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Chemical and Antimicrobial Studies of the Roots of *Tephrosia villosa* (L) Pers

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Tephrosia villosa (L) Pers roots on chemical examination and sequential chromatography gave four compounds, stigmasterol, lupeol, rotenone and dehydrorotenone. Their structures were determined by various spectral techniques (^{1}H NMR, IR, UV, MS) and elemental analysis. When the extracts were subjected to antimicrobial studies, a moderate activity was observed on tested bacteria and fungi.

Key Words: *Tephrosia villosa*, Rotenone, Antimicrobial activity.

INTRODUCTION

Tephrosia villosa (L) Pers a member of Leguminosae is a small perennial herb, distributed throughout the plains of India. Genus *Tephrosia* is estimated to contain 300 species. Most of which are herbs and few are shrubs. *Tephrosia* species are distributed chiefly in the warmer areas of Asia and Africa of which 24 species are found in India. The juice of the leaves of *T. villosa* is used traditionally for dropsy and diabetes[1,2] by the local tribes. The various species of the genus are used for stupefying and catching fish. The pisicidal and insecticidal properties were due to the presence of rotenoids[3]. Earlier *T. villosa* is reported to contain rotenoids and flavonoids[4-10].

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 and thin layer chromatography 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 roots of the *T. villosa* (1.5 Kg) were collected from Andhra University campus, Visakhapatnam during september 2003 and they were authenticated by Dr. M. Venkaiah, Taxonomist, Department of Botany Andhra University, Visakhapatnam. A voucher specimen (SG/TV/9/2003) is deposited in the institution herbarium. The material was shade dried and coarsely powdered. About 1 Kg of the powdered roots were extracted with hexane, ethyl acetate and methanol successively, the extracts were concentrated under reduced pressure to get corresponding residues of 7.5, 17.8 and 27.2 g, respectively. The ethyl acetate and methanol residues of *T. villosa* 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 four compounds. Which were designated as TVR-I, TVR-II, TVR-III, TVR-IV. The compound TVR-I was obtained from 5 % ethyl acetate in hexane which on crystallization gave feathery needles of m.p. 169-170 ºC. The compound showed yellow colour with conc. sulphuric acid, play of colours with Leibermann-Burchard test, deep red with Salkowski reaction. The another compound TVR-II was obtained from 10 % ethyl acetate in hexane which on crystallization gave white amorphous solid, m.p. 212-214 ºC. It gave yellow colour with Salkowski and rose red colour with L.B. reaction. The compound TVR-III was obtained from 50 % ethyl acetate in hexane which on crystallization gave fine colourless needles of m.p. 164-166 ºC. It gave deep red colour with Durham's test. Orange colour with shinoda's test. The compound TVR-IV was obtained from 90 % ethyl acetate in hexane which on crystallization gave colourless needles of m.p.218-219 ºC from chloroform: methanol (1:9). It gave deep red colour with Durham's reagent.

RESULTS AND DISCUSSION

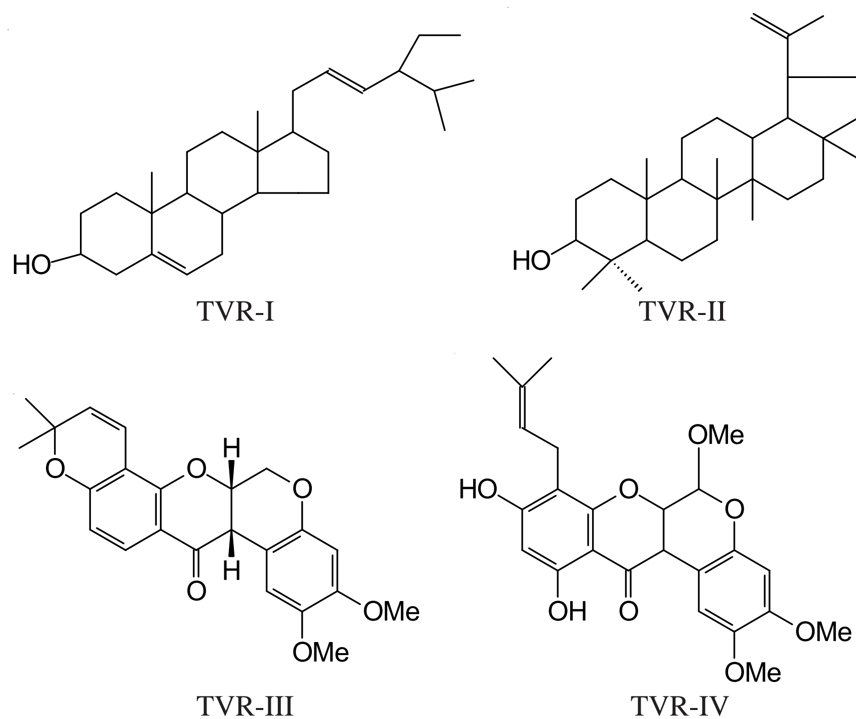
Phytochemical examination of the roots of *T. villosa* has been carried out. The combined root extacts of ethyl acetate and methanol on column chromatography afforded four compounds, TVR-I, TVR-II, TVR-III and TVR-IV. These compounds were identified as stigmasterol, lupeol, rotenone and 6a,12a-dehydro-2,3,6-trimethoxy-8-(31,31-dimethyl allyl)-9,11-dihydroxyrotenone.

Stigmasterol (TVR-I) feathery needles, m.p. 169-170 ºC, $[M]^+$ m/z: 412, $[\alpha]_D^{90}$ +36º (C, 0.97 in chloroform), $C_{29}H_{48}8O$, Found (%): C, 84.3; H, 11.9. Requires (%): C, 84.4; H, 11.7, IR (KBr, ν_{max}, cm^{-1}): 3450, 1170, 1132, 1074, 991, 971 ^{1}H NMR: δ 0.86, 1.25 (Me), 3.47 (1H, broad, 3-H) and 5.32 (1H, m, 5-H), the identity of TVR-I was confirmed by comparison with authentic sample through m.m.p and Co-TLC.

Lupeol (TVR-II) colourless needles, m.p. 212-214 ºC, $[M]^+$ m/z : 452, $[\alpha]_D^{30}$ + 38º (C, 0.93 in chloroform), Found (%): C, 84.5; H, 11.4, Requires (%) C, 84.5; H, 11.8, m.f. $C_{32}H_{52}O$, IR (KBr, ν_{max}, cm^{-1}): 3540, 3010, 2860, 1610, 1425, 1380, 1320, 1355, 165, 1080, 1018, 890, ^1H NMR ($CDCl_3$, 90 MHz, δ): δ 0.76 (d, 3H), 0.78, 0.80, 0.90, 1.02 (s, 15H), 1.63 (s, 3H), 0.91 (s, 6H), 3.18 (m, 1H), the identity of TVR-II was confirmed by comparison with authentic sample through m.m.p and Co-TLC.

Rotenone (TVR-III) fine colourless needles, m.p. 164-166 ºC, $[M]^+$ m/z: 455, $[\alpha]_D^{27}$ + 112.08º (C, 0.816 in chloroform), $C_{30}H_{47}O_3$, Found (%): C, 70.01; H, 5.50; Requires (%): C, 70.05, H, 5.38, IR (KBr, ν_{max}, cm^{-1}): 1680, 1580, 1560, 1550, 1530. ^1H NMR ($CDCl_3$, 90 MHz, δ): δ 6.77, 6.78, 6.50, 3.80, 3.90. The identity of TVR-IIl was confirmed by comparison with authentic sample through m.m.p and co-TLC.

6a,12a-Dehydro-2,3,6-trimethoxy-8-(31,31-dimethylallyl)-9,11-dihydroxyrotenone (TVR-IV) fine colourless needles, m.p.218-219 ºC, Found (%): C, 65.15; H, 5.54, Requires (%): C, 65.45; H, 5.54, $[M]^+$ m/z: 440, $C_{30}H_{47}O_3$, UV λ_{max} MeOH n., : 224, 277, 325, IR (KBr, ν_{max}, cm^{-1}): 3400, 1650, ^1H NMR (D_2O 90 MHz, δ): 1.76, 1.86 (s, 3H), 3.49 (d, 2H, = 7.0 Hz, H-1^1), 5.24 (t, 1H, J = 7.0 Hz, H-2^1) 3.60, 3.91, 3.96 (s, 3H), 8.44, 6.65 (s, 1H), 12.81 (s, 1H), 6.03 (s, 1H, 5.74 (s, 1H)., The data is well in accordance with the reported data.



Antimicrobial activity: Antibacterial and antifungal activities were studied by agar cup plate method. Two concentrations (100 µg/mL, 300 µg/mL) of ethyl acetate and methanolic extracts were evaluated for their antibacterial and antifungal activities. Benzyl penicillin and ketoconazole were used as standards (10 µg/mL). Methanolic extract showed activity against *Bacillus pumilis, Staphylococcus aureus, Eschirechia coli* and *Pseudomonas vulgaris*, mild activity against *Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus vulgaris*. Where as ethyl acetate extract showed moderate activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogens, Proteus vulgaris, Pseudomonas vulgaris*. Both the extracts exhibited moderate antifungal effect on *Aspergillus niger, Saccharomyces cerviceae, Candida albicans, Rhizopus oryzae*.

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE ETHYL ACETATE AND METHANOLIC EXTRACTS OF THE ROOTS OF *T. villosa*

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	07	10	11	15	12	09	12	09	13	11	14
Ethyl acetate extract (300 µg/mL)	19	10	13	17	18	13	11	13	12	20	16	16
Methanolic extract (100 µg/mL)	11	16	17	13	19	14	17	12	12	10	12	12
Methanolic extract (300 µg/mL)	16	20	21	16	22	15	21	16	16	17	14	16
Benzyl penicillin (10 µg/mL)	26	25	23	22	23	18	20	23	-	-	-	-
Ketaconazole (10 µg/mL)	-	-	-	-	-	-	-	-	19	17	22	18
Methanol (Control)	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate (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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