The aim of the study was to evaluate the antibacterial evaluation of root extracts of Juglans regia against Extended Spectrum Beta Lactamase (ESBL) producing E. coli and Klebsiella pneumonia in Bombay Hospital and Research Centre Jabalpur. The antibacterial activity of, ethyl acetate and methanol root extracts of Juglans regia was determined by disk diffusion method. The antibacterial activity was calculated based on the minimum inhibitory concentration using Mueller–Hinton broth in a tube-dilution method. The best antibacterial activity, calculated as minimum inhibitory concentration values, against ESBL was shown by the methanol root extract Juglans regia (25 mg/mL) for both isolated organisms and ethyl acetate (25mg/mL) against E. coli. The methanol extract showed zone of inhibition in the range of 17-26mm as compared to ethyl acetate extract which showed zone of inhibition in the range of 11-16mm against the uropathogens. The zone of inhibition ranged from 17 mm to 26 mm and MIC was 25 mg/mL. This effect is comparable to antibiotics. The results obtained in the present study suggest that Juglans regia have the potential to be developed as antibacterial agents against ESBL producing UTI bacteria strain. Further investigations are needed to identify the active compounds and their mechanism of action.

Original article
A simple and low cost mitigation of extended-spectrum beta-lactamase producing pathogenic bacteria causing urinary tract infections using Juglans regia extracts

Aashaq Hussain Allae
t, Junaid Ahmad Ahangar
t, Kownsar Jan
t, Amrit Kour	 and R.P.Mishra

*Department of PG. Studies & Research in Biological Sciences, R. D. University, Jabalpur,
†Department of Microbiology & Pathology, Bombay Hospital & Research Centre, Jabalpur,
‡Department of Microbiology, Govt. Medical College, Baramulla (J&K)

Copyright 2011. CurrentSciDirect Publications. IJBMR - All rights reserved.
Material methods:

Collection and preparation of plant material: Juglans regia, the plant investigated in the present work is widely found in Jammu and Kashmir, India. Roots of Juglans regia were collected from villages in and around Anantnag District, J&K India. After collection, the roots were sun dried for 7 days and pounded using pestle and mortar and stored at 35-37°C until required.

Soxhlet extraction: The extraction of plant under study was done according to the standard scientific method reported by (Kianbakht and Jahaniani 2003). To obtain the ethyl acetate and methanol extracts, dried and finely powdered roots of J. regia (about 20 gms each) homogenized using 250 ml ethyl acetate and methanol were added to Soxhlet apparatus for successive extraction. The boiling point was set at 40°C. The solvent was recycled. The obtained liquid extracts was subjected to rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle (Kianbakht and Jahaniani 2003).

Test bacteria

The uropathogenic bacteria included in our study were isolated from urine samples are two Gram negative bacteria that is E. coli, and K. pneumoniae from patients suffering with urinary tract infections (UTI).

Collection of urine specimen

The clean catch mid-stream technique was employed to collect two hundred urine samples from a Bombay Hospital & Research Centre Jabalpur (India) (Forbes et al. 2007).

Transport of urine specimen

Approximately 20 ml of urine will be collected aseptically in a sterile wide mouthed container. Each sample in the container was properly labeled with patients name, ID number etc. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 hour after collection (Collee et al. 1996).

Isolation and identification of bacteria from urine samples

For the isolation of UTI causing organisms, a loopful of each urine sample was streaked on the blood and Mac Conkey agar medium and incubated at 37°C for 24 hrs (Inabo and Obanivi 2006). After incubation, colonies were selected and characterized on the basis of morphological, and biochemical tests (Mac Faddin 2006).

Inoculums preparation

The UTI, ESBL-producing bacteria were taken from isolated specimens which exhibited resistance to some antibiotics in hospitalized patients. The bacteria were cultured over night (18–24 h) at 37 °C on nutrient broth for the preparation of cell suspensions. The bacteria cell suspensions were homogenized and adjusted to 0.5 McFarland standards (5 × 105 CFU/mL) using spectrophotometry.

Antibiotic susceptibility testing:

The antibiotic susceptibility testing disk was procured from local market manufactured by Hi-media. Susceptibility pattern was done by DISK diffusion method. All the isolated organisms were put into appropriate media for antibiotic susceptibility test by Bauer-Kirby disc diffusion technique (Bauer et al. 1966). Disk diffusion tests were performed and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (Clinical Laboratory Standards Institute 2014). 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 antibiotic discs having susceptibility activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The susceptibility activity of the antibiotic agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Screening for ESBL producers by disk diffusion

Method

The screening was done by disk diffusion test as recommended by the CLSI (Wayne 1998; Wayne 2000). Cefotaxime 30mcg was used as the indicator drug. Zone diameter ≥ 20mm was suspected to be due to possible ESBL producers.

Double disk synergy test (DDST)

The disk of cefotaxime (30μg) and cefotaxime (30μg) were placed 16 to 20 mm apart from the augmentin disc (centre to centre). After incubation (37°C for 24 hrs) the zone of cephalosporin disc towards the clavulanic acid disc was considered as ESBL producers (Jarlier et al.1988).

Phenotypic disc confirmatory test (PDCT)

The test was performed as recommended by CLSI (Clinical and Laboratory Standards Institute). Disks of cefotaxime (CA) 30μg and cefotaxime-clavulanic acid (CAC) 20+10μg or cefotaxime (CE) 30 μg and cefotaxime clavulanic acid (CEC) 20+10 μg were placed on MHA at a distance of 30mm between each other. Increase in zone diameter (=5mm) for CAC versus CA or CEC versus CE is confirmed as ESBL producing organisms (Jarlier et al.1988).

Antibacterial activity of extracts

The antibacterial potential of root extracts of Juglans regia was tested by disc diffusion method (Bauer et al. 1966). Mueller-Hinton agar plates were used for determining the antibacterial activity. The plant extract residues (100 mg) were re-dissolved in 5 ml of each solvent, sterilized through Millipore filter (0.22 mm) then loaded over sterile filter paper discs (6 mm in diameter) to obtain final concentration of 10mg/disc. 20 ml of Mueller-Hilton agar medium was poured into sterile Petri dishes. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 h. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The presence of inhibition zones were measured by inhibition zone scale, recorded and considered as indication for antibacterial activity.

Minimum Inhibitory Concentration

Nutrient broth (0.5ml) was added in a series of sterile tubes and a same amount of adjusted isolated organisms (0.5% Mc Farland) was inoculated into the tubes Different concentrations (25μl, 50μl, 75 μl, 100 μl and 125 μl) of the extracts were added and the tubes were incubated at 37°C for 24 hrs and examined for turbidity (Hassan et al. 2008).
Results and discussion

A total 300 urine samples were analyzed for isolation of notable bacterial pathogens. The most common isolates were Escherichia coli 140 and Klebsiella pneumonia 50 the organisms were isolated and identified using cultural characteristics and biochemical tests (Table 1-3).

ESBL positive organisms are now a global health threat (Ahmed et al. 2010) These organisms have implications on physicians and patients; they are associated with treatment failure, increased morbidity and mortality, prolonged hospital stay, poor outcome and increased health care costs (Fennell et al. 2012). In current study, it was seen that, E. coli 75 (53.57%) and Klebsiella pneumonia 20 (40%) were positive for ESBL. This frequency is much higher than previous four reports from Bangladesh (Lina et al. 2014; Biswas et al. 2014; Shilpi et al. 2013; Masud et al. 2014).

In our study ESBL producing E. coli were found susceptibility to ciprofloxacin (40%) imipenem (38%) ampicillin (30 mm). The susceptible patterns of K pneumoniae, shows highest susceptible to ciprofloxacin (38%) imipenem (36%) and levofloxacin (34%) least was found to other antibiotics.

E. coli shows susceptibility 5% susceptibility to ceftriaxone and 63% to cefazidime reported by (Jesus et al. 2010) Also in his study, 21% susceptibility of E. coli was found for ceftriaxone and cefazidime and 73.9% susceptibility to imipenem. It was found that 78.2% isolates of E. coli were positive for ESBL enzymes. World wide data shows that there is an increasing resistance among UTI pathogens to conventional antibiotics (Shafaq et al. 2011).

The soxhlet methanol and ethyl acetate extracts of J. regia roots were tested for antibacterial activity against the isolated organisms. The maximum antibacterial activity was observed of crude extract of Juglans regia against two gram negative bacteria strains. methanol extracts shows maximum activity against all pathogenic bacteria, E. coli shows (26 mm) followed by Klebsiella pneumonia (17 mm). While the ethyl acetate extracts of Juglans regia shows susceptibility against E. coli (16 mm) and Klebsiella pneumonia, (11 mm). The diameter of the zones of inhibition is shown in (Table 6). The extract of methanol showed higher activity against ESBL producing E. coli and K. pneumonia as compared to ethyl acetate (Table 6). The antibacterial activity of J. regia extract may be due to the presence of phytoconstituents. The ethyl acetate and chloroform extracts exhibited antimicrobial activities against B. subtilis, S. aureus, P. aeruginosa and E. coli. (Zhang et al. 2014). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic anti microbial (Tamanna 2010). The MIC and MBC of the extracts against the isolated bacteria were determined by the tube-dilution method. Methanol root extract of J. regia was highly effective against E. coli and K. pneumonia (MIC value of 25μg/ml) (Table 7). The result of MIC suggested that methanol root extracts of J. regia could possibly act as a bactericidal agent against ESBL E. coli and ESBL K. pneumonia. For Juglans regia to be effective antimicrobial agent it must be used in a solvent which is soluble in methanol and ethyl acetate. Thus aqueous extract of J. regia can be effectively used in various chemotherapeutic agents to inhibit growth of predominant bacteria (Kulkarni et al. 2001).

Conclusion

The results obtained in the present study suggest that Juglans regia have the potential to be developed as antibacterial agents, especially the methanol, ethyl acetate extracts exhibited significant activity against ESBL producing UTI bacteria strain, but there is also a possible synergistic action with the antibiotics. Further investigations are needed to identify the active compounds and their mechanism of action. Based on antibacterial result, the methanol extract possessed very good activity when compared with ethyl acetate extracts. Hence the further studies were carried out by using methanolic extracts of J. regia.

Funding

Yes

Authors contributions

All the authors have contributed equally.

Conflict of interests

Declared none

Acknowledgements

This study was supported by Department of Pathology & Microbiology, Bombay Hospital & Research Centre, Jabaipur; M.P. Authors are thankful to MPCST Bhopal, India to grant necessary funds for the work.

References


