ANTIBACTERIAL ACTIVITY OF RHIZOME EXTRACTS FROM *Podophyllum hexandrum* ROYALE

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Abstract

Microbial infections are the major cause of morbidity and mortality throughout the world and medicinal plants continue to act as repository of novel drug leads with novel mechanism of action. The present study evaluated the antibacterial activity of rhizome extracts of *P. hexandrum*. Dried rhizomes of *P. hexandrum* were subjected to solvent extraction by hexane, ethyl acetate, chloroform and methanol. All the extracts were tested for antibacterial activity against five pathogenic strains of gram positive and gram negative bacteria. The antibacterial activity was assessed by disc diffusion method. Methanolic extract showed good antibacterial activity against all the five bacterial strains, followed by hexane extract. The minimum inhibitory concentration (MIC) values, determined by serial tube dilution method, of methanolic extract were 250, 31.25, 7.81, 31.25 and 62.5µg/ml against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae* and *Enterococcus faecalis* respectively. This study confirms the antibacterial potential of the plant and will act as a base line for future studies in the development of novel antibacterial drug(s) from the plant.

Keywords: *Antibiotic resistance; May-apple; Disc diffusion assay; MIC*

Introduction

The striking rise in the prevalence of bacterial infections currently poses a serious threat to public health worldwide. The problem is worsened by antibiotic resistances coupled with the emergence of new pathogens with the potential for rapid global spread (Dar *et al.*, 2012), boosting the search for new bioactive agents. No doubt, there are so many drugs available today against all bacterial diseases, but unfortunately all of them have significant negative side effects, reducing their use in certain segments of the population (Pathak *et al.*, 2005). Therefore, there is a perpetual and urgent need to discover the new antibacterial agents with high safety index (Erturk *et al.*, 2006). Historically, medicinal plants have been a source of inspiration for novel drug leads. Plant derived medicines have made large contributions to human health and well being. Phytochemicals may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new

drug with novel mechanism of action. Many laboratories across the globe are involved in systematic screening of plant species for discovering new bioactive compounds, but still a very meager portion of this tremendous potential drug repertoire has been scientifically screened. Hence, there is a need for scientific evidence based validation of bioactive phytochemicals (Dar *et al.*, 2012).

Podophyllum hexandrum Royale syn. *P. emodi* Wall (Himalayan Mayapple; family: Berberideceae), a high altitude perennial herb, dates back to ancient times (Farkya *et al.*, 2004). The plant has been described as 'Aindri'– a divine drug in the traditional Indian System of Medicine – the Ayurveda (Dar *et al.*, 2016), and has also been used in traditional Chinese System of Medicine (Wang *et al.*, 2012). *P. hexandrum* has been extensively used in Ayurveda for treatment of several ailments like septic wounds, genital warts, constipation, cold, biliary fever, inflammation, burning sensation, mental disorder, monocytoid leukemia (Beutner, 1996). There are also many scientific reports regarding some pharmacological activities of the plant like antirheumatic, antiviral, pesticidal, anticancer and antimitotic (He *et al.*, 2013; Dar et al., 2016), but there is a scanty date regarding the antibacterial activity of the plant. Therefore, the aim of current study was to evaluate the antibacterial activity of rhizome extracts of *P. hexandrum*.

Materials and methods

Plant material and extraction

The fresh rhizomes of *P. hexandrum* were collected from the shady and hilly slopes of Dawar, Gurais (34°38′N 74°50′E), Jammu and Kashmir, India in the month of July, 2012. The plant material was authenticated by the Centre of Biodiversity & Taxonomy (CBT), University of Kashmir, India and a voucher specimen (KASH-1752) has been deposited. After being macerated to fine powder, 1 Kg dried rhizome powder was extracted successively with hexane, chloroform, ethyl-acetate and methanol for 16 h using soxhlet apparatus (Dar *et al.*, 2013). The extracts were filtered through a Buchner funnel using Whatman no. 1 filter paper and were concentrated to dryness under vacuum using Heidolph rotary evaporator, yielding 3.4, 71.73, 16.53 and 97.77 g of hexane, chloroform, ethyl-acetate and methanol extracts, respectively.

Microbiologic material

The standard bacterial strains used for testing antibacterial activity include *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (ATCC 19430), *Klebsiella-pneumoniae* (ATCC 15380) and *Enterococcus faecalis* (ATCC 29212).

Antibacterial activity

The agar diffusion assay was performed according to European Pharmacopoe (1997). One loopful of each test organism was suspended in 3 ml 0.9% NaCl solution separately. Nutrient agar was inoculated with this suspension of the respective organism and poured into a sterile petri dish. Sterile filter paper discs with 6 mm diameter impregnated with 2000 µg of each extract were transferred onto these prepared petri dishes as per standard procedures (Dar *et al.*, 2013). Gentamycin (30 µg/disc, Merck) was used as a positive, the solvent of each extract (DMSO) as a negative control. A pre-diffusion for 3 h was guaranteed. All experiments were carried out five times and results were recorded by measuring the zones of growth inhibition around the discs after 18 h incubation at 26 °C.

Minimum inhibitory concentration (MIC)

The MIC values were determined by standard serial broth microdilution assay (European Pharmacopoe, 1997). Ten serial dilutions of stock, ranging from concentration of 1000 µg to 0.97 µg/ml were prepared in the test tubes. The tubes were inoculated with 100 µl of bacterial strain inoculums at a concentration of 10^6 cell/ml. Standard antibiotic gentamycin was included in the assay for comparison. Nutrient broth with the inoculums only was used as growth control. All experiments were carried out in triplicates. The tubes were incubated aerobically at 37 °C for 12-18 h; after which 50 µl of 0.2 mg/ml 2-(4-iodophenyl)3-(4-nitrophenyl)-5 phenyltetrazolium chloride (INT) solution was added to each test tube, the tubes were tested for color change (Anders *et al.*, 2002). The concentration at which a decrease in red color is apparent compared to the next dilution was taken as MIC value. Bacterial growth is indicated by the red color of INT reduced to formazan.

Results and discussion

All the solvent extracts of *P. hexandrum* were tested for antibacterial activity against five pathogenic strains. The highest antibacterial activity was shown by methanolic extract of *P. hexandrum* (*MEPH*) followed by hexane extract (*HEPH*) as shown in Table 1. The antibacterial activity produced by *MEPH* was comparable to that of the standard antibiotic gentamycin. The minimum inhibitory concentration (MIC) values of *MEPH* were 125, 31.25, 7.81, 31.25 and 62.5 µg/ml against the pathogenic strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Enterococcus faecalis* respectively (Table 2).

Table 1: Antibacterial activity of rhizome extracts of *P. hexandrum*

Extract treated	HE	CE	EE	ME	Control	Standard
Microorganism	**DIZ (mm)**					
P. aeruginosa	10 ± 0.5	0.00	0.00	16 ± 0.5	0.00	22.66 ± 0.6
S. aureus	10.66 ± 0.3	0.00	0.00	17.66 ± 0.3	0.00	20.66 ± 0.3
S. typhi	11.66 ± 0.8	0.00	0.00	19.66 ± 0.8	0.00	25.33 ± 0.3
K. pneumoneae	12 ± 0.5	0.00	0.00	17.33 ± 0.3	0.00	24.66 ± 0.3
E. faecalis	11 ± 0.5	0.00	0.00	14 ± 0.5	0.00	18.33 ± 0.3

HE: hexane extract, CE: chloroform extract, EE: ethyl acetate extract, ME: methanol extract, standard: gentamycin, control: dimethyl sulphoxide (DMSO). The values are mean of six replicates ± standard error.

Table 2: Minimum inhibitory concentration of methanolic extracts of *P. hexandrum* against the tested strains.

Test strain	MIC range (µg/mL)	Kanamycin (µg/mL)
Pseudomonas aeruginosa	250	< 7.81
Staphylococcus aureus	31.25	< 7.81
Salmonella typhi	7.81	< 7.81
Klebsiella pneumoneae	31.25	< 7.81
E. faecalis	62.5	< 7.81

The data showed that *HEPH* and *MEPH* contained ingredients that were active against both Gram positive and Gram negative pathogens. There are a number of reports of methanolic extracts of plant possessing antibacterial activity (Elzaawely *et al.*, 2005). These results indicate that the extracting solvent has a definite effect on bioactive principles. *MEPH* exhibited more antibacterial activity as compared to *HEPH* in agar diffusion assay. Usually, the extract having large inhibition zone diameter with low MIC can be recognized as more potent drug than that of small inhibition zone diameter and high MIC (Semwal *et al.*, 2009). The extracts can be considered actives when the MIC is less than 100 µg/ml (Picão *et al.*, 2009). The MIC of less than 250µg/ml of *MEPH* against the tested strains indicates its promising potential as an alternative for the treatment of infectious diseases caused by these strains, since most of them have developed resistance against the known antibiotics (Singleton, 1999).

Conclusions

MEPH showed good antibacterial activity against all the tested pathogenic strains of bacteria, which justifies the traditional use of the plant to treat infectious diseases and hence reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances. The extract showed better activity against the gram negative bacteria as compared to gram positive. Owing development of multidrug resistant strains treating gram negative bacterial infections is more difficult, therefore, it will be interesting to fractionate and isolate the active antibacterial compounds from the plant.

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