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Isolation and Identification of Flavonoid "Quercetin" from *Citrullus colocynthis (Linn.)* Schrad.

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Abstract : *Citrullus colocynthis* (Linn.) Schrad. is an important medicinal plant belonging to family Cucurbitaceae. Flavonoid "quercetin" was isolated from *in vivo* (leaf, stem, fruit and root) and *in vitro* callus of the species. The dried samples were separately soxhlet extracted in 80% methanol and then reextracted with petroleum ether, diethyl ether and ethyl acetate. The fraction was concentrated and subjected to TLC. The R_f value of isolated quercetin and standard quercetin was calculated. The purified material was subjected to its IR spectra, HPLC and identified as "quercetin". This study is also of practical importance because quercetin is an important ingredient of *Citrullus colocynthis*.

Key words : *Citrullus colocynthis*, Flavonoid, *In vivo* and *in vitro*, Quercetin.

Introduction

Plants have potent biochemicals and have components of phytomedicine. Since time immemorial. Man is able to obtain from them a marvellous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001) *i.e*. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups and are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999). Arid and semi arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress *e.g.* scarcity of water, salinity, pathogens etc. They are also important for the primary metabolism of plants. These compounds include alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc. Man has been exploiting these natural plant products for use in medicines, cosmetics, dyes, flavors and foods.

Flavonoids are a group of about 4000 naturally occuring polyphenolic compounds, found universally in foods of plant origin (Harborne, 1986). These are primarily recognized as the pigments responsible for the colours of leaves, especially in autumn. Flavonoids are widely distributed in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers as well as tea and red wine. They are usually subdivided according to their

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substituents into flavanols (kaempferol, quercetin), anthocyanins, flavones, flavonones and chalcones. These flavonoids display a remarkable array of biochemical and pharmacological actions *viz*., antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities (Middleton and Kandaswami, 1993). These compounds appear to play vital roles in defence against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy (Winkel-Shirley, 2002). They are synthesized from phenyl propanoid and acetate derived precursors. Flavonoids are important for human beings due to their antioxidative and radical scavenging effects as well as their potential estrogenic and anticancer activities (Springob and Saito, 2002). Quercetin belongs to this group of plant pigments called flavonoids that are largely responsible for the colours of many fruits, flowers and vegetables. Quercetin works as antiinflammatory, antioxidant, anticancer agents (Lamson and Brignale, 2000). *Citrullus colocynthis* (Linn.) Schrad (Cucurbitaceae), one of the most important medicinal plant species is locally known as Tumba or Indrayan.

The present study deals with the isolation and identification of flavonoid "quercetin" from *in vivo* (leaf, stem, fruit and root) and *in vitro* callus samples of *Citrullus colocynthis* (Linn.) Schrad.

Materials and Methods

In vivo

Different plant parts of *Citrullus colocynthis* (stem, leaf, fruit and root) collected locally were dried, powdered and used for extraction along with the *in vitro* tissue samples.

In vitro

Six week old callus tissue (callus induced from nodal stem explant) of *Citrullus colocynthis* grown on MS-medium supplemented with BAP (2.0 mg/l) and NAA (2.0 mg/l) was dried in an oven at 100°C for 15 min. to inactivate enzymes, followed by 60°C till a constant weight was achieved. Tissue samples were powdered and used for extraction.

Extraction procedure

The dried samples were separately soxhlet extracted in 80% methanol (100 ml/ gm dry weight) on a water bath for 24 hrs (Subramanian and Nagarajan, 1969). Each of the extracts was concentrated and reconcentrated in petroleum ether (40°-60°C) (fraction-I), ethyl ether (fraction-II) and ethyl acetate (fraction-III) in succession. Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was rejected since it was rich in fatty substances whereas fraction II was analysed for the free flavonoids in each of the samples.

Fraction III of each of the test samples was hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 5 hours. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried *in vacuo*. The residues were taken up in small volumes of ethanol separately and then subjected to various tests for quercetin.

(i) Thin layer chromatography (TLC)

The glass plates (20 x 20 cm) coated with silica gel 'G' (0.2-0.3 mm thick and 30 gm/60 ml distilled water) were dried at room temperature. The dried plates were activated

at 100°C for 30 minutes in an oven and cooled at room temperature. Ethyl ether and ethyl acetate fractions from each samples were separately applied 1 cm above the edge of the plates along with standard reference compound (quercetin). These glass plates were developed in an airlight chromatography chamber containing about 200 ml of solvent mixture of n-butanol, acetic acid and water (4: I : 5, upper layer).

Several other solvent mixture such as ethyl acetate saturated with acetic acid: water (6 : 4 v/v) and forestal system (acetic acid, concentrated hydrochloric acid, water, 10 : 3 : 30 v/v) were also tried. The solvent mixture of n-butanol, acetic acid and water (4: 1 : 5, upper layer v/v) gave the best results in all the samples examined.

The developed plates were air dried and visualized under UV light which showed fluorescent spots in both the fractions II and III in all the instances coinciding with those of the standard samples of quercetin (blue, R_f 0.82).

The plates were then placed in a chamber saturated with ammonia vapours to observe the colour of spots (quercetin deep yellow) and plates were also placed in a chamber saturated with I_2 vapours to observe the colour of spots (yellow brown). The developed plates were sprayed with 5% ethanolic ferric chloride solution to observe the colour of the spots (in both the fractions II and III). R_f values were calculated for isolated samples and compared with coinciding standard.

(ii) Preparative thin layer chromatography (PTLC)

Glass plates (20 x 20 cm) thickly coated (0.4-0.5 nm) with silica gel 'G' (45 gm/80 ml water) and activated at 100°C for 30 minutes and cooled at room temperature were used for preparative thin layer chromatography (PTLC). The extract of both the fractions (II and III) were applied on separate plates and developed plates were air dried and visualized under UV light.

Each of the fluorescent spots coinciding with those of standard reference compound of quercetin were marked. The marked spots were scrapped and collected separately along with the silica gel 'G' and eluted with ethanol. Each of the elutes was then crystallized with chloroform. The purified material was subjected to its IR spectral analysis. The compounds thus isolated were subjected to colorimetry and infra red spectral studies. This purified material was also subjected to HPLC studies (Water associates, column-microporasil, 80% hexane and 20% ethyl acetate, chart spectra 1 cm/min, 0.5 ml/min UV detector at 254 nm).

Results

When the developed plates were sprayed with 5% ethanolic ferric chloride solution it showed spots which coincided with that of the reference quercetin (bluish grey, Fig. A) when plates were placed in a chamber saturated with ammonia vapours, it also showed deep yellow colour of quercetin. R_f value (0.82) of quercetin isolated from the samples coincided with the R_f value of standard quercetin.

The plates developed under UV light showed fluorescent spots in both the fraction II and III coinciding with the standard sample of quercetin (Blue). The characteristic IR spectral peaks were found to be superimposable with those of their respective

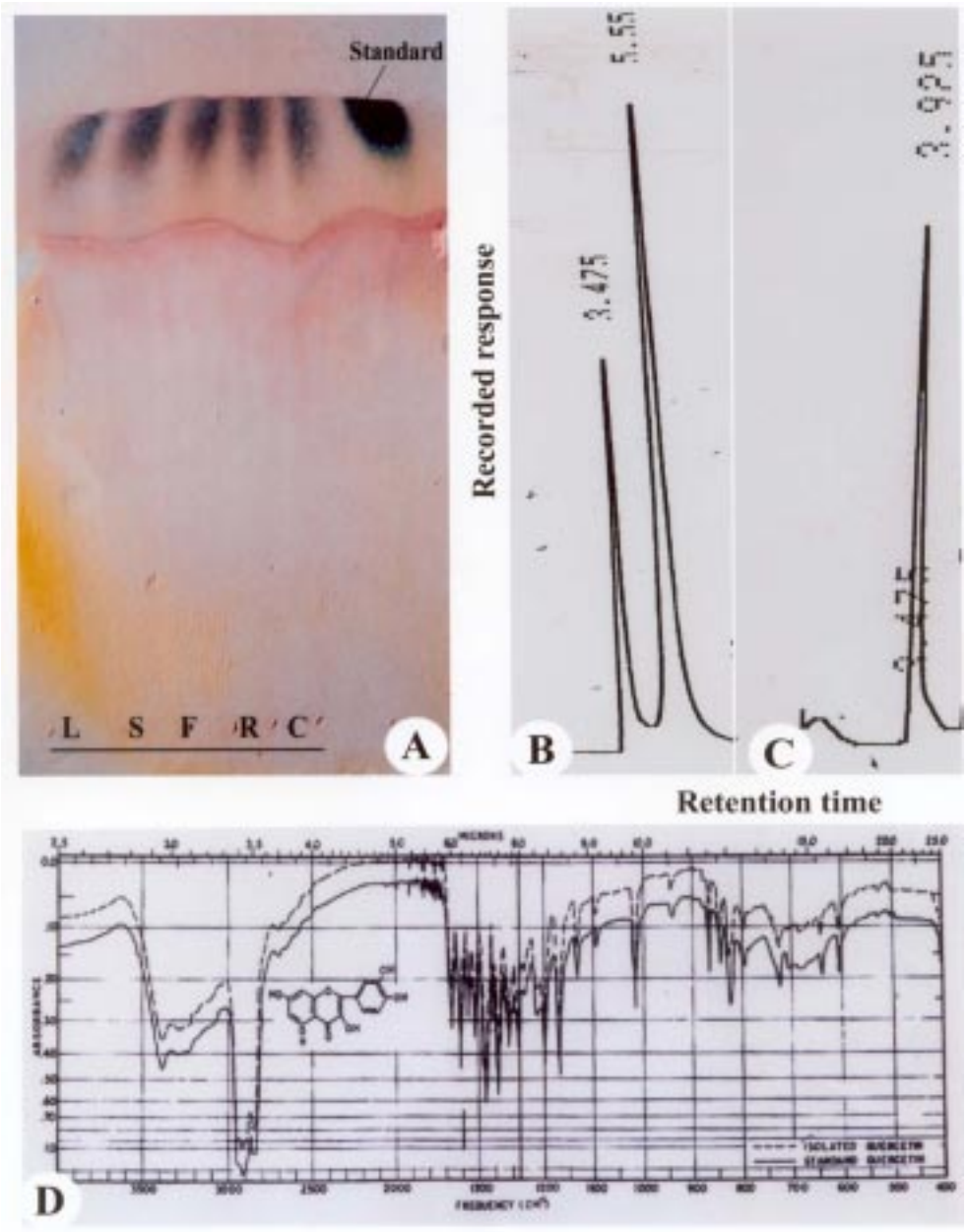

Fig. (A) TLC plates showing presence of quercetin of isolated *in vivo* and *in vitro* samples of *Citrullus colocynthis*.
(*In vivo* L= Leaf, S=Stem, F= Fruit, R=Root and C= *In vitro* callus)
(B) HPLC standard (C) HPLC isolated
(D) Superimposed IR spectra of isolated quercetin and standard querceti

standard reference compounds of quercetin (Fig. D). When isolated quercetein was subjected to HPLC, it showed retention time 3.475 min which coincided with that of standard quercetin (Figs. B & C).

Quercetin was detected in all the five *in vivo* (leaf, stem, fruit and root) and *in vitro* callus samples of *Citrullus colocynthis*.

Discussion

More than 2000 flavonoids have been reported among woody and non-woody plants (Harborne, 1980). Biosynthesis, isolation techniques and preparative chromatography (Casteel and Wender, 1953). TLC, UV and IR spectral studies have provided new dimensions to the chemistry of flavonoids to such an extent that their presence have become important taxonomically (Smith, 1969). Presence of flavonoids has been reported from many plant species like *Lycium barbarum* (Harsh *et al*., 1983); *Passiflora plamer* (Ulubelen *et al*., 1984); *Cassia angustifolia* (Goswami and Reddi, 2004); *Jatropa curcas* L. (Saxena *et al*., 2005) Quercetin has been reported from many plant species like *Cicer arietinum* Linn.(Joshi, 1985) and *Acacia catechu* (Jain *et al*., 2007). As mentioned earlier, since quercetin has anti-inflammatory, antioxidant and anticancer properties, isolation and extraction of this compound *in vivo* (leaf, stem, fruit, root) and *in vitro* callus from *Citrullus colocynthis* and its enhancement by addition of elicitors in culture, can be exploited further for large-scale production of this medicinally important compound.

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