Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (2): 124-131, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.124.131

Antibacterial Activity of *Psoralea corylifolia* L. Seed and Aerial Parts with Various Extraction Methods

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ABSTRACT

Aim of the present study was to screen thirteen plants for their *in vitro* antibacterial potentiality. The antibacterial activity of aqueous and methanolic extracts of the plants was evaluated against 5 microorganisms by agar well diffusion method. The screening experiments showed that 92% of the plants were active against gram positive bacteria while only 54% of plants were active against Gram negative bacteria. Amongst the 13 plants screened, *Psoralea corylifolia* showed best antibacterial activity and hence this plant was selected for further studies. The seed and aerial parts of *Psoralea corylifolia* was extracted successively using a series of various organic solvents. The antibacterial activity of these extracts was done against 5 microorganisms by agar disc diffusion method. All the extracts of seed and aerial parts were active against *S. epidermidis* and *P. morganii* while none of the extracts were active against *A. fecalis*. Maximum antibacterial activity was shown by dioxan extract of the seed. The present findings suggest that the dioxan extract of seed of *P. corylifolia* can be used as a promising novel antibacterial agent in the near future.

Key words: Antibacterial activity, solvent extraction, aqueous extraction, successive extraction, *Psoralea corylifolia*, Gram negative bacteria

INTRODUCTION

Plants produce a diverse range of bioactive molecules making them a rich source of different type of medicines. Antimicrobial resistance is a problem in the majority of hospitals worldwide and treatment of infection due to resistant microorganism is often difficult because many strains are resistant to most available antimicrobial agents. Two hundred and fifty years ago there were few or no synthetic medicines. The 250,000-300,000 species of higher plants were the main sources of drug for the world population. Much attention have been focused on phytochemicals as potential sources of functional substances such as antioxidants (Esaki *et al*., 1999), antiplague substances (An *et al*., 1998), antimutagenicities (Karakaya and Kawas, 1999), enzyme inhibitors (Choi *et al*., 1997) and antimicrobial substances (Yim *et al*., 1999; Rojas *et al*., 2003; Duraipandiyan *et al*., 2006).

Microorganisms have developed resistant to many antibiotics and this created immense clinical problem in the treatment of infectious diseases (Davis, 1994; Ordonez *et al*., 2003). This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the

treatment of infectious disease. Methods to identify medicinal plant leads from tropical areas include random screening, taxonomic collecting (sampling by botanical family), or ethnobotanical collecting. It has been shown that ethnobotanically-derived compounds have greater activity than compounds derived from random screening and therefore a greater potential for product development (Balick, 1990). A systematic screening of traditional medicines may result the discovery of novel effective compounds. The need of the hour is to screen a number of medicinal plants for promising biological activity. A number of such studies have been done in various places of the world (Barbour *et al*., 2004; Nair *et al*., 2008; Ding *et al*., 2008; Tanna *et al*., 2009). The aim of the present study was to conduct a preliminary screening of a few plants for their potential antibacterial activity and to select the most potent among them for further studies.

MATERIALS AND METHODS

Collection: The medicinal plants were collected from the Anand Agriculture University and Pavagadh hills, Gujarat, in the month of July, 2004. The vernacular names, family and voucher number of various screened plants is given in Table 1. The plants were thoroughly washed, cleaned air dried and then crushed in a homogenizer to fine powder and stored in air tight bottles.

Crude solvent extraction: Crude solvent extract of the plants was prepared by taking 10 g of dried powder in 100 mL of methanol in a 150 mL conical flask and shaking the mixture on a rotary shaker for 24 h (Nair *et al*., 2005). The extracts were then filtered, centrifuged at 5000 g for 10 min and the supernatant was collected and air dried under reduced pressure to obtain the extractive compounds which were stored in airtight bottles at 4°C.

Crude aqueous extraction: Crude aqueous extraction was done as described earlier (Nair *et al*., 2005). Ten grams of dried (plant or plant part) material was taken in distilled water in 500 mL

Table 1: Ethnobotanical information of the plants screened for antibacterial activity

Plant name	Family	Vernacular name	Part used	Medicinal uses	Voucher No.
Curcuma amada Roxb.	Zingiberaceae	Jangali haldar	Leaf	Skin diseases, Hic cough	PSN718
Merremia turpethum (L.) Shah and Bhatt.	Convolulaceae	Nashotar	Leaf+stem	Fever, lucoderma, Jaundice.	PSN524
Psoralea corylifolia L.	Papilionaceae	Babchi	Leaf+stem	Psoriosis and leprosy	-
Hemidesmus indicus (L.) Schult.	Periplocaceae	Upalsari, Sariya, Kapoori-Madhuri	Leaf+stem	Urinary, dysentery	PSN465
Convolvulus microphyllus Sieb ex Spr.	Convolulaceae	Shankhavali	Leaf+stem	Brain tonic	PSN495
Indigofera tinctoria L.	Papilionaceae	Gali	Leaf+stem	Diuretic, breathing problems, skin diseases	PSN202
Asteracantha longifolia (L.) Nees.	Acanthaceae	Ankhro	Leaf+stem	Rheumatism, gonorrhea, genital urinary tract infections	PSN586
Hibiscus abelmoschus Linn.	Elatinaceae	Musk dana	Leaf+stem	NF	PSN40.1A
Morinda tomentosa Heyne ex Roth.	Rubiaceae	Aal	Intoto	NF	PSN356
Cissampelos pareira L.	Menispermaceae	Venivel	Intoto	Leprosy, cough, cold, indigestion.	PSN5
Hibiscus subderiffa Linn.	Elatinaceae	Khati Bhindi	Leaf+stem	Diuretic, sedative	-
Euphorbia dracunculoids Lam.	Euphorbiaceae	Ubhi dudheli	Leaf+stem	Remove warts	-
Alangium salvifolium (L.f.) Wang.	Alangiaceae	Ankol	Leaf	Dysentery	PSN346

NF: Not found

beaker and was slowly heated on low flame for 2 h. The decoction thus prepared was filtered through 8 layer of muslin cloth and supernatant was centrifuged at 5000 g for 5 min. The supernatant was collected. The residue was again taken in distilled water and this whole procedure at slow heating was repeated twice and all the supernatants were pooled together. The amount of distilled water used varied from plant to plant. The supernatant collected was air dried under reduced pressure to obtain the extractive compounds which were stored in airtight bottles at 4°C.

Successive extraction: The most potent plant was selected and then extracted successively using a series of solvents with increasing polarity (Wiart *et al*., 2004). The solvents used here for successive extraction were petroleum ether, 1, 4-dioxan, acetone, methanol and N, N-dimethylformamide (DMF).

Tested bacterial strains: The bacterial strains were obtained from National Chemical Laboratory (NCL), Pune. The microorganisms used were *Proteus morganii* NCIM2040, *Alcaligenes fecalis* ATCC8750, *Enterobacter aerogenes* ATCC13048, *Staphylococcus epidermidis* ATCC12228 and *Bacillus megaterium* ATCC9885.

Determination of antibacterial activity: A loop full of the strain was inoculated in 25 mL of nutrient broth in a conical flask and incubated at room temperature on a rotary shaker for 24 h to activate the test bacteria. The inoculum size was 1 X 10^8 cells. Mueller Hinton Agar No. 2 was used for the antibacterial susceptibility study. The bacterial assay was performed by agar disc diffusion method and agar well diffusion method.

The antibacterial activity of solvent extracts was done by agar well diffusion method (Perez *et al*., 1990; Parekh and Chanda, 2007). The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (200 µL) was inoculated into the media (inoculum size 10^8cells mL^{-1}) when the temperature reached 40-42°C. Care was taken to ensure proper homogenization. After the medium was solidified; a ditch was made in the plates with the help of a cup-borer (8.5 mm). The test compound (100 µL) (from stock solution of 2 mg mL) was introduced into the well and the plates were incubated overnight at 37°C. The experiment was performed under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition. The results shown in the table are Mean±SEM of the activity obtained in triplicates which is subtracted from the control.

Agar disc diffusion method (Bauer *et al*., 1966; Vaghasiya *et al*., 2007) was employed for the antibacterial assay of selected medicinal plant. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (200 µL) was inoculated into the media (inoculum size 10^8 cells mL^{-1}) when the temperature reached 40-42°C. The test compound (20 µL) (from stock solution of 3 mg mL^{-1}) was impregnated in to sterile discs (7 mm) (Hi-Media) and was the n allowed to dry. The disc was then introduced into medium with the bacteria. The plates were incubated overnight at 37°C. The experiment was performed under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition.

RESULTS AND DISCUSSION

The extractive value and the percentage yield obtained for different plants screened is shown in Table 2, the extractive yield obtained for all the plants screened with distilled water ranged from 16-57% while for methanol it ranged from 4-16%. The maximum extractive yield obtained with

Table 2: Extractive value and percentage yield of the plants screened

Name of the plants	Raw material (g)	Extraction value		(%) Yield (w/w)	
		Aq (g)	Me (g)	Aq	Me
Curcuma amada	5	1.36	0.56	27.20	11.22
Merremia turpethum	5	2.23	0.47	44.60	09.44
Psoralea corylifolia	5	2.69	0.62	57.30	12.34
Hemidesmus indicus	5	0.83	0.84	16.52	16.82
Convolvulus microphyllus	5	1.27	0.45	25.46	9.06
Indigofera tinctoria	5	1.27	0.41	25.34	8.16
Asteracantha longifolia	5	1.66	0.48	33.20	9.58
Hibiscus abelmoschus	5	1.95	0.37	39.02	7.44
Morinda tomentosa	5	1.11	0.72	22.40	14.32
Cissampelos pareira	5	1.06	0.66	21.16	13.14
Hibiscus subderiffa	10	3.35	0.46	33.52	09.24
Euphorbia dracunculoids	10	3.14	0.65	31.40	13.04
Alangium salvifolium	5	1.45	0.25	28.90	4.72

Aq: Aqueous extract, Me: Methanol extract

distilled water was from *Psoralea corylifolia* (57.3%) and minimum yield obtained was from *Hemidesmus indicus* (16.52%). The maximum extractive yield obtained with methanol was from *Hemidesmus indicus* (16.82%) and minimum yield obtained was from *Alangium salvifolium* (4.72%). It appears from these results that polar compounds are more than non polar compounds. Similar results are reported earlier (Yang *et al*., 2007; Baravalia *et al*., 2009).

Thirteen medicinally important plants were screened for antibacterial activity against 5 medically important bacterial strains. The detailed results of the antibacterial study are shown in Table 3, amongst the 13 plants screened for antibacterial activity 92% plants showed activity against *E. aerogenes*, while none of the plants showed activity against *A. fecalis*. The second susceptible bacterium was *B. megaterium* which was susceptible to 54% of the plants screened. Amongst the 13 plants screened the methanolic extract of *P. corylifolia* was active against *S. epidermidis, P. morganii, B. megaterium* and *E. aerogenes* while its aqueous extract was active only against *B. megaterium* and *E. aerogenes*. The methanolic extract of *H. indicus* was active against *P. morganii* and *E. aerogenes*, while aqueous extract was active only against *E. aerogenes*. The methanolic extract of *A. longifolia* was active against *S. epidermidis* and *E. aerogenes*, while its aqueous extract was active against *B. megaterium* and *E. aerogenes*. The screening showed that 92% of plants screened were active against Gram negative bacteria (*E. aerogenes*) and 54% of plants screened were active against Gram positive bacteria (*B. megaterium).* Amongst the 13 plants screened *P. corylifolia* was most potent and showed activity against 4 of the 5 bacterial strains, so this plant was selected for further studies.

From the preliminary antibacterial screening of the 13 plants, *Psoralea corylifolia* was selected for detailed study. Seeds (SE) and leaf plus stem (taken together as aerial parts LSE) were separately extracted with petroleum ether, 1, 4-dioxan, acetone, methanol and DMF. The selection of solvents were done on the basis of their polarity, petroleum ether was used for defatting before going for other successive extraction. The extractive values and percentage yield of these parts extracted in different solvents is shown in Table 4. The percentage yield was more in seed as compared to aerial extract. Amongst the five solvents used, percentage yield was maximum in methanol, in both seed and aerial extracts.

Table 3: Antibacterial activity of medicinal plants screened

Plant	Solvent	SE	PM	BM	EA	AF
C. amada	AQ	-	-	-	9.17±0.44	-
	ME	-	-	-	11.00±0.58	-
M. turpethum	AQ	-	-	-	9.83±0.73	-
	ME	-	-	-	11.66±0.33	-
P. corylifolia	AQ	-	-	17±0.58	9.83±0.72	-
	ME	14±0.58	12±0.58	10±0.58	9.17±0.44	-
H. indicus	AQ	-	-	-	9.16±0.44	-
	ME	-	12±0.58	-	9.83±0.73	-
C. microphyllus	AQ	-	-	-	9.83±0.72	-
	ME	-	-	-	9.83±0.73	-
I. tinctoria	AQ	-	-	-	11.17±1.37	-
	ME	-	-	-	9.83±0.73	-
A. longifolia	AQ	-	-	10±0.58	12.0 ±0.58	-
	ME	9.83±0.72	-	-	10.00±0.58	-
H. abelmoschus	AQ	-	-	10±0.58	12.00±0.58	-
	ME	-	-	11±0.58	10.00±0.58	-
M. tomentosa	AQ	-	-	9.17±0.44	11.00±0.58	-
	ME	-	-	11±0.29.	9.00±0.29	-
C. pareira	AQ	-	-	-	9.17±0.44	-
	ME	-	-	-	-	-
H. subderiffa	AQ	-	-	10.67±0.33	9.17±0.44	-
	ME	-	-	-	11.00±0.58	-
E. dracunculoids	AQ	-	-	9.83±0.72	9.17±0.44	-
	ME	-	-	9.75±0.14	11.50±0.29	-
A. salvifolium	AQ	-	-	10±0.58	-	-
	ME	-	-	9.75±0.14	-	-

Mean±SEM, n = 3, Inhibition zone includes well diameter 8.5 mm, SE: *Staphylococcus epidermidis*, PM: *Proteus morganii*, BM: *Bacillus megaterium*, EA: *Enterobacter aerogenes*, AF: *Alcaligenes fecalis*, AQ: Aqueous extract, ME: Methanol extract, -: No activity

Table 4: Extractive value and percentage yield of *Psoralea corilyfolia* aerial part and seed

Solvent	Raw material (5 g)	Extractive value	% yield (w/w)
Pe	Aerial part	0.094	1.88
	Seed	0.372	7.44
Di	Aerial part	0.167	3.34
	Seed	0.238	4.76
Ac	Aerial part	0.044	0.88
	Seed	0.148	2.96
Me	Aerial part	0.176	3.52
	Seed	0.480	9.60
DMF	Aerial part	0.055	1.10
	Seed	0.086	1.72

Pe: Petroleum ether, Di: 1-4 Dioxan, Ac: Acetone, Me: Methanol, DMF: N, N- dimethylformamide

None of the extracts were active against *A. fecalis*, while all the extracts were active against *S. epidermidis* and *P. morganii* (Table 5); the DMF extract of LSE was inactive against *B. megaterium*, while all the other extracts of LSE and SE were active against *B. megaterium*. In LSE acetone, methanol and DMF extracts; and in SE methanol extract was inactive against *E. aerogenes*, while all other extracts of both LSE and SE were active against *E. aerogenes*.

Table 5: Antibacterial activity of *Psoralea corylifolia* extracts (seed and aerial part)

Extract	SE	PM	BM	EA	AF
Pe (LSE)	7.66±0.33	8.00±0.57	8.00±0.57	8.33±0.33	-
Pe (SE)	9.33±0.33	10.33±0.33	12.00±1.15	8.66±0.33	-
Di (LSE)	10.66±0.33	8.00±0.57	8.33±0.33	9.33±0.33	-
Di (SE)	11.33±0.33	12.66±0.33	14.00±1.15	11.33±0.33	-
Ac (LSE)	8.66±0.33	8.66±0.88	10.66±0.66	-	-
Ac (SE)	10.33±0.33	9.66±0.33	9.00±0.57	9.33±0.33	-
Me (LSE)	8.0±0.57	8.00±0.57	7.66±0.33	-	-
Me (SE)	8.0±0.57	8.00±0.57	8.33±0.33	-	-
DMF (LSE)	8.66±0.88	7.66±0.33	-	-	-
DMF (SE)	9.33±0.33	8.66±0.66	8.33±0.33	8.66±0.33	-

Mean±SEM, n = 3, Inhibition zone includes disc diameter 7 mm, LSE: aerial extract, SE: Seed extract, Pe: Petroleum ether, Di: 1-4 Dioxan, Ac: Acetone, Me: Methanol, DMF: N, N-dimethylformamide, -: No activity. SE: *Staphylococcus epidermidis*, PM: *Proteus morganii*, BM: *Bacillus megaterium*, EA: *Enterobacter aerogenes*, AF: *Alcaligenes fecalis*

Maximum activity against all the tested bacterial strains was shown by dioxan extract of seed. In general the activity exhibited by the SE extracts were more than the LSE extracts.

The screening showed that 92% of plants screened were active against Gram negative bacteria and 54% of plants screened were active against Gram positive bacteria. Similar results are reported earlier also (Parekh and Chanda, 2007; Enwuru *et al*., 2008; Shahwar and Muhammad, 2009). This is in contrast to the general belief that Gram positive bacteria are more susceptible to plant extracts (Yaghoubi *et al*., 2007; Kiran *et al*., 2008; Chanda and Baravalia, 2010). This kind of screening work is very much important for the selection of appropriate plant to isolate promising antibacterial agent. Amongst the 13 plants screened *P. corylifolia* was most potent and showed activity against 4 of the 5 bacterial strains studied. Different parts (aerial and seed) of *P. corylifolia* were extracted with different solvents (petroleum ether, dioxan, acetone, methanol and DMF) and amongst them; the extracts of seed (SE) showed more promising activity than the extracts of leaf plus stem (LSE). From this it can be concluded that seeds should be further studied to identify the active principle which shows the antibacterial property. Amongst the five extracts of seed studied, the 1,4-dioxan extract showed maximum activity. This extract should further be screened against fungal strains to check its antifungal activity.

The seed extract of dioxan of *P. corylifolia* can be used as a promising novel antibacterial agent in the coming years. It should be studied further to elucidate and determine structural identification of the active principle.

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