SHORT NOTES

ANTIMICROBIAL INVESTIGATION OF *AMMANIA BACCIFERA* LINN. AGAINST SOME URINARY AND GASTRO-INTESTINAL TRACT INFECTION CAUSING PATHOGENS

ABSTRACT

The plant *Ammania baccifera* Linn., commonly known as Jangli mendi, belonging to the family *Lythraceae* was investigated for its antimicrobial activity against some selected urinary tract (UT) and gastrointestinal tract (GIT) infection causing pathogens and human pathogenic fungi. The ethanolic extract of the whole plant as well as its leaves, stems and roots were screened to evaluate the antibiogram pattern followed by High Performance Thin Layer Chromatography (HPTLC) study. The extracts were then screened for their antibacterial activity against some UTI and GIT infection causing pathogens viz. *Staphylococcus aureus* MTCC*1430, *Enterococcus faecalis* MTCC 2729, *Escherichia coli* MTCC 118, *Pseudomonas aeruginosa* MTCC 1035, *Klebsiella pneumoniae* MTCC 109, *Proteus mirabilis* MTCC 743, *Salmonella typhi, Vibrio cholerae* and antifungal activity against some human pathogenic fungi viz. *Aspergillus niger* MTCC 1344, *Candida albicans* MTCC 3017, *Candida tropicalis, Candida krusei* by disc diffusion assay method. The minimum inhibitory concentration (MIC) was evaluated by two fold serial dilution assay method. The HPTLC study of the ethanolic extract of whole plant revealed the presence of eight number of components in the chromatogram developed at 254 nm. The ethanolic extract of. whole plant of A. baccifera showed highest antibacterial efficiency followed by leaf, stem and root extracts respectively. However, ethanolic extracts of whole plant and leaves exhibited moderate antifungal potency followed by stem and root extracts, respectively. The results of MIC indicated that the whole plant extract inhibited *P. mirabilis, K. pneumoniae, S. typhi and A. niger* at a concentration of 62.5 µg/ml thus exhibited broad spectrum of inhibition.

Keywords: *Ammania baccifera Linn.,* HPTLC, Antimicrobial activity, Disc diffusion assay, MIC.

INTRODUCTION

Ammania baccifera Linn. belonging to the family Lythraceae, is a branching herb distributed in rice fields and marshy localities throughout India. It is a annual herbs found growing in the marshy places in the tropics and temperate regions of the world.[1] The plant is popularly known as *Jangli mendi* and has many enthomedicinal uses. The plant is reported to have antisteroidogenic [2], analgesic[3], antiurolithiatic[4], antipyretic and diuretic[5] properties. It is also reported to be used in skin diseases, appetizer, laxative, stomachic, as antityphoid and antitubercular agents.[6] The plant is reported to contain hentriacontane, dotriacontanol, β-sitostrol- β-D-glucoside, betulinic acid, lupeol, ellagic acid and vitamin C. [7] The present study is intended to potency of the plant against some selected urinary tract (UT) and gastrointestinal tract (GIT) infection causing bacteria and fungal pathogens and compare with potent standard antibacterial and fungal agents[8,9].

MATERIALS AND METHODS

Collection and processing of the plant material

The plant was collected from the rice field and marshy localities of Kanchipuram district, Tamilnadu, India. It was authenticated in the Plant Anatomy Research Centre (PARC), Chennai and the voucher specimen (PARC/2007/88) was deposited in the herbarium. Then the plant was collected in bulk and washed with tap water to remove the soil and dirt particle. The whole plant, as well as its leaves, stems and roots were separately shade dried and powdered with a mechanical grinder. The powder was passed through sieve number 40 and stored in an airtight container for further use.

Table I: *In vitro* Antibacterial activity of *Ammania baccifera* extracts against UT and GIT pathogens by Disc diffusion assay method

Organisms	Conc. (µg/ disc)	Zone of Inhibition (in mm)				Standard
		Ew	El	Er	Eb	
1	a	18.2±0.71	17.3±0.38	10.1±0.13	--	(CF)
	b	12.7±0.75	11.8±0.17	--	--	26.4 ± 0.45
2	a	22.7±0.47	18.1±0.27	15.7±0.53	18.4±1	28.0 ± 0.15
	b	16.1±0.65	10.4±0.31	12.1±0.37	13.0±0.08	(Cf)
3	a	19.2±0.9	16.8±0.13	15.8±0.33	20.1±0.31	24.5 ± 0.47
	b	11.9±0.76	11.0±0.25	10.6±0.46	15.7±0.27	(Cf)
4	a	20.1±0.66	15.3±0.69	15.1±0.36	16.1±0.21	25.4 ± 0.35
	b	13.9±0.29	9.5±0.16	8.0±0.16	10.6±0.49	(Cf)
5	a	14.4±0.17	10.9±0.11	11.8±0.79	--	25.3 ± 0.35
	b	10.8±0.47	--	8.8±0.48	--	(Cf)
6	a	12.9±0.18	--	10.7±0.31	--	13.7± 0.7
	b	9.1±0.58	--	--	--	(Cf)
7	a	20.6±0.36	22.0±0.8	18.5±0.27	15.2±0.15	27.3± 0.69
	b	15.3±0.41	14.0±0.5	13.6±0.45	9.1±0.49	(Cf)
8	a	17.7±0.39	14.5±0.13	13.9±1.0	11.3±0.49	25.8±0.5
	b	12.4±0.48	10.1±0.5	10.3±0.31	9.4±0.61	(Cf)
9	a	21.9±0.52	23.7±0.38	19.8±0.73	20.0±0.57	23.1 ± 0.11
	b	15.1±0.71	17.2±0.42	13.4±0.46	12.8±0.48	(F)
10	a	17.5±0.32	11.8±0.19	13.6±0.08	12.5±0.35	13.2 ± 0.27
	b	12.4±0.57	--	9.4±0.65	--	(F)
11	a	24.6±0.63	21.3±0.68	18.7±0.17	22.7±0.62	10 ± 0.05
	b	19.1±0.39	16.1±0.46	16.8±0.25	18.3±0.07	(F)
12	a	26.0±0.54	27.3±0.09	22.9±0.05	24.4±0.69	22.2 ± 0.2
	b	18.8±0.17	19.0±0.4	20.1±0.27	20.2±0.76	(Am)

- Indicates no zone of inhibition. a and b indicates the concentrations of the extracts at 1000 and 125 µg/ disc respectively.

*1. Pseudomonas aeruginosa MTCC 1035; 2. Escherichia coli MTCC 118; 3. Proteus mirabilis MTCC 743; 4. Klebsiella pneumoniae MTCC 109; 5. Staphylococcus aureus MTCC*1430; 6. Enterococcus faecalis MTCC 2729; 7. Salmonella typhi; 8. Vibrio cholerae; 9. Candida albicans MTCC 3017; 10. Candida tropicalis; 11. Candida krusei and 12. Aspergillus niger MTCC 1344.* Ew, El, Er and Eb stands for ethanolic extract of whole plant, leaf, root and bark, respectively. CF, F and Am stand for ciprofloxacin 25 µg/disc, fluconazole 10 µg/disc and amphotericin B 100 units/disc, respectively.

*All the values are mean ± standard deviation of three determinations.

Table II: The MIC values of *Ammania baccifera* extracts against the microorganisms tested by Two Fold Serial Dilution Assay

Micro organisms	MIC (µg /ml)			
	Ew	El	Er	Eb
1	125	250	nf	nf
2	62.5	500	250	250
3	62.5	1000^{50}	500	250
4	125	500	500	500
5	1000^{50}	nf	1000^{50}	nf
6	1000^{50}	nf	nf	nf
7	62.5	125	125	500
8	125	500	250	500
9	250	500	500	500
10	500	1000^{50}	1000^{50}	1000^{50}
11	125	500	125	250
12	62.5	62.5	125	125

*1. Pseudomonas aeruginosa MTCC 1035; 2. Escherichia coli MTCC 118; 3. Proteus mirabilis MTCC 743; 4. Klebsiella pneumoniae MTCC 109; 5. Staphylococcus aureus MTCC*1430; 6. Enterococcus faecalis MTCC 2729; 7. Salmonella typhi; 8. Vibrio cholerae; 9. Candida albicans MTCC 3017; 10. Candida tropicalis; 11. Candida krusei and 12. Aspergillus niger MTCC 1344.* Ew, El, Er and Eb stands for ethanolic extract of whole plant, leaf, root and bark, respectively.

nf - not found with in the concentration range of extract 7.8-1000 µg/ml

^{50} – MIC at which approx. 50% of the organisms were inhibited.

Extraction

The air dried powered materials of the whole plant and its various parts were extracted separately with ethanol (95 %) in Soxhlet apparatus[10]. The solvent was completely removed under reduced pressure and stored in vacuum dessicator.

HPTLC study

The ethanolic extracts were subjected for the development of HPTLC finger printing patterns. High Performance Thin Layer Chromatography (HPTLC) study was carried out on silica gel 60 F_{254} aluminium plate prewashed with methanol and activated at 50ºC for 30 min. 5 µl of the sample solution was applied as 6 mm band by linomat 5 applicator, under inert gas flow, positioned 15 mm from bottom. The plate was developed with n-butanol: propanol: acetic acid: water, (2:1:1:1, v/v/v/v) in ascending mode upto 85 mm. The mobile phase was removed from the plate with stream of hot air and it was scanned by Camag TLC scanner 3 with winCATs software (version 1.3.4) in absorbance-reflectance scan mode.

Antimicrobial evaluation

The in-vitro screening was carried out using selected urinary tract (UT) and gastrointestinal tract (GIT) infection causing pathogens which include *Pseudomonas aeruginosa* 1035, *Escherichia coli* 118, *Proteus mirabilis* 743, *Klebsiella pneumoniae* 109, *Staphylococcus aureus**1430, *Enterococcus faecalis* 2729, *Candida albicans* 3017, and *Aspergillus niger* 1344 procured from Microbial Type Culture Collection centre (MTCC) & Gene Bank, Chandigarh, India. Other strains which include *Salmonella typhi, Vibrio cholerae, Candida tropicalis* and *Candida krusei* which were obtained from Post Graduate Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India. These organisms were identified by standard microbiological methods[11]. The antibacterial and antifungal screening of the extracts were carried out by determining the zone of inhibition using disc diffusion method[12-13]. The minimum inhibitory concentration (MIC) was studied by two fold serial dilution method[14].

RESULTS

From the HPTLC study, the mobile phase n-butanol: propanol: acetic acid: water (2:1:1:1, V/V/V/V) was found as an optimized solvent system which enable satisfactory separation of components

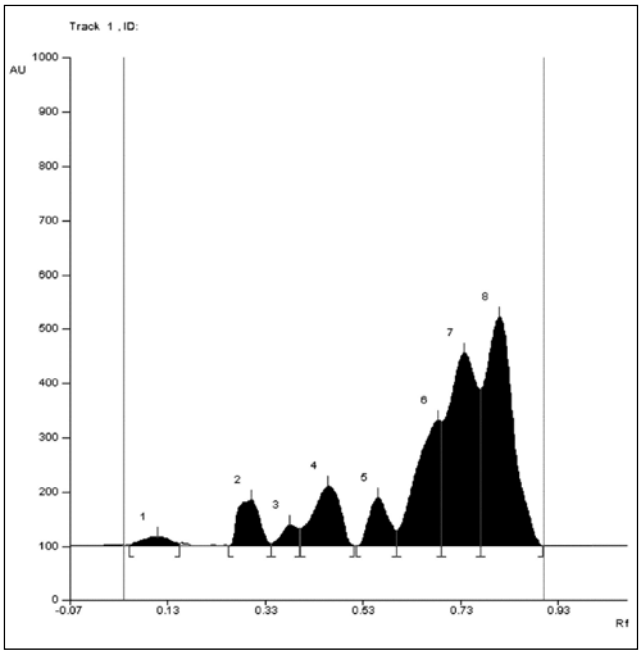

Fig. 1: HPTLC Chromatogram of *A. baccifera* at 254 nm

in the extract with well resolved peaks (Fig. 1). The wavelength 254 nm was found suitable to obtain symmetrical chromatogram with maximum number of peaks with Rf values: 0.81, 0.74, 0.68, 0.56, 0.46, 0.38, 0.30 and 0.11.

The efficacy of *A. baccifera* extracts to that of standard antibacterial (ciprofloxacin 25 µg/disc) and antifungal (fluconazole 10 µg/disc) in terms of zone of inhibition and their MIC values are tabulated Table I and II.

DISCUSSION

Ethanolic extract of whole plant of *A. baccifera* showed highest antibacterial efficiency followed by ethanolic extract of leaves, stems and roots, respectively. However, ethanolic extract of whole plant and leaves of *A. baccifera* exhibited moderate antifungal potency followed by stem and root extracts, respectively.

Ethanolic extract of whole plant showed highest and lowest (22.7±0.47 and 12.9±0.18 mm) zone of inhibition against *E. coli* and *E. faecalis*, respectively. The ethanolic extract of leaf of *A. baccifera* exhibited highest and lowest (22.0±0.8 and 10.9±0.11 mm) inhibition against *S. typhi* and *S. aureus*, respectively. *S. typhi* was also strongly inhibited (18.5±0.27 mm) whereas *E. coli* inhibited poorly (10.1±0.13 mm) by its root extract. Stem extract showed significant inhibition of *P. mirabilis* (20.1±0.31 mm) and least activity against *V. cholerae* (11.3±0.49 mm). *E. coli*, *S. aureus* and *E. faecalis* were not inhibited by stem extract. Leaf and root extract did not respond to *E. faecalis* growth.

Ethanolic extract of whole plant of *A. baccifera* showed highest zone of inhibition against *C. tropicalis* and *C. krusei* where as the leaf extract inhibited *C. albicans* and *A. niger* strongly as shown in Table I.

It was evident from the results that ethanolic extracts of *A. baccifera* under investigation when tested at higher concentrations (1000 µg/disc) exhibited significant zone of inhibition against *C. krusei* compared to standard antifungal agent fluconazole (10 µg/disc) and equivalent inhibitory zones against *A. niger* when compared to amphotericin B (100 units/disc).

The results of MIC tabulated in Table II indicated that the whole plant extract of A. baccifera inhibited *P. mirabilis, K. pneumoniae, S. typhi and A. niger* at a concentration of 62.5 µg/ml thus exhibited broad spectrum of inhibition.

The results of extensive antimicrobial study revealed that ethanolic extract of *A. baccifera* whole plant showed highest antimicrobial efficiency that prompted the authors to conduct future studies on the isolation, purification and characterization of the phytoconstituents responsible for the said activity.

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