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Original Article

ISOLATION AND IDENTIFICATION OF FLAVONOIDS FROM PROSOPIS JULIFLORA

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ABSTRACT

Objective: The Flavonoids are remarkable and important secondary metabolites, found in plants. The present study was carried out to investigate the flavonoid content present in the leaves, stem, and pods of *Prosopis juliflora*. **Methods:** The established protocol of Subramanian & Nagarajan, 1969 was used for isolation of flavonoids. The structure of the isolated compounds was established on the basis of physical and chemical test and spectroscopic evidences (TLC, IR and GC- MS). **Results:** The study was revealed that the three types of flavonoids Quercetin, Kaempferol and Luteolin were found in *Prosopis juliflora*. Out of these three flavonoids, Quercetin was found as a major flavonoid. Kaempferol and luteolin were found as minor flavonoids. **Conclusion:** The present flavonoids may be responsible for various activities found in this plant, like- Antioxidant activity, anti-microbial activity etc.

Key words: Quercetin, Kaempferol, Luteolin, GC-MS.

INTRODUCTION

Plants, as the source of medicine, have been playing an important role in the health services around the globe. About three-quarters of the world's population relies on plant and their extracts for health care[1]. *Prosopis juliflora* is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world. All parts of *P. Juli flora* have a wide range of uses [2]. It is an important species because of its high nitrogen fixing potential in very dry areas and in drought seasons and also because of it provides shelter and food to many species of animals on its nectar, pollen, leaves and fruits [3]. *Prosopis juliflora* is highly esteemed for windbreaks, soil binders, sand stabilizers, living fences, fuel wood, bee plants and animal feed [4]. It is also known for its ethnomedicinal properties, mainly used for boils, rheumatic pain, digestive disturbances [5]. There are many natural products help to improve and care human health as crude drugs and herbal medicines [6]. Analgesic drugs from Prosopis juliflora are used in single or in combination to affect peripheral or central nervous system (CNS) to decrease pain sensation [7].

MATERIALS AND METHOD

Different plant parts of *P. Juli flora viz*. Leaves, Stem, Pods were collected from Chimanpura (Jaipur), air dried and powdered, separately. Each of these extracted separately with 80% methanol on a water bath for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) separately. Each step was repeated thrice for complete extraction, fraction 1 was discarded in each case because it contained the fatty substance, whereas fraction II and fraction III were concentrated and used for determining flavonoids.

Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid ($10mLg^{-1}$ plant material for 2 h), filtered and the filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings and concentrated *in vacuo* [8].

Thin Layer Chromatography (TLC)

Thin glass plates (20x20 cm) were coated with Silica gel G (250mμ thick). The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100^0C for 30 minutes to activate and then cooled to room temperature. The freshly prepared and activated plates were used for analysis. Each of the extracts was co- chromatographed with authentic flavonoid as a marker. (quercetin, luteolin, and kaempferol). These plates were developed in an air tight chromatographic chamber saturated with a solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3. The developed plates were air dried and visualized under UV light by exposure to ammonia fumes. The developed plates were also sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber separately. The colored spots thus developed were noted and the Rf value of each spot was calculated [9].

Preparative Thin Layer Chromatography (PTLC)

PTLC of aforementioned flavonoid extracts was carried out using silica gel G coated plates (BDH; 500mμ in thickness) by spotting the extract as well as standard markers (luteolin, kaempferol and quercetin). These plates were developed in the solvent mixture of benzene, acetic acid, and water (125:72:3), air dried and examined under UV light. Each of spots corresponding to the standard markers was marked, scraped from 200 plates, and eluted with 50% methanol. The eluted fractions were filtered, air dried and again co-chromatographed along with standard markers to test their purity. The eluted fractions were subjected to crystallization separately and melting point (mp), mixed melting point (mmp) was determined. The isolates were also subjected to ultraviolet and infrared spectral studies [10].

RESULTS

In the present investigation, three types of flavonoids (Quercetin, kaempferol, and luteolin) were found in the plant *Prosopis cineraria*.It was observed that quercetin was major flavonoid while kaempferol and luteoline were minor flavonoids. Total flavonoid content (free+bound) was highest in leaves (8.7 mg/dw) and lowest of that was in the stem (5.6 mg/dw). The maximum amount of free flavonoids was found in leaves (4.5 mg/dw) and minimum amount was found in the stem (2.1 mg/dw). The highest amount of bound flavonoids was found in pods (6 mg/dw) and lowest amount was found in the stem (3.5 mg/dw)(Table -1). The IR figures of Quercetin, Kaempferol and Luteolin are given respectively Fig-1, Fig-2, and Fig-3.

Table 1: Total Flavonoid content in *Prosopis juliflora* L. (in mg/gdw)

Plant Parts	Free Flavonoids				Bound Flavonoids				Total Flavonoids(Free+Bound)			
	Q	K	L	Total	Q	K	L	Total	Q	K	L	Total
Leaf	2.1±0.07	1.8±0.06	0.6±0.02	4.5±0.12	2.9±0.08	1.1±0.02	0.2±0.007	4.2±0.09	5±0.19	2.9±0.08	0.8±0.03	8.7±0.29
Stem	1.2±0.04	0.6±0.02	0.3±0.009	2.1 ±0.07	1.0±0.04	1.1±0.02	1±0.04	3.5±0.08	2.6±0.08	1.7±0.065	1.3±0.03	5.6±0.20
Pod	1.1±0.03	0.6±0.02	.3±0.009	2±0.069	3.9±0.10	2±0.06	.1±0.002	6±0.22	4.8±0.11	2.6±0.06	0.4±0.009	8±0.24

Where Q= Quercetin, K= Kaempferol , L= Luteolin.All values are mean using standard deviation. Values are the mean ± SEM (n = 3 replicates in each group). *P < 0.05; **P < 0.001 compared with the control ; P < 0. 001

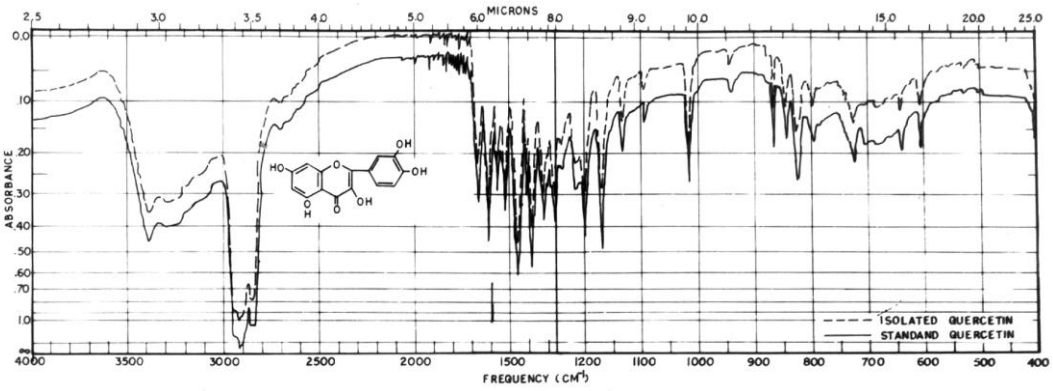

Figure 1: Infra-red Spectra of Isolated and Standard Quercetin.

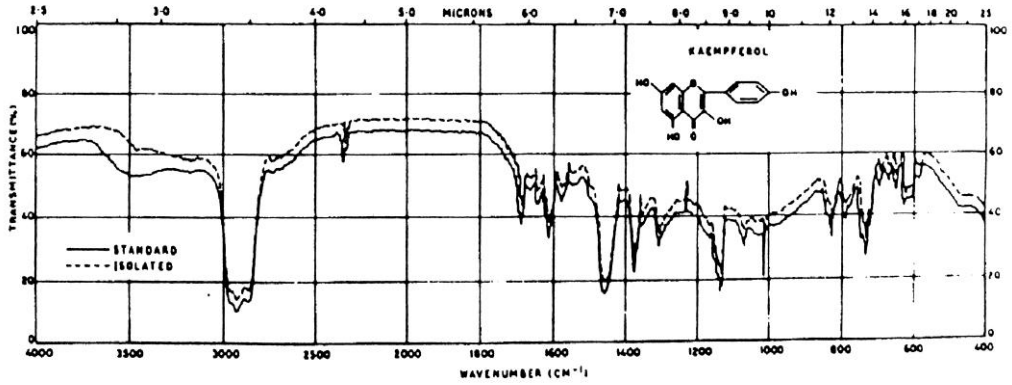

Figure 2: Infra-red Spectra of Isolated and Standard Kaempferol

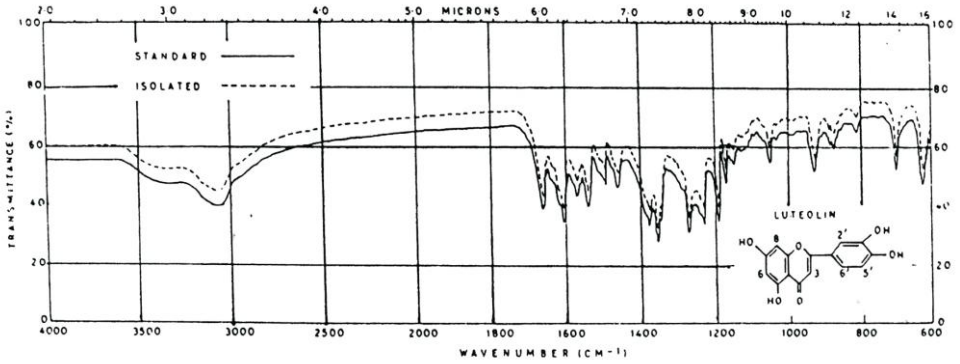

Figure 3: Infrared Spectra of Isolated and Standard Luteolin

DISCUSSION

Flavonoids are used commercially as biologically active compounds and generally high value-low volume products than the primary metabolites, which are used in drug manufacture by the pharmaceutical industries. Flavonoids are compounds found in fruits, vegetables and certain beverages that have diverse beneficial biochemical and antioxidant effects. More than 2000 flavonoids have been identified from plants [11]. Hence the above study concluded that quercetin, kaempferol, and luteolin are present in the parts of *Prosopis juliflora.*

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