BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 348-352
ISSN: 1991-0088

Phytochemical investigation and *in vitro* antimicrobial activity of *Richardia scabra*

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Article Info

Received: 22 August 2015
Accepted: 15 September 2015
Available Online: 20 March 2016

DOI: 10.3329/bjp.v11i2.24666

Cite this article:
Poonkodi K, Ravi S. Phytochemical investigation and *in vitro* antimicrobial activity of *Richardia scabra*. Bangladesh J Pharmacol. 2016; 11: 348 -52.

Abstract

The present study was aimed to evaluate the phytochemical screening and antimicrobial activity of the petroleum ether and methanol extracts from the mature leaves of *Richardia scabra* from India. Disc diffusion method was used to determine the zone inhibition of the tested samples for antibacterial and agar plug method was used to determine the antifungal activity, while the microtube-dilution technique was used to determine the minimum inhibitory concentration. Both extracts showed significant antibacterial and antifungal activities when tested against 10 bacterial and four fungal strains. The minimum inhibitory concentrations of the methanol extract of *R. scabra* ranged between 12.5–100 µg/mL for bacterial strains. Alkaloids, steroids, flavonoids, fatty acids, terpenoids and simple sugar were detected as phyto-constituents of extracts. To the best of our knowledge, this is the first report against antimicrobial activity of common weed species *R. scabra* found in India.

Introduction

Indigenous herbal remedies are widely used against many infectious diseases from long back. The plant and plant products are known to posses excellent antimicrobial properties and play a significant role in preventing infectious diseases (Vineet et al., 2010; Rios and Recio, 2005). In recent years, more number of plant based antibiotics are emerging, but the resistance developed by bacteria against antibiotics when used for long run leads to develop new drugs with affordable cost and no adverse effects.

Therefore, it is of great interest to carryout antimicrobial potential of unexplored plant *Richardia scabra* Linn belongs to the family *Rubiaceae*. Most of the members of the plants are mainly distributed in the tropical and subtropical regions with a few exceptions in temperate regions. Several plants in this family contain alkaloids as the main source such as coffee and quinine. Plants like *Spermacoce hispida* (Kaviarasan et al., 2008), *Randia dumetorium* Lamk, *Anthocephalus cadamba* Linn (Chandrashekar andPrasanna, 2009), *Ixora brachiata*, *Mitracarpus villosus* (Irobi and Daramola, 1994) and *Borreria hispida* (Kottai Muthu et al., 2010) have anti-oxidant, anti-inflammatory, antibacterial, anti-diabetic and antifungal properties.

So far, a few species of genus *Richardia* are evaluated for their phytochemical and pharmacological studies. In this view our tested plant *R. scabra* commonly known as Florida pusley or rough Mexican clover is native to North America and is considered as a weed species. It is also found in tea and maize fields in south India. There are very little information in literature about medicinal properties of this plant. However, oral reports from local herbal medical practitioners indicate that the extract of this plant is used to cure skin diseases, wound healing (Ayyanar and Ignacimuthu, 2005) posses diaphoretic properties (Senthil Kumar et al., 2006),

urinary tract infection, tonic, asthma, emetic, dermatitis and stomachache. It contains emetine alkaloid and is used as substitute for ipecac as one of the emetine rich plant (Pullaiah, 2006). Literature survey showed that so far only one report for isolation of lipids. To the best of our knowledge, no information is available on the antimicrobial nature of this plant.

Materials and Methods

Plant collection and extraction

The plant was collected locally in Pollachi, Tamil nadu, South India. The species for this study was identified as *R. scabra* by the Botanical survey of Coimbatore, Tamilnadu. The plant was shade dried and coarse powdered plant material (1.5 kg) was subjected to exhaustive maceration with petroleum ether for 72 hours. This process was repeated until maximum extraction of the phytochemicals, then the extract was dried by rotary vacuum evaporator, the jelly like mass obtained was weighed and stored in desicator until analysis. The procedure was repeated for methanol extract.

Preliminary phytochemical screening

Petroleum ether and methanol extracts of *R. scabra* were subjected to qualitative chemical analysis by using standard procedure (Kayani et al., 2007) to identify the nature of phytochemical constituents present in it.

Collection and maintenance of test organisms

The organisms used were clinical isolates of *Staphylococcus aureus, Bacillus subtilis, Rhodospirillum, Salmonella paratypi, Kiebsiella pneumoniae, Bacillus lintus, Vibrio cholerae, Staphylococcus albus, Escherichia coli and Pseudomonas aureginosa* and four fungal strains *Candida albicans, Aspergillus fumigate, Dreschlera turcica* and *Fusarium verticillioides* (from the KMCH Hospital, Coimbatore). They were collected in McCartney bottles containing nutrient agar slants.

Antibacterial activity

The antibacterial activity of *R. scabra* was determined by disc diffusion method. The inoculums for the experiment were prepared in fresh nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture was adjusted by the addition of sterile saline or broth.

This method depends on the diffusion of the extract from a cavity through the petri dish, to an extent such that growth of the added microorganism was prevented entirely in circular area or zone around the cavity containing extract. The standardized inoculums was inoculated in the plates by dipping a sterile in the inoculums, removing excess of inoculums by pressing and rotating the swab firmly against the side of the culture tube before the plates are seeded above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60° after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed (Bauer et al., 1966; Murray et al., 1995).

Petri dishes were soaked overnight in *R. scabra* extract solution (100 µg/mL) and one quadrant for standard ciprofloxan (5 µg/disc) was placed with the help of sterile forceps. Then petri dishes were placed in the refrigerator at 4°C or at room temperature for 1 hour for diffusion. After the incubation period (at 37°C for 24 hours), diameter of zone of inhibition in mm obtained around the well was measured. The diameter obtained for the petroleum and methanol extracts were compared with that of the diameter produced by ciprofloxacin. The diameter of zone of inhibition was proportional to the antibacterial activity of the extract.

Minimum inhibitory concentrations

The minimal inhibitory concentration (MIC) was studied by broth dilution method. The methanol extract was dissolved in 10% dimethyl sulfoxide (DMSO) and was first dilute to the highest concentration (100 µg/mL) to be tested and than 2-fold dilutions of the test antimicrobial agent were made in a concentration range from 1.56-100 µg/mL in sterile test tubes containing Muller Hinton Broth. Overnight culture was grown at 37°C based on Kirby-Bauer procedure and diluted to Muller- Hinton Broth. The sterile tubes were labeled 1-8 and 8th tube was taken as control. 1 mL of Muller Hinton Broth was transferred to all tubes except 6th and 7th. These two tubes were added with 0.1 mL of broth. 1 mL of *R. scabra* extract was added to 1st tube and mixed well, from this tube 1 mL of solution was transferred to remaining tubes up to 6th tube. From this 6th tube 0.5 mL was taken and transferred to 7th tube, and all the tubes were added with 0.1 mL of culture and then incubated at 37°C for 24 hours. After incubation observe the turbidity by spectrophotometric method. The lowest concentration of test extracts and reference antibiotic which caused complete inhibition of growth of organism was taken as MIC.

Antifungal activity

The activity of the plant extracts on various fungal strains were assayed by agar plug method and spore germination inhibition assay. The fungicidal effect of the plant extracts can be assessed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the disc or the wells.

Potato dextrose agar medium was prepared and poured on to the petri plates. A fungal plug was placed in the center of the plate. Sterile discs immersed in the plant extracts were also placed in the plates. Flucanazole was

used as antifungal control. The plates were then incubated at room temperature for 3 days at 28°C for fungal pathogens. The zone of inhibition in diameter was observed and recorded in mm.

Results

The phytochemical investigation of both extracts of *R. scabra* revealed the presence of steroid, triterpenoid, phenol, flavonoid, tannins, fatty acid, alkaloid, furanoid and coumarin (Table I).

The petroleum ether extract exhibited moderate to feeble inhibition against all test bacteria with maximum against *S. aureus* and *S. paratypi* (16 mm) and minimum against *Rhodospirillum* (8.5 mm) (Table II). The methanol extract was able to inhibit all the organisms and showed the zone of inhibition in the range of 15-24 mm. It authenticates that the entire tested micro-organism were susceptible to both extracts and degree of susceptibility was in the decreasing order of *S. aureus, S. albus* > *K. pneumonia* > *S. paratypi* > *P. aureginosa* > *B. subtilis* >> *B. lintus* > *V. cholera* > *Rhodospirillum* > *E. coli.* The maximum antibacterial activity was shown in terms of zone of inhibition by *S. albus and S. aureus* (24 mm), but least sensitive micro-organisms were *E. coli* and *Rhodospirillum* (15.7 and 16.3 mm respectively).

Table I

Phytochemical investigation of *R. scabra*

Phytochemicals	Methanol extract	Petroleum ether extract
Alkaloids	+	+
Tannins	+	-
Flavonoids	+	-
Steriods	+	+
Terpenoids	+	-
Simple sugar	+	+
Furanoids	+	-
Fatty acid	-	+

+ indicates present, - indicates absent

The results of broth dilution test for MIC of methanol extract has been given in Table III. It was found that the MIC for methanol leave extract of the plant against *S. aureus, K. pneumoniae and S. albus* was 12.5 µg/mL, whereas for *S. paratypi, B. lintus, P. aureginosa and V. cholerae* were inhibited at 25 µg/mL. From this result the extracts of *R. scabra* showed potent antibacterial activity towards all the 10 investigated phytopathogenic bacteria. The highest antibacterial activity was shown towards *S. aureus* and *K. pneumoniae*. The results indicated that the methanol extract was more effective than the petroleum ether extract. This might be due to the fact that methanol extract had more antibacterial constituents. The extracts were active against both Gram positive and Gram negative bacteria.

Antifungal activity

The antifungal activity of petroleum ether extract of *R. scabra* exhibited moderate activity against *C. albicans* with zone of inhibition of 10 mm followed by *A. fumigate* 14 mm, *D. turcica* 12 mm and *F. verticillioides* 13 mm (Table IV). The methanol extract of *R. scabra* showed better antifungal activity against all tested micro-organisms with zone of inhibition of 14 mm for *C. albicans*, 21 mm for *A. fumigate*, 20 mm for *D. turcica* and 18 mm for *F. verticillioides*. The results are comparable with the standard flucanazole.

Table II

Antibacterial activity of *R. scabra*

S. No	Organisms	Zone of inhibition (mm)		
		Petroleum ether extract	Methanol extract	Ciprofloxacin (5 µg/disc)
1.	*S. aureus*	15	24	27
2.	*B. subtilis*	10	19	28
3.	*S. paratypi*	16	22.4	27
4.	*K. pneumoniae*	10	23	27
5.	*S. albus*	14	24	29
6.	*V. cholerae*	12	17	29
7.	*Rhodospirillum*	8.5	16.3	26
8.	*E. coli*	13	15.7	29
9.	*B. lintus*	9	18.6	27
10.	*P. aureginosa*	9	21	24

Table III

MIC of each bacterial strain

SL. No.	Organisms	MIC (µg/mL)
1	*S. aureus*	12.5
2	*B. subtilis*	50
3	*S. paratypi*	25
4	*K. pneumoniae*	12.5
5	*S. albus*	25
6	*V. cholerae*	25
7	*Rhodospirillum*	50
8	*E. coli*	50
9	*B. slintus*	25
10	*P. aureginosa*	12.5

Discussion

From the preliminary test, the R. *scabra* extract have many important secondary metabolites, and an attempt was made to isolate the active phytoconstituents, we isolated stigmasterol, β-sitosterol, quercetin, oleanolic acid and heraclenin from the plant very first time. The results are good agreement with R. *grandiflora* which revealed the presence of two phenolic compounds, *m*-methoxy-*p*-hydroxybenzoic acid, hydroxybenzoic acid, two steroids in the mixture (β-sitosterol and stigmasterol) and chlorophyll derivative (pheophytin *a*). The phytochemicals investigation performed with *R. brasiliensis* revealed it had different classes of metabolites like coumarins, flavonoids, triterpenes, sterols and phenolic acids (Pinto et al., 2008).

Many *Rubiaceae* plant species have potent antimicrobial nature (Parthasarathy et al., 2009; Giang et al., 2007; Toure et al., 2011; Irobi et al., 1994). The ethanol and hexane extracts of *R. brasiliensis* has potent antimicrobial and modulating action. So far, there is no report on isolation and biological activities of *R. scabra* plant for comparison. The present study indicates that the extracts of *R. scabra* displayed concentration-dependent antibacterial activity. The significant antibacterial activity of the active plant extract was comparable to ciprofloxacin (5 µg/disc) and also has sufficient antifungal activities. The results are comparable with flucanazole. This result also stands as a scientific support for the usage of this plant for treating skin disease in traditional medicine. It is due to the presence of steroids, flavonoids and terpenoids of this plant extracts.

Conclusion

Petroleum ether and methanol extracts of R. scabra leaves have great potential as antibacterial and antifungal potentials along with many important phytochemicals.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

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Table IV

Antifungal activity of *R. scabra*

SL. No.	Organism	Zone of inhibition (mm)		Standard (Flucanazole)
		Petroleum ether extract	Methanol extract	
1	*Candida albicans*	10	14	20.5
2	*Aspergillus fumigates*	14	21	22.2
3	*Dreschlera turcica*	12	20	23.4
4	*Fusarium verticillioides*	13	18	25

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