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Research Article

ISOLATION AND IDENTIFICATION OF FLAVONOIDS FROM *Sesamum indicum*

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ABSTRACT

Natural substances have long served as sources of therapeutic drugs. Many substances have been derived from traditional medicines. The plants are rich in secondary metabolites. The medicinal properties of these plants have been attributed to the biochemicals present in the plant materials. In addition to their role in human and animal nutrition, knowledge of micronutrients and phytochemical composition is fundamental to the understanding of modes and mechanisms of action of medicinal plants in general. In the present investigation, quercetin and kaempferol have been isolated and identified from stem, leaves and unorganized cultures of *Sesamum indicum* and maintained by frequent subculturings on Murashige and Skoog's medium (1962) supplemented with NAA+BAP(5.0+0.5mg/L). The study showed that maximum content of quercetin and kaempferol was observed in 6 weeks old calli and minimum in stem of *S. indicum.* The structure of the isolated compound was established on the basis of physical, chemical test and spectroscopic evidences.

Key words: Flavonoids, quercetin, kaempferol, *Sesamum indicum*

INTRODUCTION

Flavonoids demonstrate a wide range of biochemical and pharmacological effects including anti-oxidation, anti-inflammation, anti-platelet, anti-thrombotic action, and anti-allergic effects. They can inhibit enzymes such as prostaglandin synthase, lypoxygenase, and cyclooxygenase, closely related to tumorigenesis (Baumann *et al*., 1980; Laughton *et al*., 1991), and induce detoxifying enzyme systems such as glutathione S-transferase (Smith and Yang, 1994). Quercetin inhibited oxidation and cytotoxicity of low-density lipoprotein *in vitro* (De Whaley *et al*., 1990), and can reduce risk for coronary heart disease or cancer (Yoshida *et al*., 1990). The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research (Kokate, 1994). The plants are rich in secondary metabolites (Butenandt *et al*., 1940; Bonner and varner, 1965; Sarin, 2005; 2009). These include flavonoids, alkaloids, pyrethrins, sterols etc. Therefore, a number of analytical techniques have been utilized to evaluate the metabolism and bioavailability of these substances *in vitro* and *in vivo* (Kulling *et al*., 2002; Heinonen *et al*., 1999;).

Sesamum indicum is an erect tropical annual plant growing up to 100 cm tall. It is an ancient cultigen. Today, it is mostly grown in India and the Far East Asia Countries (China, Korea), but its origin is probably tropic Africa although some other sources seem to favour an Indian origin. The seeds of *S. indicum* contain about 50 to 60% of fatty oil, which is characterized by two alkaloids, sesamin and sesamolin. Sesame seed is a source of edible oil and is also used as a spice. They are rich in powerful antioxidants. Sesame seeds have been used as a medicine since antiquity. They are considered to be antioxidant, anticancer, demulcent, emollient and laxative properties. So in this regard an attempt has been made to isolate the compound quercetin and kaempferol from this plant.

METHODOLOGY

Plants were collected from field conditions at Jaipur. Plant parts were cleaned and oven dried at 35ºC for 30 min and then at 25^{0}C till constant weight was achieved and then powdered. The voucher specimen of experimental plant was deposited in Herbarium at Department of Botany, University of Rajasthan, Jaipur (RUBL NO. 20628).

Cotyledonary leaves (derived from mature seed germination) were surface sterilized with mercuric chloride ($HgCl_2$) solution (0.1; w/v) for 2 min and subsequently rinsed thrice with sterile distilled water and treated with antibiotic (ciprofloxacin, 250mgL^{-1}) prior to inoculation in order to remove any kind of microbial interactions. Surface sterilization was done in a horizontal Laminar flow hood fitted with ultraviolet light. Cotyledonary leaves were inoculated in the flasks containing culture medium aseptically supplemented with NAA+BAP (5.0+0.5 mg/L). Cultured flasks were incubated in culture camber. The cultures were observed and examined every week and final morpho-genetic data were recorded. The maintained calli were harvested regularly at the transfer age of 2, 4, 6, 8 weeks. Each of the callus samples was harvested and their growth indices (GI) were calculated on fresh weight basis.

$$GI = \frac{\textit{Final wt. of the tissue} - \textit{Initial wt. of the tissue}}{\textit{Initial wt. of the tissue}}$$

Flavonoid extraction

Each of the shade dried and powdered plant material (leaves, stem and calli) of selected plants were Soxhlet's extracted with 80% methanol (50 g.) for 24 hours on water bath separately (Subramanian and Nagarajan, 1969). The methanol soluble fractions were filtered, concentrated *in vacuo* and the aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr-l), ethyl ether (Fr-II) and ethyl acetate (Fr-III) separately. Each step was repeated three times 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.

Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10 mL/g plant material for 2 hours), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled together separately, neutralized by distilled water with repeated washings, and concentrated *in vacuo*. Both fraction II and fraction III were taken up in small volume of ethanol (2-5 mL) before chromatographic examination.

Thin layer chromatography

Thin glass plates (20X20 cm) were coated with Silica Gel G (250 mμ thick). The freshly prepared plates were dried at room temperature; thereafter theses were kept at 100 C for 30 min to inactivate the enzymes and then cooled at room temperature. Each of the extract was co-chromatographed with authentic samples of flavonoids (kaempherol and quercetin) as markers. These plates were developed in an air-tight chromatographic chamber saturated with solvent mixture .The developed plates were air dried and visualized under UV light and by exposure to ammonia fumes. The mouth of a 100 mL bottle containing concentrated NH_4OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. 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. Several other solvent systems such as *n*-butanol, acetic acid, water (4:1:5, upper layer), *n*-butanol, acetic acid, water (3:1:1) were also tested, but the solvent system containing benzene, acetic acid and water (125:72:3) gave better results.

Preparative thin-layer chromatography (PTLC)

PTLC of above mentioned flavonoids extracts was carried out using silica gel-G coated plates (BDH; 500 mμ in thick) by spotting the extract as well as standard markers (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 the spots corresponding with the standard markers was marked, scraped from 200 plates and eluted with 50% methanol. The eluted fractions were filtered, dried and again co-chromatographed along with standard markers to test their purity. The eluted fractions were subjected to crystallization separately and the melting point (mp), mixed melting point (mmp) was determined. The isolated flavonoids were also subjected to ultraviolet and infrared spectral studies.

Table I. Approximate growth indices of tissue culture of *S. indicum*

S. No.	Age of tissues (weeks)	*S. indicum* (GI)
1	2	0.72
2	4	1.34
3	6	2.23
4	8	1.62

Table II. Callus production from cotyledonary leaves explants of *S. indicum* on MS (1962) medium with varied concentrations and combinations of growth hormones. Values represent treatment of three replicates ± SE

Growth hormone	Concentration (mg/L)	Callus Formation (%)			
		BAP (mg/L)			
		0.5	1.0	3.0	5.0
IAA (mg/L)	0.5	13.5±0.9	15.74±1.23	16.3±1.08	13.1±1.21
	1.0	22.45±0.39	25.6±2.6	28.4±2.36	29.89±1.32
	3.0	31.24±1.7	33.0±1.69	42.9±2.3	41.7±1.3
	5.0	14.3±1.7	17.89±1.76	22.28±1.63	29.56±1.55
IBA (mg/L)	0.5	48.3±0.36	49.57±0.42	51.0±1.86	39.3±1.62
	1.0	49.82±2.83	52.6±1.90	53.41±1.79	40.31±1.41
	3.0	51.0±2.84	41.24±1.26	55.68±0.54	56.77±1.29
	5.0	55.34±1.26	57.33±2.84	58.2±3.1	59.69±2.12
NAA (mg/L)	0.5	76.0±1.90	82.0±1.24	72.11±0.98	74.85±2.76
	1.0	86.0±1.83	87.13±2.55	86.2±3.44	86.8±0.66
	3.0	90.6±0.54	94.3±0.98	83.0±1.88	84.0±1.56
	5.0	95.78±2.05	97.4±3.2	86.53±3.5	88.75±2.06

RESULT AND DISSCUSION

Tissue culture

The callus induction was observed within 20-22 days after inoculation of explant from the cotyledonary leaves on modified MS medium supplemented with different concentration of growth hormones. A combined effect of different growth hormones (cytokinins and auxins) in various combinations was also studied. The callus formation was observed in all the concentration of growth hormo-nes tried but maximum callus growth was observed when MS medium was suppl-emented with NAA+BAP (5.0+0.5 mg/L) (Table I).

The maximum GI (2.23; based on dry weight) was found in tissue growth on MS medium supplemented with NAA+BAP (5.0+0.5 mg/L). At the transfer age of 6 weeks (42 days), which decreased subsequently in 8 weeks old tissue. Minimum GI (0.72; based on dry weight) was observed in 2 weeks old callus culture (Table II).

Flavonoid identification

In *S. indicum,* it is observed that the total flavonoid content (free + bound) was higher in leaf (1.13 mg/g.dw) (Table III) followed by stem (0.98 mg/g.dw). Total flavonoids in their bound form were found to be maxmium in leaf (0.46 mg/g.dw) and lowest in stem (0.39 mg/g.dw). The total free and bound kaempferol content was higher in leaf (0.62 mg/g.dw) as compared to stem (0.53 mg/g.dw). The total free and bound quercetin content was also higher in leaf (0.51 mg/g.dw) as compared to stem (0.45 mg/g.dw) (Table III).

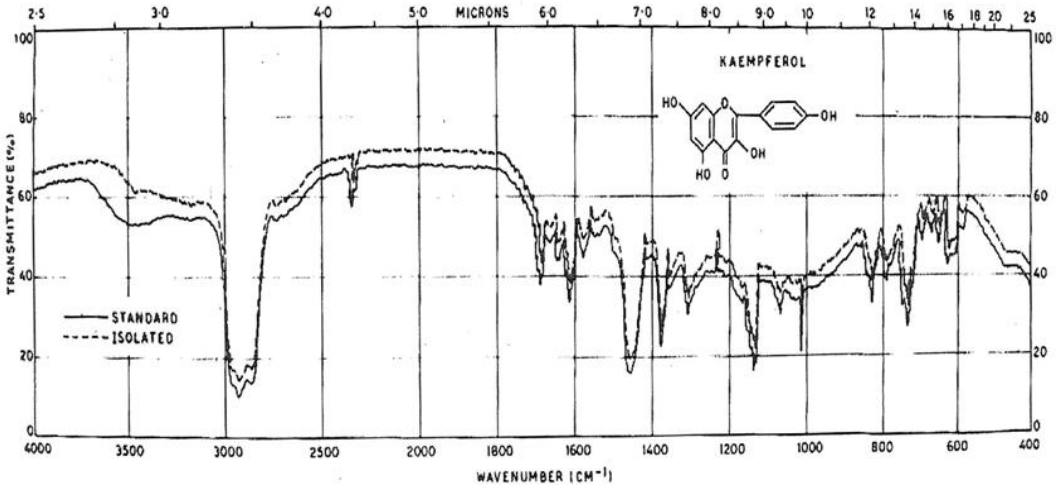

Figure1. Infrared spectra of standard and isolated quercetin

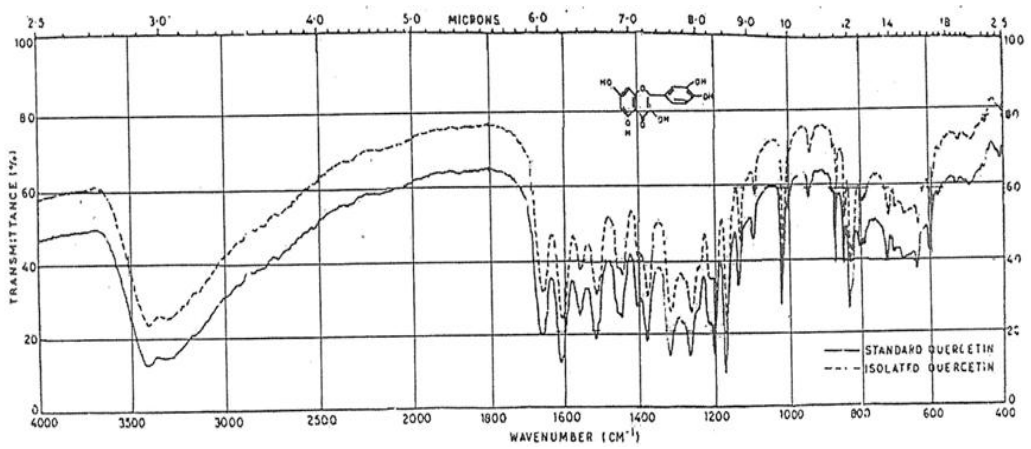

Figure 2. Infrared spectra of standard and isolated kaempferol.

In vitro studies showed maximum amount of total flavonoid content in 6 weeks old calli (1.54 mg/g.dw) (Table IV) and minimum in 2 weeks old calli (0.81 mg/g.dw). The total free and bound kaempferol content was highest in 6 weeks old calli (0.85 mg/g.dw) and lowest in 2 weeks old calli (0.45 mg/g.dw). The total free and bound quercetin content was highest in 6 weeks old calli (0.69 mg/g.dw) and minimum in 2 weeks old calli (0.36 mg/g.dw) (Table IV).

CONCLUSION

Flavonoids are low molecular weight secondary metabolites that, unlike primary metabolites, are not essential for plant survival. Nevertheless, they are bioactive across kingdoms with over 9000 structural variants known (Williams and Grayer 2004). One of their most important roles is to influence the transport of the plant hormone, auxin (Peer and Murphy, 2007).

From the above study, quercetin and kaempferol were isolated and characterized from *S. indicum* plant parts and callus culture with IR spectral studies (Figure 1 and 2). Quercetin and kaempferol has anticancer, antiinfla-mmatory, antiviral, fibromyalgia, metabolic syndrome etc. Quercetin is frequently used therapeutically in allergic conditions, including asthma and hayfever, eczema, and hives. Additional clinical uses

Table III. Yield of flavonoids isolated from various plant of *S. indicum*

Plant parts	Free flavonoids (mg/g. dw)			Bound flavonoids (mg/g. dw)			Total flavonoids (mg/g. dw)		
	Q	K	T	Q	K	T	Q	K	T
Leaf	0.30	0.37	0.67	0.21	0.25	0.46	0.51	0.62	1.13
Stem	0.28	0.31	0.59	0.17	0.22	0.39	0.45	0.53	0.98

Abbreviations: K: Kaempferol: Q: Quercetin and T: Total Flavonoids

Table IV. Yield of flavonoids isolated from *in vitro* cultures of *S. indicum*

Age of tissue	GI	Free flavonoids (mg/g. dw)			Bound flavonoids (mg/g. dw)			Total flavonoids (mg/g. dw)		
		Q	K	T	Q	K	T	Q	K	T
2	0.72	0.19	0.24	0.43	0.17	0.21	0.38	0.36	0.45	0.81
4	1.34	0.26	0.30	0.56	0.20	0.26	0.46	0.46	0.56	1.02
6	2.23	0.37	0.48	0.85	0.32	0.37	0.69	0.69	0.85	1.54
8	1.62	0.28	0.33	0.61	0.22	0.30	0.52	0.50	0.63	1.13

Abbreviations: K: Kaempferol: Q: Quercetin and T: Total Flavonoids; GI : Growth Index

include treatment of gout, pancreatitis and prostatitis, which are also, in part, inflammatory conditions. The common link is its ability to mediate production and manufacture of proinflammatory compounds.

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