



PRELIMINARY SCREENING AND ANTIMICROBIAL ACTIVITY OF *PICRORHIZA KURROA* ROYLE ETHANOLIC EXTRACTS

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ABSTRACT

The present study was conducted to investigate antimicrobial activity of *Picrorhiza kurroa* ethanolic extract against strains of Gram-positive, Gram-negative bacteria and fungi. *Picrorhiza kurroa* rhizome is used traditionally for the treatment of allergic disorders namely psoriasis, eczema, bronchial asthma, etc. The extract was tested for its antimicrobial activity against Gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria like *Pseudomonas aeruginosa* and *Escherichia coli* and three fungi viz. *Aspergillus niger*, *Candida albicans* and *Malassezia furfur*. Inhibition of microbial growth was investigated using agar well-diffusion method. The extract was active against all assayed organisms with minimal inhibitory concentration (MIC) values ranging from 65 to 260 mg/ml.

Keywords: *Picrorhiza kurroa*, Antimicrobial, Well diffusion method, Pharmacognostical.

INTRODUCTION

The antibiotic era started in the 1950s, and from then onwards the use of plant antimicrobials declined¹, although it was not the case as far as the traditional healing systems that heavily rely on the medicines from the natural sources, especially plants, are concerned. The emergence and spread of microbial resistance is growing each day, thereby necessitating the development of new antimicrobials of natural or synthetic origin². As far as the natural sources are concerned, apart from the microbial sources, plants appear to be valuable antimicrobial resources. Plants can produce a large number of secondary metabolites that may exceed a hundred thousand molecules³, all of these don't have antimicrobial potential, but some of them can produce significant activity against the human pathogens. One of the species that emerged from such an inventory is *P.kurroa* Royle⁴. The rhizome of *Picrorrhiza kurroa* (*Scrophulariaceae*) was utilized clinically in Ayurveda for treatment of hypertension and cardiac disorders. It is also well known drug for treatment of fever and several ailments of the liver and spleen. Distribution of species is restricted to the Himalayan region and China. While *P.kurroa* occurs mainly in the Western Himalaya at an altitude of 3000-4300 meters⁵. Dry Rhizome contains Kutkin 3.4%w/w. Kutkin is the stable mix crystal of two C-9 iridoid glycosides of Picroside I and Kutkoside. Kutkin, a bitter glycosidal principle, is reported⁶. Iridoids represent a large and still expanding group of cyclopentan-(c) – pyran monoterpenoids⁷. The aim of this study was the antimicrobial activities of ethanolic extract of *P.kurroa* against a range of food borne pathogenic and spoilage bacteria, evaluating minimal inhibitory concentrations, and kinetic parameters, in an attempt to contribute to the

use of these as alternative products for microbial control and food preservation.

MATERIALS AND METHODS

Plant Material

The rhizomes of *P. kurroa* were collected from local market, Mumbai. The drug was authenticated at the Agharkar Research Institute, Pune India. A Voucher specimen (AHMA R 095) has been deposited Agharkar Research Institute, Pune India.

Preparation of the Extracts

The shade dried, powdered rhizomes (250gm) of *P. kurroa* were defatted by extracting with Petroleum ether (60-80°C), followed by extraction with ethanol using Soxhlet extractor. The ethanolic extract was then concentrated using rotary flash evaporator to a syrupy consistency. The residual solvent was removed by drying the extract in vacuum oven (yield - 25.5gm).

Microorganisms

The following bacterial strains were used in the antimicrobial tests. Gram positive bacteria were *Staphylococcus aureus* (ATCC 6538P) and *Bacillus subtilis* (ATCC 6633). Gram negative bacteria were *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027). Yeast like fungus used was *Candida albicans* (ATCC 10231). Other fungi like *Aspergillus niger* (ATCC 16404) and *Malessezia furfur* (ATCC 1734) were also used. All microbial strains were obtained from the M. K. Rangnekar's Laboratory, Mumbai, India. In vitro antibacterial activity was determined by using Nutrient agar (Himedia Laboratories Pvt. Ltd., Mumbai; 28g/l), McConkey's agar (Himedia Laboratories Pvt. Ltd., Mumbai; 51.5g/l), Vogel Johnson's agar (Himedia



Laboratories Pvt. Ltd., Mumbai; 61g/l) and Sabouraud's dextrose agar (Himedia Laboratories Pvt. Ltd., Mumbai; 47g/l). Each medium was autoclaved at 121°C, 15 psi for 15 min before inoculation. The bacteria used in the tests were obtained from 24 h cultures, whereas *Candida albicans*, *Malessezia furfur* and *Aspergillus niger* inocula were prepared by suspending colonies from 48 and 72 h cultures respectively and suspended in sterile saline solution to obtain concentrations of approximately 3×10^8 CFU/ml by comparison to the Mc Farland standard no 0.5⁹.

Antimicrobial Activity

Antimicrobial activity of ethanolic extract was determined using agar well diffusion method¹⁰. About 15ml of sterilized selective agar based mediums were added aseptically to sterile plates to prepare a basal layer. The plates were incubated at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ for 24 hrs. The basal layer was seeded the next day with 7ml of sterilized selective agar based medium containing 1ml of suspension of standard inoculums. The plates were allowed to set. Each petridish was divided into four sectors, and in each sector a bore of 6mm diameter was made using sterilized borer in the solidified medium. Using sterilized dropping pipettes, each bore in different sector was carefully loaded with 75µl of test compound and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for 24 h for bacteria and 28°C for 48 h and 72 h for *Candida albicans*, *Malessezia furfur* and *Aspergillus niger* respectively. The ethanolic extract was 2 fold serially diluted in dimethyl sulphoxide (DMSO) to obtain concentrations from 5 mg – 20 mg/ 75 µl. Wells with equal volume of DMSO were used as negative controls.

Results of the quantitative screening were recorded as the average diameter of the inhibition zone surrounding the wells containing the test solution. The zone of inhibition of growth of microorganisms around the well was measured in mm, with the help of a scale. The minimum inhibitory concentration (MIC) of the ethanolic extract against microorganisms was also determined. This was done by loading different concentrations of the ethanolic extract in the well using the same method of agar well diffusion method. Zones of inhibition in mm were also measured. The experiments were carried out in triplicate and the mean of the diameter of the inhibition zones was calculated.

TLC and HPTLC Fingerprinting

Methanolic extract of *P. kurroa* and Arogyawardhini Bati was compared for the presence of Kutkoside, and Picroside through HPTLC fingerprinting using parameters like *R_f*, wavelength and peak area.

The label of claim of Arogyawardhini Bati is as follows- Shudda parad 6mg, Shduddha Gandhak 6 mg, lauha Bhasma 6 mg, Abhrak Bhasma 6mg, Tamara Bhasma 6mg, Harre 12mg, Awala 12mg, Shuddha Shilajit 18mg, Chitrak Mool 24mg, Shuddha Guggul 24mg, Kutaki 132mg, etc.

RESULTS AND DISCUSSION

Antimicrobial Activity

The minimum inhibitory concentration (MIC) of the ethanolic extract against different microorganisms is tabulated in Table 1. The studied concentrations of the ethanolic extract, 20 to 5 mg exhibited antimicrobial activity against the test microorganisms with zone sizes ranging from 7 to 12mm. Among the microorganisms studied, the most susceptible microorganism was *E.coli* and the most resistant microorganism was *Aspergillus niger*. The minimum inhibitory concentrations of the ethanolic extract ranged from 65 to 260 mg against the different test organisms. The photographs of the plates exhibiting antimicrobial activity against the different test microorganisms are shown in Figure 1,2,3,4.

Table 1: Antimicrobial Activity of ethanolic extract against tests organisms

Organism	Minimum inhibitory concentration (MIC) mg/ml.	Zone of inhibition (mm) at MIC
Bacteria(Gram Positive)		
<i>Bacillus subtilis</i>	65	9
<i>Staphylococcus aureus</i>	195	8
Bacteria (Gram negative)		
<i>Pseudomonas aeruginosa</i>	65	8
<i>Escherichia coli</i>	65	12
Fungi		
<i>Aspergillus niger</i>	260	7
<i>Candida albicans</i>	130	7
<i>Malasseiza furfur</i>	65	8

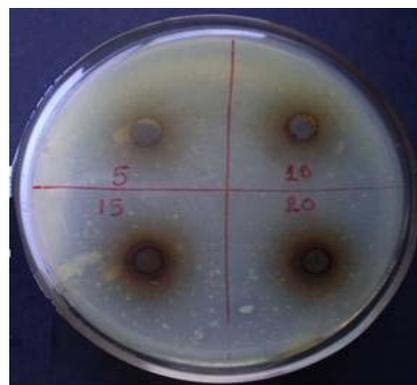


Figure 1: Ethanolic Ext. of *P. kurroa* (5mg, 10mg 15 mg, 20mg/75 µl) on *M. furfur*



Figure 2: Ethanolic Extract of *P. kurroa* (5mg, 10mg 15 mg, 20mg/75 µl) on *E. Coli*

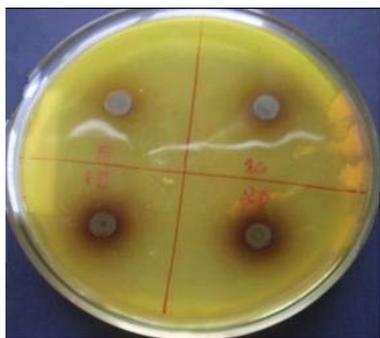


Figure 3: Ethanolic Extract of *P. kurroa* (5mg, 10mg 15 mg, 20mg/75 µl) on *S. aureus*

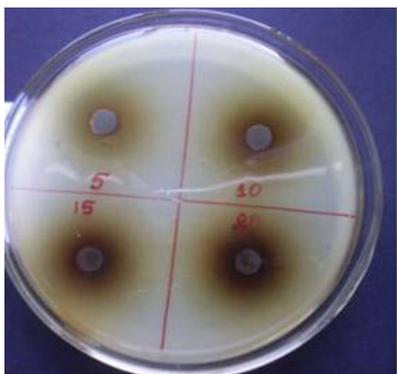


Figure 4: Ethanolic Extract of *P. kurroa* (5mg, 10mg 15 mg, 20mg/75 µl) on *B. subtilis*



Figure 5: HPTLC Plate image at visible light developed with Vanillin-Sulphuric acid Reagent.

1 & 2: Arogyawardhini Bati methanolic extract (50 µl), 3 & 4- methanolic extract of *P. kurroa* (20 µl).

HPTLC Analysis

HPTLC analysis of the methanolic extract of *P. kurroa* for the glycosides revealed presence of two major spots. These spots were visualized by spraying with Vanillin-Sulphuric acid, lead to formation of bluish-brown color. Based on the reaction given by the constituents with Vanillin-Sulphuric acid and Rf value, the spots were identified on the Preliminary basis to be picroside and kutkoside, by comparing with the reported data⁸.

Table 2: Chromatographic data of HPTLC analysis of methanolic extracts of *P. kurroa* and Arogyawardhini Bati

Track	Start position (Rf)	Start height (AU)	Max position Rf	Max height (AU)	Max %	End position Rf	End height (AU)	Area (AU)	Area %
1	0.23	77.8	0.31	212.7	9.12	0.37	17.6	12478.4	16.24
2	0.21	39.5	0.27	137.3	8.05	0.34	2.1	7085.7	15.19
3	0.19	67.4	0.30	431.6	23.08	0.34	17.6	24806.9	40.14
4	0.20	65.8	0.31	427.0	23.14	0.38	13.0	25760.5	40.63

Tracks 1 & 2: Arogyawardhini Bati (50 µl), Tracks 3 & 4- methanolic extract of *Picrorhiza kurroa* (20 µl)

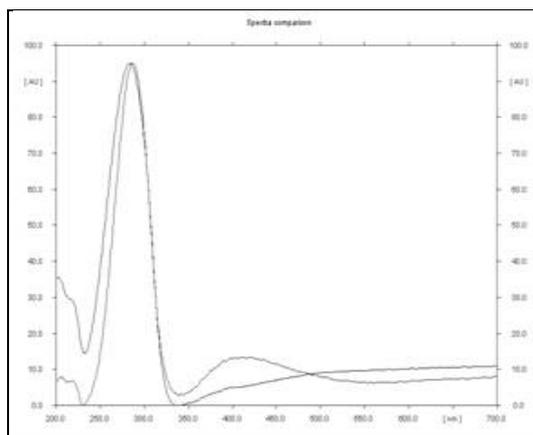


Figure 6: Wavelength scans of spot with Rf 0.24 of methanolic extract of *P. Kurroa* (A) and Arogyawardhini Bati (B)

Further, the presence of kutkoside could be confirmed through carrying out wavelength scan of the spot (figure 6) corresponding to Rf value (0.24), without derivatizing with Vanillin-Sulphuric acid. The wavelength scan revealed that the constituent has λ_{max} at 295nm which

correspond to kutkoside (295nm) as reported in literature. Analysis of kutkoside using the external reference standard of kutkoside could not be carried out as the reference standard was not affordable.

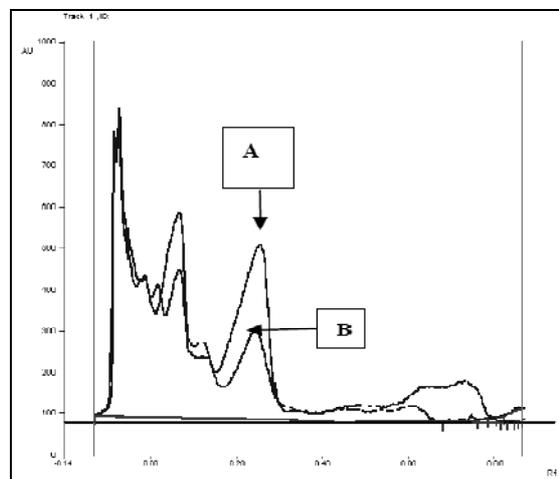


Figure 7: Fingerprint of methanolic extracts of *P. kurroa*. (A) and Arogyawardhini Bati. (B)

CONCLUSION

Presence of *P.kurroa* in the Arogyawardhini bati was confirmed through HPTLC fingerprinting of the formulation. HPTLC Chromatogram of Arogyawardhini bati indicated allied spots with Rf values and the reaction to Vanillin-Sulphuric acid with the constituents of *P.kurroa* shown in figure 7 and table 2.

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