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In vivo and *in vitro* antimicrobial efficacy of *Mimosa hamata*

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Mimosa hamata Willd. (*Mimosaceae*) has demonstrated pronounced bioefficacy against the selected bacteria (*Escherichia coli, Klebsiella aerogenes, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa*), fungi (*Aspergillus flavus, Fusarium moniliforme* and *Rhizoctonia bataticola*) and viruses (*Herpes simplex, Poliomyelitis* and *Vesicular stomatitis*).

Keywords: *Mimosa hamata*, cell cultures, antibacterial, antifungal, antiviral efficacies
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Introduction

Mimosa hamata Willd. (Vern. Jinjani; *Mimosaceae*), a much branched, armed shrub, is commonly distributed along the open sandy places, often gregarious and abundant throughout the arid zones of Rajasthan, Punjab, Central and South India. Its pounded seeds boiled in buffalo milk are taken as a tonic against weakness[1]. A crude alcoholic extract of its aerial parts shows antibacterial properties[2-5]. Leaves contain ethyl gallate and gallic acid. The roots contain saponins and show pharmacological activity[6-8]. The present work deals with the antimicrobial bioefficacy and the possible active principles against bacteria, fungi and virus of *M. hamata*.

Material and Methods

Plant Material

Whole plants of *M. hamata* were collected from the fields (Jaipur-Ajmer Road) near Ajmer, Rajasthan, during July-December and identified from the Herbarium, Department of Botany, University of Rajasthan, Jaipur (Herbarium Sheet No. RUBL 9565).

Tissue Cultures

The seeds, pre-treated in 10% H_2SO_4 for 12-16 hrs followed by several rinses with distilled water, were surface-sterilized with 0.1% $HgCl_2$ solution for 2-3 min, rinsed (3X) in sterile distilled water and aseptically inoculated in 100 ml flasks containing ~ 30 ml of MS[9] medium containing 10 mg/l indole-3-acetic acid and 0.4 mg/l kinetin. The flasks were kept in the aseptic culture conditions (26±1°C; 55% relative humidity and 300 lux light) continuously, where the seeds started sprouting within a period of 3-4 days of the inoculation. After 3-4 weeks, the seedlings were transferred to Revised MS medium[10] supplemented with 1% agar and 6 ppm of 2,4-dichlorophenoxyacetic acid for better induction of the callus tissue. The callus cultures were maintained for a period of over 18 months by periodic subculturing (6-8 weeks) onto fresh MS medium. Subsequently, the callus was harvested at the transfer age of 6-8 weeks, kept at 100°C (5 min) so as to inactivate the enzymatic activity followed by continuous drying at 60°C, till a constant weight was obtained and then used for further studies.

Preparation of Test Extracts

Dried whole plants (900 g) as also the callus (500 g) were powdered and exhaustively extracted with 95% ethanol (3X) separately. The extracts were concentrated, successively fractionated in different solvents and dried *in vacuo* (petroleum ether - 2.1 g, 1.2 g; benzene - 15.6 g, 7.3 g; chloroform - 26.2 g, 15.4 g and water - 14.5 g, 9.1 g, respectively). Later, each of these fractions was subjected for its bioefficacy against the selected test microbes and based on the results, potent active fraction(s) were further investigated for the characterization of their bioactive constituents (Table 1).

Test Microorganisms

Pure bacterial cultures, namely *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris,*

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Table 1—Antimicrobial efficacy of *M. hamata in vivo* and *in vitro*

Nature of extract		Bacteria					Fungi			Virus		
		EC	KA	PV	SA	PA	AF	FM	RB	HSV	Polio	VSV
WHOLE PLANT												
Ethanolic	IZ	12	12	13	13	9	26	14	28	1000-500: N, T	1000-100: N	1000-100 : N
	AI	0.55	0.55	0.62	0.56	0.43	1.18	0.61	1.27	100 : RF 10^2	500 : RF 10^4	100 : T
										50-10 : RF1	100-10 : RF1	500-100 : RF 10^4*
												50-10 : RF1
Petroleum ether	IZ	-	±	-	±	±	11	8	11	1000-100 : N	100-25 : N	100 - 50 : N
	AI						0.5	0.35	0.50	500 : T	100 : RF10	RF 10^5*
										100 : RF 10^2	50-1 : RF1	25 : RF 10^2
										50 : RF 10		10 : 1 : RF1
										10 : RF1		
Benzene	IZ	±	±	7	6	±	7	±	8	1000-50 : N	100 : N	100-50 : N
	AI			0.33	0.26		0.32		0.36	500-100 : T	100-1: RF1	100 : RF10
										50 : RF10		50-1 : RF1
										10 : RF1		
Chloroform	IZ	6	±	8	6	±	7	12	9	1000-500 : N,T	1000-500 : N,T	1000-500 : N,T
	AI	0.27		0.38	0.26		0.32	0.52	0.41	100 $RF10^3$	100-10: RF1	100 : $RF10^4$
										50 : $RF10^2$		50 : $RF10^3$
										10 : RF10		25 : $RF10^2$
												10 : RF1
Aqueous	IZ	14	8	13	9	6	17	23	18	1000-500 : T	100-1 : RF1	100-1 : RF1
	AI	0.64	0.36	0.62	0.39	0.29	0.77	1.00	0.82	100-10 : RF1		
CALLUS TISSUE												
Ethanolic	IZ	10	9	9	11	7	18	12	19	1000-50 : N,T	100-1 : RF1	100 : RF 10^5
	AI	0.45	0.41	0.42	0.49	0.33	0.81	0.52	0.86	100 : $RF10^2$		50:$RF10^2$
										50 : RF10		25: RF10
										10 : RF1		10-1: RF1
Petroleum ether	IZ	±	+	-	±	±	7	8	8	1000-500 : N	100-25 : N	500 :T
	AI						0.32	0.35	0.36	100 : RF10	100 : RF10	250:$RF10^4$*
										50-10 : RF1	50-10 : RF1	125: $RF10^3$
												50: RF10
												25 : RF1
Chloroform	IZ	6	8	±	8	9	8	9	10	1000-500 : N	1000-50 : N	1000-100 : N
	AI	0.27	0.36		0.35	0.43	0.36	0.39	0.45	1000 : T	100 : RF10	100:$RF10^3$
										500 : RF10	50-10 : RF1	50: RF10
										100-10 : RF1		10: RF10
Aqueous	IZ	12	7	14	7	8	11	14	16	1000-500 : T	100-1 : RF1	100-1 : RF1
	AI	0.64	0.32	0.66	0.30	0.38	0.5	0.61	0.73	100-10 : RF1		

EC, *Escherichia coli*; KA, *Klebsiella aerogenes*; PV, *Proteus vulgaris*; SA, *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; AF, *Aspergillus niger*, FM, *Fusarium moniliforme*; RB, *Rhizoctonia bataticola*; HSV, *Herpes simplex* Type 1; Polio, *Poliomyelitis* Type 1; VSV, *Vesicular stomatitis*,

IZ, Inhibition zone (in mm) including the diameter of disc (6 mm); AI, Activity index = Inhibition area of the test sample / Inhibition area of the standard (amikacin, 10 μg/disc - bacteria; mycostatin, 100 units/disc - fungi); -, Not measurable; ±, Trace activity;

N, Precipitation of substances in growth medium; NC, Crystal formation; T, Cell monolayer disrupted (complete lysis of cells, cell death); T/2, Cell monolayer affected (complete growth stop); T/4, Cell monolayer intact (cell growth and/or morphology affected); RF, Titer reduction factor = The ratio between virus titer of control and sample dilution;

* = Complete titer reduction: Control titer / RF = 1; + = Product concentration in μg/ml.

Staphylococcus aureus and *Pseudomonas aeruginosa*, obtained through the courtesy of the SMS Medical College, Jaipur, India, were maintained on nutrient broth medium while the test fungi (*Aspergillus flavus, Fusarium moniliforme* and *Rhizoctonia bataticola*) obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, were maintained on potato dextrose agar medium at 37°C for 24 h and 27°C for 48 hrs, respectively.

Similarly, different virus stocks (*Herpes simplex* Type 1 and *Vesicular stomatitis*) were grown in VERO cells, whereas *Poliomyelitis* Type1 were prepared in HeLa suspension cells. Stock cultures of VERO cells (25×10^6 cells/bottle) were maintained in IL Roux bottles containing tissue culture medium[11].

Protocols for Biological Testings

For antibacterial and antifungal efficacies, disc diffusion method[12] was adopted on Whatman No. 1 paper discs (6 mm diam) containing 500 mg/disc of test extracts and control (amikacin, 10 μg/disc; mycostatin, 100 units/disc) as reference drugs. Later their inhibition zone and activity index were calculated.

The antiviral activity of plant extracts was confirmed by the plaque inhibition method[13-15]. Serial 10-fold dilutions (50 μl) of virus grown in the tissue culture medium M-2 and 2% newborn-calf serum were used to inoculate pre-emptied confluent VERO monolayers in microtiter plates. The antiviral effect of the plant extracts was determined as the reduction factor of the viral titer.

Results and Discussion

The ethanolic extract inhibited growth of almost all the select bacteria and fungi. The petroleum ether extract exhibited almost no activity against any test bacteria but was effective against the three select fungi. The aqueous fraction was, however, active against all the test bacteria and fungi but efficacy-wise it was less active than the ethanolic extract. It is noteworthy that varied fractions obtained from the seedling callus also exhibited similar pattern of bioactivity against both the bacteria and fungi, the efficacy was, however, lower (Table 1).

While comparing antiviral efficacy, different test fractions exhibited a significant fall of the viral titer of infected monolayer of one or the other viral types (reduction factors of 1×10^2 to 1×10^5; Table 1). Interestingly, ethanolic extract was active against all the three test viruses, while petroleum ether fraction exhibited pronounced effect against *V. stomatitis* as also chloroform fraction, which was equally effective against *H. simplex*. However, none of these fractions was appreciably effective against *Poliomyelitis*. It was also revealed that in the bioefficacy of the fractions obtained from callus tissue the activity was lower than the extracts of whole plant (Table 1).

Conclusion

From the bioefficacy data, it is evidenced that—i) In antibacterial and antifungal efficacies the alcoholic extract and aqueous fraction was more effective; ii) Antiviral efficacy was more pronounced than other bioactivities studied; and iii) The chloroform fraction had more effectivity against the test viruses, which may be attributed to the presence of saponins, mimonosides[8] A, B and C. Further, based on these studies, potentiality of the callus cultures to biosynthesize similar compounds is established.

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