ANTIMICROBIAL PRINCIPLES IN *MIMOSA HAMATA*

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Mimosa hamata Willd. (Mimosaceae) is widely distributed in Pakistan and India (1). A crude alcoholic extract of its aerial parts was found to possess antibacterial properties (2) of a broad nature; however, no chemical investigation on the plant has been reported in the literature. The present communication describes the isolation and characterization of the major active principles, ethyl gallate and gallic acid. Ethyl gallate has also been found in *Acacia* species (3), *Arbutus unedo* of the *Ericaceae* family (4), in seeds of *Bombax malabaricum* (5) and *Rhynchosia phaseoloides* (6) and in panicles of *Mangifera Indica* (7). It was isolated as the antimicrobial principle from the aquatic plant *Nymphaea tuberosa* (8) being strongly active against *Mycobacterium smegmatis*, moderately active against *S. aureus* and *E. coli* and less active against *C. albicans*. Gallic acid has been reported to inhibit *S. aureus* slightly (9). It has been isolated as one of the antimicrobial principles from leaves of *Lawsonia inermis* L. (10).

EXPERIMENTAL[1]

PLANT MATERIAL.—Fresh aerial parts of the plant *Mimosa hamata* Willd[2] were collected while in the flowering stage. Dried plant material has also been used without any significant difference in activity or chemical composition.

ANTIMICROBIAL TESTING.—*In vitro* antimicrobial activity was tested by the agar cup-plate method (11) against *Salmonella typhosa*, *S. para* A, *S. para* B, *Shigella dysenteriae*, *S. flexneriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Vibrio cholera ElTor*, *V. C. inaba*, *Staphylococcus aureus*, *S. albus*, *Diplococcus pneumoniae*, *Corynebacterium diptheriae*.[3]

For this purpose 20 ml of molten sterile agar was poured into sterile Petri dishes, 8.5 to 9.0 cm in diameter. After the agar had solidified it was swabbed with a 24-hr old broth culture of the organism. A cavity 0.8 cm in diameter was made in the center with the help of a sterilized cork borer. The cavities were filled with 0.3 ml of a 2% solution of the substance being tested in 90% ethanol. Controls were run with all the organisms, using 90% ethanol. The inoculated Petri dishes were incubated at 37° and the zones of inhibition were measured after 24 hr. For comparison, 0.3 ml of 0.00333% aqueous solution of streptomycin equivalent to 10 μg was used.

ISOLATION OF THE ANTIMICROBIAL COMPOUNDS.—Plant material (5 kg) was cut into small pieces and extracted three times with 95% ethanol. The extracts were pooled and concentrated *in vacuo* at 40° to a thick semisolid mass (413 g) which showed antimicrobial activity as given in table 1. When the concentrated extract was repeatedly extracted with acetone it yielded acetone soluble and acetone insoluble portions. The acetone soluble portion, after removal of the solvent, was further divided into petroleum ether soluble and insoluble fractions. The acetone insoluble and petroleum ether soluble fractions showed negligible antimicrobial activity and were not studied further.

[1]Equipment: uv, Unicam SP500; ir, Perkin-Elmer 237; melting point, Kofler block; elemental analysis, micro-analytical section PCSIR Laboratories, Karachi. The composition of chromatography fractions and the purity of crystalline phenolic compounds were checked by single and two dimensional paper chromatography on Whatman No. 1 filter paper in the upper layer of *n*-butanol-acetic acid-water (BAW) (4:1:5) and 2% acetic acid. The chromogenic reagents used were (1) 0.3% potassium ferricyanide-0.3% ferric chloride (1:1), freshly mixed; and (2) 1% silver nitrate solution in acetone followed by treatment with 0.5% ethanolic sodium hydroxide and, finally, 20% sodium thiosulphate solution for washing the paper; and (3) 1% alcoholic ferric chloride.

[2]Identified by Botany Department, University of Karachi.

[3]The test organisms were obtained from the University of Karachi. The isolation of the active principles was followed by observation of the antimicrobial activity. In addition to antimicrobial activity, a phenolic test ($FeCl_3$) was also used since the activity was found to be due to the phenolic components.

The petroleum ether insoluble fraction (40 g) was chromatographed on silica gel (400 g) which was eluted with solvent mixtures of benzene, chloroform, and ethanol by a gradient technique. A total of 100 fractions of 250 ml each were collected. Fractions showing similar constituents by paper chromatography, in the upper layer of BAW, were combined. Fractions 1–20 (4 g) contained non-phenolic and inactive components; 21–30 (8.0 g) showed a single spot; 31–36 (4 g) showed two spots; 37–40 (1.7 g) showed a single spot and 41–68 (5.5 g) gave more than two spots and also gave a faintly phenol test; 69–100 (6.8 g) were inactive. Only those fractions which showed a single spot and exhibited antimicrobial activity against all the test organisms were worked on further.

TABLE 1. Antimicrobial activities of ethanolic extract of *Mimosa hamata* Willd. aerial parts (2%, w/v in 90% ethanol).

Organisms	Zone of inhibition in mm	
	Extract of *Mimosa hamata*	Streptomycin 10 μg
1. *Salmonella typhosa*	28	23
2. *S. para* A	25	19
3. *S. para* B	35	17
4. *Shigella dysenteriae*	32	18
5. *S. flexneriae*	24	12
6. *Escherichia coli*	32	22
7. *Klebsiella pneumoniae*	25	19
8. *Streptococcus faecalis*	28	13
9. *Vibrio cholera ElTor*	40	13
10. *V. C. inaba*	28	14
11. *Staphylococcus aureus*	26	21
12. *S. albus*	35	20
13. *Diplococcus pneumoniae*	30	14
14. *Corynebacterium diphtheriae*	28	20

IDENTIFICATION OF ETHYL GALLATE.—Fractions 21–30, eluted with chloroform-ethanol mixture 9:1, were combined (8.0 g) and rechromotographed on a silica gel (160 g) column developed with petroleum ether followed by a mixture of petroleum ether and ether. Eluates with petroleum ether and ether (1:1) were combined to yield a solid (2.2 g) which, on repeated crystallization with petroleum ether and ether, gave colorless needles, mp 154.7° [reported mp, 158° (13)]. The acetyl derivative of the compound was prepared with acetic anhydride/pyridine at room temperature. It was recrystallized with ethanol, mp 134°. [reported mp, 138° (13)].

The ultraviolet spectrum of the compound showed λ max (EtOH) 277 and 218 mμ. An infrared spectrum showed absorptions at 3400 (m, OH), 1750 (s, C-O), 1650 (s), 1560 (s), 1470 (s), 1300 (s), 1260 (m), 1220 (m), 1110 (m), 1040 (m, C-OH), 975 (s), 870 (s), 769 (s), and 725 (m) cm^{-1}. The mass spectrum showed a molecular ion M$^+$ at *m/e* 198, and the molecular formula as determined by elemental analysis and molecular weight was found to be $C_9H_{10}O_5$. *Anal.* calc. for $C_9H_{10}O_5$; C, 54.54; H, 5.05; O, 40.41. Found: C, 54.60; H, 5.09; O, 40.31. An nmr of its acetate showed a singlet at 2.2 τ which integrated for two equivalent aromatic protons, a quadruplet at 6.65 τ which integrated for two methylene protons, a singlet at 7.72 τ which integrated for nine phenolic acetoxy protons, and a triplet at 8.7 τ which integrated for three methyl protons. The compound gave intense blue coloration with $FeCl_3$, no coloration or precipitate with vanillin- HCl and gelatin solution and had R_f 0.78 and 0.5 on paper in BAW and 2% acetic acid respectively. Based on the above physical data and comparison with an authentic sample prepared according to the general method (12) of esterification of gallic acid, the compound was identified as ethyl gallate.

IDENTIFICATION OF GALLIC ACID.—Fractions 37–40, eluted with a mixture of chloroform-ethanol (80:20), yielded 1.7 g of solid, which was rechromatographed on silica gel (15 g) in benzene followed by benzene with increasing amounts of ethyl acetate. Eluates from a solvent mixture of benzene and ethyl acetate (1:1) yielded a solid (0.7 g) which on repeated recrystallization with hot water gave brownish crystals, mp 234°, [reported 235–40 (13)]. The acetyl derivative of the compound was prepared with aqueous NaOH/acetic anhydride at 0°.

After recrystallization with hot water, [reported 171–2° (13)] its melting point was 168°. The uv spectrum of the compound showed λ max (EtOH) 274 and 218 mμ. An infrared spectrum showed absorptions at 3480 (m, OH), 1650 (s, C=O), 1600 (s), 1549 (s), 1425 (s), 1350 (s), 1275 (w), 1150 (m), 1025 (m, C=OH), 900 (m), 860 (s), 760 (s) and 735 (s) cm^{-1}. The compound gave blue coloration with $FeCl_3$, pink coloration with KCN, and yellow coloration with aqueous alkali and no precipitate with gelatin solution. It had R_f 0.65 in BAW (4:1:5) and 0.33 in 2% acetic acid. Based on the above physical data and comparison with an authentic sample, the compound was identified as gallic acid.

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