

Phytochemical and Antimicrobial Screening of *Teramnus Mollis* Aerial Parts

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Abstract : The chloroform and methanolic extracts of aerial parts of *Teramnus mollis* were subjected to column chromatography which afforded five compounds, β - sitosterol, lupeol, β -amyrin, diadzin and vitexin. The extracts were also screened for antimicrobial activity and the chloroform extract showed strong antibacterial activity against *Staphylococcus aureus* and methanolic extract exhibited moderate antibacterial activity. Further the chloroform extract displayed moderate antifungal effect whereas the methanol extract showed strong antifungal activity against *Candida albicans*.

Key words- *Teramnus mollis*, aerial parts, phytoconstituents, antimicrobial activity

INTRODUCTION:

In tropical countries large segment of the population rely on traditional medicines for their health needs (1). Over 80% of population in the developing world makes use of medicinal plants extracts to provide health (2). The searches for new compounds with antimicrobial activity from plants have been the subject for intense research in recent years (3-5). This is due to the fact that plants are widely used in folk medicine to combat various diseases in human caused by pathogenic organisms (6-8).

Teramnus mollis belonging to the family Fabaceae is a small herb grown as covering crop. It is used in rheumatism, TB, nervous affection and cataract. Fruits are used as astringent. (9)

MATERIALS AND METHODS:

Column chromatography and TLC were carried out using silica gel (60-120 mesh) and silica gel G (Acme) respectively. Visualization of the TLC plates was done by spraying 5% methanolic sulphuric acid. Melting points were recorded by Boietus melting point apparatus. UV spectra were obtained on systronics UV spectrophotometer, IR spectra were recorded on BUCK scientific -500 spectrophotometer using KBr pellets. ¹HNMR spectra were taken on BRUKER AM 400 with TMS as an internal standard.

EXPERIMENTAL

Collection of the plant material:

The plant material was collected from Vepada, Vizianagaram District and the identity was established by Dr. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam.

Extraction of the plant material:

Air dried powdered roots (1.5 Kg) of *Teramnus mollis* was subjected to extraction with chloroform and methanol. The procedure was repeated for 3 times. The extracts were concentrated and dried under vacuum to get a residue of 22 and 14gms respectively. The extracts were then subjected to preliminary phytochemical screening followed by column chromatography.

Antimicrobial screening of the plant extract

Screening of the plant extract for antimicrobial activity was done by agar diffusion method. Neomycin and Nystatin were used as a positive control for antibacterial and antifungal activities respectively.

Test microorganisms

Bacterial cultures of *Bacillus subtilis*, *Bacillus pumilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteases vulgaris* and fungal cultures *Aspergillus niger*, *Penicillium chrysogenum* and *Candida albicans* were used as test organisms for the antimicrobial studies.

Antibacterial activity:

Nutrient agar medium was inoculated with 24 hours old stock cultures of the above mentioned test organism and were transferred into sterile 15cm diameter petri dishes. The medium in the plates were allowed to set at room temperature for about 30minutes to solidify in laminar air flow unit. In each plate 4 cups of 6mm diameter were made in each plate at equal distance. Stock solutions of the test extracts were prepared in concentrations of 100mg/ml and 300mg/ml. 50 μ l of the extract was placed in the cups by sterile pipettes. In each plate one cup was used for Neomycin (10 μ g/ml) which served as a positive control and one cup for methanol which served as a negative control. The petridishes thus prepared were incubated for 24 hrs at 30 $^{\circ}$ C and were later examined by measuring the zones of inhibition and the results were tabulated.

Antifungal activity:

Potato dextrose agar medium (PDA) was prepared and inoculated with 5 μ l of aqueous suspension of the above mentioned test organisms, which were prepared from 48 hrs cultures, are transferred into sterile petri dishes. The medium in the plates were allowed to set at room temperature for about 10 minutes. In each plate 4 cups of 6mm diameter were made in each plate at equal distance. Stock solutions of the test extract were prepared in concentrations of 100mg/ml and 300mg/ml. 50 μ l of the extract was placed in the cups by sterile pipettes. In each plate one cup was used for Nystatin (10mg/ml) which served as a positive control and one cup for methanol which served as a negative control. The petri dishes thus prepared were incubated for 24 hrs at 30 $^{\circ}$ C and were later examined by measuring the zones of inhibition and the results were tabulated.

RESULTS AND DISCUSSION

Phytochemical screening

The chloroform extract gave positive test for Liebermann-Buchard test for the presence of terpenes, the methanolic extract gave positive test for flavonoids (Shinoda test).

Characterization of the isolated compounds:

β - sitosterol: It was crystallized from petroleum ether as colorless needles, M.P- 134-136 $^{\circ}$ C, gave positive color reaction with Liebermann -Buchard test. The IR spectrum showed bands 2970, 2950, 2880, 1470, 1385 and 1055 cm^{-1} . The ^1H NMR spectrum showed peaks at δ 0.83-1.01(methyl's), 3.47 (1H broad $\text{C}_3\alpha\text{-H}$) and 5.35(1H, m, $\text{C}_5\text{-H}$).

Lupeol: crystallized from chloroform-petroleum ether as needles, M.P -195 $^{\circ}$ C, gave pink color with Liebermann- Buchard test and yellow colour with tetranitromethane test. The IR spectrum showed absorption bands at 2923, 2854, 1536, 1454, 1392, 1380, 1259, 1143, 770 cm^{-1} and ^1H NMR spectrum (300MHz, CDCl_3 , δ) : 0.76-1.02 (18H, S, 6xMe), 1.65(3H,S,=CCH $_3$), 2.25(1H,d,19-H), 3.15(1H,m,3 α -H), 4.6-4.7(2H,d,=CH $_2$, Vinylic protons).

β - amyrin: It was crystallized from petroleum ether as white needle shaped crystals, m. p 195-197 $^{\circ}$ C, gave positive color reaction with Liebermann- Buchard test (pink) for triterpenes. A hydroxyl and tri substituted double bond was noticed in IR spectrum at 3500, 2250, 1380, 1030, 820. The ^1H NMR spectrum showed peaks at δ 1.51, 1.01, 0.99, 0.95, 0.88, 0.82 and 0.90 (all s) and multiplet at δ 5.23 was indicative of the olefinic proton.

Diadzin: The compound appeared as a white crystalline solid from aqueous methanol, m.p: 232-233 $^{\circ}$ C. The IR spectrum showed bands at 3372 (hydroxyl), 1623(carbonyl), 1514and 1445 cm^{-1} (aromatic). The proton NMR (500MHz, DMSO) spectral data contained six aromatic protons constituted by a singlet at δ 8.36(1H) and an ABX spin system, characteristic of 1,2,4- trisubstituted phenyl unit (8.04 (1H, d, J= 8.8Hz), 7.14(1H,dd, J=2.2, 8.8Hz) and 7.22(1H,d,J=2.1Hz) and an AA'BB' spin system (7.40 (2H,D, J=8.6Hz) and 6.81(2H,D,J=8.6Hz) attributable to a para- disubstituted phenyl unit. The above indicated the presence of an isoflavanoid skeleton. The spectrum also showed a group of signals between 3.10 and 3.70, in addition to anomeric proton signal at 5.10(1H, d, J=7.6Hz) suggestive of a sugar unit. The ^{13}C NMR [125MHz,d $_6$ -DMSO] spectrum showed six quaternary carbons (d 118.5, 122.0, 123.7,157.0, 157.2 and 161.0) three aromatic carbons(d 103.4, 115.6 and 127.0), a β – olefinic carbon (d 153.3) and a carbonyl carbon resonating at d 174.8. The signals at d 60.7, 69.7, 73.2, 76.5, 77.2 and 100.0 are attributable to a sugar moiety. The ^{13}C NMR chemical shifts of the sugar moiety matched well with those recorded for glucose unit and the data matched with that of diadzin and the identity was confirmed by comparison with authentic sample through m.p and co-TLC.

Vitexin: The compound was obtained as a yellow solid, mp: 275-276 $^{\circ}$ C. The IR spectrum showed bands at 3381(hydroxyl), 1652(carbonyl), 1568, 1501 cm^{-1} (aromatic). The ^1H NMR and spectrum showed a chelated hydroxyl proton (d 13.18, 1H, s), an aromatic singlet (d 6.28,1H, s), a downfield signal (d 6.28,1H, s) suggestive of a flavonoid and an AA'BB' spin system (d 8.30, (2H, d, J=8.5Hz) and 6.09 (2H, d, J=8.5Hz) attributable to a para- disubstituted phenyl unit. In addition, the ^1H NMR showed a series of signals between d 3.19 and 3.98 (6H), characteristic of sugar unit. The absence of O-glycosidic proton signal and the presence of signal at d 4.69 (1H, d, J=9.88 Hz) indicated the presence of a C-glycoside. The above data matched well with that of Vitexin and the identity was confirmed by comparison with an authentic sample through m.p and co-TLC.

Antimicrobial activity of *Teramnus mollis* extracts:

The chloroform and methanol extracts of aerial parts of *Teramnus mollis* showed antimicrobial activity against gram (+) ve organisms and gram (-) ve organisms and the activity increased with concentration. The chloroform extract showed strong antibacterial activity against *Staphylococcus aureus*, whereas the methanolic extract showed moderate antibacterial activity. The chloroform extract showed moderate antifungal effect whereas the methanol extract showed strong antifungal activity against *Candida albicans*.

TABLE 1: Antibacterial activity of the chloroform and methanolic extracts of leaves of *T.mollis*

S.No	Name of the extract	Diameter of zone of inhibition				
		Gram (+) ve bacteria			Gram (-) ve bacteria	
		<i>B.s</i>	<i>B.p</i>	<i>S.a</i>	<i>E.c</i>	<i>P.v</i>
1	Chloroform extract of <i>T.mollis</i> (100mg/ml)	11	9	9	9	11
2	Chloroform extract of <i>T.mollis</i> (300mg/ml)	13	10	16	12	11
3	Methanol extract of <i>T.mollis</i> (100mg/ml)	8	9	-	8	9
4	Methanol extract of <i>T.mollis</i> (300mg/ml)	11	10	-	10	11
5	Neomycin(10µg/ml)	16	14	16	16	14
6	Control (Chloroform/Methanol)	-	-	-	-	-

TABLE 2: Antifungal activity of the chloroform and methanolic extracts of leaves of *T.mollis*

S.No	Name of the extract	Diameter of zone of inhibition		
		<i>A.n</i>	<i>P.c</i>	<i>C.a</i>
1	Chloroform extract of <i>T.mollis</i> (100mg/ml)	13	11	12
2	Chloroform extract of <i>T.mollis</i> (300mg/ml)	13	12	12
3	Methanol extract of <i>T.mollis</i> (100mg/ml)	9	9	9
4	Methanol extract of <i>T.mollis</i> (300mg/ml)	9	10	19
5	Nystatin(10µg/ml)	12	14	12
6	Control (Chloroform/Methanol)	-	-	-

CONCLUSION:

The chemical examination of chloroform and methanolic extracts of the aerial parts of *Teramnus mollis* afforded five compounds, β -sitosterol, lupeol, β -amyryn, diadzin and vitexin which were characterized by spectroscopy.

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