African Journal of Biomedical Research, Vol. 9 (2006); 89 -93
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

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Full Length Research Article

In-vitro Antimicrobial Activities of Extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae)

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Received:
November, 2005
Accepted *(Revised)*:
April, 2006
Published
May 2006

ABSTRACT

The aqueous and ethanolic extracts of Launaea procumbens Roxb. (Labiateae), Vitis vinifera L. (Vitaceae) and Cyperus rotundus L. (Cyperaceae) were evaluated for antimicrobial activity against clinically important bacteria viz. Alcaligenes faecalis ATCC8750, Bacillus cereus ATCC11778, Bacillus subtilis ATCC6633, Enterobacter aerogenes ATCC13048, Escherichia coli ATCC25922, Klebsiella pneumoniae NCIM2719, Proteus mirabilis NCIM224, Proteus vulgaris NCTC8313, Pseudomonas aeruginosa ATCC27853, Pseudomonas pseudoalcaligenes ATCC17440, Salmonella typhimurium ATCC23564, Staphylococcus aureus ATCC25923, Staphylococcus epidermidis ATCC12228, Staphylococcus subfava NCIM2704 and Candida tropicalis ATCC4563. The in vitro antimicrobial activity was performed by agar disc diffusion and agar well diffusion method. The ethanolic extracts of all the plants were active against all the investigated bacterial strains while all the aqueous extracts were inactive except for Vitis vinifera L. S. typhimurium was the most resistant bacterial strain against all the extracts ***(Afr. J. Biomed. Res. 9:89 – 93, May 2006)***

Keywords: antimicrobial activity, aqueous extract, ethanolic extract, *Launaea procumbens* Roxb., *Vitis vinifera* L. and *Cyperus rotundus* L.

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Abstracted by:
African Index Medicus (WHO), CAB Abstracts, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Farnsworth and Loub, 1983). Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds (Janovska et al., 2003). Further, scientific investigation and information of the therapeutic potential of the plant material is limited.

Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). There is an urgent need to systematically evaluate the plants used in traditional medicine. Such research could lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatment. Now a day a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects.

The need of the hour is to screen a number of medicinal plants for promising biological activity. Considering the aforesaid, the three traditionally used medicinal plants belonging to three different families were screened for their antimicrobial properties. *Launaea procumbens* Roxb. (Labiateae) is used as coolent, diuretic, demulcent, allergic infections. *Vitis vinifera* L. (Vitaceae) is used in conditions like burning sensations, haemorrhages, anaemia, leprosy, skin diseases, syphilis, asthama, jaundice, bronchitis (Anjaria et al., 2002; Sriram et al., 2004). *Cyperus rotundus* L. (Cyperaceae**)** is used in stomach disorders, diarrhoea, dysentery. The roots and tubers are analgesic, antibacterial, antispasmodic, antitussive, aromatic, astringent, carminative, diaphoretic, diuretic, emmenagogue, litholytic, sedative, skin, stimulant, stomachic, tonic and vermifuge (Singh and Kachroo, 1976; Lassak and McCarthy, 1978; Yeung, 1985; Bown, 1995). An essential oil in the tubers has antibiotic activity and has been shown to arrest the growth of *Micrococcus pyrogenes* (Chopra et al., 1986). The purpose of this study was to screen for the aqueous and ethanolic extracts of these medicinal plants that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

MATERIAL AND METHODS

Plant material: Fresh plant or plant parts were collected randomly from the semi-arid region of Rajkot Gujarat, India. Whole plant of *Launaea procumbens* Roxb., leaves of *Vitis vinifera* L. and whole plant of *Cyperus rotundus* L. were taken for investigation of antimicrobial property. The taxonomic identities of these plants were confirmed by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot and the voucher specimen numbers of the plants were preserved. Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts: For aqueous extraction, 10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours, was pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh et al., 2005). It was then autoclaved at 121 °C temperature and at 15 lbs pressure and stored at 4 °C.

For solvent extraction, 10 g of air-dried powder was taken in 100 ml of organic solvent (methanol or ethanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh et al., 2005) and stored at 4°C in airtight bottles.

Microorganisms: *In vitro* antimicrobial activity was examined for aqueous and ethanol extracts from three medicinal plants used by traditional healers. Microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India.

Microorganisms were maintained at 4°C on nutrient agar slants (for bacteria) and MGYP slants (for yeast).

Antimicrobial assay: The antimicrobial assay was performed by two methods viz. agar disc diffusion method (Bauer et al., 1966) for aqueous extract and agar well diffusion method (Perez et al., 1990) for solvent extract. The molten Mueller Hinton Agar was inoculated with the 100 µl of the inoculum (1 x 10^8 Cfu) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85cm). 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter (Table-1). The experiment was done three times and the mean values are presented.

RESULTS

The data reported in Table 1 presents the antimicrobial activity of the aqueous and ethanolic extracts of *Launaea procumbens* Roxb., *Vitis vinifera* L. and Cyperus *rotundus* L. The results indicate that the extracts from the medicinal plants studied showed inhibition of growth of some of the tested micro organisms with to various degrees.

Table 1.

In vitro Antimicrobial activity of aqueous and ethanolic extracts of *Launaea procumbens* (Roxb.) (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae).

Microorganisms	*L. procumbens Roxb.*		*Vitis vinifera* L.		*Cyperus rotundus* L.		*Antimicrobics*	
	Ae	Ee	Ae	Ee	Ae	Ee	C	Ap
	Zone of inhibition (mm)*							
A. fecalis *ATCC8750*	-	10	10	10	-	12	17	-
B. cereus ATCC11778	-	25	13	21	-	18	17	-
B. subtilis ATCC6633	-	10	10	12	-	12	16	-
E. aerogenes ATCC13048	-	11	12	14	-	11	20	-
E. coli ATCC25922	-	10	-	15	-	-	22	-
K. pneumoniae NCIM2719	-	14	14	16	-	15	32	-
P. mirabilis NCIM224	-	17	10	16	-	15	18	-
P. vulgaris NCTC8313	-	-	-	11	-	11	21	-
P. aeruginosa ATCC27853	-	-	-	11	-	-	10	-
P.pseudoalcaligenes ATCC17440	-	-	17	13	-	11	28	-
S. typhimurium ATCC23564	-	-	-	-	-	-	25	-
S. aureus ATCC25923	-	30	10	15	-	13	20	-
S. epidermidis ATCC12228	-	21	11	12	-	14	19	-
S. subfava NCIM2704	-	19	10	14	-	13	18	-
C. tropicalis ATCC4563	-	18	-	-	-	-	-	7

Ae: aqueous extract; Ee: ethanolic extract; Disc diameter -7mm and cork-borer diameter – 8.5 mm, Positive control: C: Chloramphenicol (30 mcg/disc); Ap: Amphotericin B (100 units/disc)

**Values are mean of three replicates; --: no inhibition zone*

The ethanolic extract was found to be the most effective antimicrobial agent as compared to the aqueous extract. The ethanolic extract of *Launaea procumbens* Roxb. was active against more than 70 percent of microorganisms investigated while its aqueous extract was totally inactive against all the microorganisms investigated. The ethanolic as well as the aqueous extract of *Vitis vinifera* L. was active against more than 85 and 65 per cent of the studied bacterial strains respectively. Both, ethanolic and aqueous extracts of Cyperus *rotundus* L. showed similar activity as that of *Launaea procumbens* Roxb. Amongst all the three plant species, *Launaea procumbens* Roxb. exhibited remarkable activity against some microorganisms.

B. cereus was the most susceptible gram-positive bacteria followed by *S. aureus* and *S. epidermidis* while *B. subtilis* was the least susceptible gram-positive bacteria. *S. typhimurium* was the most resistant gram-negative bacterial strain followed by *P. aeruginosa*, *P. vulgaris* and *E.coli*. against all the extracts. *K. pneumoniae* was the most susceptible gram-negative bacteria. None of the extracts except ethanolic extract of *Launaea procumbens* Roxb. exhibited anticandidal activity against *C. tropicalis*. The inhibitory activities of all the extracts reported in Table 1 are comparable with standard antimicrobics chloramphenicol (30 mcg/disc) and amphotericin B (100 units/disc).

DISCUSSION

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that methanol extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent (de Boer et al., 2005). These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. Lin et al. (1999) reported that Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study.

Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts as compared to gram-negative bacteria. This is in agreement with previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Vlietinck et al, 1995; Rabe and Van Staden, 1997).

The results of present study supports the traditional usage of the studied plants and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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