcus saprophyticus* (18.12%)

Table 5: Biochemical tests of recovered clinical isolates

Catalase	Indole	Oxidase	Citrate	MR	VP	Urease	Organism confirmed
+	+	_	_	+	_	_	*Escherichia coli*
+	_	+	+	+	_	_	*Proteus mirabilis*
+	_	_	+	_	+	±	*Klebsiella pneumoniae*
+	_	+	+	_	_	_	*Pseudomonas aeruginosa*
+	_	_	+	_	_	+	*Staphylococcus saprophyticus*

+= positive, - = negative, ± = Some species are positive some negative

The results of inhibitory effect of root extract of *Juglans regia are* shown in Table VII

Table 6: Antibacterial activity of root extract of *Juglans regia* against selected UTI pathogens

Bacterial species	Solvents used	Standard
	Hexane	Ciprofloxacin
Escherichia coli	22	36
Proteus mirabilis	17	32
Klebsiella pneumoniae	16	39
Pseudomonas aeruginosa	12	35
Staphylococcus saprophyticus	07	38

The results indicate that five bacterial species exhibit different sensitivities towards the extract. The extract was found to be inhibitory to both bacterial isolates but with variable extent. The order of activity against selected bacteria was *E. coli*> *P. mirabilis* > *K. pneumoniae* > *P. aeruginosa* >*S. saprophyticus* >. The obtained crude extract of root was tested against different bacterial strains and compared to that of antibacterial antibiotic, ciprofloxacin. The results of the sensitivity test are shown graphically in (Fig. 4).

Inhibition zones noticeable for hexane extract derived from *Juglans regia* against *E. coli* (22 mm), *Pseudomonas aeruginosa*, (12 mm) *Klebsiella pneumonia*, (16 mm) *Proteus mirabilis*, (17mm) and minimum activity was observed against *Staphylococcus saprophyticus* (07 mm). The diameter of the zones of inhibition is shown in (Table VII). The diameters of the zones of inhibition with the standard drug used were 36 mm, 32 mm, 39mm, 35 mm, and 38 mm for the five strains respectively.

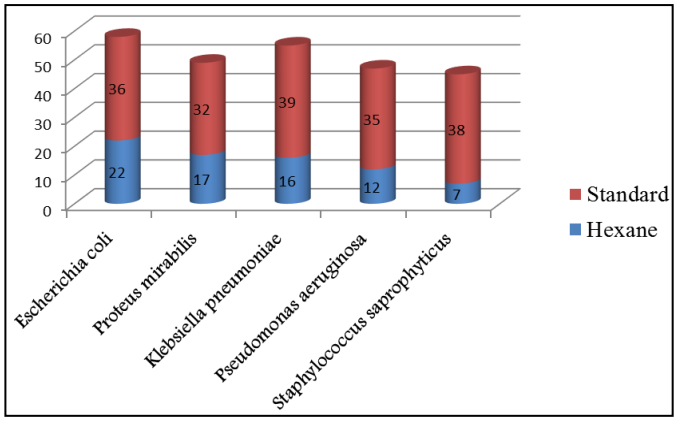

Fig 4: Antibacterial screening of hexane root extract of *J. regia*

Urinary Tract Infections remains the most common bacterial infection in human population and is one of the most frequently occurring nosocomial infections (Ronald *et al*., 2001) [15]. Urinary tract infection occurs more frequently in female than men. This is remaining a major clinical problem over 50 years after the introduction of antimicrobial chemotherapy (Mohammad and Kambiz, 2010) [12]. From this present study, a total of 300 urine samples were collected. Among them, 160 samples showed the presence of *E. coli*, *K. pneumoniae*, *P. mirabilis P. aeruginosa* and *S. saprophyticus*. The data obtained in this study is similar to those obtained from the study of the leaves and seeds of *P. biglobosa* (Jacq.) The henanic root extract of *Juglans regia* gave prominent activity against most of the frequently encountered isolates of uropathogens causing community and hospital acquired urinary tract infections Since, in the present study we have tested most of the common causative agents of UTI, we were able to detect a wide spectrum of antibacterial activity of this plant extract against common uropathogens. Hence, it may be used in future to obtain novel therapeutic compounds in the treatment of UTIs caused by organisms which show resistance to the currently available antimicrobial agents. Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and could serve as a source of both traditional and orthodox medicine (Akinyemi *et al*., 2007) [2]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds. Medicinal plant extracts, therefore offer considerable potential for the development of new agents effective against infections currently difficult to treat.

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Conclusion

The results of the present study suggest that the extract of studied plant can be used as potential leads to discover new antibacterials to control UTI caused by bacterial organisms.

So, the medicinal plants are the best alternate for treating such uti causing bacteria because antibiotics are expensive to use, in our study plant hexanic extract showed remarkable antibacterial activity against all five tested bacteria (*E. coli, K. pneumoniae, P. mirabilis, S. saprophyticus and P. aeruginosa*) and thus justifying its use in traditional medicine. This study revealed that the edible plant sources can also be effective as the modern medicine to inhibit the growth of pathogenic urinary tract bacteria and devastating the antibiotic resistance. It is also envisaged that further work should be done in this direction.

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