Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/hermed

Original Research Article

Cyperus rotundus: A potential novel source of therapeutic compound against urinary tract pathogens

*Anjana Sharma**, *Rani Verma*, *Padmini Ramteke*

Bacteriology Laboratory, Department of Post Graduate Studies and Research in Biological Science, R.D. University, Jabalpur 482001, India

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form
14 December 2013
Accepted 19 January 2014
Available online 25 January 2014

Keywords:
Cyperus rotundus
High performance liquid chromatography
Method validation
Minimum inhibitory concentration
Thin layer chromatography
Thin layer chromatography bioautography

ABSTRACT

Bioactive compounds from medicinal plants offer a potentially beneficial alternative to synthetic antibiotics. The crude extract of *Cyperus rotundus* was screened for antiuropathogenic activity using the disc diffusion method, which indicated its broad-spectrum antibacterial activity against urinary tract infection (UTI) pathogens. The minimum inhibitory concentrations of the ethanolic extract ranged from 2.5 to 10 mg/mL against different uropathogens. Phytochemical analysis of *C. rotundus* showed the presence of tannins and saponins. Thin layer chromatography (TLC) analysis of the ethanolic extract indicated the presence of two spots. The TLC bioautography confirmed the antibacterial activity of the spot with a resolution front (R_f) of 0.435, which indicated its bioactive property. High performance liquid chromatography (HPLC) was validated using parameters such as linearity, accuracy, precision, limit of detection and limit of quantification. HPLC analysis of the active spot indicated the presence of saponins only, with a retention time of 16.102 min. Quantitative studies revealed that 75.6373 mg/mL of saponin was present in the ethanolic extract of the rhizome of *C. rotundus*. Therefore, the study scientifically validated the use of *C. rotundus* plant for the treatment of UTIs by tribal populations and advocated the potential application of the bioactive compounds from *C. rotundus* for the treatment of UTIs.

1. Introduction

Urinary tract infection (UTI) is one of the most common human diseases, affecting people throughout their life span from neonate to the geriatric age group (Barnett and Stephens, 1997; Kunin, 1997; Foxman, 2002; Raju and Tiwari, 2004). The term UTI covers a variety of clinical entities, ranging from cystitis and prostatitis to pyelonephritis (Huethers and Mccance, 2000; Wagenlehner et al., 2008). Worldwide, about 150 million people are diagnosed with UTIs per year (Akram et al., 2007). It represents the most commonly acquired bacterial infection, accounting for 25% of all infections, among which 80–85% infections are caused by Gram-negative bacteria, whereas 15–20% are caused by Gram-positive bacteria (Gales et al., 2002). Members of the Enterobacteriaceae family including *Escherichia coli*, *Klebsiella* spp., *Enterococcus* spp., *Citrobacter* spp., *Pseudomonas* spp. and *Proteus* spp. are the

* *Corresponding author at*: Department of Post Graduate Studies and Research in Biological Science, R.D. University, Jabalpur, MP 482001, India. Tel.: +91 761 2416667/9425155323; fax: +91 761 2603752.
E-mail addresses: anjoo1999@gmail.com (A. Sharma), rani.verma786@gmail.com (R. Verma), Padmini.ramteke@gmail.com (P. Ramteke).
2210-8033/$ – see front matter © 2014 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.hermed.2014.01.003

primary causative agents of UTIs (Farrell et al., 2003; Ronald, 2003; Nicolle, 2008).

Since the 1990s the management of UTIs has become more complicated due to increasing antimicrobial resistance, especially towards β-lactams and trimethoprim/sulphamethoxazole (TMP/SMX) (Gupta et al., 2001; Hooton, 2003). The efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003), and has necessitated the search for new antimicrobials from alternative sources. One way to prevent antibiotic resistance is by using new compounds that are not based on the existing synthetic antimicrobial agents (Shah, 2005). Synthetic drugs are not only expensive and at times ineffective for the treatment of diseases, but are also often associated with side effects (Shariff, 2001).

Medicinal plants for healthcare have a traditionally important socio-cultural and spiritual position in the lives of many rural and tribal populations throughout the world. Natural products from medicinal plants, either pure compounds or standardized plant extracts due to their wide range of chemical diversity, provide unlimited opportunities for the development of new antimicrobial agents (Cos et al., 2006; O'Bryan et al., 2008). The development of alternative antimicrobial agents in order to treat infection is an urgent requirement and one of the possible strategies towards this objective is the rational investigation of bioactive plant phytochemicals (Cordell, 2000).

In the Indian system of medicine, the rhizome of *C. rotundus* has been recommended for the treatment of various clinical conditions, such as diarrhoea, dysentery, leprosy, bronchitis, amenorrhea, dysmenorrhea, fever, arthritis and blood disorders (Umerie and Ezeuzo, 2000; Uddin et al., 2006). Therapeutic actions of the rhizome include antipyretic, anti-inflammatory, sedative, anti-arthritic and diuretic but there are no reports of its antiuropathogenic properties (Singh et al., 2012). *C. rotundus* is commonly used by tribal populations for the treatment of UTIs. Therefore, the present study was undertaken to validate the knowledge of the tribal people using chromatographic techniques.

2. Materials and methods

2.1. Reagents and chemicals

High performance liquid chromatography (HPLC)-grade methanol, acetonitrile and acetic acid were purchased from Merck, Mumbai, India. Analytical-grade ethanol was purchased from Beijing Chemical Reagents Company, China. HPLC-grade water was purchased from Fisher scientific; India and antibiotic discs were purchased from Himedia, Mumbai, India.

2.2. Plant material

The rhizome of *C. rotundus* was collected from the tribal region of Dindori (Madhya Pradesh) during 2008 and 2009, based on the information provided by the ethnobotanical survey of India and local medicine men of this region (Fig. 1). The

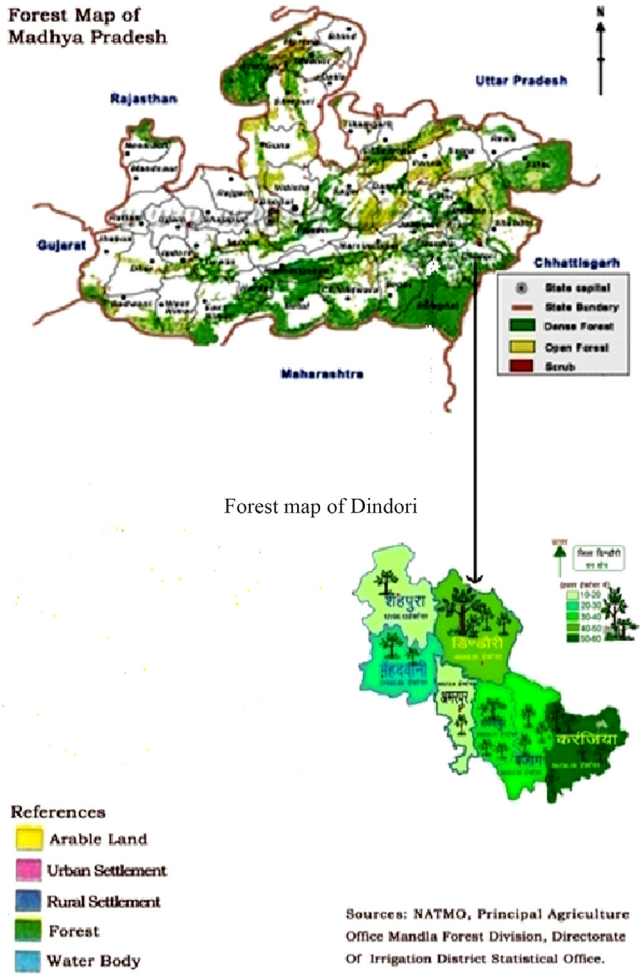

Fig. 1 – Sampling sites of Dinbori (Madhya Pradesh).

specimens were labelled, numbered and annotated with the date of collection and the locality. Identification of the plant was authenticated by the late Dr. J.L. Shrivastava, State Forest Research Institute (SFRI), Jabalpur (MP) India. The plant material was first dried at room temperature, followed by drying in an oven at 50 °C for 48 h. It was then reduced to coarse powder using a grinder and stored at room temperature (Tetyana et al., 2002).

2.3. Extraction

2.3.1. Aqueous extraction

10 g of coarse powdered plant material as described in Section 2.2 was dissolved in 100 mL of distilled water and kept in a boiling water bath for 6 h; then filtered through eight layers of muslin cloth and centrifuged at 10,000 rpm for 15 min. The supernatant was collected and concentrated up to one quarter of the original volume at 40 °C and stored in a screw cap bottle at 4 °C (Nair et al., 2005).

2.3.2. Solvent extraction

Organic (acetone, chloroform, ethanol and methanol) extracts of the plant material were prepared according to the method described by Nair et al. (2005), with certain modifications.