



CHEMICAL EXAMINATION OF THE ROOTS OF *POLYGONUM PLEBIEUM* R.BR.

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ABSTRACT

Chemical analysis of *Polygonus plebieum* roots (Polygonaceae) afforded Stigmasterol, 7-4-dimethoxy quercetin, Kaempferol, Quercetin, Myrecetin and Scutellarein from chloroform extract. The compounds were identified by chemical tests, chromatographic analysis and spectroscopy.

INTRODUCTION

Polygonum plebieum (Polygonaceae), common name "Bishkatali" is an annual odoriferous herb (50-90 cm) indigenous to Nepal and is widely distributed in Bangladesh, north-east India, China and Japan. The genus *Polygonum* is well-known for producing pharmacologically active substances and also for its therapeutic use in Oriental traditional medicine systems. Ethanolic extract of *Polygonum plebieum* is known to have anti-bacterial properties. Sesquiterpenes and flavonoid glycosides isolated from the plants are reported to have several pharmacological actions¹. Several biologically active substances were reported from the whole herb such as

viscoazusone, viscoazulone, viscoazulenic acid, viscoazulenic acid methyl ether, viscoazucine, viscozucenic acid, polygosomic acid, viscosomic acid, 3',5'-dihydroxy-3,4',5',7-tetramethoxyflavone, quercetin-3-O - (6''-caffeoyl) -β- D galactopyranoside, quercetin-3-O-(6''-feruloyl)-β-D-galactopyranoside and quercetin-3-O-(6''-galloyl)-β- D-galactopyranoside²⁻⁵. As a part of the ongoing chemical and bioactivity studies on the *Polygonum* genus, the author has taken up the roots of *Polygonum plebieum* for its bioactive constituents. The plant material was collected from north coastal districts of Andhra Pradesh, India and was extracted with chloroform (3 x 1.5 L)

for 24 hours. The combined extract was concentrated under reduced pressure, 14 g brown residue was yielded. The residue gave pink colour with Liebermann-Burchard test for triterpenoids and sterols, olive green colour with ferric chloride for phenols and magenta colour with Shinoda's test for flavonoids. On TLC the chloroform extract displayed prominent spots in methanol: chloroform (1:99) system after spraying with 5% ethanolic sulphuric acid. The extract (12 g) was chromatographed over silica gel following gradient elution technique successively using n-hexane, chloroform and methanol (each 200 ml fraction). While eluting the column, six compounds were obtained and were designated as **PPRC-1** to **PPRC-6**.
Characterization of the compounds
PPRC- 1 (0.01 g, Stigmasterol)

It was crystallized from hexane as white needles, m.p. 139-140°C. It was analyzed for C₂₉H₄₈O. It gave positive play of colour (pink-blue-green) to Liebermann- Burchard test for sterol. Molecular mass 412.37 requires positive API-ES, m/z (rel. int.): 413.3 [M+H]⁺ (15). The m/z values (rel. int.) of different fragments as 301.3 (16), 260.3 (18), 218.3 (30), 175.3 (12) were also displayed in the spectrum. The ¹³C NMR spectrum displayed two signals at δ 11.87 (C-26) and 11.98 (C-18) which are highly shielded by the surrounding methyl, methylene and methine groups. Signals at δ 18.80 (C-28) and 19.40 (C-29) indicate the two methyl groups of isopropyl moiety. δ value at 21.12 indicates the methyl carbon at 19-position. Signal at δ 23.13 reveals the presence of carbon at 21-position. Olefinic carbons showed peaks at δ 140.81 (C-5), 121.71 (C-6), 138.27 (C-22) and 129.34 (C-23). A characteristic signal at δ 71.84 was observed in the spectrum, which indicates the attachment of one

hydroxyl group at 3-position. All spectral data are shown in figure 4.1 and 4.10. All the spectral characteristics of the compound were in close agreement with those of stigmasterol. Identity of the compound was further confirmed by comparison with authentic sample through m.m.p. and co-TLC.

PPRC-2 (0.02 g, 7,4'-dimethoxyquercetin)

It was recrystallized from mixture of methanol: chloroform 19:1 as yellow crystals, m.p. 238-240°C and ¹H NMR was analyzed for the formula C₁₇H₁₄O₇. UV δ 9.76 (1H, s, H-3- OH); 12.51 (1H, s, H-5-OH); 6.32 (1H, d, 1.6 Hz, H-6); 3.90 (6H, s, H-7- and 4'-O-Me); 6.78 (1H, d, 1.6 Hz, H-8); 7.79 (1H, d, 1.6 Hz, H-2'); 9.53 (1H, s, H-3'-OH); 6.90 (1H, d, 8Hz, H-5'); 7.76 (1H, dd, 8, 1.6 Hz, H-6'). From the above spectral characteristics, PPRC-2 was identified as 7,4'-dimethoxyquercetin. Further identity was confirmed by comparison with an authentic sample through m.m.p. and co-TLC.

PPRC-4 (0.02 g, Quercetin)

It was crystallized from methanol as yellow crystals, m.p. 318-320°C and was analyzed for the formula C₁₅H₁₀O₇. It gave magenta colour in Shinoda's test and dense green colour with ferric chloride. UV (nm): 257, 267 (sh), 301 (sh) and 370; MeOH/AlCl₃ 265, 301 (sh), 359 and 425. A bathchromic shift of 8 and 58 nm in MeOH/AlCl₃ suggested the presence of chelated and free hydroxyl groups at 5- and 3-positions respectively. Positive API-ES, m/z (rel. int.): 85.3 (18), 107.3 (100) and 301.3 (9). The properties of the compound PPRC-4 closely approached to those of quercetin.

PPRC-5 (0.03 g, Myrcetin)

It was crystallized from methanol as pale yellow needles, m.p. 357-359°C. It was

analyzed for the formula $C_{15}H_{10}O_8$. It showed magenta colour in Shinoda's test and olive green with ferric chloride. The 1H NMR (400 MHz, DMSO- d_6) showed signals at δ 12.61 (1H, *s*, H-5-OH); 6.18 (1H, *d*, 1.6 Hz, H-6); 10.30 (1H, *s*, H-7-OH); 6.38 (1H, *d*, 1.6 Hz, H-8); 7.29 (2H, *s*, H-2' and -6'); 8.4 (3H, *br s*, H-3'-, H-4'- and H-5'-OH). From the above spectral characteristics, PPRC-5 was identified as myrcetin. Further identity was confirmed by comparison with an authentic sample through m.m.p. and co-TLC.

PPRC-6 (0.02 g, Scutellarein)

It was crystallized from methanol as pale yellow crystals, m.p. 327-329°C and analyzed for the formula $C_{15}H_{10}O_6$. It gave deep green colour with ferric chloride indicating the presence of phenolic hydroxyl group. UV (1H, *s*, H-4'-OH). From the above properties, PPRC-6 was identified as scutellarein. Further identity was confirmed by comparison with an authentic sample through m.m.p. and co-TLC.

EXPERIMENTAL

Plant material

The Plant material *Polygonum plebium* was collected from forest Pilak, India. Authentication of the plant specimen (SD001) was done by scientist Dr. P.V. Prasanna at BSI, Deccan Regional Centre, Hyderabad. A voucher specimen (SD001) was deposited at Herbarium, of the University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

EXTRACTION

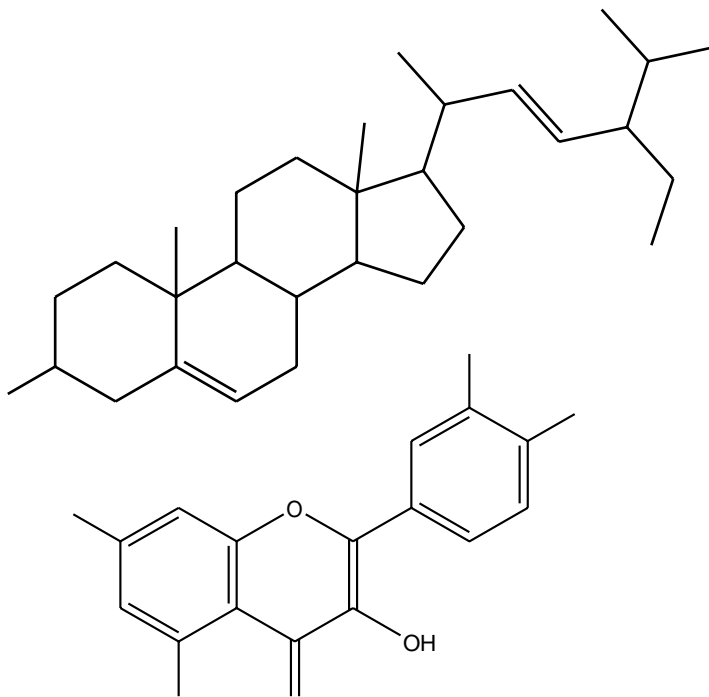
1 kg of dried root powder was extracted for 24 hours with chloroform (3 x 1.5 L). TLC examination of the residue showed number of prominent

spots (methanol:chloroform 1:99). The pooled extract was concentrated under reduced pressure and yielded 14 g brown residue. The extract was chromatographed on silica gel and successively eluted (each 200 ml fraction) with n-hexane, chloroform and methanol.

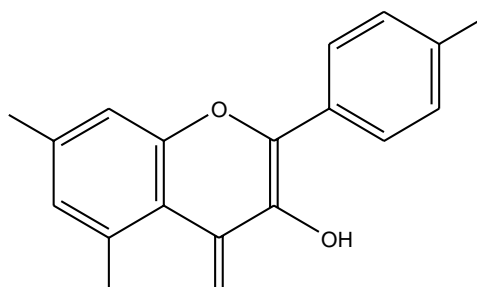
ELUTION AND ISOLATION:

Elution of the chromatogram with chloroform: hexane (25:75) (fractions 43-51) obtained white amorphous powder which on repeated crystallization from hexane afforded white needles of PPRC-1 (0.01 g) and was identified as stigmasterol.

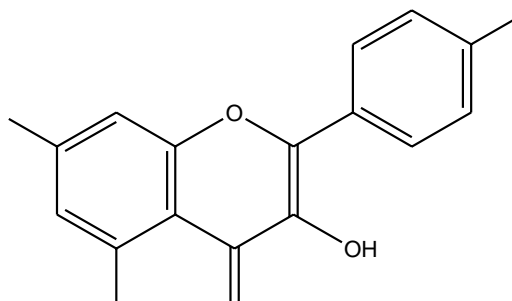
On continuation of elution with a solvent system of methanol: chloroform (5:95) (fractions 124-127), it yielded dark yellow solids which on repeated crystallization from a mixture of methanol: chloroform (19:1) obtained yellow crystals of PPRC-2 (0.02 g) and was identified as 7,4'-dimethoxyquercetin. Elution with methanol: chloroform (5:95) (fractions 128-131) yielded yellow solid. Repeated crystallization from methanol, it obtained pure yellow crystals of PPRC-3 (0.02 g) and was identified as kaempferol. Further elution with methanol: chloroform (10:90) (fractions 132-135) yielded yellow amorphous mass which on recrystallization from methanol, afforded yellow needles of PPRC-4 (0.02 g) and was identified as quercetin. Elution with methanol: chloroform (15:85) (fractions 139-142) yielded another dark yellow solid which on repeated crystallization from methanol afforded yellow needles of PPRC-5 (0.03 g) and was identified as myrcetin.



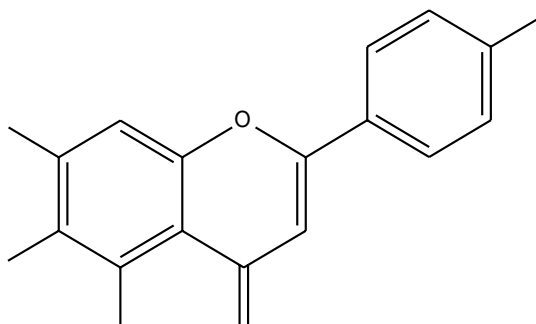
7,4'-dimethoxyquercetin



Kaempferol



Quercetin



Scutellarein

Further elution with methanol: chloroform (20:80) (fractions 143-148) yielded clump of yellow mass which on subsequent recrystallization from methanol, obtained pale yellow crystals of PPRC-6 (0.02 g) and was identified as scutellarein.

RESULTS AND DISCUSSION

Separation by conventional gradient chromatographic elution of chloroform extract of *Polygonum plebieum* root afforded six compounds namely stigmasterol (PPRC-1), 7,4'-dimethoxyquercetin (PPRC-2), kaempferol (PPRC-3), quercetin (PPRC-4), myrcetin (PPRC-5) and scutellarein (PPRC-6). All the six compounds were identified by chemical and spectral analysis. A variety of bioactive compounds were recorded from *Polygonum* genus ranging from flavonoids, sesquiterpenes, anthraquinones, stilbene glycosides, terpenoids, coumarins and esters. Earlier from *P. viscosum*, sesquiterpenes such as viscoazusone, viscoazulone, viscoazulenic acid, viscoazulenic acid methyl ether, viscoazucine, viscozucenic acid, polygosomic acid, viscosomic acid etc. and flavonoids such as 3', 5'-dihydroxy-3,4',5',7'- tetramethoxyflavone, quercetin-3-*O*-(6''-caffeoyl)- β -D-galactopyranoside, quercetin-3-

O-(6''-feruloyl)- β -D-galactopyranoside and quercetin-3-*O*-(6''-galloyl)- β -D-galactopyranoside were reported^{183,193,194}. Among the six compounds isolated and characterized in present chemical examination, stigmasterol (PPRC-1) was not reported earlier from *P. viscosum*. 7,4'-dimethoxy quercetin (PPRC-2), quercetin (PPRC-4)⁹⁻¹² and scutellarein (PPRC-6) were reported from *P. hydropiper*. Kaempferol (PPRC-3) from *P. chinensis*¹²⁻¹⁵ and myrcetin (PPRC-5) from *P. cuspidatum*¹³ were also reported earlier. This is the occurrence of the six compounds for the first time.

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