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Research Article

Studies on Antimicrobial and Antioxidant Potentials of Triterpenoidal Saponins from *Mimosa Hamata* Willd

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Abstract

Mimosa hamata Willd. (Family: Mimosaceae) is a flowering shrub belonging to family Mimosaceae native to the Thar desert of Indian sub-continent. The methanolic extract of its roots afforded five triterpenoidal saponins which were identified as Mimonoside A(3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabino pyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyloleanolic acid); Mimonoside B (3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic acid); Mimonoside C (3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyl machaerinic acid); Saponin A (3-*O*-D-glucopyranosyl-(1→2)-L-rhamnopyranosylmorolic acid) and Saponin B (3-*O*- L-rhamnopyranosyl-(1→2)- D-glucopyranosylmorolic acid).
On screening for antibacterial activity it was observed that the compounds were more effective against the Gram –ve bacteria viz. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with saponins A and B exhibiting best activity. In antifungal activity, amongst the selected fungi the saponins were effective against *Rhizoptonia bataticola* only, with mimonoside C demonstrating greater efficacy. In antioxidant assay, saponins A and B only exhibited appreciable potential.

1. INTRODUCTION

Mimosa hamata Willd. is a flowering shrub belonging to family Mimosaceae native to the Thar desert of Indian sub-continent. The decoction of the plant is used as a tonic for general weakness and in urinary complaints. A paste of the leaves is applied over glandular swellings and is used in dressing for piles, sinus and sores . Its seeds are used as a blood purifier while the roots possess contraceptive efficacy[1, 2]. Phytochemical analysis have led to the isolation of gallic acid, ethyl gallate and 4-ethyl gallic acid from flowers[3] and triterpenic saponins mimonosides A, B and C and saponins A and B from the roots[4,5]. Pharmacological screening of the various extracts viz. antifungal activity of deproteinized leaf extract[6, 7], antibacterial activity of alcoholic extract of aerial parts[8], antimicrobial[9] and antoxidant[10, 11] and pharmacological evaluation[12] of the root extract has been carried out.
Saponins are widely distributed in the plant kingdom and reported to demonstrate diverse range of biological activities viz. anti-inflammatory, antibacterial, antifungal, antiviral, haemolytic and cytotoxic[13, 14]. As a part of our research program to identify new natural antimicrobial and/or antioxidant agents and since no such studies of the isolated triterpenoidal saponins from the roots of *M. hamata* have been carried out so far, hence the present work was undertaken.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant materials

The roots of *M. hamata* were collected from Ajmer-Jaipur highway between June-September and authenticated (RUBL 9565) by one of the authors, Prof. S.C. Jain.

2.2 Isolation of saponins

The roots were shade-dried, powdered and exhaustively extracted with methanol. The concentrated methanolic extract was suspended in water and fractionated with pet. ether, when three layers were formed, upper pet. ether layer, lower aqueous layer and in between was the middle layer which appeared to be a sort of emulsion. The lower aqueous layer was extracted with n-butanol and this layer was precipitated with acetone. The residue on CC afforded mimonoside A (3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyloleanolic acid, **I**); mimonoside B (3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic acid, **II**); mimonoside C (3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyl machaerinic acid, **III**). The middle layer on column chromatography (CC) over silica gel afforded saponin A (3-*O*-D-glucopyranosyl-(1→2)-L-rhamnopyranosylmorolic acid, **IV**) and saponin B (3-*O*- L-rhamnopyranosyl-(1→2)- D-glucopyranosylmorolic acid, **V**) [Figure 1]. The isolated saponins were identified on the basis of spectral data (IR, 1H NMR, 13C NMR and Mass)[4, 5].

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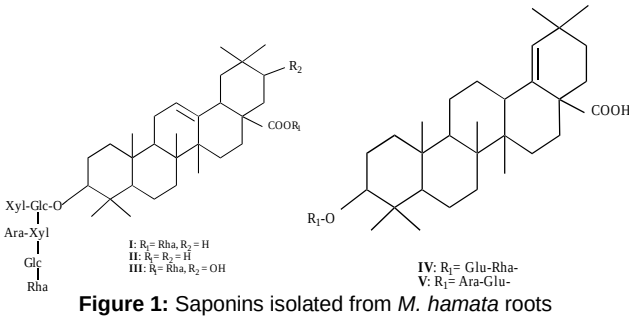

Figure 1: Saponins isolated from *M. hamata* roots

2.3 Microbial strains used

Antimicrobial effect was determined against 6 bacteria viz. *Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 13098, and *Staphylococcus aureus* ATCC 9144 as Gram +ve while *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25998 and *Klebsiella pneumoniae* ATCC 11298 as Gram – ve bacteria and 4 fungi viz. *Aspergillus flavus* ATCC 16870, *A. niger* ATCC 322, *Candida albicans* ATCC 4718 and *Rhizoctonia bataticola*. The pure cultures of bacteria and fungi were obtained from IMTECH, Chandigarh and IARI, New Delhi, respectively.

2.4 Antimicrobial assay

Bacterial cultures were grown and maintained on nutrient broth (NB) medium at 27°C for 48 hrs and fungal cultures on Sabouraud dextrose agar medium at 37°C for 48 hrs. Antimicrobial assay was performed using agar well diffusion method [15]. Inoculums were prepared by suspending the bacteria in nutrient broth medium and fungus in Sabouraud dextrose agar medium overnight at 37°C (10^6 – 10^7 CFU/ml concentration). 20 µl of bacterial and 80 µl of fungal suspensions were inoculated in nutrient agar and Sabouraud dextrose agar plates respectively. 10 µg/ml of the saponin was used for each well. To ensure diffusion of sample into agar, the plates were incubated at 37°C for bacteria and 25°C in case of fungi for appropriate time periods under aerobic conditions. The diameter of the inhibition zone around each well was measured. Gentamycin (10 µg/ml) in case of bacteria and ketoconazole (100 units/ml) in case of fungi were used as standard antibiotics.

2.5 Statistical analysis

All experiments were performed in triplicate and data were analyzed according to one-way ANOVA method using IBM-SPSS version 20. Mean values (three replicates ± S.E.) were statistically compared with Duncan test, at probability level P=0.05.

2.6 Antioxidant activity

Free radical scavenging activity employing DPPH was carried out following the method of Khalaf et al (16). A solution (2.5 ml) of 2×10–3 mg/ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was mixed with equal volume of test compound/ascorbic acid (standard) solution in methanol and kept in dark for 30 min. The absorbance at 517 nm was monitored at different concentrations (10, 20, 40, 60, 80 µg/ml) using UV-Vis spectrophotometer. Blank was also carried out to determine the absorbance of DPPH, before interacting with the extract. The capability to scavenge the DPPH radical was calculated using following equation:

$$\% \text{ Inhibition} = 1 - (OD_{Sample} / OD_{Control}) \times 100$$

where, $OD_{Control}$ is the absorbance of the control (containing all reagents except the test compounds) and OD_{Sample} is the absorbance of test sample. The optical density of control was considered as 100% unreduced DPPH and IC_{50} values were determined as the concentration of the extract required to achieve 50% reduction in DPPH radicals.

3. RESULTS AND DISCUSSION

The antibacterial and antifungal activities of the isolated saponins were carried out by agar well diffusion method and the results are shown in Table 1.

In antibacterial activity, it was observed that in general the compounds were more effective against Gram -ve bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*) demonstrating the activity index equivalent and/or even more to the standard, gentamycin in *K. pneumoniae* and *P. aeruginosa*. On comparing the activity of these compounds, it has been noticed that the saponins A and B (compound IV and V) having the triterpenoidal moiety, morolic acid and lesser sugar moieties are more active. In Gram +ve bacteria the isolated compounds were found to be only moderately active.

In fungi, all the saponins exhibited pronounced activity against *R. bataticola* only, where mimonosides C (compound III) demonstrated greater activity (AI = 1.02) than the standard, ketoconazole. None of the saponins were active against *A. flavus*, *A. niger* and *C. albicans*.

In DPPH free radical scavenging activity (Table 2) appreciable antioxidant potentials were demonstrated by compounds IV and V as compared to the control, quercetin.

Table 1: Antibacterial and antifungal activity of saponins

Microorganism		Saponins				
		I	II	III	IV	V
B. subtilis	IZ	10.00 ± 0.57	13.33 ± 0.32	13.66 ± 0.66	14.00 ± 0.00	11.66 ± 0.74
	AI	0.45	0.60	0.62	0.63	0.58
E. aerogenes	IZ	11.33 ±0.87	14.66 ± 0.67	13.66 ± 0.66	14.00 ± 0.57	13.66 ± 0.33
	AI	0.59	0.76	0.71	0.73	0.71
E. coli	IZ	15.66 ± 0.66	17.33 ± 0.57	15.66 ± 0.66	16.66 ± 0.66	16.00 ± 0.00
	AI	0.78	0.86	0.78	0.83	0.80
K. pneumoniae	IZ	14.66 ± 0.57	15.00 ± 1.73	16.00 ± 0.00	19.66 ± 0.57	24.66 ± 0.57
	AI	0.60	0.61	0.66	0.81	1.01
P. aeruginosa	IZ	10.33 ± 0.32	11.33 ± 0.32	14.00 ± 0.57	15.33 ± 0.32	16.66 ± 0.66
	AI	0.73	0.80	1.00	1.09	1.19
S. aureus	IZ	11.00 ± 0.57	10.33 ± 0.32	11.33 ± 0.76	10.00 ± 1.00	10.33 ± 0.87
	AI	0.52	0.49	0.53	0.47	0.49
A. flavus	IZ	12.00 ± 0.00	11.66 ± 0.74	10.00 ± 0.00	14.66 ± 0.66	14.33 ± 0.32
	AI	0.44	0.43	0.37	0.54	0.53
A. niger	IZ	12.66 ± 0.88	16.33 ± 0.87	15.33 ± 0.67	18.33 ± 0.32	16.33 ± 0.67
	AI	0.46	0.60	0.56	0.67	0.60
C. albicans	IZ	10.33 ± 0.32	14.33 ± 0.32	13.66 ± 0.88	12.00 ± 0.00	11.00 ± 0.57
	AI	0.46	0.65	0.62	0.54	0.50
R. bataticola	IZ	14.33 ± 0.57	15.66 ± 0.67	16.33 ± 0.87	11.66 ± 1.52	14.00 ± 0.57
	AI	0.89	0.97	1.02	0.72	0.87

Table 2: Free radical scavenging activity of saponins

Compound	IC_{50}	% Inhibition (ug/ml)				
		10	20	40	60	80
I	0.45	43.57	43.64	44.64	63.32	64.34
II	0.55	44.18	48.76	48.76	49.68	52.13
III	0.60	43.57	43.64	44.46	50.75	52.13
IV	0.085	66.49	68.25	70.30	73.19	74.49
V	0.10	66.49	68.25	70.30	73.19	75.93
Quercetin	0.06	62.42	80.58	93.38	93.82	94.71

4. CONCLUSION

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuous search for new and potent antibiotics.

Our study further strengthens the fact that the triterpenoids play a potent inhibitory role against the microbes and also possess antioxidant potential. The results also support the traditional use of this plant and scientific validation for use of *M*. *hamata* in various infections and its nutraceutical value.

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REFERENCES

1. Nadkarni AK, Nadkarni KM, Indian Materia Medica, Popular Book Depot, Bombay, 1953.
2. Satyavati GV, Gupta AK, Tandon N, Medicinal Plants of India. Vol. II, ICMR, New Delhi, 1987, 254.
3. Mehta BK, Sharma S, et al "Ethylgallic acid from two *Mimosa* species" *Phytochem*, 1998, 27: 3004-3005.
4. Jain R, Arora R, "3-0-L-Arabinosyl-D-glucosyl morolic acid from *Mimosa hamata*" *J Med. Arom. Plant Sci*, 1997, 29: 22-23.
5. Jain R, Arora R, et al "Saponins from the roots of *Mimosa hamata* Willd" *Indian J Chem*, 1997b, 36B: 61-64.
6. Mukadam DS, Balkhande LD, et al "Antifungal activities in deproteinized leaf extract of weeds and nonweeds" *Indian J Microbiol*, 1976, 16: 78-79.
7. Umalkar GV, Mukadam DS, et al "Fungistatic properties of some deproteinized leaf extract" *Sci & Cult*, 1977, 43: 437.
8. Hussain N, Modan MH, et al "Antimicrobial principles in *Mimosa hamata*" *J Nat Prod*, 1979, 42: 525-527.
9. Jain SC, Jain R, et al "*In vivo* and *in vitro* antimicrobial efficacy of *Mimosa hamata*" *Indian J Biotech*, 2004, 3: 27-273.
10. Jain SC, Jain R., et al "Antioxidant activity of mimonosides isolated from *in vitro* regenerated plants of *Mimosa hamata* Willd" *Indian J Pl Physiol*, 2009, 14: 124-129.
11. R Singh, Jain SC, Jain R. "Antioxidant activity of some medicinally important arid zone plants" *Asian J Exp Sci*, 2009, 23(1): 215-221.
12. Jain R, Arora R, et al "Pharmacological evaluation of *Mimosa hamata* roots" *Fitoterapia,* 1997, 68: 377-378.
13. Challinor VL, Stuthe MU, et al "Structure and bioactivity of steroidal saponins from the roots of *Chamaelirium luteum* (False unicorn)" *J Nat Prod*, 2012, 75(8): 1469-1479.
14. Amin Elham, El-Hawary SS, et al "Triterpenoidal saponins: Bioactive secondary metabolites from *Zygophyllum coccineum*" *Planta Med*, 2011, 77(5): 488-491.
15. Boyanova L, Gergova G, et al "Activity of Bulgarian propolis against 94 Helicobacter pylori strains *in vitro* by agar well diffusion, agar dilution and disc diffusion methods" *J Med Microbiol*, 2005, 54(5): 481-483.
16. Fogliano V, Verde V, et al "A method of measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines" *J Agric Food Chem*, 1999, 47: 1035-1040.