World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Constituents from the Leaves of *Tecoma stans* Juss

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Received: 20-10-2016 / Revised: 16-11-2016 / Accepted: 20-11-2016 / Published: 26-11-2016

ABSTRACT

Tecoma stans Juss (Bignoniaceae) has wide range of therapeutic uses and is used in the treatment of diabetes, digestive problems, infections, diuretic and vermifuge. In an attempt to isolate the phytoconstituents from the leaves of *Tecoma stans*, chloroform and methanolic extracts were subjected to column chromatography which resulted in two iridoid glycosides, 5-deoxystansioside, plantarenaloside apart from β-sitosterol, lupeol, luteolin and ursolic acid. Screening of the methanolic extract for antimicrobial activity showed moderate antimicrobial activity.

Key Words: *Tecoma stans*, Bignoniaceae, Leaves, Iridoids, Triterpenes, Antimicrobial

INTRODUCTION

Tecoma stans Juss is a flowering perennial shrub that grows to a height of 5-7.6mts. Many biologically active compounds and their extracts have been used in traditional medicine [1,3]. Two alkaloids tecomanine and tecostanine were reported and identified as hypoglycemic agents [4-8]. Phytosterols, monoterpenes, triterpenes, flavonoids, phenols, saponins, and iridoid glycosides were reported from the leaves and roots of *T. stans*[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. ^{1}HNMR spectra were taken on BRUKER AM 400 with TMS as an internal standard.

Collection of the plant material: The plant material was collected from Guntur, Andhra Pradesh and the identity was established by Dr.M.Venkaiah, Department of Botany, Andhra University, Visakhapatnam.

***Extraction of the plant material and isolation of phytoconstituents*:** The freshly collected leaves (1kg) of *T. stans* were dried, powdered and extracted with petroleum ether, chloroform and methanol separately for 6 hours respectively. The extracts were concentrated under vacuum to give the corresponding residues. The TLC profile of chloroform and methanolic extracts were similar, so the chloroform and methanol extracts were combined and column chromatographed over silicagel (100-200) and eluted with petroleum ether, chloroform and methanol in order of polarity and 250ml fractions were collected. Each fraction after concentration was monitored on TLC. Six compounds namely β-sitosterol, lupeol, luteolin, 5-deoxystansioside, plantarenaloside, and ursolic acid, were isolated and identified by chemical tests and spectral means.

Antimicrobial screening of the plant extract: Screening of the plant extract for antimicrobial activity was done by agar well diffusion method. chloramphenicol and fluconazole were used as a positive control for antibacterial and antifungal activities respectively.

Test microorganisms: Bacterial cultures of *Staphylococcus aureus, Streptococcus anginosus, Micrococcus luteus, Lactobacillus acidophilus, Streptococcus mutans, Proteus vulgaris, Erwinia carotovora, Enterobacter aerogens, Escherichia coli, Pseudomonas aeuriginosa, Klebsiella*

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pneumoni and fungal cultures *Aspergillus niger 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 was 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 extract were prepared in concentrations of 100mg/ml and 300mg/ml. 50µl of the extract was placed in the cups by sterile pipettes. In each plate one cup was used for chloramphenicol (1mg/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 30^0C 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 5µl 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. 50µl of the extract was placed in the cups by sterile pipettes. In each plate one cup was used for fluconazole (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 30^0C and were later examined by measuring the zones of inhibition and the results were tabulated.

RESULTS AND DISCUSSION

Characterization of the isolated compounds:
β-Sitosterol: crystallized from hexane as colorless fine needles, m.p-134º-136ºc, gave positive color reaction for sterols with Liebermann Buchard test. The IR spectrum showed absorption bands at 3440, 2970, 2050, 1470, 1385, 1055 cm^{-1}. The ^{1}H NMR spectrum showed peaks at δ 0.80-1.25 (methyls), δ 3.45(1H broad C_3α-H) and δ 5.30 (1H, m, C_5-H).

Lupeol: crystallized from chloroform-petroleum ether as needles, m.p -195^0C,gave pink colour 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 ^{1}HNMR spectrum (300MHz,$CDCL_3$,δ):O.76-1.02(18H,s,6xMe), 1.65(3H,S,=CCH_3),2.25(1H,d,19-H),15(1H,m,3α-H), 4.6-4.7(2H,d,=CH_2, Vinylic protons).

Luteolin: crystallized in chloroform-methanol as yellow needles, m.p.333-334^0C. It gave green color with ferric chloride test and orange color with shinoda test. λ_{max} MeOH nm: 243sh,254,266sh and 352. $AlCl_3$:264sh, 328sh, 402, $AlCl_3$:/HCl, 276sh, 276, 296sh, 356, 385NaOAc:269,326sh, 387.

5-Deoxystansioside: crystallized in chloroform-methanol as yellow needles, m.p 146-147^0C. It gave blue colour with Wieferring test. IR: 3450, 2950, 2910, 2850, 1630, 1365, 1320, 1220, 1030, 900, 840. ^{1}HNMR (90MHz,D_2O,):9.18 (1H,S,H-II), j.45(IH,s,H-3),5.54(1H,d,J1-2=7.5Hz,H-1), 3.00(1H,m,H-5),2.5-1.2(6H,C-9,C-8,C,V,C-6),1.15(3H,d,$J_{10.8}$=6.0Hz,Me-10)

Plantarenaloside: was crystallized in chloroform-methanol as yellow needles, m.p146-147^0C. It gave blue colour with Wieferring test. λ_{max} MeOH nm: 241 nm. ^{1}HNMR (90MHz,D_2O,): 5.93(H-1,s), 7.56(H-3,s) 0.90(H-10,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), 1954.6(C-11), 99.4(C-1^1), 73.2(C-2^1), 76.1(C-3^1), 61.5(C-6^1).

Ursolic acid: was crystallized in chloroform-methanol as yellow needles, m.p285^0C. It gave pink color with Libermann –Buchard test. IR v Max (KBr) cm^{-1} 3390, 2944, 2857, 1696, 1455, 1386, 1259, 1143, 1172.^{1}HNMR (300MHz,$CDCl_3$,δ):3.2(1H,s,br,H-3), .4(1H,s,br,H-12),2.7(1H,m,H-18), 1.26(3H,s,h-23), 1.01(3H,s,H-24) 0.84(3H,s,H-25), 1.04(3H,s,H-26) 1.26(3H,s,H-27), 0.98 (3h,d,H-29), 0.91(3H,d,H-30).

Antimicrobial activity: The methanolic extract of the leaf of *Tecoma stans* showed moderate activity against Gram (+) ve and Gram(-) ve bacterial and fungal strains . The activity of the extracts increased with increasing concentrations.

CONCLUSION

The methanolic extract of the leaf of *Tecoma stans* resulted in isolation of in two iridoid glycosides, 5-deoxystansioside, plantarenaloside apart from β-sitosterol, lupeol, luteolin and ursolic acid. The methanolic of the extract leaf of *Tecoma stans* showed moderate antimicrobial activity.

TABLE 1: Antibacterial activity of the methanolic extract of leaves of *Tecoma stans*

S. No	Name of the extract	Diameter of zone of inhibition											
		Gram (+) ve bacteria						Gram (-) ve bacteria					
		S.a	*S.an*	*B.s*	*M.l*	*L.a*	*S.m*	*P.v*	*E.r*	*E.a*	*E.c*	*P.a*	*K.p*
1	Methanolic extract of *T.stans* (100mg/ml)	12	11	20	13	10	13	15	13	12	13	12	9
2	Methanolic extract of *T. stans* (100mg/ml)	14	12	30	17	12	14	17	14	17	16	16	10
3	Standard chloramphenicol (1mg/ml)	27	34	50	30	30	35	29	15	25	20	45	45
4	Control (Methanol)	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2: Antifungal activity of the methanolic extract of leaves of *Tecoma stans*

S.No	Name of the extract	Diameter of zone of inhibition	
		A.n	C.a
1	Methanolic extract of *T.stans* (100mg/ml)	9	8
2	Methanolic extract of *T. stans* (300mg/ml)	12	10
3	Standard Fluconozole (10 mg/ml)	22	20
4	Control (Methanol)	-	-

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