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IN-VITRO AND *IN-VIVO* FLAVONOID CONTENT IN URGENIA *INDICA* (ROXB.)KUNTH

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ABSTRACT

Different plant parts as well as tissue samples (2, 4, 6 and 8 weeks old) of *Urgenia indica* were used to estimate the flavonoids . The *in vitro* studies showed that the maximum amount of total flavonoid content was in 6-week old tissue (1.22 mg/gdw) and lowest in 2 week old tissue (0.81 mg/gdw). The total Kaempferol content was highest in 6 week old tissue (0.69 mg/gdw) and lowest in 8 week old tissue (0.34 mg/gdw). However, the total Quercitin content was highest (0.40 mg/gdw) in 8 weeks old cultures

KEYWORDS :*Urgenia indica,* **flavonoids, Kaempferol, Quercitin**

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INTRODUCTION

U. indica is belongs to the family Liliaceae and native plant of Mediterranean sea cost area. It is distributed all over the world, Spain, Portugal, Morocco, Algeria, Corsica, Southern France, Italy, Malta, Dalmatia, Greece, Syria and Asia Minor. It is also found throughout India extends to the lower Himalaya regions .[1,2] *U. indica* is also documented as a good source of antimicrobial compounds [3] Flavonoids are one of the biologically active chemical constituents of plants. These natural products are readily available to man in the form of vegetables and fruits[4]. Flavonoids are polyphenolic compounds based on a C15 (C6C3C6) framework. They contain a chroman ring (C-ring) with a second aromatic ring (B-ring) at the C-2, C-3, or C-4 position. The heterocyclic six-membered C-ring is sometimes replaced by a five-membered ring (e.g., aurones) or the acyclic form (chalcones). The oxidation state of the C-ring is used to classify flavonoids into different categories, of which typical examples are flavan-3-ols, flavanones, flavones and flavonols. The term *flavonoid* can be ambiguous as it may refer either to the class of all C6C3C6 compounds, or its meaning may be restricted to 2-arylchromans with a carbonyl group at C-4 (C-ring) [5].

MATERIALS AND METHODS

(i) Collection of plant Material

U. indica (RUBL No. 20398) were collected (July-August, 2006) from botanical garden of Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni, Solan (Himachal Pradesh) and Botanical Garden of National institute oceanography Goa, India. Plant was identified by comparing with those available in the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

(ii) Development of tissue culture of U. indica

U. indica healthy mature bulb scale and leaves were used as explants for tissue culture. The dried and outer scales of bulbs were discarded and the bulb was washed for one hour under running water. These bulbs were then surface sterilized with 0.1% (w/v) mercuric chloride for 5 min. Washed thrice with sterile distilled water, dipped in 95% ethanol and flamed over sprit lamp under sterile condition. Outer scales from the bulbs were removed and the inner core was cut horizontally into explants of about 1 cm diameter and 0.5 cm thickness. The sterile explants were then aseptically inoculated in 100 ml flasks containing 35 ml of Murashige and scookg's medium [6] supplemented with different concentration of auxin and cytokinin and 0.8% Agar and growth indices was calculated on different time interval.

(iii) Extraction and Quantification of Flavonoids

(1) Extraction

Different plant parts as well as tissue samples (2, 4, 6 and 8 weeks old) of *U. indica* were air dried and powdered, separately. Each of these was extracted separately with 80% methanol on a water bath[7] for 24 hr. The methanol soluble fractions were filtered, concentrated *in vacuo* and the aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr-1), ethyl ether (Fr-II) and ethyl acetate (Fr-III) separately. Each step was repeated thrice for complete extraction, fraction I was discarded because it contained fatty substances, whereas fraction II and III were concentrated and used for determining free and bound flavonoids respectively. Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10 ml/g plant material for 2 hr), filtered and filtrate was extracted thrice with

ethyl acetate. All ethyl acetate layers were pooled together separately, neutralized by distilled water with repeated washings, and concentrated *in vacuo*. Both fraction II and fraction III were taken up in small volume of ethanol (2-5 ml) before chromatographic examination.

(***2). Identification***

The further identification of the isolated flavonoids (Kaempherol and quercetin) was done by mp, mmp performed in capillaries (Toshniwal Melting Point Apparatus), IR (Infra-red spectrophotometer; Perkin, Elmer 337, Grating Infra-red spectrophotometer), UV (Ultraviolet and visible spectrophotometer; Carl Zeiss, Jena, DDR, VSU-ZP spectrophotometer) analysis along with their respective authentic samples.

RESULT AND DISCUSSION

The maximum growth index was observed in 6 weeks old tissues grown as static cultures in both the plants, which decreased subsequently in 8 weeks old tissues *U. indica* the maximum growth index (1.12) was found in 6 weeks old and minimum (0.25) in 2-week old cultures. Quantitative data revealed that the total (free + bound) flavonoid content was highest in leaf (0.57 mg/gdw), lowest in bulb (0.48 mg/gdw) and total kaempferol content was highest in leaf (0.36 mg/gdw) and lowest in bulb (0.21 mg/gdw) (Table 1, Fig.1.) The *in vitro* studies showed that the maximum amount of total flavonoid content was in 6-week old tissue (1.22 mg/gdw) and lowest in 2 week old tissue (0.81 mg/gdw). The total kaempferol content was highest in 6 week old tissue (0.69 mg/gdw) and lowest in 8 week old tissue (0.34 mg/gdw). However, the total Quercitin content was highest (0.40 mg/gdw) in 8 weeks old cultures (Table 2, Fig. 2).

Table 1

Growth indices (GI) of tissue culture of U. indica

S. No	Age of tissue (in weeks)	Growth Indices
1	2	0.25
2	4	0.56
3	6	1.12
4	8	0.96

Table 2

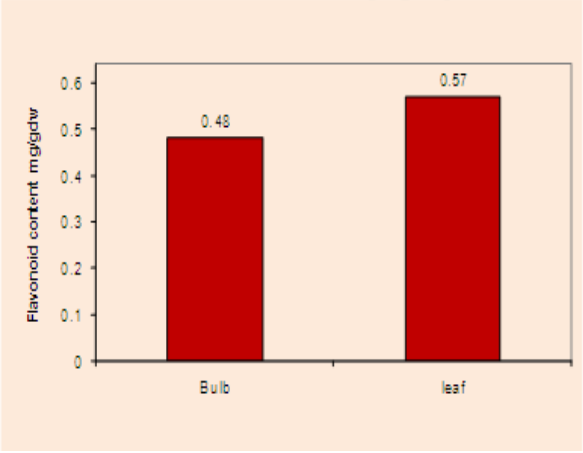
Total flavonoid content (free + bound) (mg/gdw) in various plant parts of U. indica

S.No	Plant parts	Free Flavonoids (mg/gdw)			Bound Flavonoids (mg/gdw)			Total Kaempferol (mg/gdw)	Total Quercetin (mg/gdw)	Total Flavonoids (Free +Bound) (mg/gdw)
		Kaempferol	Quercetin	Total	Kaempferol	Quercetin	Total			
1	Bulb	0.13	0.08	0.21	0.18	0.09	0.27	0.31	0.17	0.48
2	Leaf	0.17	0.11	0.28	0.19	0.10	0.29	0.36	0.21	0.57

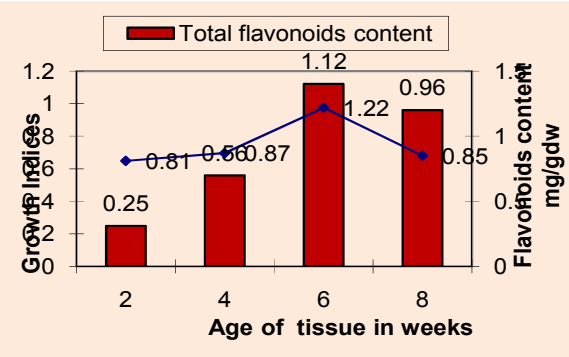
Table 3
Growth indices and total flavonoid content in vitro (free + bound) (mg/gdw) in various plant parts of U. indica

S.No.	Age of Tissue in Weeks	Growth Indices	Free Flavonoids (mg/gdw)			Bound Flavonoids (mg/gdw)			Total Kaempferol (mg/gdw)	Total Quercetin (mg/gdw)	Total Flavonoids (Free +Bound) (mg/gdw)
			Kaempferol	Quercetin	Total	Kaempferol	Quercetin	Total			
1	2	0.25	0.22	0.18	0.40	0.25	0.16	0.41	0.47	0.34	0.81
2	4	0.56	0.24	0.21	0.45	0.29	0.13	0.42	0.53	0.34	0.87
3	6	1.12	0.38	0.26	0.64	0.31	0.27	0.58	0.69	0.53	1.22
4	8	0.96	0.24	0.20	0.44	0.19	0.22	0.41	0.43	0.42	0.85

Graph 1
Total flavonoid (free + bound) content (mg/gdw)



Graph 2
Growth indices and total flavonoid content (mg/gdw) in various age of tissue cultures of U. indica



CONCLUSION

Flavonoids are naturally occurring phenolic compounds which are widely distributed in plants and have been found in a number of tissue cultures,[8] reported Flavonoids in *Gloriosa superba* L. tissue culture which belongs to family liliaceae .[9] studied flavonoids of some species of Liliaceae family using paper and thin-layer chromatography. Studies of aglycone and glycoside fractions of flowers and leaves of 60 species of Liliaceae proved the presence of these compounds in the following groups: Melanthioideae, Asphodeloideae, Lilioideae, Scilloideae, Allioideae and Smilacoideae.

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