Arabian Journal of Chemistry (2012) **xxx**, xxx–xxx

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

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

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Received 30 March 2011; accepted 26 February 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

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Peer review under responsibility of King Saud University.
http://dx.doi.org/10.1016/j.arabjc.2012.02.009

ELSEVIER **Production and hosting by Elsevier**

effectiveness of the extracts was less than the conventional antibiotic, ciprofloxacin. Further the 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

Please cite this article in press as: Rathee, D. et al., Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. Arabian Journal of Chemistry (2012), http://dx.doi.org/10.1016/j.arabjc.2012.02.009