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Original Article

In-vitro Antimicrobial Activity of Roots of *Rauwolfia serpentina* L. Benth Kurz

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Abstract

Microbial pathogens develop resistance to antibiotics after repeated administration during the treatment of infectious diseases. Therefore, it is necessary to find alternative antimicrobial drugs and the present trend is focused on medicinal plants. The hereby research work was carried out to investigate the antimicrobial activity of solvents as well as aqueous extracts of *Rauwolfia serpentina* roots. The extracts were tested against *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* by agar well diffusion method. It was observed that methanol extract showed the highest antimicrobial activity against multi drug resistance *S. aureus* at 100 mg/ml concentration, while *S. aureus* was the most susceptible bacterium to all extracts. However, *E. faecalis*, *M. luteus* and *S. pneumoniae* were also susceptible to the experimented solvents and extracts. On the other hand, *K. pneumoniae* was resistant against the solvent and aqueous extracts. The present study suggested that methanol extracts of *R. serpentina* roots would be helpful in treating diseases caused by human pathogenic bacteria and fungi. In particular, based on the results obtained in the current experiment, it can be recommended for the control of infectious Gram-positive bacteria.

Keywords: medicinal plant, multidrug resistance, reserpine, sarpagandha, solvent extract

Introduction

Medicinal plants and their products have been used extensively to treat medical problems. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cure the microbial pathogens in developing countries and moreover, the use of herbal remedies has risen in the developed countries in the last decades. In this connection, medicinal plants are an important source of potentially useful structures for the development of new chemotherapeutic agents against multi drug resistance pathogens. The presence of antimicrobial substances in higher plants is well established (Srinivasan, 2011).

Rauwolfia serpentina L. Benth Kurz (Apocynaceae), commonly known as Sarpagandha, is an important medicinal plant of Indian subcontinent and South East Asian countries. It is a small, woody, perennial medicinal shrub. The International Union for the Conservation of Nature and Natural Resources (IUCN) has assigned an endangered status to *R. serpentina*.

It is a medicinally famous herb in Ayurveda, Siddha, Unani and Western system of medicines (Ajayi *et al*., 2011). It has been reported to contain 50 indole alkaloids that are mainly localized in the root. The roots are tuberous, irregularly nodular, with pale brown bark. They are of immense medicinal value and have steady demand in both domestic and international markets. In action, the root is bitter, acrid, laxative, diuretic, antidote to snake venom, expectorant and febrifuge. In folk and tribal medicine, the root of this plant is used during delivery to stimulate uterine contractions and promote the expulsion of the fetus. Crying babies are put to sleep by working mothers by making them to suck the breasts, which are smeared with the root-paste. It is also a valuable remedy in the treatment of painful affections of the bowels. Roots are used for treating various CNS disorders.

The root of this plant contains several alkaloids; the major and most potent alkaloid is 'reserpine', which is very much useful in insomnia and reducing blood pressure. The root extracts are used for treating intestinal disorders, particularly diarrhea and dysentery and also used as anthelmintic. It is used for the treatment of cholera, colic and fever. The juice is used as a remedy for opacity of the cornea. The total root extracts exhibits a variety of effects viz., sedation, hypertension, brodyeardia, myosis, ptosis and tremors which are typical of reserpine (Mulliken and Crofton, 2008; Selvam, 2012). Pharmacological studies demonstrate that Rauwolfia possesses cardiovascular (Anitha and Kumari, 2006), antihypertensive (Von Poser *et al*., 1990), antiarrhythmic (Kirillova *et al*., 2001), antiinflammatory (Rao *et al*., 2012), antipyretic (Amole and Onabanjo, 1999), antidiabetic (Campbell *et al*., 2006), anticancer (Bemis *et al*., 2006), hypoglycaemic and hypolipidemic (Qureshi *et al*., 2009), hepatoprotective (Gupta *et al*., 2006), sedative (Weerakoon *et al*., 1998), antihistaminase (Sachdev *et al*., 1961), mosquito larvicidal (Das and Chandra, 2012), antidiarrhoeal (Ezeigbo *et al*., 2012) and antimicrobial activities (Ahmed *et al*., 2002).

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Hence, the aim of the present study was to investigate the antimicrobial activity of *R. serpentina* roots and to investigate which extracts might be a possible source for new antimicrobial substances against human pathogenic microorganisms.

Materials and Methods

Root collection

The roots of *R. serpentina* were collected from Kambalakonda forest area, Visakhapatnam, Andhra Pradesh, India. The specimen was authenticated by Prof. Vatsavaya S. Raju, Plant Systematics Lab, Kakatiya University, Warangal and voucher specimen (L. Mutyala Naidu – 2501) was deposited in the Herbarium of Botany Department (BDH), Andhra University, Visakhapatnam.

Root extract

The dugout roots were cleaned and dried in shade (25-28 °C) for a month. The dried roots were grounded using a mechanical grinder. Sequential extraction of it was carried out using the solvents hexane, chloroform and finally methanol (Aniel Kumar *et al*., 2014). The filtrates were concentrated by removing the solvents under reduced pressure at 40 °C using a rotary evaporator. The concentrated crude extracts were labelled and stored at 4 °C.

Simultaneously, the aqueous extract of the root was prepared by adding boiled water to the powder in a beaker on water bath with occasional stirring for 4 hours. The aqueous extract was then filtered and reduced under pressure. At the time of testing known quantity of crude extracts (50 mg/ml, 75 mg/ml and 100 mg/ml) were dissolved in DMSO (Dimethyl sulfoxide).

Microbial strains

The following strains were collected from microbial type culture and collection (MTCC), Chandigarh, India. Seven bacterial strains namely *Bacillus subtilis* MTCC B2274, *Enterococcus faecalis* MTCC B3159, *Escherichia coli* MTCC B1560, *Klebsiella pneumoniae* MTCC B4030, *Micrococcus luteus* MTCC B1538, *Pseudomonas aeruginosa* MTCC B2297, *Proteus vulgaris* MTCC B7299, *Staphylococcus aureus* MTCC B3160, *Streptococcus pneumoniae* MTCC B2672, and three fungal strains such as *Aspergillus niger* MTCC F4325, *Candida albicans* MTCC F7315 and *Saccharomyces cerevisiae* MTCC F2567, were tested.

Antimicrobial screening

The lyophilized culture was sub-cultured and concentration of working stock culture was assessed as 10^{-6} CFU/ml. Specified quantity of nutrient agar was prepared and plated in aseptic conditions. The agar well diffusion technique was followed for antimicrobial susceptibility test for crude extracts and DMSO (negative control), whereas agar disc diffusion method was followed for antimicrobial susceptibility test for standard antibiotic disc. The extracts were dissolved in DMSO to get the known concentrations of 50 mg/ml, 75 mg/ml and 100 mg/ml. The activity was compared with tetracycline disc (10 µg/disc). After 24 h of incubation at 37 °C the zone of inhibition was measured by using an antibiotic zone reader scale (HiAntibiotic ZoneScale-c) and tabulated.

For the antifungal activity, the same method as for bacteria was adopted of nutrient agar, whereas saboraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for the *C. albicans*, *S. cerevisiae* and three days for *A. niger* (Aniel Kumar *et al*., 2010). About 500 µg of fluconazole was dissolved in 1 ml of sterile deionized water, about 10 µl of 0.5 mg/ml fluconazole (equivalent to 5 µg dose).

The extracts that exhibited inhibition zones were subjected to minimum inhibition concentration (MIC) assay by using serial two-fold dilution method (Aniel Kumar and Naidu, 2011). A quantity of 0.6 g of each extract was dissolved in 300 ml sterile nutrient broth which yields initial concentration of 2,000 µg/ml. Subsequently, two-fold serial dilution was made from the stock to obtain 1,000; 500; 250; 125; 62.5; 31.2 µg/ml concentrations. One ml of standardized inoculums of each test organism was introduced into each extract nutrient broth mixture and then incubated at 37 °C. The lowest concentration inhibiting growth was regarded as the MIC of the extracts. For the fungi, the inoculated medium was incubated at 25 °C for two (*C. albicans*, *S. cerevisiae*) to three (*A. niger*) days.

The tubes that showed no visible growth were streaked on fresh nutrient agar plates (for bacteria) and on Sabouraud plates (for fungi). The plates were incubated at 37 °C for 24 hours and examined for growth. Minimum inhibitory concentration was regarded as the lowest concentration of the extracts that prevent the growth of the bacterial or fungal colony on solid medium.

Statistical analysis

Each experimental data from triplicates were subjected to one way ANOVA using Minitab version 15. A significant level of $p < 0.01$ was used for all statistical analyses.

Results and Discussion

The results are summarized in Table 1, demonstrating that the methanol extracts exhibited high antimicrobial activity, while chloroform and aqueous extracts showed moderate activity and hexane extract shown low activity. Methanol extract exhibited the highest inhibition zone value against *S. aureus,* followed by *B. subtilis* at the concentration of 100 mg/ml when compared with the rest. The observed zone of inhibition was 26 ± 0.2 mm against *S. aureus* and 24 ± 0.9 mm for *B. subtilis*. Chloroform and hexane extracts were shown within the high inhibition values against *E. faecalis* whereas hexane extracts was efficient against *S. pnemoniae*. Among the bacterial species, *M. luteus* and *S. aureus* were highly sensitive to all tested extracts, whereas *K. pneumoniae* was found to be highly resistant. Moreover, hexane extract did not exhibit antimicrobial action against *B. subtilis, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, A. niger* and *C. albicans*.

The negative control of DMSO had no effect on microbial growth. The absence of inhibition zones confirmed that DMSO could not act as antimicrobial agent. The positive controls have varied the antimicrobial activity

Table 1. The antimicrobial activity of *Rauwolfia serpentina* against various pathogens

Organisms	Zone of inhibition (mm)[a]												S	D
	Hexane extract			Chloroform extract			Methanol extract			Aqueous extract				
	50	75	100	50	75	100	50	75	100	50	75	100		
B. subtilis	–	–	–	12±0.1	14±0.9	17±0.4	19±0.1	19±0.9	24±0.9	–	–	–	18^{T}	–
E. faecalis	10±0.2	10±0.4	16±0.4	14±0.7	20±0.5	22±0.2	17±0.5	19±0.9	20±0.5	–	–	–	21^{T}	–
M. luteus	10±0.1	13±0.1	13±0.9	13±0.2	14±0.1	16±0.5	10±0.4	12±0.1	16±0.4	9±0.1	11±0.5	13±0.1	24^{T}	–
S. aureus	12±0.9	12±0.3	14±0.9	14±0.4	15±0.1	15±0.2	24±0.4	24±0.7	26±0.2	15±0.9	16±0.9	20±0.9	24^{T}	–
S. pneumoniae	–	13±0.4	13±0.2	14±0.9	14±0.7	15±0.9	14±0.2	17±0.4	20±0.5	20±0.9	20±0.4	22±0.9	22^{T}	–
E. coli	–	–	10±0.9	10±0.6	12±0.4	14±0.4	10±0.1	12±0.5	14±0.2	12±0.4	14±0.1	16±0.2	22^{T}	–
K. pneumoniae	–	–	–	–	–	–	11±0.2	13±0.1	13±0.1	–	–	–	24^{T}	–
P. aeruginosa	–	–	–	–	–	–	11±0.5	12±0.6	14±0.2	14±0.7	16±0.6	17±0.1	25^{T}	–
P. vulgaris	–	–	–	10±0.2	12±0.2	14±0.2	12±0.1	14±0.5	15±0.1	–	–	–	22^{T}	–
A. niger	–	–	–	14±0.4	16±0.2	18±0.4	10±0.5	12±0.2	14±0.5	–	–	–	18^{F}	–
C. albicans	–	–	–	–	10±0.4	12±0.5	10±0.2	12±0.5	14±0.2	–	–	–	23^{F}	–
S. cerevisiae	8±0.7	10±0.1	12±0.1	13±0.9	15±0.2	17±0.2	13±0.1	15±0.9	17±0.2	–	–	–	20^{F}	–

[a]: Each value is the mean of three replicates with standard deviation; *P* value is < 0.01 extremely significant when compared with standard, S: Standard antibiotics, [T]: Tetracycline; [F]: Fluconazole, D: DMSO, –: No activity

Table 2. MIC values of *Rauwolfia serpentina* root extracts against bacteria and fungi

Organisms	Hexane extract	$ChCl_3$ extract	Methanol extract	Aqueous extract
B. subtilis	>1,000	>1,000	62.5	>1,000
E. faecalis	500	>1,000	500	>1,000
M. luteus	1,000	125	500	1,000
S. aureus	1,000	62.5	31.2	250
S. pneumoniae	1,000	1,000	1,000	1,000
E. coli	250	>1,000	1,000	>1,000
K. pneumoniae	>1,000	1,000	>1,000	1,000
P. aeruginosa	>1,000	>1,000	500	500
P. vulgaris	>1,000	1,000	500	1,000
A. niger	>1,000	>1,000	125	>1,000
C. albicans	125	1,000	500	500
S. cerevisiae	>1,000	>1,000	1,000	>1,000

Table 3. Phytochemical constituents of *Rauwolfia serpentine* roots

Phytochemicals	Hexane extract	$ChCl_3$ extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+	+
Aminoacids	-	-	-	-
Anthraquinone	-	-	-	-
Carbohydrates	+	+	+	+
Cardiac glycosides	-	+	+	-
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Phenols	-	+	+	-
Saponins	-	+	+	-
Steroids	+	+	+	+
Tanins	-	-	+	+
Terpenoids	+	+	+	-
Volatile compounds	-	-	-	-

with inhibition zones ranging between 18-25 mm. Methanol extract exhibited a broad spectrum of antimicrobial activity against *B. subtilis* and *S. aureus*, while chloroform extract against *E. faecalis* and *A. niger*; and aqueous extract against *S. pneumoniae*.

The MIC of the *R. serpentina* root extracts are presented in Table 2. The MIC, MBC and MFC values for all extracts were tested for all inhibition zone values. From the MIC tubes, inoculations were made on the agar plates to test the bactericidal and fungicidal concentrations. The results indicated that the strongest MIC was against *S. aureus* for methanol and chloroform extracts, with 31.2 µg/ml to 62.5 µg/ml, respectively. Earlier, it was reported that the crude extracts are generally a mixture of active and non-active compounds and MIC of less than 100 µg/ml, demonstrating good antimicrobial activity (Webster *et al*., 2008). In the present study hexane, chloroform, methanol and aqueous extracts had shown lower MIC values, of 125 µg/ml, demonstrating the strong antimicrobial activity of *R. serpentina* roots.

In the present work, it was estimated that both solvent and aqueous extracts contain alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids (Table 3) and the antimicrobial activity may be a result of individual or a combination of these bioactive compounds. It can be a source of newer useful drugs and of great pharmacological importance. Methanol extract of *R. serpentina* roots were found to possess the maximum inhibitory effects against tested bacteria and fugal species when compared to aqueous, chloroform and hexane extracts. This may be due to the presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins etc., which warrants *R. serpentina* could be subjected to extensive experimental studies in the future, so that to treat certain diseases caused by pathogenic bacteria and fungi.

In the present study, it was observed that *R. serpentina* roots extracts have been more active against Gram-positive bacteria than against Gram-negative bacteria. It was reported that Gram-positive bacteria should be more susceptible, since they have only an outer peptidoglycan layer which is not an effective barrier. The Gram-negative bacteria have an outer phospholipidic membrane that make the cell wall impermeable to liphophilic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. Many results confirmed these observations, thus some plant extracts were found to be more active against Gram-positive bacteria than Gram-negative ones (Kelamanson *et al*., 2000; Masika and Afolayane, 2000).

Conclusions

The present study suggested that methanol extract of *Rauwolfia serpentina* roots would be helpful in treating diseases caused by human pathogenic bacteria and fungi. In particular, it can be recommened that the *R. serpentina* roots to be used for the control of infectious diseases caused by multidrug resistant *Staphylococcus aureus*. It might turn out to be a good candidate in the search for effective and efficient antimicrobial agents.

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