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Iridoid Compounds and Antimicrobial Activity of the Roots of *Tecoma stans* (L) Juss

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The combined ethyl acetate and methanolic extracts of the roots of *Tecoma stans* gave two iridoid compounds, 5-deoxystantioside, plantarenaloside when subjected to chromatographic techniques. In addition to iridoid compounds luteolin, lupeol acetate and β-sitosterol were also isolated. The structures of these compounds were determined by spectral means (^{1}H NMR, IR, UV, MS). Further the extracts and the isolates were subjected to antimicrobial studies, which showed moderate activity.

Key Words: Iridoids, *Tecoma stans*, 5-Deoxystantioside, Plantarenaloside.

INTRODUCTION

Tecoma stans (L) Juss (Bignoniaceae) is an ornamental tree, commonly known as yellow elder, distributed throughout the tropical parts of India. The roots of *T. stans* are used by the folk as a remedy for snake and rat bites, scorpion sting[1,2]. The plant was also reported to have antidiabetic and hypoglycemic activity[3-7]. Chemically the plant contains monoterpene alkaloids[8,9], tecomine, tecostanine which were found to be responsible for the hypoglycemic activity[10-14]. In the present study two iridoid compounds, 5-deoxystantioside (TSR-IV), plantarenaloside (TSR-V) were isolated from the roots of *T. stans* apart from luteolin (TSR-III), lupeol acetate(TSR-II) and β-sitosterol(TSR-I).

EXPERIMENTAL

UV Spectra were obtained on Systronics UV Spectrophotometer, IR spectra were recorded on BUCK Scientific-500 spectrophotometer using KBr pellets. Melting points were determined using Boeitus micro melting point apparatus and are uncorrected. The ^{1}H NMR spectra were taken on Bruker AM400 spectrophotometer with TMS as an internal standard. The mass spectra were taken on MAT-95 mass spectrophotometer. Column

chromatography (CC) and TLC were carried out on silica gel (60-120 mesh, Acme) and Silica gel G (Acme), respectively. The visualization of TLC was done by spraying 5 % sulphuric acid reagent in methanol. All the solvents (Merck) used were distilled prior to use.

Plant material: The roots of *T. stans* were collected (2 Kg) from Andhra University campus, during December 2003 and authenticated by Dr. M. Venkaiah, taxonomist, Department of Botany, Andhra University, Visakhapatnam, a voucher specimen (SG/TS/12/2003) was deposited in the institution herbarium. The material was shade dried and coarsely powdered. About 1.5 Kg of the powder was extracted using soxhlet apparatus successively using petroleum ether (60-90 ºC), ethyl acetate and methanol and concentrated under reduced pressure to get residues of 7.2, 12.3 and 15.4 g, respectively.

Isolation of compounds: The ethyl acetate and methanol residues of *T. stans* were combined as they were similar on TLC (chloroform:methanol, 9:1) and column chromatographed over silica gel (60-120 mesh, Acme) using gradient elution which yielded five compounds and were designated as TSR-I, TSR-II, TSR-III, TSR-IV and TSR-V. The compound TSR-I was obtained from 5 % ethyl acetate in petroleum ether which on crystallization gave colourless needles of m.p. 134-136 ºC, showed positive Leibermann-Burchard test as green colour. Another compound TSR-II was obtained from 20 % ethyl acetate in petroleum ether which on crystallization gave colourless flakes of m.p. 214 ºC and pink colour with Leibermann-Burchard test. The other compound TSR-III was obtained from 75 % ethyl acetate in petroleum ether which on crystallization gave yellow needles of m.p. 333-334 ºC. On TLC a purple spot was observed under UV and intensified when exposed to ammonia, showed green colour with ferric chloride and orange colour with shinoda's test. The compound TSR-IV was obtained from 2 % methanol in ethyl acetate which on crystallization gave pale yellow flakes, m.p. 146-147 ºC. It showed blue colour with Wieferring test. The compound TSR-V was obtained from 5 % methanol in ethyl acetate which on crystallization gave buff coloured amorphous powder. It showed blue colour with Wieferring test indicating iridoids.

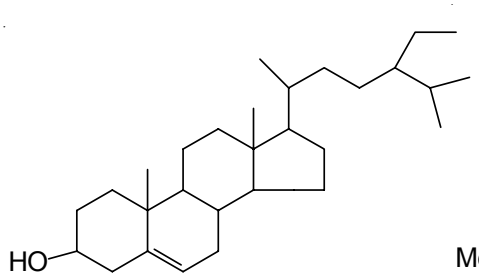
RESULTS AND DISCUSSION

Phytochemical examination of the roots of *T. stans* has been carried out. The combined root extacts of ethyl acetate and methanol on column chromatography afforded five compounds, TSR-I, TSR-II, TSR-III, TSR-IV and TSR-V. These compounds were identified as β-sitosterol, lupeol acetate, luteolin, 5-deoxystantioside and plantarenaloside by elemental analysis, spectal studies, m.m.p and co.TLC. The isolates 5-deoxystantioside and plantarenaloside were reported for the first time from the roots of *T. stans*.

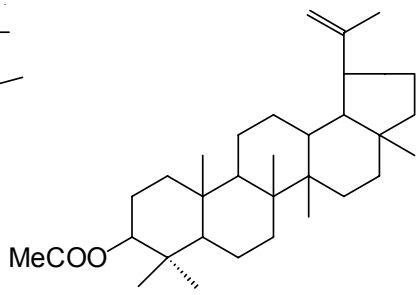
β-Sitosterol (TSR-I) colourless needles, m.p. 134-136 ºC, $[M]^+$ m/z: 414, $[\alpha]_D^{90}$ + 36º (chloroform), $C_{29}H_{50}O$, Found (%): C, 83.07; H, 12.7 Requires (%): C, 83.0; H, 12.2, IR (KBr, ν_{max}, cm^{-1}): 3440, 2970, 2880, 2050, 1470, 1385, 1055, ^1H NMR: δ 0.86, 1.25 (Me), 3.47 (1H, broad, 3-H) and 5.32 (1H, m, 5-H), the identity of TSR-I was confirmed by comparison with authentic sample through m.m.p and Co-TLC.

Lupeol acetate (TSR-II) colourless flakes, m.p. 214 ºC, $[M]^+$ m/z: 468, $C_{32}H_{52}O_2$, Found (%): C, 82.3; H, 10.8, Requires (%): C, 82.0; H, 11.2, $[\alpha]_D^{30}$ + 37º (C, 0.93, in chloroform), IR (KBr, ν_{max}, cm^{-1}): 2910, 2840, 1720, 1630, 1440, 1382, 1380, 1240, 1010, 970, 880, ^1H NMR ($CDCl_3$, 90 MHz, δ): δ 0.76-1.02 (18H, 6XCH_3), 1.65 (3H, s, CH_3-C=CH_2), 1.94 (3H, s, 3βOAc), 4.3 (1H, m, 3α-H) 4.52 (2H, d, =CH_2), the identity of compound **II** was confirmed by comparison with authentic sample through m.m.p and Co-TLC.

Luteolin (TSR-III) yellow needle, m.p. 333-334 ºC, $[M]^+$ m/z: 286, $C_{15}H_{10}O_6$, λ_{max} MeoH n.m: 243 sh, 254, 266, 292 sh, 352, $AlCl_3$: 264 sh, 328 sh, 402, $AlCl_3$/HCl: 276 sh, 276, 296 sh, 356, 385, NaOAc: 269, 326 sh, 387, the identity of TSR-III was confirmed by comparison with authentic sample through m.m.p and Co-TLC.



TSR-I



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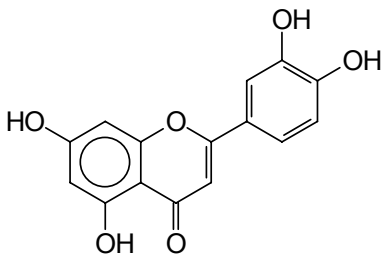
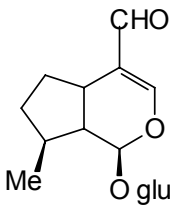
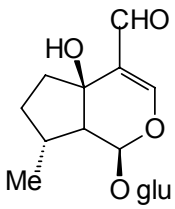


TSR-III



TSR-IV



TSR-V

5-Deoxystantioside (TSR-IV) pale yellow flakes, m.p.146-147 ºC, $[M]^+$ m/z: 344, $C_{16}H_{24}O_8$, Found (%): C, 55.4; H, 7.07, Requires (%): C, 55.8; H, 7.02 $[\alpha]_D^{30}$ -117º, λ_{max} MeOH n.m: 249, IR (KBr, ν_{max}, cm^{-1}): 3450, 2950, 2910, 2850, 1630, 1365, 1320, 1220, 1030, 900, 840, ^1H NMR (D_2O, 90 MHz, δ): δ 9.18 (1H, s, H-11), 7.48 (1H, s, H-3), 5.54 (1H, d, $J_{1.9}$ = 1.5 Hz, H-1), 4.88 (1H, d, $J_{1.2}$ = 7.5 Hz, H-1), 3.00 (1H, m, H-5), 2.5-1.2 (6H, C-9, C-8, C-7.C-6), 1.15 (3H, d, $J_{10.8}$ = 6.0 Hz MeOH). The data is well in accordance with the reported data.

Plantarenaloside (TSR-V) buff coloured amorphous powder, $[M]^+$ m/z : 360, $C_{16}H_{24}O_9$ $[\alpha]_D^{25}$ -188.8º, λ_{max} MeOH nm: 241, IR (KBr, ν_{max}, cm^{-1}): 3450, 2950, 2910, 2850, 1630, 1365, 1320, 1220, 1030, 900, 840, ^1H NMR (D_2O, 90 MHz, δ): δ 5.93 (H-1, s), 7.56 (H3, s), 0.90 (H10, d, 7.0), 9.23 (H-11, s), 4.83 (H-1^1, d.7.5), D_2O 97.3 (C-1), 165.5 (C-3), 125.0 (C-4), 72.9 (C-5), 38.3 (C-6), 32.2 (C-7), 34.2 (C-8), 51.7 (C-9), 15.9 (C-10), 194.6 (C-11), 99.4 (C-1^1), 73.2 (C-2^1), 76.1 (C-3^1), 61.5 (C-6^1). The data is well in accordance with the reported data.

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE TWO ISOLATES AND ETHYL ACETATE, METHANOLIC EXTRACTS OF THE ROOTS OF *T. stans*

Extracts	Zone of Inhibition											
	Gram +ve bacteria				Gram –ve bacteria				Fungi			
	B.s	*B.p*	*S.a*	*S.p*	*E.c*	*Pr.v*	*P.a*	*Ps.v*	*A.n*	*C.a*	*S.c*	*R.o*
Ethyl acetate extract (100 µg/mL)	13	10	17	12	14	13	12	11	09	10	12	09
Ethyl acetate extract (300 µg/mL)	17	15	20	16	19	18	15	20	14	12	15	11
Methanolic extract (100 µg/mL)	20	18	18	19	16	15	19	16	14	11	12	13
Methanolic extract (300 µg/mL)	25	23	21	24	21	22	24	22	17	15	15	16
Benzyl penicillin (10 µg/mL)	27	27	26	29	26	25	26	27	-	-	-	-
Ketaconazole (10 µg/mL)	-	-	-	-	-	-	-	-	23	20	20	21
TSR-IV (10 µg/mL)	19	17	16	18	15	14	17	16	14	12	15	13
TSR-V (10 µg/mL)	16	15	13	14	16	15	13	16	15	13	14	15
Ethyl acetate (Control)	-	-	-	-	-	-	-	-	-	-	-	-
Methanol (Control)	-	-	-	-	-	-	-	-	-	-	-	-

#Values are the average of triplicate, includes cup diameter (6 mm);
B.s = Bacillus subtilis; E.c = Escherichia coli; A.n = Aspergillus niger; B.p = Bacillus pumilis; P.a = Pseudomonas aeruginosa; C.a = Candida albicans; S.p = Streptococcus pyogens; Pr.v = Proteus vulgaris; S.c = Saccharomyces cerviceae; S.a = Staphylococcus aureus; Ps.v = Pseudomonas vulgaris; R.o = Rhizopus oryzae

Antimicrobial activity of the two isolates and the root extract of *T. stans*: Antibacterial and antifungal activity were studied by agar cup plate method. Two concentrations (100 µg/mL, 300 µg/mL) of ethyl acetate, methanolic extracts of *T. stans* roots and 10 µg/mL concentration of TSR-IV and TSR-V were evaluated for their antibacterial and antifungal activity. Benzyl penicillin and ketaconazole were used as standards (10 µg/mL). Methanolic extract exhibited antibacterial activity against *B. subtilis*, *S. pyogens*, *B. pumilis*, *Ps. vulgaris*, *Pr. vulgaris*, *P. aeruginosa* and it was dose dependent. It also showed considerable activity on the tested fungi, *S. cerviceae*, *R. oryzae*. Whereas ethyl acetate extract exhibited moderate activity against *S. aureus*, *E. coli*, *Pr. vulgaris* and mild antifungal activity on the tested fungi. Among the compounds tested, the compound TSR-IV exhibited considerable activity against *B. subtilis*, *S. pyogens*, *B. pumilis* and *Ps. vulgaris* and showed moderate activity against other organisms. Whereas TSR-V exhibited moderate activity against *B. subtilis*, *E. coli* and *Pr. vulgaris*, mild activity against *P. aeruginosa*, *S. pyogens* and *B. pumilis*.

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