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IN-VITRO ANTI-BACTERIAL ACTIVITIES OF *PICRORHIZA KURROA* RHIZOME EXTRACT USING AGAR WELL DIFFUSION METHOD

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ABSTRACT

The present investigation focuses on the antimicrobial potential of acetone, ethanol, methanol, aqueous and hexane extracts of rhizome of *Picorhhiza kurroa* against selected bacterial strains belonging to two different genera of gram positive and gram negative bacteria. Antibacterial studies were done by agar well diffusion technique. Ethanol rhizome extract of *Picrorhiza kurroa* showed high antibacterial activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi* and *S.pyogens*. The methanol rhizome extracts showed high antibacterial activity against *S.aureus* and *P.aeruginosa*, whereas acetone and hexane extract showed intermediate activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *P.aeruginosa* and *S.pyogens*. Aqueous rhizome extract did not show antibacterial activity against the tested bacterial strains. The present study suggests that the ethanol and methanol extracts of rhizome of *Picrorhiza kurroa* contain compounds that can form the basis for the development of a novel broad spectrum antibacterial formulation.

Keywords: *Picrorhiza kurroa*, antibacterial activity, ethanol extract, methanol extract, acetone extract, hexane extract.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources[1]. India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species[2]. Many plants have been sources of medicines since ancient times. According to World Health Organization, 80% of the population of the world depends on traditional medical practitioners for their medicinal needs. Yet a scientific study of plants to determine their antimicrobial active compounds[3] is a comparatively new field. Numerous surveys on biological important medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrence of active compounds in higher plants[3].

Picrorhiza kurroa (Scrophulariaceae) is a small perennial herb that grows in northwest India on the slopes of the Himalayas between 3000 and 5000 meters. It is an important herb in the traditional Ayurvedic system of medicine and has been used to treat liver and bronchial problems. Other traditional uses include treatment of dyspepsia (Similar to gentian in its bitter quality), bilious fever, chronic dysentery and scorpion sting. The most important active constituents of *Picrorhiza kurroa* are the cucurbitacin glycosides, apocynin, drosin, iridoid glycoside picrosides and kutkin[4,5]. *Picorrhiza kurroa* has hepatoprotective effect against Amanita poisoning[6] Carbon tetra chloride[7], and Aflotoxin B1[8]. Bioactivity studies on *Picrorhiza kurroa* established its anti-infamammatory[9], immunomodulatory[10] and hydrocholeretic effects in rats and dogs[11] and antiviral activity on vaccina virus[12]. The present study was carried out to test the antibacterial efficacy of the rhizome extract of *Picrorhiza kurroa* with reference to bacteria spp.

MATERIALS AND METHODS

Plant material

Picrorhiza kurroa Royle ex Benth roots growing in the Himalayan region at an attitude of 2700-4500 m were identified and collected under the supervision of a botanist. They were cleaned with distilled water and shade dried at room temperature. The plant material was authenticated and a voucher specimen of the plant was kept at the Department of Botany (CAHC 110), C.Abdul Hakeem College, Melvisharam, Vellore, Tamilnadu, India.

Preparation of extracts

The powdered roots (230 g) of *Picrorhiza kurroa* Royle ex Benth were extracted separately to exhaustion in a Soxhlet apparatus using acetone, ethanol, methanol, aqueous and hexane solvent systems. All the extracts were filtered through a cotton plug followed by Whatman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get. 3.82 g, 2.37g, 3.02g, 4.87g and 4.32g yield from acetone, ethanol, , methanol, aqueous and hexane fractions respectively. The extracts were preserved in airtight containers and kept at 4-5°C until further use. All the extracts were tested for antibacterial activity against the bacterial spp.

Test organisms

The bacterial spp used for the test were *E.coli* (MTCC 443), *Bacillus cersus*, *Salmonella typhi* (MTCC 733), *Klebsiella Pneumoniae*(MTCC139), *Pseudomonas aeuriginosa(*MTCC741), *Staphylococcus aureus* (MTCC 2940), *Streptococcus pyogens*(MTCC 442) and *Proteus mirabilis* (MTCC 1429). All the stock cultures were

obtained from Microbial Type Culture Collection (IMTECH, India).

Culture media and inoculums preparation

Muller Hinton agar / Nutrient broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loop full of all the bacterial cultures were inoculated in the Nutrient broth (NB) at 37°C for 72 hrs.

Antibacterial activity study

Agar Well diffusion method

The extracts obtained from the rhizome were used for studying their antibacterial activity. A loop full of bacterial strain was inoculated in 30 ml of Nutrient broth in a conical flask and incubated for 72 hrs to get active strain by using agar well diffusion method[13]. Muller Hinton Agar was poured into Petri dishes. After solidification 0.25 ml of test strains were inoculated in the media separately. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm).The extract compound (50 µl) was introduced into the well and plates were incubated at 37°C for 72 hrs. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition[14]. A control with Chloromphenical was kept for all test strains and the control activity was deducted from the test and results were recorded.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

Antibacterial activity was measured using a dilution technique[15]. The plant extract (100 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially two fold diluted Nutrient broth (Himedia, India) to obtain a concentration range of 15.6-1000 mg/ml. Nutrient broths containing only DMSO diluted in the same way, which did not influence bacterial growth, were included as controls. The bacterial strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1 X 10^6 CFU/ml). This suspension was used as the inoculums for the test in the agar plates.

Bacterial suspensions (100µl) were inoculated using a micropipette. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the bacteria in tubes. The minimal bactericidal concentration (MBC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the bacteria on solid media in Petri plates that were incubated at 37°C for 72 hrs.

Statistical analysis

Data are expressed as means ±SEM. Statistical analysis was performed with SPSS (8th version). Difference on statistical analysis of data were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

In the present study the antibacterial activity of the acetone, ethanol, methanol, aqueous and hexane plant extracts was evaluated against eight bacterial spp. (Table1, Fig.1).In the first stage, acetone, ethanol, methanol, aqueous and hexane rhizome extracts of *Picrorhiza kurroa* were applied on each bacterial species. Ethanol rhizome extract of *Picrorhiza kurroa* showed high antibacterial activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi* and *S.pyogens*. The methanol rhizome extracts showed high antibacterial activity against *S.aureus* and *P.aeruginosa* whereas acetone and hexane extract showed intermediate activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *P.aeruginosa* and *S.pyogens*. Aqueous rhizome extract did not show antibacterial activity against the tested bacterial strains. The inhibitory activities of all the extracts reported in Table 1 are comparable with standard antimicrobics Ampicillin (10µg).

Table.1: Antibacterial activity of *Picrorhiza kurroa* by agar well diffusion method

Organism	Mean zone of Inhibition (in mm)					Ref drug
	AE	ET	MT	AQ	HE	
S.aureus	7.16 ± 0.08	13.2 ± 0.17	12.1 ± 0.13	-	9.06 ± 0.12	23.1 ± 0.13
S.pyogenes	-	10.13 ± 0.08	6.06 ± 0.12	5.16 ± 0.11	9.03 ± 0.07	19.16 ± 0.08
B. cereus	6.10 ± 0.05	14.0 ± 0.08	11.03 ± 0.07	-	11.16 ± 0.08	26.10 ± 0.05
E.coli	6.05 ± 0.12	12.23 ± 0.13	10.26 ± 0.14	-	9.10 ± 0.05	25.03 ± 0.14
K.pneumoniae	9.10 ± 0.05	16.16 ± 0.07	11.16 ± 0.08	-	10.26 ± 0.13	22.13 ± 0.07
P.mirabilis	-	-	-	-	-	24.13 ± 0.06
S.typhi	8.13 ± 0.07	13.26 ± 0.03	9.16 ± 0.23	-	8.26 ± 0.13	19.4 ± 0.20
P.aureginosa	6.06 ± 0.12	7.13 ± 0.18	13.06 ± 0.15	-	6.13 ± 0.07	22.96 ± 0.07

AE- Acetone, ET – Ethanol, MT –Methanol, AQ- Aqueous, HE- Hexane, (-) no zone of inhibition, Ref drug – Ampicillin.

Successful extraction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that the ethanol and methanol extracts of these plants were much better and powerful. This may be due to the better solubility of the active components in organic

solvent[16]. In the present study the ethanol rhizome extract revealed higher degree of antibacterial activity for S.aureus, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi* and *S.pyogens* when compared to that of other bacterial spp tested. However, the antibacterial activity of methanol, acetone and hexane rhizome extract was found to be less potent in comparison to ethanol rhizome extract. Similar studies elsewhere recorded antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Salmonella typhimurium*[17].The antibacterial activity of *Picrorhiza kurroa* against test strains such as *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *P.aeruginosa*, *Proteus mirabilis* and *S.pyogens* compared to control can be attributed to the chemical profile of the extracts containing saponins, alkaloids etc.

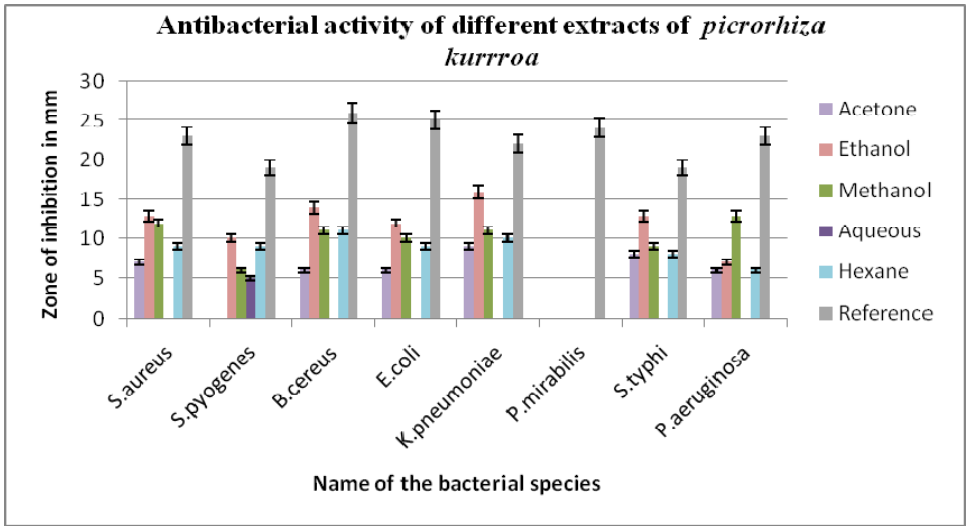

Fig. 1: In-vitro anti-bacterial activities of *Picrorhiza kurroa* rhizome extract using agar well diffusion method.

CONCLUSION

The results of present study support the traditional usage of the studied plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials to carry out further pharmacological evaluation.

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