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Research Article

Antimicrobial Screening of *Picrorhiza kurroa* Royle Ex Benth Rhizome

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ABSTRACT

The methanol extract of Picrorhiza kurroa rhizome was found to be active against the bacterial strain and the aqueous extract is active against the fungal strain used for the study. The 10 mg/ml stock solution of different extracts viz. chloroform, methanol and water extract were used for the screening of the antimicrobial activity by using Cup plate method and Minimum inhibitory concentration. The result showed that a methanol extract exhibited a significant activity against the bacterial strain when compare with Ciprofloxacin a standard drug by cup plate method and aqueous extract are active against the fungal strain when compared to Fluoconazole a standard drug used for fungi. Hence this study prove that Picrorhiza kurroa possess antimicrobial activity.

Keywords: Picrorhiza kurroa; Antimicrobial activity; Scrophulariacae; Cup plate.

INTRODUCTION

India has an ancient heritage of traditional medicine with great wealth of traditional knowledge and wisdom1. A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. Microbes are directly related with the health and welfare of human beings. Some are valuable and some are injurious. According to World Health Organization, medicinal plants would be the source of a diversity of drugs. Many efforts have been made to discover new antimicrobial compounds from varied kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines2. In increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced propensity to antibiotics raises the

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Table No.1. Zone of inhibition of bacteria and fungi (in mm^2) of *Picrorhiza kurroa* rhizome.

Sr. No.	Extract	*S. aureus*	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
1	Petroleum ether	_	_	_	_	_
2	Chloroform	_	_	_	_	_
3	Methanol	18	20	16	_	_
4	Aqueous	_	_	_	10	12
5	Ciprofloxacin (10 µg/ml)	26	28	24	_	_
6	Fluconazole (10 µg/ml)	_	_	_	20	22

Table No. 2. Minimum Iinhibitory Concentration (MIC) of *Picrorhiza kurroa* rhizome.

Microorganism	MIC of Standard Drug		Extract	Serial dilution (µg/ml)				
				50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.2 µg/ml
E. coli	Ciprofloxacin µg/ml	0.156	Pet ether	-	-	-	-	-
			Chloroform	-	-	-	-	-
			Methanol	+	+	-	-	-
			Aqueous	-	-	-	-	-
B. subtilis	Ciprofloxacin µg/ml	0.156	Pet ether	-	-	-	-	-
			Chloroform	-	-	-	-	-
			Methanol	+	+	-	-	-
			Aqueous	-	-	-	-	-
S. aureus	Ciprofloxacin µg/ml	0.156	Pet ether	-	-	-	-	-
			Chloroform	-	-	-	-	-
			Methanol	+	+	-	-	-
			Aqueous	-	-	-	-	-
A. niger	Fluconazole µg/ml	0.312	Pet ether	-	-	-	-	-
			Chloroform	-	-	-	-	-
			Methanol	-	-	-	-	-
			Aqueous	+	-	-	-	-
C. albicans	Fluconazole µg/ml	0.156	Pet ether	-	-	-	-	-
			Chloroform	-	-	-	-	-
			Methanol	-	-	-	-	-
			Aqueous	+	-	-	-	-

specter of fatal bacterial infections and adds exigency to the search of new infection-fighting strategies3. In contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side ef¬fects and have an enormous therapeutic potential to heal many infectious diseases4. The materia medica of India provides great information of folklore and traditional aspects of therapeutically importance of natural origin products. A large number of lead

compound have come out from Ayurveda. In fact, more and more people are becoming interested in Ayurveda now a day's worldwide5. The complementary and alternative medicines are becoming more widely accepted and there is increasing belief in efficacy and safety of herbal remedies6. The Medicinal plants are back bone of the traditional medicine. In many countries of World, especially the poor and less developed countries still used folklore medicinal plant for the treatment of disorder or ailments7. Literature survey has revealed that amongst many unexplored plants, Picrorhiza kurroa Royle ex Benth (Scrophulariacae) is an important medicinal plant used traditionally as well as in modern medicine. In Ayurveda, P. kurroa Royle is called 'Katuka'. It is also described by Susruta as jvaraghna antipyretic and visaghna a detoxifying. Picrorhiza kurrooa Royle is a small reputed alpine herb. It is an endemic to Himalayan region of Pakistan, India, Nepal and China. It used in treatment of liver disorder, fever, asthma, jaundice caused by environmental pollution, industrial toxicants, food adulteration, malnutrition, excessive consumption of alcohol and certain infection8. The present paper deals with the antimicrobial evaluation of Picrorhiza kurroa by Cup Plate method and Minimum inhibitory concentration against some microbes.

Plant material- The rhizomes of Picrorhiza kurroa Royle ex Benth. were procured from local market of Hisar in June 2010 and authenticated by Dr H.B. Singh, Head Raw Material Herbarium & Museum, Ref. NISCAIR/RHMD/Consult-2010-11/11/1413/11. A voucher specimen has been submitted in Department of Pharmaceutical Science, G.J.U.S & T, Hisar. The plant material was air-dried at room temperature and then powdered.

Preparation of extracts- The dried powder of rhizomes of Picrorhiza kurroa was exhausted successively by pet ether, chloroform and methanol by hot extraction process and then aqueous extract was prepared by maceration in distilled water for 18 hrs. The liquid extract so obtained was concentrated in vacuum at 40oC. These extracts were stored in refrigerator at 4oC until used for experiment reported in this study.

Micro-organisms used- The following strains of bacteria were used: Escherichia coli MTCC1652, Bacillus subtilus MTCC 2063 and Staphylococcus aureus MTCC 2901. The fungal strain used in this study were Candida albican MTCC 227 and Aspergillus niger MTCC 8189.

Preparation of test inoculums- The various strains of microorganism were inoculated in sterile nutrient broth (Hi media). This medium was incubated at 37°C ± 1°C for 24 hours and sterilized. The inoculum was used for antimicrobial assay.

Antimicrobial assay- Agar well diffusion method: The antimicrobial activity was tested against (methanol, chloroform, petroleum ether and aqueous extract) rhizome of P. kurroa.

The inoculation of microorganism was prepared from bacterial culture and fungal culture. About 15 to 20 ml of Muller-Hinton agar and SAB agar medium was poured in the sterilized Petri dishes and allowed to solidify. One drop of bacterial and fungal strains was spread over the medium by a rod. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. The plant extracts 10mg/ml were added to the wells. Plates were incubated at 37°C for 24 h. The standard and test compounds (extracts of petroleum ether, chloroform, methanol and water) solution were prepared in dimethyl sulphoxide (DMSO) at the concentration of 10 mg/ml respectively. Standard drugs used in the study were Ciprofloxacin for bacterial assay and Fluoconazole for assay of fungi. Antimicrobial activities were evaluated by measuring the inhibition zone diameters9,10,11.

Minimum Inhibitory Concentration- The MIC was conducted according to two fold serial dilution method. The stock solutions of test solution (extracts) were prepared at concentration of 100 µg/ml in nutrient broth serially diluted at up to five times. Six assay tubes were taken for screening minimum inhibitory concentration of each strain. In the 1st tube 1ml of the seeded broth is inoculated and then 1ml of the test compound solution was added and thoroughly mixed to concentration of 50µg/ml. To make further dilution of this solution 1 ml from first tube is inoculated into 2nd assay tube serially and was done in duplicate. The procedures were conducted under aseptic conditions. The inoculated tubes were kept at 37°C ± 1°C at 24 hours for bacterial assay and 7 days for Aspergillus niger fungi and 3 days for Candida albicans fungi are inoculated, after incubation period tubes were removed and observed any deposits or turbidity in the solution12.

RESULTS AND DISCUSSION

In present study the methanol extract of P. kurroa rhizomes possess significantly antimicrobial activity when compared with standard drug. It is evident from the studies presented in Table 1 and Table 2 that the methanol extract possesses antimicrobial activity in cup plate method and minimum inhibitory concentration study. The cup plate method result showed of diameter was found to be 18mm, 20 mm and 16 mm diameter of methanol extract in S. aureus, B. subtilus and E. coli a bacterial strain used. The aqueous extact showed diameter 10mm, 12mm in A. niger and C.albicans a fungal strain, when compared with standard drug Ciprofloxacin of diameter 26 mm, 28 mm and 24 mm against bacterial strain S. aureus, B. subtilus and E. coli. The standard drug Fluoconazole showed 20mm and 22mm diameter against fungal strain A. niger and C. albicans in aqueous extract. The result are reported according to the liquid dilution screening of antimicrobial activity of higher

plants13,14,15. The methanol extract possess a MIC 25 µg/ml in bacterial strain and 50 µg/ml in fungal strain when compared with standard drug Ciprofloxacin and Fluoconazole of MIC 0.I56 µg/ml, 0.156 µg/ml and 0.312 µg/ml against bacterial and fungal strain used. Hence the present study proves that methanol extract gave highest activity against bacteria and aqueous extract against fungi.

CONCLUSION

The antimicrobial evaluation of this plant are drawn by using a standard procedure which is helpful in authenticate the traditional potential of such plant species. This is first such report on this plant using these strains.

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Competing interests- The authors declare that they have no competing interests.

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