Arabian Journal of Chemistry (2016) 9, S1307–S1313

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea

Deepti Rathee [a], Permender Rathee [b], Sushila Rathee [b], Dharmender Rathee [a,*]

[a] *Department of Pharmacognosy and Phytochemistry, JCDM College of Pharmacy, Sirsa, India*
[b] *Department of Analytical Chemistry & Natural Products, PDM College of Pharmacy, Bahadurgarh, India*

Received 30 March 2011; accepted 26 February 2012
Available online 10 March 2012

KEYWORDS

Antimicrobial;
Iridoid glycosides;
Phytochemical screening;
HPTLC;
Picrorrhiza kurroa

Abstract Phytochemical screening of the rhizomes of *Picrorrhiza kurroa* Benth revealed the presence of some bioactive components, which have been linked to antimicrobial properties. Various chemical tests and TLC studies showed the presence of glycosides, sterols and phenolic compounds when tested on different extracts of *P. kurroa* rhizomes. The major chemical constituents found in this plant were iridoid glycosides and cucurbitacins (triterpenoids) present in the methanolic extract. The effects of methanolic and aqueous extracts on some pathogenic bacterial and fungal strains viz.: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*, *Escherichia coli* and *Candida albicans*, *Aspergillus niger*, respectively, showed that the plant part can be used to treat infections caused by these bacteria and fungi. The aqueous and methanolic extracts showed antibacterial activity but the significant antimicrobial activity was shown by methanolic extract only, against *P. aeruginosa* and *S. aureus;* while moderate activity against *E. coli, B. subtilis* and *M. luteus*. The effectiveness of the crude extract confirmed its use in traditional medicine to treat skin, urinary tract, diarrheal infections and gastrointestinal infections. The aqueous extract was less effective against the microbial strains and no activity against fungal strains. The MICs of the methanolic extract against the test bacteria were high and correlate with sensitivity test results. The effectiveness of the extracts was less than the conventional antibiotic, ciprofloxacin. Further the

* Corresponding author. Address: Department of Pharmacognosy and Phytochemistry, JCDM College of Pharmacy, Sirsa, Haryana, India. Tel.: +91 9050352764; fax: +91 01666 23100.
E-mail address: rathee_dharmender04@yahoo.com (D. Rathee).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2012.02.009

HPTLC studies were performed to estimate the content of iridoids and it was found to be 3.66 ± 0.11 and 4.44 ± 0.02 for picroside I and kutkoside, respectively.

1. Introduction

Despite the existence of a wide variety of antimicrobial agents, the search for new ones is of great importance. Microbial infections are the cause of a large burden of diseases and bacteria are listed in the first position among the common microorganisms responsible for opportunistic diseases occurring associated with AIDS. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics (Keasah et al., 1998; Marimoto and Fujimoto, 1999). Many people still use traditional herbs to treat a variety of diseases including bacterial infections. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents (Chopra et al., 1996; Baquero, 1997) that lead to repeated use of antibiotics and insufficient control of the disease (NCID, 2002). New prototype antimicrobial agents are needed to address this situation and plants are among the most important common sources of potentially valuable new drugs. There is, therefore, an urgent need to investigate the biological properties of additional medicinal plants in order to develop new drugs. This prompted us to evaluate plants as a source of potential chemotherapeutic agents for antimicrobial activity based on their ethnomedical use. Till date no reports exist on the antimicrobial activity of *Picrorrhiza kurroa* rhizomes. *P. kurroa* Benth. a well-known herb in the Ayurvedic System of Medicine and has traditionally been used to treat disorders of the liver and upper respiratory tract, to treat dyspepsia, chronic diarrhea, scorpion sting and also reduce fever and dyspepsia (Nadkarni and Nadkarni, 1976). It has been extensively used in Oriental medicine for a variety of conditions, including liver and lung diseases, fever, skin lesions, worm infections, rheumatic diseases, urinary disorders, heart failure and snake and scorpion bites. The root of the plant is considered to be a valuable bitter tonic, antiperiodic, cholagogue, stomachic, laxative in small doses and cathartic in large doses and useful in gastrointestinal and urinary disorders, leukoderma, snake bite, scorpion sting and inflammatory affections (Kirtikar and Basu, 1935; Chopra et al., 1958; Pandey, 1979; Dey, 1980; Jayaweera, 1982).

Kutkin is the active principle of *P. kurroa* and is comprised of kutkoside and the iridoid glycoside picrosides I, II, and III. Other identified active constituents are apocynin, drosin, and nine cucurbitacin glycosides (Weinges et al., 1972; Stuppner and Wagner, 1989). Apocynin is a catechol that has been shown to being a powerful anti-inflammatory agent, (Simons et al., 1990) while the cucurbitacins have been shown to be highly cytotoxic and possess antitumor effects (Stuppner and Wagner, 1989). Iridoids comprise part of a group of plant metabolites based on a monoterpene structure with a cyclopenta[c]pyranoid skeleton. The chemical studies on the *P. kurroa* rhizomes revealed the presence of iridoids (Basu et al., 1971), acetophenones (Stuppner et al., 1990) and cucurbitacins (Gupta, 2001; Stuppner et al., 1991; Ji and Zhang, 1998). The bioassays of the constituents from the rhizomes were focused on hepatoprotective, antioxidant and immune-modulating activities (Junior, 1990).

The present paper concentrates on antimicrobial potential of *P. kurroa* rhizomes. In this study the antimicrobial activity of methanolic and aqueous extracts of *P. kurroa* rhizomes was tested against a battery of microorganisms including Gram-positive and Gram-negative bacteria and fungi. The extracts were tested *in vitro* against 5 strains of bacteria and 2 strains of fungi: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*, *Escherichia coli* and *Candida albicans*, *Aspergillus niger*, respectively.

Iridoid glycosides (Puri et al., 1992; Garg et al., 1994) constitute an important class of compounds among natural products, employed for medicinal purpose from time immemorial to relieve various ailments and possess wide spectrum of biological activities viz. immunomodulatory (Dorsch et al., 1991), antiasthmatic (Dhawan, 1995), hepatoprotective (Mizoguchi et al., 1992), choleretic (Trovato et al., 1993), hypoglycemic and hypolipidemic (Ivanovska et al., 1996), anti-inflammatory (Ortiz de urbina et al., 1994), antispasmodic (Hansal et al., 1965), etc. Iridoid glycosides such as agnusides and negundoside have been used extensively in Chinese herbal medicine to cure ailments like chronic bronchitis, rheumatic difficulties, bacterial dysentery, cough, cold, burns, scalds and gonorrhea (Trease and Evans, 2002).

2. Material and methods

2.1. Selection and collection of plant material

The rhizomes of *P. kurroa* were selected on the basis of traditional claims for its anti diarrheal activity and purchased from local market in Sirsa district of Haryana. It was authenticated from the Dept. of Botany, Punjab University, Chandigarh against voucher specimen PK-1. The rhizomes of *P. kurroa* were subjected to shed drying and further crushed to powder and then the powder was passed through the mesh 40.

2.2. Preparation of extracts

The dried and ground plant material (1.0 kg) was successively extracted with methanol and water for 72 h each. The extracts were concentrated to dryness under reduced pressure. The obtained extracts were stored in a refrigerator at 4 °C until use.

2.3. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne (1984), Brunton (1995), Wagner et al. (1984). The shade dried and powdered rhizomes of *P. kurroa*, were subjected to maceration with different solvents like petroleum ether (60–80 °C), chloroform, ethyl acetate, methanol and finally macerated with water so as to get respective extracts. All extracts were filtered

individually, evaporated to dryness. After drying, the respective extracts were weighed and percentage yields were determined separately and stored in freeze condition for further use. The qualitative chemical tests, for identifying the presence of various phytoconstituents, were carried out on various extracts of *P. kurroa* rhizomes. The extracts were screened for the presence of tannins, saponins, sterols/triterpenes, alkaloids, cucurbitacin glycosides, flavonoids, polyphenolic compounds, protein/amino acids, carbohydrates and iridoid glycosides on Silica gel G (Merck) plates (0.25 mm thickness) Stahl, 1969. Development was carried out with various solvent systems viz.: ethyl acetate:formic acid:methanol (6:0.6:0.4 v/v/v), ethyl acetate:methanol:water (10:1.3:1.0 v/v/v), ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.7 v/v/v), chloroform:methanol:water (6.4:5.0:1.0 v/v/v), benzene:ethyl acetate (8.6:1.4 v/v) and ethyl acetate:methanol:water:acetic acid (6.5:1.5:1.5:1.0 v/v/v/v). After development in the different solvents, the plates were sprayed with Dragendorrf's reagent, $AlCl_3$, hydroxylamine–ferric chloride, ninhydrin and antimony trichloride reagents for the discovery of alkaloids, flavonoids, proteins/amino acids and sterols/triterpenes, respectively. Detection of cucurbitacin glycosides, saponins, tannins, and carbohydrate is carried out using, KOH, anisaldehyde–sulfuric acid, ferric chloride, and naphthoresorcinol reagents, respectively. To visualize TLC of iridoid glycosides a non specific reagent is used, Vanillin in the presence of sulfuric acid or hot HCl is used. Reagents were prepared according to Stahl (Inouge, 1991). Detection was carried out visually in visible light and under UV light (λ = 366 nm). The literature cited the major presence of iridoids in *P. kurroa* rhizomes so that the detection of iridoid glycosides in the drug is done by Trim & Hill color reagent: 1 g of powered drug was taken in a test tube and placed with 5 ml of 1% aqueous HCl, after 3–6 h 0.1 ml of the macerate is decanted into another tube containing 1 ml of the Trim–Hill reagent (made up from 10 ml acetic acid, 1 ml of 0.2% copper sulfate ($CuSO_4$, $5H_2O$) in water and 0.5 ml conc. HCl). When the tube is heated for a short time in flame, a color is produced if certain iridoids are present. Further, iridoid glycosides presence can be confirmed by performing TLC identity test by visualizing under UV light 254 nm and by spraying the plate with anisaldehyde–sulfuric acid reagent, before spraying anisaldehyde–sulfuric acid a strong fluorescent quenching is visible in case of iridoid glycosides (Wagner et al., 1984).

2.4. HPTLC studies

Densitometric HPTLC (High Performance Thin Layer Chromatography) analysis was performed to confirm the presence and to estimate the content of iridoid glycoside Kutkin which is a mixture of picroside I and kutkoside in the ratio of 1:1.5 in methanolic extract of rhizomes by co-chromatography with authentic sample (RRL, Jammu, India). Quantification of Kutkin was carried out for the methanolic extract of rhizomes. The linearity of the HPTLC method was investigated for Kutkin in the range of 300–1800 ng/spot at three concentration levels using the Camag Linomat V applicator onto the precoated silica gel plate (Merck). The plate was then eluted with ethylacetate:formic acid:methanol (6:0.6:0.4 v/v/v) and scanned densitometrically at 290 nm in absorbance mode using tungsten lamp. The percentage of Kutkin in the methanolic extract was calculated by calibration curve using peak height and peak area ratio. ICH guidelines were followed for the validation of the analytical methods developed (CPMP/ICH/281/95 and CPMP/ICH/381/95) for precision, repeatability and accuracy (data not shown).

2.5. Microbial strains

Five strains of bacteria and two strains of fungi were procured from the Microbial Type Culture Collection (MTCC, IMTECH), Institute of Microbial Technology, Chandigarh, India, and were tested: *P. aeruginosa* (MTCC 1688), *S. aureus* (MTCC 737), *B. subtilis* (MTCC 441), *M. luteus* (MTCC 106), *E. coli* (MTCC 443), *C. albicans* (MTCC 3017), and *A. niger* (MTCC 1344). All the strains were stored at freeze temperature until use.

2.6. Culture media

Nutrient agar (NA) (Himedia) containing bromocresol purple was used for the activation of *Bacillus* species, while NA was used for other bacteria. Sabouraud glucose agar (SDA) (Himedia) was used for the activation of the fungi. The NA was used in sensitivity assay and also used for minimum inhibitory concentration (MIC) determination.

2.7. Chemicals for antimicrobial assay

Ciprofloxacin and Nystatin (Central Drug House (P). Ltd., India) were used as positive reference standards (RA) for all bacterial and fungal strains, respectively. The dimethylsulfoxide (DMSO) (Qualigens) was used as the solvent for the tested samples.

2.8. Preparation of inoculums

Bacterial inoculums were prepared by growing freeze-dried cells in NA for 24 h at 37 °C. Slants were prepared by streaking of these cell suspensions and sub culturing was done by using the same broth to provide initial cell counts of about 10^4 CFU/ml and incubated at 37 °C for required time. The filamentous fungi were grown on SDA slants at 25 °C for seven days and the spores were collected using sterile double distilled water and homogenized.

2.9. Preparation of test sample

The aqueous and methanolic extracts were dissolved in 10% aqueous DMSO to obtain different concentrations (10 mg, 25 mg, 50 mg and 100 mg per ml). Negative controls used were 10% aqueous DMSO (solvent control). Ciprofloxacin and Nystatin were used as positive reference standards having a concentration of 5 μg/ ml for all bacterial and fungal strains.

2.10. Antimicrobial assays

The antimicrobial activity was evaluated by Cup-Plate method and MIC values were also determined.

2.10.1. Cup-plate method assay

Petri plates were prepared by pouring 30 ml of NA medium for all the bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and

allowed to dry for 10 min. Three wells or cavities were made in agar containing each petri dish by a sterilized steel borer. To these cavities standard and test compound solutions were filled. All the works were carried out under aseptic conditions for microbial assay. The plates for the bacteria were incubated at 37 °C ± 1 °C for 24 h. The fungal strains *C. albicans* and *A. niger* were incubated at 25 °C for 72 h and seven days, respectively. The antimicrobial potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was carried out in the triplicate form.

2.10.2. Minimum inhibitory concentration (MIC)

The experiment was according to twofold serial dilution method. The stock solution of test solutions (extracts) was prepared at the concentration of 100 µg/ml in nutrient broth and serially diluted up to five times. Six assay tubes were taken for screening MIC of each strain. In the first tube 1 ml of the sterilized nutrient broth was inoculated and then 1 ml of the test compound solution was added and thoroughly mixed to a concentration of 50 µg/ml. Further dilutions of this solution were made by inoculating 1 ml from first tube into second assay tube serially and 0.1 ml of each test inoculums was added in each tube and was done in duplicate. The procedures were conducted under aseptic conditions. The inoculated tubes were kept at 37 °C ± 1 °C at 24 h for bacterial assay and seven days for fungi (*A. niger*) and three days for fungi (*C. albicans*) at 25 °C ± 0.1 °C during the incubation period. After the incubation period, tubes were removed and observed for any deposits or turbidity in the solution and shaken to suspend bacteria and fungi that might have been settled down. These concentrations were observed and assumed as MIC.

3. Results and discussion

3.1. Preliminary phytochemical screening

The tested plant showed positive results for variable amounts of iridoid glycosides, cucurbitacins, unsaturated sterols/triterpenes, and polyphenols. In the methanolic extract glycosides, sterols or triterpenoids and phenolic compounds are present in considerable amounts. The presence of iridoid glycosides was further confirmed by the Trim–Hill reagent. The dark greenish brown color was obtained after the test was performed and this confirmed the presence of iridoids. Cucurbitacin glycosides (triterpenoids) were present in methanolic extract in good amount. Traces of glycosides and phenolic compounds were detected in the aqueous extract of *P. kurroa* rhizomes. Alkaloids, flavonoids, tannins, saponins, proteins/aminoacids and carbohydrates were not found in any of the tested extracts. The presence of these constituents was further confirmed by TLC studies on various extracts. TLC showed the presence of iridoid glycosides mainly in methanolic extract, two prominent spots appear after derivatization with vanillin in the presence of sulfuric acid or hot HCl. Presence of glycosides, sterols and polyphenols was confirmed in methanolic extract after spraying of KOH and anisaldehyde–sulfuric acid reagent, five spots appeared on the plate. Glycosides and polyphenols were also found in aqueous extract, while all other phytoconstituents were absent from all extracts.

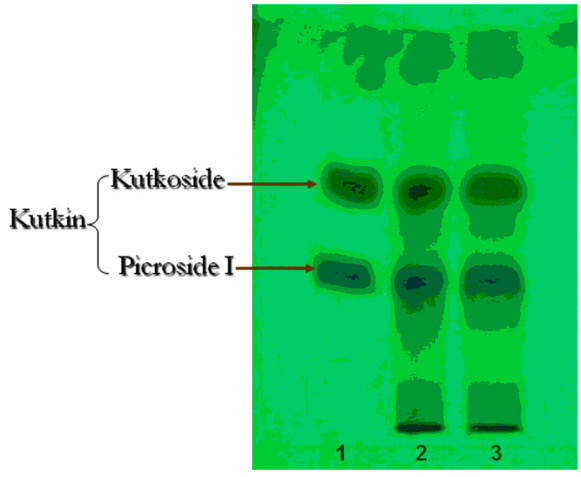

Figure 1 TLC profile of test solution of *Picrorrhiza kurroa* rhizome extract.

3.2. HPTLC studies

The densitometric HPTLC analysis confirmed the presence and estimated the content of Kukin in methanolic extract of plant rhizomes. Kutkin present at R_f [picroside I (0.51), kutkoside (0.63)] (Fig. 1) and content in the plant drug was found to be 3.66 ± 0.11% and 4.44 ± 0.02%, respectively for picroside I and kutkoside (Fig. 2).

3.3. Antimicrobial assays

Aqueous and methanolic extracts were screened against bacteria and fungi. The aqueous and methanolic extracts show activity but significant activity was shown by methanolic extract against *P. aeruginosa* and *S.* aureus; while moderate activity against *E. coli, B. subtilis* and *M. luteus* (Table 1).

The aqueous extract shows no activity against any of the fungi, while methanolic extract shows activity only against *C. albicans* but no activity against *A. niger* (Table 2). The results of both the extracts were correlated with the standard drug and show that the methanolic extract shows good activity against all bacteria while moderate activity was shown by aqueous extract against all bacterial strains.

3.4. Minimum inhibitory concentration

The MIC of methanolic extract against bacterial strains was found to be 12.5 µg/ml for *S. aureus, E. coli, Bacillus subtilus*;

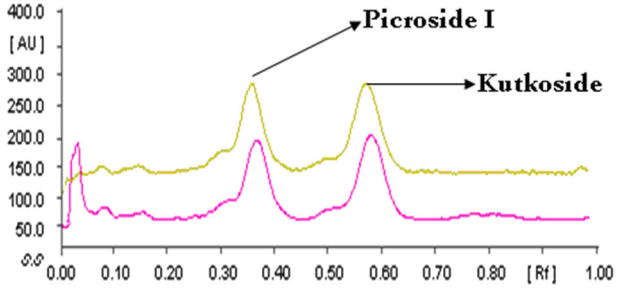

Figure 2 TLC densitometric scan at 290 nm of test solution of *Picrorrhiza kurroa* rhizome.

Table 1 Antibacterial activity of the aqueous and methanolic extracts of *P. kurroa* on various strains of bacteria at various concentrations.

Extracts	Conc. (mg/ml)	Cup-plate method (inhibition zone, mm)				
		S. A.	M. L.	B. S.	P. A.	*E. coli*
Aqueous	10	3.0 ± 0.1	–	–	1.3 ± 0.15	3.67 ± 0.058
	25	8.0 ± 0.1	4.0 ± 0.1	4.3 ± 0.05	9.0 ± 0.1	5.67 ± 0.058
	50	10.0 ± 0.1	6.0 ± 0.1	6.33 ± 0.05	11.3 ± 0.21	8.33 ± 0.058
	100	12.7 ± 0.05	8.0 ± 0.1	7.0 ± 0.1	13.0 ± 0.1	11.3 ± 0.058
Methanolic	10	4.7 ± 0.2	–	–	1.0 ± 0.17	2.7 ± 0.05
	25	8.0 ± 0.17	2.1 ± 0.05	4.0 ± 0.1	8.3 ± 0.05	4.0 ± 0.1
	50	12.7 ± 0.12	6 ± 0.1	6.0 ± 0.11	10.7 ± 0.15	8.3 ± 0.05
	100	16.0 ± 0.17	11 ± 0.1	8.33 ± 0.05	16 ± 0.1	14.0 ± 0.1
Ciprofloxacin	5 µg/ml	26 ± 0.05	14 ± 0.06	32 ± 0.02	25 ± 0.03	22 ± 0.056

S. A. – *Staphylococcus aureus*, M. L. – *Micrococcus luteus*, B. S. – *Bacillus subtilus*, P. A. – *Pseudomonas aeruginosa*, *E. coli* – *Escherichia coli*; – sign shows no zone of inhibition.

Table 2 Antifungal activity of methanolic extract of *P. kurroa* on two fungal strains at various concentrations.

Fungi	Conc. (mg/ml)	Extracts (zone of inhibition, mm)		
		Aqueous extract	Methanolic extract	*N* 5 µg/ml
Candida albicans	10	–	1.3 ± 0.058	12.2 ± 0.087
	25	–	2.67 ± 0.058	
	50	–	3.7 ± 0.058	
	100	–	5.0 ± 0.058	
Aspergillus niger	10	–	–	11.47 ± 0.065
	25	–	–	
	50	–	–	
	100	–	–	

N – Nystatin; – sign shows no zone of inhibition.

Table 3 The results of MIC for antibacterial activity of aqueous and methanolic extracts of *P. kurroa* at various doses on bacterial strains.

Microorganism	Name of extracts	Serial dilution (µg/ml)					
		50	25	12.5	6.25	3.12	1.56
Staphylococcus aureus	Methanolic	–	–	–	+	+	+
	Aqueous	–	–	+	+	+	+
Micrococcus luteus	Methanolic	–	–	–	+	+	+
	Aqueous	–	–	+	+	+	+
Pseudomonas aeruginosa	Methanolic	–	–	–	–	+	+
	Aqueous	–	–	–	+	+	+
Bacillus subtilus	Methanolic	–	–	–	+	+	+
	Aqueous	–	–	–	+	+	+
Escherichia coli	Methanolic	–	–	–	+	+	+
	Aqueous	–	–	–	+	+	+

– No growth; + growth; stock solution = 100 µg/ml; Std. drug – ciprofloxacin.

while for *M. luteus* and *P. aeruginosa* it was found to be 6.25 µg/ml and the MIC of aqueous extract against *S. aureus, M. luteus* and *E.* coli bacterial strains was observed as 8.5 µg/ml, and for *P. aeruginosa, and Bacillus subtilus* it was found to be 4.25 µg/ml (Table 3). The MIC of the methanolic extract against *C. albicans* was found to be 6.25 µg/ml and no MIC was found against *C. albicans* for aqueous extract. Negative MIC was found against *A. niger* of both the extracts (Table 4).

Table 4 The results of MIC for antifungal activity of *P. kurroa* methanolic extract on fungal strains at various doses.

Microorganism	Name of extracts	Serial dilution (μg/ml)					
		50	25	12.5	6.25	3.12	1.56
Candida albicans	Methanolic	−	−	−	−	+	+
Aspergillus niger	Methanolic	+	+	+	+	+	+

− No growth; + growth.

4. Conclusion

In conclusion, the antimicrobial activities displayed by the methanolic extract of *P. kurroa* rhizomes are significant, while the aqueous extract also shown some moderate antibacterial activity. The significant antimicrobial activity was only shown by methanolic extract in comparison to aqueous extract. Preliminary phytochemical screening showed the presence of iridoid glycosides and it was confirmed by Trim–Hill reaction which showed the presence of iridoids. The HPTLC studies further confirmed the presence of iridoid glycosides and the content of Kutkin was also estimated and is present in a very good amount in the rhizomes of this plant. Methanolic extract shown considerable activity against both the bacterial and fungal strains and also the presence of iridoids was confirmed in the methanolic extract of *P. kurroa* rhizomes, so it might be concluded that the activity may be due to the iridoid glycosides present in this plant. The results reported here, render this species interesting for further mechanism based research.

Acknowledgements

Authors are very thankful to the Dept. of Botany, Punjab University, Chandigarh for authentication of the plant and also thankful to the administration of both the colleges for providing facilities.

References

Baquero, F., 1997. Gram-positive resistance: Challenges for the development of new antibiotics. J. Antimicrob. Chemother. 39, 1–6.

Basu, K., Dasgupta, B., Bhattacharya, S.K., Debnath, P.K., 1971. Chemistry and pharmacology of apocynine, isolated from *Picrorhiza kurroa*. Curr. Sci. 22, 603–604.

Brunton, J., 1995. Text Book of Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept Limited. Springer, London, pp. 592–593.

Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, L.D., 1958. Chopra's Indigenous Drugs of India, second ed. Dhur and Sons, Calcutta, pp. 181–183.

Chopra, I., Hodgson, J., Metcalf, B., Poste, G., 1996. New approaches to the control of infections caused by antibiotic resistant bacteria. An industry perspective. J. Am. Med. Assoc. 275, 401–403.

Dey, A.C., 1980. Indian Medicinal Plants Used in Ayurvedic Preparations. Bhishen Singh Mahendra Pal Singh, Dehra Dun, India, p. 8 I.

Dhawan, B.H., 1995. Med. Chem. Res. 5, 595–605.

Dorsch, W., Stuppner, H., Wagner, H., Gropp, M., Demoulin, S., Ring, J., 1991. Int. Arch. Allergy Appl. Immunol. 95, 128–133.

Garg, H.S., Bhandari, S.P.S., Tripathi, S.C., Patnaik, G.K., Puri, A., Saxena, R.P., 1994. Phytother. Res. 8, 224–228.

Gupta, P.P., 2001. Picroliv: hepatoprotectant, immunomodulator. Drugs Future 1, 25–31.

Hansal, R., Leuckert, C., Rimpler, H., Schaaf, K.D., 1965. Phytochemistry 4, 19.

Harborne, J.B., 1984. Phytochemical methods, second ed. Springer, Chapman and Hall, New York, London and New York, p. 288.

Inouge, H., 1991. Triddon in methods on plant biochemistry. In: Charlwood, D.V., Banthaope, D.V. (Eds.), . In: Terpenoids, vol. 7. Academic Press, London, pp. 99–143.

Ivanovska, N., Iossifova, T., Kostova, I., 1996. Phytother. Res. 34, 2375–2379.

Jayaweera, D.M.A., 1982. Medicinal Plants Used in Ceylon, Part 5. The National Science Council of Sri Lanka, Colombo, Sri Lanka, p. 76.

Ji, W.L., Zhang, C.H., 1998. Research progress on *Picrorhiza kurroa*. Zhongcaoyao 29, 54–56.

Junior, P., 1990. Planta Med. 56, 1.

Keasah, C., Odugbmi, T., Ben Redjeb, S., Boye, C.S., Dosso, M., The Members of Palm Project, 1998. Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Eight African Hospitals and Malta. Poster E.093, 38th ICAAC, San Diego, September 24–28.

Kirtikar, K.R., Basu, B.D., 1935. In: Indian Medicinal Plants, vol. III. Lalit Mohan Basu, Allahabad, pp. 1825–1826.

Marimoto, K., Fujimoto, M., 1999. Report of questionnaire survey for methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* in the Kinki district. Kansenshogaku Zasshi 73, 584–592.

Mizoguchi, Y., Sagasami, C., Ichikawa, Y., Kobbayashi, S., 1992. Planta Med. 58, 394–397.

Nadkarni, K.M., Nadkarni, A.K., 1976. The Indian Materia Medica. Popular Prakashan, Bombay, India, pp. 953–955.

National Center for Infectious Diseases (NCID), 2002. Campaign to Prevent Antimicrobial Resistance in Healthcare Settings. Centre for Disease Control and Prevention. Available from <http://www.cdc.gov/drugresistacne/healthcare/problem.htm>.

Ortiz de urbina, A.V., Martin, M.L., Fernandez, B., San Roman, L., Cubillo, L., 1994. Planta Med. 60, 512–515.

Pandey, G.S., 1979. Bhavaprakasa Nighantoo (Indian Materia Medica). Chaukhamba Sanskrit Sansthan, Varanasi, India, pp. 70–71.

Puri, A., Saxena, R.P., Guru, P.Y., Kulshreshtha, D.K., Sexena, K.C., Dhawan, B.N., 1992. Planta Med. 58, 528–532.

Simons, J.M., Hart, B.A., Vai, I., Ching, T.R., 1990. Metabolic activation of natural phenols into selective oxidative burst agonists by activated human neutrophils. Free Radic. Biol. Med. 8, 251–258.

Stahl, E., 1969. Thin Layer Chromatography: A Laboratory Handbook. Springer International, New York.

Stuppner, H., Wagner, H., 1989. New cucurbitacin glycosides from *Picrorhiza kurroa*. Planta Med. 55, 559–563.

Stuppner, H., Kaehlig, H., Seligmann, O., Wagner, H., 1990. Minor cucurbitacin glycosides from *Picrorhiza kurrooa*. Phytochemistry 29, 1633–1637.

Stuppner, H., Mueller, E., Wagner, H., 1991. Cucurbitacins from *Picrorhiza kurrooa*. Phytochemistry 30, 305–310.

Trease, G.E., Evans, W.C., 2002. Text Book of Pharmacognosy. Bailliere Tindall, Eastbourne, UK.

Trovato, A., Forestieri, A.M., Iauk, L., Barbera, R., Momtforte, M.T., Galati, M.E., 1993. Plant. Med. Phytother. 26, 300–308.

Wagner, H., Baldt, S., Zgainski, E.M., 1984. Plant Drug Analysis. Springer Verlag, Berlin/New York.

Weinges, K., Kloss, P., Henkels, W.D., 1972. Natural products from medicinal plants. XVII. Picroside-II, a new 6-vanilloylcatapol from *Picrorhiza kuroa* Royle and Benth. Justus Liebigs Ann. Chem. 759, 173–182.