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Chemical and Antimicrobial Evaluation of the Leaves of *Talinum triangulare* (Jacq) Willd

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The methanolic extracts of *Talinum triangulare* on chromatography yielded five compounds, which were identified as β-sitosterol, oleanolic acid, oleanolic acid glycoside, oleanolic acid rhamno glucoside and β-sitosterol-3-β-D-glucoside. These were characterized by chemical tests and spectral means (UV, IR, MS, ^{1}H NMR). The methanolic extract of *Talinum triangulare* was also screened for antimicrobial studies. A moderate activity was observed on tested organisms.

Key Words: *Talinum triangulare*, Methanolic extract, Antimicrobial activity.

INTRODUCTION

Talinum triangulare (Jacq) Willd. (Portulacaceae) is a green leafy vegetable[1-3] commonly grown in south India. It is known as Ceylon spinach in western countries. *Talinum* is one of the largest genus of portulacaceae family and consists of 25 species world wide. Out of these only four species are available in India. The herb is medicinally used by local tribes to cure inflammations, ulcers, diarrhea and dysentery. It is reported to have antioxidant and cytoprotective activity. The herb is reported to contain saponin glycosides[4-6].

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.

The leaves of *T. triangulare* were collected from in and around Andhra University campus, during september 2003 and authenticated by Dr. M. Venkaiah, taxonomist, Department of Botany Andhra University, Visakhapatnam, a voucher specimen (SG/TT/9/2003) was deposited in the institution herbarium. The fresh leaves of *T. triangulare* of about 1.5 Kg were extracted with methanol and concentrated under vaccum to get the corresponding residue of 40 g.

The methanolic residue was column chromatographed on silicagel (60-120 mesh, Acme) using gradient elution which afforded five compounds, which were designated as TTL-I, TTL-II, TTL-III, TTL-IV and TTL-V. The compound TTL-I was obtained from 50 % chloroform in hexane which on crystallization gave colourless needles, m.p. 136-138 ºC. It gave yellow colour with concentrated sulphuric acid, play of colours with Libermann Burchard test and deep red colour with Salkowski reaction. The compound TTL-II was obtained from pure chloroform which on crystallization gave fine needles of m.p. 272-274 ºC. It gave pink colour with Libermann Burchard test. Another compound TTL-III was obtained from 2 % methanol in chloroform which on crystallization gave white flakes, m.p. 306-308 ºC. It gave (+) ve Molisch's test for glycosides and Libermann Burchard test. The compound TTL-IV was obtained from 5 % methanol in chloroform which on crystallization gave white solid, m.p. 308-310 ºC. It gave (+) ve Molisch's test and Libermann Burchard test. The other compound TTL-V was obtained from 10 % methanol in chloroform which on crystallization gave colourless flakes, m.p. 289-292 ºC. It gave yellow colour with concentrated sulphuric acid, play of colours with Libermann Burchard test and deep red with Salkowski reaction.

RESULTS AND DISCUSSION

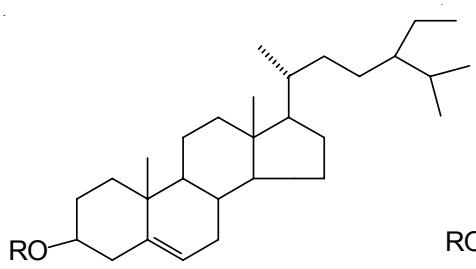
Phytochemical examination of the leaves of *T. triangulare* afforded five compounds and were identified by their spectral data and elemental analysis. They were designated as TTL-I, TTL-II, TTL-III, TTL-IV and TTL-V. β-sitosterol (TTL-I) colourless needles, m.p. 134-138 ºC, $[\alpha]_D^{30}$ + 36º (C, 1.101 in chloroform), $C_{29}H_{50}O$, Found (%): C, 83.7; H, 12.7, requires (%): C, 83.0; H, 12.2, $[M]^+$ m/z: 414, IR (KBr, ν_{max}, cm^{-1}): 3440, 2970, 2880, 2050, 1470, 1385, 1055, ^1H NMR: 0.67 (s, 3H), 0.83 (d, 6H) 0.92 (s, 6H), 3.5 (m, 1H), 5.34 (d, 1H). The identity of TTL-I was confirmed by comparison with authentic sample through m.m.p and co-TLC.

Oleanolic acid (TTL-II) fine needles, m.p.272-274 ºC, $[\alpha]_D^{90}$ + 64º (C, 0.5 in methanol), $C_{30}H_{47}O_3$, Found (%): C, 78.5; H, 10.6, Requires (%): C, 78.6; H, 10.5, $[M]^+$ m/z: 455, IR (KBr, ν_{max}, cm^{-1}): 3470, 1693, ^1H NMR ($CDCl_3$, 90 Hz, δ): 1.03 (s, 3H), 0.98 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H), 0.68 (s, 3H), 0.65 (s, 3H). The identity of TTL-II was confirmed by comparison with authentic sample through m.m.p and co-TLC.

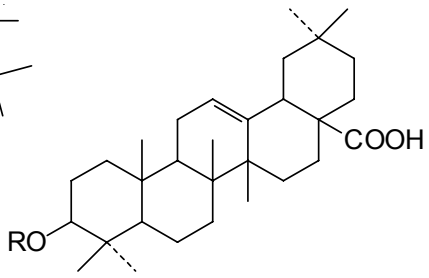
Oleanolic acid glycoside (TTL-III) white flakes, m.p. 306-308 ºC, $[\alpha]_D^{30}$ + 67º (C, 0.5 in methanol), $C_{36}H_{59}O_9$, Found (%): C, 68.2; H, 9.1, Requires (%): C, 68.0; H, 9.2, $[M]^+$ m/z: 635, IR (KBr, ν_{max}, cm^{-1}): 3470, 1693, ^1H NMR ($CDCl_3$, 90 Hz, δ): 1.03 (s, 3H), 0.98 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H), 0.68 (s, 3H), 0.65 (s, 3H), 5.30-5.40 (m, glycosidic), 5.30 (m, 1H). The identity of TTL-III was confirmed by comparison with authentic sample through m.m.p and co-TLC.

Oleanolic acid rhamno glycoside (TTL-IV) white solid, m.p. 308-310 ºC, $[\alpha]_D^{90}$ + 67º (C, 0.5 in methanol), $C_{30}H_{47}O_3$, Found (%): C, 64.7; H, 8.5, Requires (%): C, 64.9; H, 8.7, $[M]^+$ m/z: 635, IR (KBr, ν_{max}, cm^{-1}): 3470, 1693, ^1H NMR ($CDCl_3$, 90 Hz, δ): 1.03 (s, 3H), 0.98 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H), 0.68 (s, 3H), 0.65 (s, 3H), 5.30-5.40 (m, glycosidic), 5.30 (m, 1H). The identity of TTL-IV was confirmed by comparison with authentic sample through m.m.p and co-TLC.

β-Sitosterol-3-O-β-D-glucoside (TTL-V) colourless flakes, m.p. 289-292 ºC, $[\alpha]_D^{90}$ - 27.5, (C, 1.101 in pyridine), $C_{25}H_{60}O_6$, Found (%): C, 74.1; H, 10.8, Requires (%): C, 74.3; H, 11.2, $[M]^+$ m/z: 456, IR (KBr, ν_{max}, cm^{-1}): 3440, 2970, 2880, 2050, 1470, 1385, 1055, ^1H NMR ($CDCl_3$, 90 Hz, δ): 0.67 (s, 3H), 0.83 (d, 6H), 0.92 (s, 6H), 3.5 (m, 1H), 5.34 (d, 1H). The aglycone was identified by its ^1H NMR data, the sugar was identified in descending paper chromatography by eluting with solvent system butanol: pyridine:water (6:4:3) and by spraying 1 % silver nitrate in acetone and sodium hydroxide. Its identity was further confirmed by comparison with authentic sample.



TTL-I, R = H
TTL-V, R = Glu



TTL-II, R = H; TTL-III, R = Glu
TTL-IV, R = Rham-glu

Antimicrobial activity: Antibacterial and antifungal activities were studied by agar cup plate method. Two concentration (100 µg/mL, 300 µg/mL) of methanolic extract of *T. triangulare* were evaluated for their antibacterial and antifungal activities. Benzyl penicillin and nystatin (10 µg/mL) were used as standards. The methanolic extract showed considerable

activity against *Staphylococcus aureus, Bacillus subtilis* and no activity against *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Pseudomonas vulgaris*. The extract exhibited very mild antifungal activity against *Candida albicans* and *Rhizopus oryzae* (Table-1).

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE METHANOLIC EXTRACT OF THE LEAVES OF *T. triangulare*

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*
Methanolic extract (100 µg/mL)	12	13	14	10	–	–	–	–	8	8	7	9
Methanolic extract (300 µg/mL)	17	16	18	14	–	–	–	–	11	12	10	13
Benzyl penicillin (10 µg/mL)	22	19	24	20	26	24	22	22	–	–	–	–
Nystatin (10 µg/mL)	–	–	–	–	–	–	–	–	19	20	19	21
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*

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