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Research Article

DETERMINATION OF QUERCETIN IN EXTRACT OF *ELAEOCARPUS GANITRUS* ROXB. SEEDS BY USING HPTLC METHOD

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ABSTRACT

Herbal medicines have good efficacy, safety, and lesser side effects. They have great demand in developed world for primary health care. India has rich traditional knowledge, heritage of herbal medicines and large biodiversity but despite it India has dismal share of world market. Many Pharma companies marketed herbal preparation as neutraceutical and took excuses from quality control parameter set by W.H.O. India has thousands of medicinal plants but in Indian Pharmacopoeia. Quercetin, one of the most abundant natural flavonoids, presents in daily food. Quercetin is of interest because of its pharmacological function. The quantitative determination of flavonoids compound in seeds of *Elaeocarpus ganitrus* was carried out in high performance thin layer chromatography. Concentration of quercetin in *Elaeocarpus ganitrus* seeds was calculated based on calibration curve.

Keywords: *Elaeocarpus ganitrus* Seeds, HPTLC, Quercetin

INTRODUCTION

Plants of the genus *Elaeocarpus* have been reported to be of use as traditional medicines, particularly in India. *Elaeocarpus ganitrus* (syn: *Elaeocarpus sphaericus*; Elaeocarpaceae) is a tree found in the Himalayan region of India. The fruits of this plant are commonly known as Rudraksha and have been used in Ayurvedic traditional medicine for the treatment of mental diseases, epilepsy, asthma, hypertension, arthritis and liver diseases [1, 2, 3].

Flavonoids occur, either as free molecules or as glycosides. They have widespread occurrence in plant kingdom. They occur ubiquitously in ferns and fern allies (Pteridophyta), conifers (Gymnosperms), dicot and monocots. Chemically, flavonoids show a fifteen-carbon skeleton, which consist of two phenyl rings connected by three carbon bridges. Flavonoids have been found possess a number of biological activities [4]. Flavonoids (flavus- yellow) or bioflavonoid, are a ubiquitous group of poly phenolic substances which are present in most plants, concentrated in the seeds, fruit skin, peel, bark and flowers [5]. They have been used extensively as a chemotaxonomic markers and are abundant in the polygonaceae, rutaceae, leguminosae, umbelliferae and composite. Many flavonoid-containing plants are diuretic or antispasmodic. Some flavonoids have antitumor, antibacterial or antifungal properties [6].HPTLC is now-a-days applied to obtain "Finger-print" patterns of herbal formulations, quantification of active ingredients and also detection of adulteration. HPTLC is rapidly gaining importance in biochemistry of natural products and in analysis of biofluids in the field of pharmacokinetics[4]. Densitometric HPTLC has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. Hence a densitometric HPTLC method has been developed in the present work for quantitation of quercetin from hydro alcoholic extract of dried flowers of *N. stellata*[7].

MATERIALS AND METHODS

Plant material

The Methanolic extract of seeds of *Elaeocarpus ganitrus* were obtained by Soxhlet method. The leaves and seeds of Rudraksha were collected from Bahrich in 2011 from Uttar Pradesh, India. The plant was identified and authenticated by Dr. Tariq Husain, National Botanical Research Institute, Lucknow. The voucher specimen (98159) has been deposited in herbarium.

Standards and reagents

Chloroform, methanol are used as solvent system, plant sample (1) and anisaldehyde sulfuric acid, ferulic acid (F) and quercetin (2) are used as spraying reagent. The calibration curves were constructed and shown in results.

Preparation of crude extracts

Accurately weighed 2.0 gm of the coarse powder of Plant sample extracted with methanol and sonicate for 30 minutes. The combined extracts were filtered and prepare 10mg/ml solution with analytical grade methanol.

High Performance Thin Layer Chromatography

HPTLC was performed on 20 cm x 10 cm TLC glass plates precoated with 200-µm layer thickness of silica gel $60F_{254}$ (a d fine-chem. Limited, Mumbai, India). Samples were applied as 6 mm band width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (camag, Switzerland) under a flow of N_2 gas. The linear ascending development was carried out with Toluene: Ethyl Acetate, [80:20 v/v] for plant samples as mobile phase in a Camag glass twin through chamber (20 x 10 cm). The chamber was previously saturated with mobile phase vapour for 8 minutes at room temperature (25 ± 2°C), 50 % ± 2 relative humidity and plates were developed at distances of approximately 80 mm from the point of application. After development, plates were dried through air dried and scanning were performed using Camag TLC Scanner 3 at λ_{max} 600 nm in UV absorbance mode for plant samples operated by win CATS Software [version 3.2.1]. The shift

dimensions were 4 mm x 0.45 mm and the scanning speed was 100 mm/s.

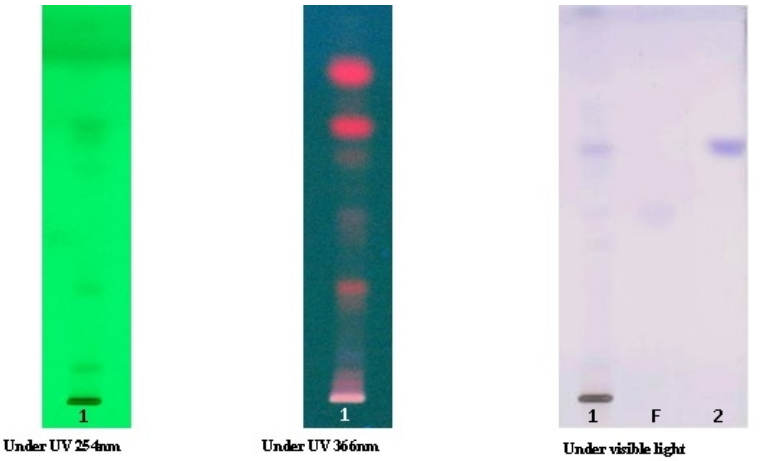

Figure: 1 Photograph of Chromatogram Obtained at, 254nm 366nm and under visible light of extract of *Elaeocarpus ganitrus* Roxb. Seeds Slides shown that -1= Plant sample, F= Ferulic acid, 2= Quercetin

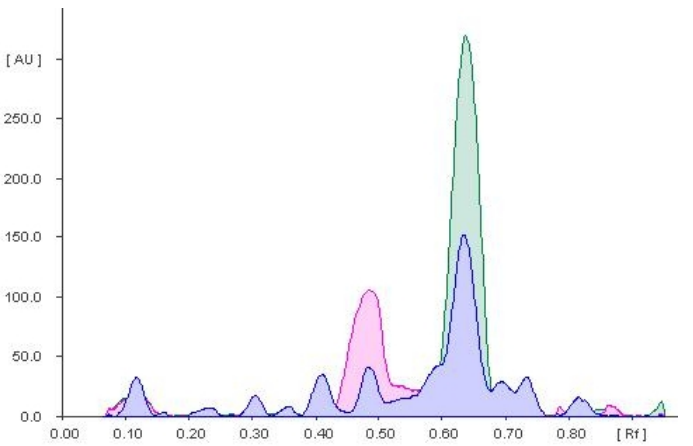

Figure: 2 Densitometric scan profile at 366nm

Solvent system: Chloroform: Methanol: 95:05
Spraying reagent: Anisaldehyde Sulfuric acid
Ferulic acid in 20 µl of sample = 415.96ng
Quercetin in 20 µl of sample = 355.39ng

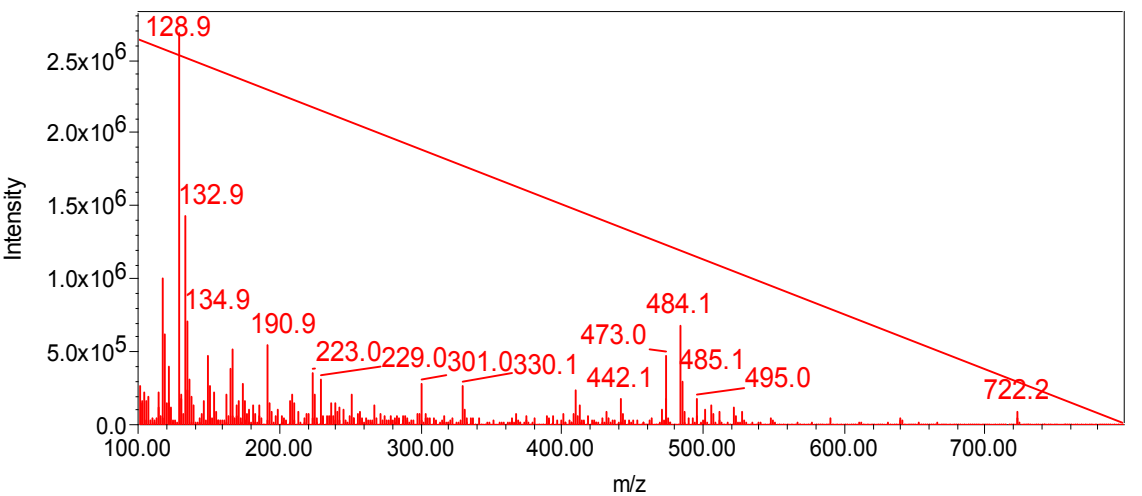

Figure: 3 ESI –MS spectrometry of Quercetin

Molecular mass -302
Negative mode 302-1= 301

RESULT AND DISCUSSION

The developed HPTLC procedure was precise, specific and accurate [8]. In the final stage of investigation we evaluated the content of flavonoids in seeds of *Elaeocarpus ganitrus* using parallel Christ-Müller's and after acid hydrolysis HPTLC methods. The content (in mg) of flavonoids according to the Christ-Müller's methods was calculated as quercetin-flavonol type compound and monoside-hyperoside. Results given as hyperoside varied from 3.1 mg/g to 5.0 mg/g in seeds. Addition of the latter two standards was necessary as isomerisation of 8-C-glycosides to their 6-C-glycosides derivatives took places during acid hydrolysis. Measurements were made by wavelength 342 nm. Standard curves were prepared for individual standards taking into consideration a relationship between peak area field and standard concentration. The obtained results for flavonoids standards indicate good precision of the method used. Kaempferol and quercetin were found to dominate in seeds of *Elaeocarpus ganitrus*. The content of C-glycosides derivatives of apigenin and quercetin in all seed samples was in a standard quantity than flavonol type compounds. On the contrary, in sample C-glycosides derivatives of apigenin and luteolin were dominant. The values obtained for flavonoids sum by Christ-Müller's method were higher than using HPTLC method after acid hydrolysis, which results from a possible reaction between $AlCl_3$ and non-flavonoid compounds present in the investigated samples.

The final result was shown in figure 1 and 2. The graph was also shown.

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