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FLAVONOIDS FROM *GLORIOSA SUPERBA* LINN. TISSUE CULTURE

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In the present investigation two flavonoids, kaempherol and quercitin have been reported from *Gloriosa .superba* L. of Liliaceae family *in vivo* and *in vitro*. Quantification data revealed that the total flavonoid content (free + bound) was higher in tissue culture than in plant parts. The *in vitro* studies showed that the maximum amount of total flavonoid content was found in 6-weeks old tissue (2.21 mg/g.d.w) and minimum (1.13 mg/g.d.w) in 2-weeks-old tissue. *In vivo* studies showed higher flavonoids content in leaf (0.84 mg/g.d.w) when compared to rhizome (0.43 mg/g.d.w).whereas the total flavonoids content in their bound form was highest in leaf (0.45 mg/g.d.w) and lowest in rhizome (0.23 mg/g.d.w).

Key words: Flavonoids, *Gloriosa superba*, Tissue culture

The plant *Gloriosa superba* Linn. is a climber which scrambles over other plants with the aid of *tendrils* at the ends of their *leaves* and can reach up to 3 meters in height. A native to tropical forest of Africa is now found growing naturally in many parts of Tropical Asia including India, Burma, Malaysia and Srilanka. Studies reveal that all parts of the plant, especially the tubers are extremely toxic due to the presence of a highly active alkaloid, Colchicine. The species also contains another toxic alkaloid, Gloriosine (Gooneratne 1966, Angunawela and Fernando 1971). However there is no report on the production of flavonoids from this plant. The present studies therefore, are undertaken to determine and compare the production of flavonoids *in vitro* and *in vivo*.

MATERIAL AND METHODS

Gloriosa superba L. (Liliaceae) plants were collected from Botanical garden of Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni, Solan (Himachal Pradesh) and Jhalana Dungari hills Jaipur (Rajasthan) India.

For *in vitro* studies terminal shoot tips, stem nodes with single auxiliary bud, dormant and non dormant rhizome were used as explants. The explants were washed with running tap water pre soaked in 0.1% liquid detergent for about 30 min , then the explants were surface sterilized with 0.1% (w/v) mercuric chloride for 3 min. followed by two to three rinses of sterile distilled water.

The basal medium contained MS (Murashige and Skoog 1962) salts, B5 (Gamborg *et al.* 1968) vitamins, 3% sucrose and 0.9% agar, Basal medium was supplemented with various concentrations and combinations of growth regulators such as 2,4-D (2,4-dichlorophenoxy acetic Acid), BAP (6-benzylamino purine), NAA (Naphthalene acetic acid), Kinetin , IBA (Indole butyric acetic acid) and IAA (Indole acetic acid). The medium was adjusted to pH 5.8 with NaOH/HCl and dispensed in culture tubes and conical flasks of 100 ml capacity. The media was sterilized by autoclaving at 1.063 Kg/cm² pressure for 20 minutes. The calluses were subcultured on fresh

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media every two weeks depending on the rate of callus growth. Explants showing no visible callus growth or with slow growing callus were transferred to fresh media every four weeks. The growth index was calculated after every two weeks time interval

All the cultures were incubated at 24 ± 2^0C and at photoperiod of 16.h provided by cool-white fluorescent light with the of intensity 3mmol m^{-2} s^{-2}.

Analysis of Flavonoids

Different plant parts as well as tissue samples (2, 4, 6 and 8 weeks old) of *G. superba* were air dried and powdered, separately. Each of these was extracted separately with 80% methanol on a water bath (Subramanian and Nagarajan 1969) for 24 h. 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.

Thin glass plates (20x20cm) were coated with Silica gel G (250 mμ thick). The freshly prepared plates were dried at room temperature; thereafter these were kept at 100°C for 30 min Each of the extract was co-chromatographed with authentic samples of flavonoids (kaempherol and quercitin) as markers. These plates were developed in an air-tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water, 125: 72: 3). The developed plates were air dried and visualized under UV light and by exposure to ammonia fumes, fluorescent spots corresponding to that of standard markers were marked.

The 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.

RESULTS

Callus induction was obtained in various concentrations of growth regulators from corm and leaf explants Various concentrations and combinations of 2, 4-D, NAA and K in B_5 and MS medium produced profuse, white, friable callus within two to three weeks. The callus initiated in the media supplemented with NAA and BA combination was compact and semi-hard. The best results obtained were in the medium supplemented with a combination of NAA 2mg/l + KIN 0.5mg/l

All the cultured explants showed callus formation. About 70 % of the non-dormant corm bud explants, 55% of the young leaf explants, 76% of the shoot bud explants, 45% of the root explants, 87% of the dormant corm explants, 66% of the stem explants and 57% of the pedicel explants have successfully produced calluses Quantification data revealed that the total flavonoid content (free + bound) was more in leaf (0.84 mg/gdw) when compared to rhizome (0.43 mg/gdw). The total flavonoids in their bound form were highest in leaf (0.45 mg/gdw) and lowest in rhizome (0.23 mg/gdw). The total free and bound kaempferol content was highest in leaf (0.52 mg/gdw) and lowest in rhizome (0.27 mg/gdw). The total quercetin content was highest (0.33 mg/gdw) in flower and lowest in rhizome (16 mg/gdw) (Table 1).

The *in vitro* studies showed that the maximum amount of total flavonoid content was in 6-week old tissue (2.21 mg/gdw) and

Table 1: Total flavonoid content (free + bound) (mg/gdw) in various plant parts of *G. superba.*

S. No.	Plant parts	Free Flavonoids (mg/gdw)			Bound Flavonoids (mg/gdw)			Total Kaempferol (mg/gdw)	Total Quercetin (mg/gdw)	Total Flavo-noids (Free + Bound) (mg/gdw)
		Kaemp-ferol	Quercetin	Total	Kaemp-ferol	Quercetin	Total			
1	Stem	0.23	0.10	0.33	0.28	0.13	0.41	0.51	0.23	0.74
2	Rhizome	0.13	0.07	0.20	0.14	0.09	0.23	0.27	0.16	0.43
3	Flower	0.22	0.18	0.40	0.24	0.15	0.39	0.46	0.33	0.79
4	Leaf	0.25	0.14	0.39	0.27	0.18	0.45	0.52	0.32	0.84

Table 2. Growth indices and total flavonoid content in vitro (free + bound) (mg/gdw) in various splant parts of *G. superba*

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 (mg/gdw) (Free + Bound)
			Kaemp-	Quercetin ferol	Total	Kaemp-	Quercetin ferol	Total			
1	2	0.26	0.45	0.13	0.58	0.46	0.17	0.63	0.91	0.30	1.13
2	4	0.65	0.49	0.16	0.65	0.55	0.12	0.67	1.04	0.28	1.32
3	6	1.27	0.89	0.25	1.14	0.68	0.09	0.77	1.57	0.34	2.21
4	8	0.86	0.69	0.21	0.90	0.46	0.19	0.65	1.15	0.40	1.35

minimum (1.13 mg/gdw) was in 2-week-old tissue. The total amount of kaempferol was maximum in 6-week old tissue (1.57 mg/gdw) and minimum in 2-week old tissue (0.91 mg/gdw). However, the total amount of quercitin was highest in 8 weeks old tissue (0.40mg /gdw) and lowest in 4 weeks old tissue (0.28 mg/gdw) (Table 2)

DISCUSSION

Since there was no report in the literature on the presence of flavonoids in *G. superba,* hence, the present study was undertaken. In the present study kaempferol and Quercetin have been confirmed both *in vivo* and *in vitro* in *G. superba*. The total flavonoids content (free + bound) was more in leaf when compared to rhizome of *G. superba* .The *in vitro* studies revealed that the highest amount of total flavonoid content is present in 6 weeks old tissue and minimum in 2 weeks old tissue.

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REFERENCES

Angunawela RM & Fernando HA 1971 Acute ascending polyneuropathy and dermatitis following poisoning by tubers of *G. superba* Linn. *Ceylon Med J* **16**(4) 233-5.

Gamborg OL, Miller RA & Ojima K 1968 Nutrient requirements of suspension cultures of soybean root cells. *Exp Cell Res* **50** 151-158.

Gooneratne BW 1966 Massive generalized alopecia after poisoning by *G. superba* Linn.*Br Med J* **231**(5494) 1023-4.

Murashige T & Skoog F 1962 Arevised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant* **15** 473-497.

Subramanian SS & Nagrajan S 1969 Flavonoids of the seeds of *Crotolaria retusa* and *C. striata*. *Curr Sci* **38** 65.