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ANTIBACTERIAL EVALUATION OF *QUASSIA INDICA (GAERTN.) NOOTEB. IN STEENIS* TOWARDS BACTERIA INVOLVED IN SKIN DISEASES

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Abstract:*Quassia indica* has been used as medication for a number of diseases in traditional systems of medicine. Leaves of *Quassia indica* evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacterial species involved in skin diseases in human beings. Antibacterial activity was evaluated by disc diffusion method. Petroleum ether and ethanol extracts were found to be active against *Pseudomonas aeruginosa.* The results indicated that the plant exhibited antibacterial activity especially towards single pathogen, *Pseudomonas aeruginosa*. Phytochemical analyses were performed to analyse the nature of compounds contained in the active extracts. Flavonoids, phenols, steroids were detected in petroleum ether and ethanol extracts. Alkaloids were not detected in any of the extracts. Active ethanol extract of the plant exhibited minimum inhibitory concentration as well as minimum bactericidal concentration of 31.25mg/ml towards *Pseudomonas aeruginosa*. The study revealed that the active ethanol extract possessed some antibacterial compounds, which could be isolated from the ethanol extract.

Keywords: *Quassia indica,*Simaroubaceae, antibacterial, disc diffusion, MIC

INTRODUCTION

Quassia indica (Gaertn.) Nooteb. in Steenis., a small tree belongs to Simaroubaceae and is commonly known as Njotta, Karinjotta in Malayalam and Niepa bark tree in English. Its synonym is *Samadera indica* Gaertn. [1]. The plant is found along backwaters and moist deciduous forests. It is indigenous to western peninsula throughout the South Konkan and Malabar and Ceylon. It is a tree, smooth in texture and growing to a height of 10 m. Bark is pale, wood is light and soft. Leaves are simple elliptic, oblong, about 20 cm wide. Flowers are numerous, pinkish yellow, on dense and paniculate short stalked umbels [2]. *In vitro* antiplasmodial activity was reported in roots and leaves of *Quassia india* [3]. Quassinoids isolated from *Quassia indiaca* were shown to exhibit significant growth-inhibitory activity against the cultured malarial parasite *Plasmodium falciparum* [4]. Samaderin B and C isolated from the seed kernels of *Samadera indica* were shown to exhibit antifeedent activity against *Spodoptera litura* [5]. Quassinoids isolated from the seeds and bark of *Samadera indica* exhibited antifeedant and growth regulatory activities against the tobacco cutworm, *Spodoptera litura* [6]. Present study is an attempt to evaluate antibacterial potential of the plant in various extracts of increasing polarity. The extracts were prepared in different solvents of increasing polarity towards pathogenic micro organisms involved in various skin diseases.

METHODOLOGY

Preparation of plant extract

Fresh specimens were collected in the month of December from Kottayam District of Kerala State, India. A voucher specimen (AJ 1240) was deposited at the herbarium of St. Thomas College Palai. The air-dried roots of the plant material (50 g) was ground and utilised for preparing extracts. Extracts of petroleum ether, acetone, ethanol and water were made successively [7].

Microorganisms used

Test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh. These include *Staphylococcus aureus* subsp *aureus* (MTCC-96), *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-741), *Serratia marcescens* (MTCC-97) and *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109). All these bacteria are involved in various skin infections [8]. The bacteria were sub cultured on nutrient agar slants, incubated at 37^{o}C for 24 hours and stored at 4^{o}C in the refrigerator to maintain the stock culture.

In vitro antibacterial assay

The disc diffusion method [9] was used to determine the growth inhibition of bacteria by plant extracts. Sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2) was transferred into sterile petridish and after solidification; the bacteria (1 ml broth of approximately 10^5 CFU) were swabbed with a sterile swab under aseptic conditions. Sterile discs prepared using Whatman No. 4 Filter Paper, of 5-mm diameter were used in the study. The original solvent in which the extract prepared was used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. Discs including controls were also prepared in the same way as those with extracts. Discs were introduced onto the surface of the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth was determined by measuring the diameter of zone of inhibition. Experiments were conducted in more than three replicates and average inhibitory zone diameter was determined along with standard deviation.

Minimum inhibitory concentration (MIC)

The MIC of the extracts was done by incorporating various amounts (250 – 0.24 mg/ml) of the extract into sets of test tubes with the culture media [10]. 50 µl of the bacterial broth culture was added into each of the test tubes. The bacterial cultures with the plant extracts were incubated at 37^{o}C for 24 hours. Test tube containing only the growth medium and each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not allow any visible growth when match up to that of the control tubes.

Minimum bactericidal concentration (MBC)

Samples from the tubes used in the MIC assays, which did not exhibit any visible growth after a period of incubation were sub cultured onto a freshly prepared nutrient medium [11]. The minimum bactericidal concentration was taken as the lowest concentration of the extract that did not give a single colony on the nutrient agar plate after 24 hours of incubation period.

Preliminary detection of photochemical

The crude samples were subjected to phytochemical screening for the detection

of alkaloid, phenolics, sugars, flavonoids using the method of Harborne [12].

Table 1: Antibacterial activity of *Quassia indica*

Quassia indica extracted in solvents	Inhibition zone (mm) Value = Mean ± SD				
	K.p (G-) MTCC-109	S.a (G+) MTCC-96	P.a (G-) MTCC-741	E.c (G-) MTCC-443	S.m (G-) MTCC-97
Petroleum ether	-	-	27.8 ± 0.64	-	-
Acetone	-	-	7.2 ± 0.39	-	-
Ethanol	-	6.4 ± 0.39	29.4 ± 0.74	-	-
Water	-	-	-	-	-

(-) = No inhibition of growth. K.p, *Klebsiella pneumoniae* subsp *pneunoniae*; S.a, *Staphylococcus aureus* subsp *aureus*; P.a, *Pseudomonas aeruginosa*; E.c, *Escherichia coli* and S.m, *Serratia marcescens,* No inhibition with negative control (solvent blank) Value = Mean ± SD; Disc diameter 6 mm; G+ Gram positive; G- Gram negative

Table 2: Phytochemicals detected in various extracts of *Quassia indica*

Extract used	Phytochemicals detected in various extracts (values: + present; - absent)			
	Flavonoids	Alkaloids	steroids	Phenolics
Petroleum Ether	+	-	+	+
Acetone	+	-	+	+
Ethanol	+	-	+	+
Water	-	-	-	+

RESULTS AND DISCUSSION

Antibacterial activity and phytochemical assessment of *Quassia indica* were conducted using petroleum ether, acetone ethanol and water as extracting solvents in the gradation of increasing polarity. The results are reported in the Table 1 and 2. The plant exhibited antibacterial activity towards *Pseudomonas aeruginosa* and *Staphylococcus aureus,* while acetone extract was acting only against *Pseudomonas aeruginosa*. The petroleum ether and ethanol extract of *Quassia indica* exhibited maximum activity when compared to others. Maximum activity was found against *Pseudomonas aeruginosa.* Petroleum ether extract of *Quassia indica* did not show any activity towards *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens* and *Staphylococcus aureus.* Water extracts did not show antibacterial activity towards the tested organisms. Ethanol extract was acting only against *Pseudomonas aeruginosa.* Antibacterial activity observed at its maximum in ethanol extract towards *Pseudomonas aeruginosa* and therefore ethanol extract was selected for detailed antibacterial evaluation tests like MIC and MBC. MIC and MBC values of 31.25 mg/ml observed towards *Pseudomonas aeruginosa*. Kitagawa *et al*., [4] reported that *Quassia indiaca* exhibited significant growth-inhibitory activity against the cultured malarial parasite *Plasmodium falciparum*. The present investigation clarified the antibacterial property of the leaves towards one of the clinically important multi-drug resistant stains, *Pseudomonas aeruginosa* in addition to antiplasmodial activity. *Pseudomonas aeruginosa* is often encountered in nosocomial infections and its infection is common in patients receiving treatment of severe burns or other traumatic skin damage and in people suffering from cystic fibrosis. This pathogen colonises the lungs of patients and increasing mortality rate of individuals with the disease [13].

The present demonstration of around 30 mm zone of inhibition of this pathogen reveals the antibacterial potential of the plant. The photochemical evaluation of *Quassia indica* showed that phenolics, steroids and flavonoids were present in active ethanol and petroleum ether extracts. None of the extracts showed the presence of alkaloids. The presence flavonoids, phenolics and steroids in active extracts might be responsible for the antibacterial activity of the active extracts. The present result supported the ethno botanical role of the plant in controlling pathogens involved in skin diseases like scabies, leprosy as reported by [14] and other diseases due to bacterial infections like erysipelas, chest infections etc [15]. Another interesting observation is that polar and non-polar extracts performed equally well towards *Pseudomonas aeruginosa*. Antibacterial principles can be isolated and purified from active ethanol extract of the plant and can be used as a phtomedicine instead of synthetic antibiotics.

CONCLUSION

Antibacterial activity of leaves of *Quassia indica* was tested towards bacteria involved in various skin diseases in human beings. Petroleum ether extract and ethanol extracts were found to be effective against *Pseudomonas aeruginosa.* Antibacterial activity was demonstrated towards single pathogen, *Pseudomonas aeruginosa*. Phytochemical analysis of active extracts indicated the presence of flavonoids, phenols and steroids. Alkaloids were not detected in any of the extracts. Active ethanol extract of the plant exhibited minimum inhibitory concentration as well as minimum bactericidal concentration of 31.25mg/ml towards *Pseudomonas aeruginosa*. In view of the analysis, the leaves can be recommended as source for isolating and characterizing new antibacterial drugs for modern medicine.

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