FLAVONOIDS FROM *TRIGONELLA POLYCERATA IN VIVO* AND *IN VITRO*

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Flavonoids viz., luteolin, kaempferol, apigenin and quercetin were isolated and identi fied from plant parts and tissue cultures of *Trigonella polycerata*. The flavonoid content was more in tissue culture than plant parts. Among the individual flabonoids luteolin was maximum whereas kaempferol was minimum. Apigenin was absent in roots.

Keywords : *Trigonella polycerata;* Flavonoids; Tissue culture.

Introduction

Flavonoids, the phenolic compounds of wide spread occurrence as natural constituents are important both physiologically and biochemically They have been reported form *Trigonella foenum-graecum* and *T. corniculata* (Varshney and Sharma, 1966; Wagner et al., 1972; Sheshadri *et al.*, 1973; Sood, 1975) but there is no report of their investigation from *T. polyeerata*, hence the present investigation was taken up.

Materials and Methods

The plants were collected from the fields in the months of February-March, identified and a museum specimen was deposited in the Herbarium of Botany Department, Rajasthan University, Jaipur (RUBL No. 9670). Unorganised static cultures of *T. polycerata* were established from seeds on MS medium which was followed formation of callus mass when transferred to revised RT medium (Khanna and Staba, 1968) supplemented with 1 ppm of 2,4-Dichlorophenoxy acetic acid and 1% agar. The static culture samles were maintained for 18 months by periodic subculturings and harvested at different time intervals of 2,4,6,8 and 10 weeks of fresh subculturing, and their grwoth index (GI) were calculated separately.

$$GI = \frac{\text{Final dry wt, — Initial dry wt.}}{\text{Initial dry wt.}}$$

Different plant parts and harvested tissue samples were kept at 120°C for 20 min. to inactivate the enzymes and at 60°C till dryness. Each of the above sample was powdered and analysed for

flavonoid contents by extracting first in 80% EtOH following method of Subramanian and Nagarjun (1969). The extract so obtained was filtered, filtrate was dried *in vacuo* reextracted separately in light petroleum ether (40–60°C), diethyl ether and ethyl acetate in succession (Fraction I, II, III respectively). Each step was repeated thrice to ensure complete extraction. Fraction I was found to be rich in fatty substances and sugars, Fraction II with free flavonoids and Fraction III with bound flavonoids. For the extraction of bound flavonoids fraction II was hydrolysed with 7% sulfuric acid (5 ml/gm residue) for 2 hrs. The mixture was filtered and the filtrate was extracted with ethyl acetate, washed with distilled water till the pH was 6, dried *in vacuo* and weighed. Fraction II and III thus obtained were analysed for free and bound flavonoids, resectively.

Thin glass plates coated with silica gel G (250 mμ wet thickness) were activated at 100°C for 30 minutes, cooled and fraction II and III residue were applied separately alongwith authentic samples (Luteolin, Quercetin, Apigenin, Kaempferol, Rutin, Quercetegetin), developed in various solvent mixture of which Benzene: Acetic Acid: Water (125:72:3) of Wong and Francis (1968) gave best results (Table 1). The developed plates were visualized under UV light and the fluorescent compounds coinciding with respective authentic samples, their Rf value and characteristic colours were noted. Later, these plates were also sprayed with 5% ethanolic ferric chloride, 0.1% aluminium chloride and iodine vapours, separately for characteristic colour development of the spots. These spots were collected from about 250-300 preparative TLC (500mμ wet thickness) plates separately eluted in ethyl acetate. The eluates were filtered dried *in vacuo*, crystalised and subjected to mp, mmp, UV and IR spectral studies alongwith their authentic samples for comparison.

The quantitative estimation of each of the flavonoid observed both in free and bound form was done by spectrophotometric method of Mabry *et al*, (1970) separately.

Results and Discussion

Five spots were visualized under UV light coinciding with their respective authentic samples in Rf value and characteristic colours coinciding with standard apigenin, luteolin, kaempferol and quercetin while one spot remained unidentified. Presence of these four compounds was further confirmed by mp, mmp (undepressed), UV spectroscopy showing characteristic absorbance maximum bands in MeOH (Table 1) and superimpossible IR spectra.

Quantification data revealed that the total flavonoid content (free + bound) was more in aerial parts (1.64 mg/gdw) as compared to roots (0.88 mg/gdw). The total flavonoids in their bound form were higher in aerial parts (1.00 mg/gdw) and minimum in roots (0.55 mg/gdw). The total luteolin content (free and bound 1.02 and 0.56 mg/gdw, respectively) was found to be maximum whereas total Kaempferol (0.09, 0.12 mg/gdw, respectively) was minimum. Both luteolin and Kaempf-

Table 1. Rf values, colour reactions and spectral data of isolated flavonoids from *T. polycerata*

Phytochemical parameters	Flavonoids			
	Apigenin	Kaempferol	Luteolin	Quercetin
Rf values				
A*	0 78	0.86	0.56	0.78
B	0.89	0.83	0.83	0.64
C	0.87	0.55	0.77	0.41
Colour reactions				
UV + ammonia	yellow green	bright yellow	yellow	yellow
I_2 uapours	brown	brown	brown	brown
Colour by chromatographic sprays				
$FeCl_3$ visual	tan	brown	tan	bright grey
UV	black	black	black	black
$AlCl_3$ visual	grey	yellow	dull yellow	dull yellow
UV	yellow green	yellow green	yellow green	yellow green
Ultraviolet absorption (in methanol)				
λmax (nm)	267, 296 sh, 336	253 sh, 266, 294 sh, 322 sh, 368	342 sh, 253, 267, 291 sh, 349	255, 269sh, 301 sh,374
Melting points (°C)	340-341	271-273	327-328	309-311

*Solvent systems A — benzeneace-tic acid-water (125:72:3),
B — n- butanol-acetic acid-water (4:1:5, upper layer),
C — n-butanol-acetic acid–water (3:1:1),

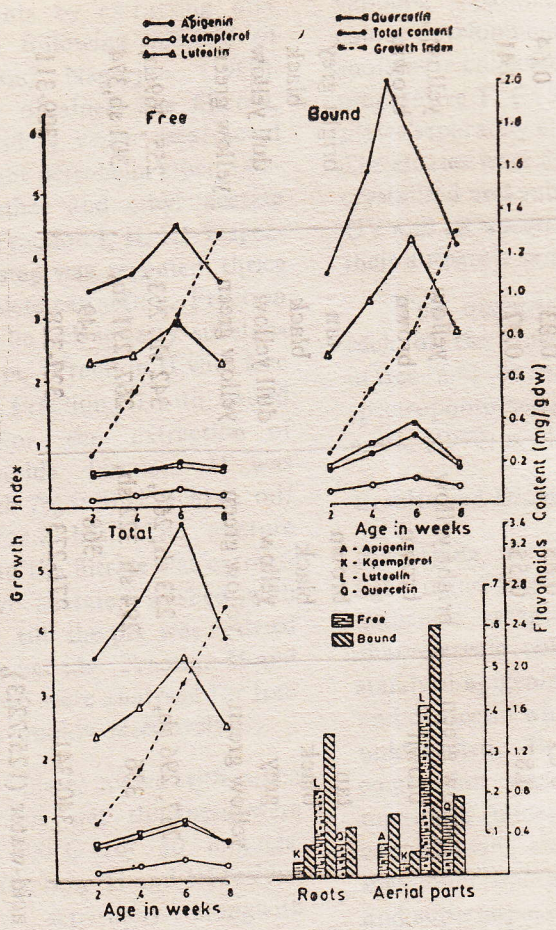

Fig. 1. Flavonoid content in *Trigonella polycerata* plant parts and callus cultures

erol were more in their bound form. In roots the total Kaempferol content (0.12 mg/gdw) was found to be little higher (0.0) mg/gdw) than aerial parts Fig.1).

In vitro studies showed that the maximum amount of total flavonoid content was in 6 week old tissue (3.43 mg/gdw) and minimum was in 8 week old tissue (2.31 mg/gdw). Individually total luteolin was maximum (.13 mg/gdw) and kaempferol was minimum (0 20 mg/gdw) in 6 week old cultures. However, flavonoids were more in their bound form than free form, whereas GI was maximum in 8 week-old culture (Fig. 1)

In the present study of *T. polycera ta* plant parts apigenin, Kaempferol, luteolin and quercetin have been confir-

med wherein total content was more in aerial parts. The absence of apigenin in roots and presence in aerial parts may be due to enzymatic degradation as observed by Hauteville *et al.*, (1978). Overall presence of more bound flavonoid than free form may be explained that flavonoids are usually present in bound form with glycosides thus giving higher concentration.

Uddin *et al*, (1977) reported quercetin, luteolin and vitexin-7-glucoside in seedling callus culture of *T. foenum-graecum* wherein total flavonoid content gradually increased up to 4 week old culture, thereafter it decreased. In *T. polycerata* cultures the total flavonoid content increased almost double in 6 week old tissues when compared with aerial parts *in vivo*, indicating that the flavonoid synthesis and recovery is much more in *in vitro* system than in *in vivo* and also that static cultures of *T. polycerata* maintained for 18 months by periodic subculturings retained their flavonoid biosynthetic potential.

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