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CHEMICAL CONSTITUENTS OF *TEPHROSIA PURPUREA* FAMILY: *LEGUMINOCAE*

V. K. AGNIHOTRY, S. P. SHARMA*, N. AGNIHOTRI and R. C. SAXENA

Department of Chemistry, S. S. L. Jain College, VIDISHA (M.P.) INDIA
[a]Pest Control & Ayurvedic Drug Research Lab., VIDISHA – 464001 (M.P.) INDIA

ABSTRACT

A triterpenoid saponin was isolated from *Tephrosia purpurea* family leguminocae. It showed maximum 79% yield in water extract over soxhletion, which afforded three fractions. Fraction CF3 was found biologically active. F2 shows 84% mass cell stabilizing activity. This fraction upon isolation and purification yielded a biologically active principles.

Key words: *Tephrosia purpurea*, Saponin, Isolation, Characterization.

INTRODUCTION

Saponin are secondary metabolite synthesize by the Angeospermic plants. They are more widely distributed among the plant of family Leguminosae. Saponin as the main indicate show soap like properties. When a small amount of powder material mix with water attach to gives foam which persist for a longer period indicating the presence of saponin. Saponin of *Tephrosia purpurea* have been shown as anti asthmatic against Horse serum induced asthma in Albino rats[1]. Similarly Soni et al. have shown the soap like property of saponin and isolated sapogenin like active principle from plant of leguminosae.

Tephrosia purpurea roots yielded compound which is a crystalline material containing isoflavon[2]. Pod of this plant *T. purpurea*. A is insecticidal[3]. Sing et al. 2007τ have reported various biological activity of plant occimum including immuno modulatory and antiinflamatory activities.

*Author for correspondence; Ph.: (M) 9827099045; E-mail: rcsvds@yahoo.com

EXPERIMENTAL

Plant material

The plant *Tephrosia purpurea* was authenticated by S. K. Jain, Department of Botany. After identification a voucher specimen was procured in herbarium record at serial No. 35.

Extraction

Extraction of the plant materials is needed for the study of phytochemical or phytopharmacological properties. The extraction of shade dried powderd material (40-60 mesh size) of plant *Tephrosia purpurea* was carried out separately by Soxhlet apparatus in the laboratory using different solvents of increasing order in polarity. The different solvent used for the extraction purpose depend on the chemical nature and corresponding the active principles of the compound to be separated out from the particular plant material. The extraction procedure adopted was given by Harborne (1984)[5]. The made of extraction of plant material depends on the texture and water content of the plant material so the substance to be extracted either in alcohol or in water. Keeping this fact in view, in the present study, the plant material was mainly extracted in 90% methanol and water. The methods used for extraction of plant materials are soxhlet extraction method.

Source of chemical

All chemicals which were used are purchased from Sigma Aldrich Limited, USA. All other reagent and chemical were of analytical grade.

Chromatographic analysis

Chromatographic analysis of crude extract was carried out using the following techniques:

(i) Column chromatography (Stock & Rice 1974)[6]

(ii) Thin layer chromatography (Brimley & Barrett)[7]

(iii) Acid hydrolysis

(iv) Methylation

Animal material

Albino rats of either sex (Sprague Dawley or Wister Strain), weighing between 200-250 g were used for studies carried out in rat models. Albino mice of either sex (Balb/c strain), weighing between 20-22 g were used for study carried out in mice model.

Animals were housed in an air conditioned room (25 ± 2°C, 30-65% RH) in plastic cages (4 animals per cage at the most) having paddy husk (Shree Dutt Agro Pvt. Ltd. Vadodara) as bedding with 12 hr light-12 hr dark cycles. They had free access to pelleted diet (Pranav Agro Foods Pvt. Ltd, Vadodara) and tap water.

All experimental protocol was approved by the IAEC as per guide line CPCSEA (Regd. No. 804/03/CA/CPCSEA).

RESULTS AND DISCUSSION

Based on the above spectral data a saponin like compound was isolated from *Tephrosia* purpure. Which showed anti asthmatic activities in Horse serum and triple antigen 0.5 induced asthma in rats. The compound isolated from the plant showed more than 79% intact mass cell against 84% in standard drug pridenesolono thus the compound isolated from *Tephrosia purpurea* caused inhibition of granulation in the mass cell which is an anti asthmatic activities. Similar antihistaminic activities has also been reported earlier[4] by Khare and Saxena[8]. Soni et al.[9] and Patel et al.[10] Recently a phyto-chemical study of antimicrobial activity at *Tephrosia purpurea* have been reported by Murganathan et al. 2007[11].

Table 1: Percentage yield of soxhlet extraction of crude extract of *Tephrosia purpurea*

Plant name	Solvent used	Weight of powdered plant material	Volume of solvent	Weight of extract	Percentage yield of extraction
Tephrosia purpurea	Chloroform	500 mg	1000 mL	14.5 g	29.0%
	Petroleum ether	500 g	1000 mL	12.4 g	24.8%
	Methanol	500 g	1000 mL	18.0 g	36.0%
	Water	500 g	1000 mL	20.5 g	41.0%

Table 2: Thin layer chromatography of 90% MeOH extracts of plant materials with R_f value and the colour characteristics

Crude extract of plant material	Solvent system	Spot	R_f value	Visual light	Iodine chamber	UV light
Tephrosia purpurea	n-Butanol NH_4OH (1 : 1)	CF_1	0.78	Dark green	Green	Yellowis green
		CF_2	0.61	Yellow	Yellowish green	Yellow
		CF_3	0.47	Light green	Light green	Green

Table 3: Column chromatography of 90% methanolic crude extract *of Tephrosia purpurea*

Solvent system	No. of fraction	Weight of fraction (g)	Colour of fractions	Fraction used
n-butanol : NH_4OH (1 : 1)	TF_1	0.60	Dark green	TF_1 & TF_3 were used for spectral analysis and antibacterial activity
	TF_2	0.59	Green	
	TF_3	0.70	Brown	
	TF_4	0.50	Dark brown	

^{1}H NMR

The ^{1}H NMR spectrum of compound from *Tephrosia purpurea* at 400.5 MHz showed the signal for different proton present in the compound, the chemical shift of range ^{1}H NMR 8.7, 2.60-1.010.

The ^{1}H NMR spectrum of **1** displayed signals for 7 protons. In the chemical shift range of 7.01 to 7.71 Hz with typical coupling constant of 6.0-8.0 Hz are appropriate the aromatic protons, indicating a doublet substituted benzene ring II, which confirmed the 3-O-glycosides structure. Acid hydrolysis with 2% HCl afforded the aglycone and two sugar moieties, removal of one galactose unit followed by one rhamnose.

Proton-NMR also supported the presence of one rhamnose and one galactose unit with the glactose at δ 5.57 indicating a β-linkage of that galactose unit having large coupling

constant (J = 7.8 Hz), ^{13}C NMR spectrum of fraction AF_2 also indicated the presence of three methyl (two methoxy and one of rhamnose).

^{1}H NMR sperum of compound indicating the presence of a methylene dioxy group at 5.575 MHz attached to an aromatic nucleus.

The ^{1}H NMR signals at δ 2.59 (1 Hm) showed the protons of the γ-dihydropyrone moiety of a hemoisaflavone. In addition two benzyl methylene protons appeared at δ 2.577 (2H-add) J = 12, 8, 4, H_2. 7.21 (2H, J, H-2-4-6) melting point range is 173-175°C. Chemical shifting in active compound was mentioned in Table 4.

Table 4: ^{1}H NMR of a *Tephrosia purpurea*

δ H (J in Hz)	Position
7.260	1HdJ = 2 H_2H-2' & H-6'
5.575	2"(t)-10
3.640	3"(6H.S.2 x 0 Me)
2.577	2HdddJ = 12, 8, 4 Hz
2.271	2" 3-H
2.043	1.2-H, S 4 XAc
1.525	3'
1.507	3H,S Me-4"
1.425	3H, S Me-5"
1.370	3H d J = 6Hz
1.333	3"
1.146	3"
1.024	5" d (7)
1.010	3H, S, H
0.998	3HdJ = 5.8Hz
0.859	3H S 4 XCH_3
0.804	3H S Me
0.692	4"'d (7)

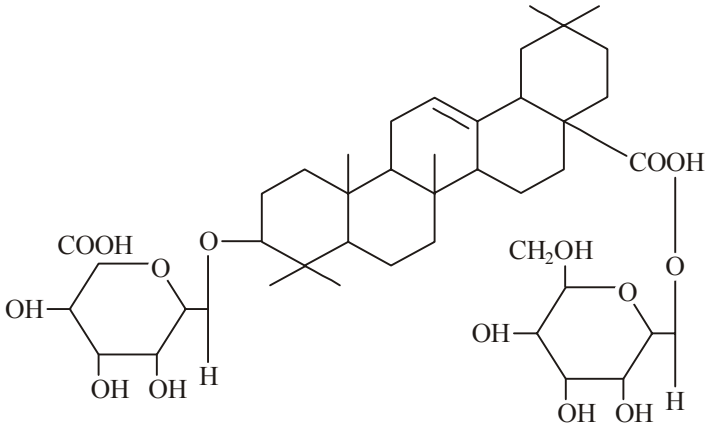
^{13}C NMR

^{13}C NMR was done at 100.60 MHz processing parameters. The ^{13}C signal at 167.1 indicated the presence of a carboxyl group, which was attached to a benzene ring II. In addition the base peak at m/z 107 in the mass spectrum confirmed the presence of a benzoyl group 'A'. The signal at d 2.046 in the ^{1}H NMR spectrum and ^{13}C NMR shift at 170.2 indicated an acetoxy group probably linked to a six membered ring II.

MASS

The FAB mass spectrum of compound obtained from *Tephrosia purpurea* showed the molecular ion peak at m/z 358 which revealed the molecular formula $C_{36}H_{45}O_{15}$ for this compound. Output range : 107 to 941. $[M^{-1}]$ 91 (C_7H_8) 154 $[C_{10}H_{18}O]^+$ 192 ($C_{12}H_2O$) + 230 $[C_{16}H_{22}O]^+$.

Structure of the active compound



Triterpenoid

Spectral data of compound

Blackish green powder m.p. 173-175°C, R_f value 0.67 (n-butanol : NH_4OH, 1 : 1) highly soluble in chloroform. IR ν_{max} KBr cm^{-1} : 3693.0, 3021, 2926.5, 2858.3, 1595.2, 1216.3, 762, 671.

Tephrosia purpurea which is locally known as Sharpankho (wild indigo) was extracted, isolated and purified using different methods[11]. Evaporation was performed under reduce pressure in Bucchi type rotator evaporator. M.P. was determined on an electro thermal micro electronic melting point apparatus. The IR spectrum was recorded on a

Shimadzu IR-470 A Spectrophotometer. The ^{1}H NMR and ^{13}C NMR, 1H-1H COSY, HSQC and DEPT spectra were taken in duterrated chloroform ($CHCl_3$) with TMS as an interned standard in a 400 MHz or 100 MHz instrument. The mass spectrum was recorded in EI ionization mode. FI mass spectrum of compound isolated from *Tephrosia purpurea* shown the molecular ion peak m/z 192. Which reveal the molecular formula $C_{36}H_{45}O_{15}$ for this compound. This showed mass cells stabilizing activities.

IR and UV

IR spectrum of compound was taken on Parkin Elmer model, shows single sharp peak at 3693 cm^{-1} indicative of the presence of O-H (Phenolic) sretching. A strong bond at 3031 cm^{-1} shows C-H stretching which showed aliphatic compound, the blunt peak as a sign 2858.3 cm^{-1} showed CH_2 stretching similarly, a blunt peak at 1595.2 cm^{-1} shows C = O stretching, sharp single peak at 762 cm^{-1} shows C-O-C stretching. IR spectrum was supported by the UV which shows the absorption for five region starting at 229.5 nm wave length to 204 nm wave length. The detail IR and UV spectrum and absorption was mentioned in Table (5) and (6).

Table 5: IR spectrum of *Tephrosia purpurea*

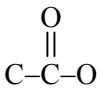
Peak	Characteristic	Wave length (cm^{-1})	Group band causing absorption
1	Single sharp short	3693.0	O-H stretching (Phenolic)
2	Single sharp long	3021.0	C-H stretching (Aromatic)
3	Single sharp	2926.5	-CH_2 Stretching (Aliphatic asymatic)
4	Blunt single	2858.3	-CH_2 Stretching
5	Blunt single	1595.2	C = O Stretching
6	Single sharp long	1216.3	
7	Blunt single	762	C-H out of plane
8	Single sharp long	671	O = C = O

Table 6: UV spectrum of *Tephrosia purpurea*

Position (nm)	Intensity
229.5	10.72
223	10
217	10
210	10
204	10

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