J. Phytol. Res. 3 (1 & 2), 1990

FLAVONOIDS FROM *IN VIVO* AND *IN VITRO* TISSUE CULTURES OF *DOLICHOS LABLAB L.* AND THEIR ANTIFUNGAL SCREENING.

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The seeds, stem, leaves and flowers of *Dolichos Lablab* L. were collected and unorganised callus tissue of *D. Lablab* raised and maintained by frequent subculturing on revised Murashige and Skoog's medium supplemented with 1 ppm of 2, 4-D and 1% Agar (RT), have been analysed for their flavonoids (Kaempferol and Quercetin) content. The isolated flavonoids were screened for their antifungai activity The maximum amount was found in the flowers.

Key words : Flavonoids; Kaempferol; Quercetin.

Introduction

Flavonoids constitute one of the most characteristic class of compounds in higher plants. These are the water soluble phenolic compounds which are known to impart colour to flowers and fruits. A number of plant species have shown the presence of flavonoids (Stickland and Sunderland, 1972; Jain and Khanna, 1974; Khanna *et. al.*, 1982; Grover, 1985; Kumar and Khanna, 1986). The antimicrobial activity of flavonoids (Khanna *et. al.*, 1980; Khanna, 1987; Khanna *et. al.*, 1989) have also been described from various plant species. Hence, keeping the importance of earlier work the various plant parts and tissues of *D. lablab* were analysed for flavonoids content.

Material and Methods

The seeds, stem, leaves and flowers were collected locally, dried and powdered. The callus tissue were initiated from seedling of *D. lablab.* The cultures were established on revised Murashige and Skoog's medium (Khanna and Staba, 1968; RT medium) supplemented with 1ppm of 2 4-D and 1% agar. The callus tissue grown on different media were harvested at the transfer age of 2,4,6 and 8 weeks and growth indices calculated separately (GI=Final dry weight – initial dry weight/initial dry weight of tissue). Five such replicates of each

of the tissue samples were examined and their mean values recorded. The different samples were dried, powdered and extracted by following the method of Subramanian and Nagarajan (1969). The various dried samples were separately Soxhlet extracted in 80% enthanol (100 ml/gm dry weight of tissue) on a water bath for 24 hours. Each of the extracts was concentrated and re-extracted with petroleum ether (40-60°C), ethyl ether and ethyl acetate in succession. Each step was repeated three times to ensure complete extraction. Petroleum ether fraction was rejected due to fatty substances whereas ethyl ether and ethyl acetate fractions were analysed for free and bound flavonoids respectively. The ethyl acetate fraction of each of the samples was hydrolysed by refluxing with 7% H_2SO_4 for 2 hr. The mixture was filtered and the filterate extracted with ethyl acetate in separating funnel. Some amount of water was also added to separate the two layers. The ethyl acetate layer (upper) was washed with distilled water to neutrality, dried *in vacuo* and was analysed for bound flavonoids.

Thin glass plates (20 x 20 cm) were coated with silica gel G and dried at room temperature activated at 100°C for 30 min. in an oven and cooled. Ethyl ether and ethyl acetate fractions were separately applied 1 cm above the edge of the plates alongwith the standard reference compounds (Kaempferol, quercetin, apigenin, luteolin and vitexin). These glass plates were developed in an air tight chromatographic chamber containing solvent mixture of n-butanol, acetic acid and water (4:1:5).

The developed plates were dried at room temperature and sprayed with 5% ethanolic ferric chloride and heated in an oven at 100°C. Two spots developed the reference values of which coincided with those of the reference standard sample of Kaempferol (Brownish, Rf 0.91) and quercetin (bluish grey, Rf 0.82).

The quantitative estimation of kaempferol and quercetin was carried out colorimetrically following the method of Mabry *et. al* (1970) However, the identity was carried out using the techniques of mp, IR spectra and HPLC.

The substances (Kaempferol and Quercetin) isolared as described above were screened against two fungal pathogen *Candida albicans* and *Penicilium puberulum* Fresh potato dextrose agar (PDA) medium was used as the growth medium for the two test fungi. The paper disc method (Gould and Bowie, 1952) was used for screening. Five replicates in each experiment were run and their average value computed.

Table 1. Flavonoids from Plant Parts and Tissue Culture of *Dolichos lablab* L.

Parts used	GI	Free (F) mg/gdw			Bond (B) mg/gdw.			Total (F+B) mg/gdw		Total
		K	Q	T	K	Q	T	K	Q	
Stem	—	0.35	0.30	0.65	0.33	0.24	0.57	0.68	0.54	1.22
Leaves	—	0.40	0.32	0.72	0.53	0.29	0.82	0.93	0.61	1.54
Flowers	—	0.38	0.41	0.79	0.56	0.26	0.82	0.94	0.67	1.61
Seeds	—	0.26	0.22	0 48	0.20	0.18	0.38	0.46	0.40	0.86
Tissue (6 weeks)	5.6	0.17	0.15	0.32	0.11	0.09	0.20	0.28	0.24	0.52

K=Kaempferol,
Q=Quercetin
g. d. w.=gram dry weight
GI (Growth Index)=(Final dry wt. of the tissue — Initial dry wt. of tissue/Initial dry wt. of the tissue)

Results and Discussion

Growth index (GI) of tissue of *D. lablab* was maximum (5.6) in six weeks old tissue which decreased during the eighth week. The presence of two flavonoids, Kaempferol and quercetin were confirmed by Co-tlc (Rf 0.91 and 0.82 respectively), mp (271–73° and 309–11°C respectively) and UV spectra (Kaempferol 253, 266; quercetin 255, 269 in methanol). Further confirmation by IR and HPLC shows that the characteristic peeks of the two isolates were found to be superimposable with those of the standard reference compounds of Kaempferol and quercetin. The total flavonoid content as also the individual flavonoid were found to be maximum in flowers (kaempferol 0.94 mg/gdw; quercetin 0.67 mg/gdw). However, the amount of the individual flavonoid varied in free and bound. In free from kaempferol was maximum in leaves (0.40 mg/gdw) whereas in bound from kaempferol was maximum in flowers (0.56 mg/gdw). The amount of quercetin in free form was highest in flowers (0.41 mg/gdw) whereas in bound form it was maximum in leaves (0.29 mg/gdw). The total flavonoid content in bound form in leaves and in flowers are similar quantitatively (0.82 and 0.82 mg/gdw) but variable in indlvidual contents. However, tissue culture studies revealed less amount as compared to plant parts (Table 1). Moreover, maximum production in flowers might be responsible for this particular organ to play an important role in providing resistance to plant against pathogens.

The isolated flavonoids (kaempferol and quercetin) were found to be active against the fungus, *Cadida albicans* and *Penicillium puberulum*. Kaempferol was found to be active against both the micro-organism whereas quercetin was active against the *P. puberulum* only when compared with standard reference disc, mycostatin.

Acknowledgements

The authors are thankful to Director, CDRI, for HPLC, Head Department of Chemistry for IR and Head, Depatment of Botany for providing necessary facilities.

Accepted July, 1990.

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