Anti-inflammation Activity of 3′-methoxyquercetin-3-O-rutinoside isolated from *Ipomoea sepiaria*

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Abstract: ***Ipomoea sepiaria (Convolvulaceae) is a source of the classical Ayurvedic medicinal plant Lakshmana. In folklore practice this herb is known as a good antidote to arsenic poisoning, uterine tonic, aphrodisiac and anti-ulcer drug. The present investigation of isolation of 3′-methoxy quercetin-3-O-rutinoside was obtained from the flowers of Ipomoea sepiaria. Moreover, the absence of toxicity of plant extracts and the isolation of active compounds are important to propose these plants as alternative approaches to resistance management and significant inhibitory effect on the oedema formation. The structure was established based on analysis of HPLC, UV, IR, ^{1}H NMR and ^{13}C NMR spectroscopy methods.***

Keywords: *Ipomoea sepiaria*, Convolvulaceae, quercetin, Anti-inflammation

1. Introduction

Natural products from plants provide a source for bioactive compounds and have the potential for developing some novel therapeutic agents. There has been an ever growing interest of drugs originating from plants which have been found to form an important class for disease control[1]. Flavonoids are polyphenols widely distributed in plants. There are about 5000 kinds of flavonoids, including flavones, biflavonoids, flavanones, flavanonols, isoflavones, flavan-3-ols, chalcones, flavan-3,4-diols, dihydrochalcones, anthocyanidins, etc., which have the function of protecting cardiovascular, antioxidant, anti-tumor, anti-inflammation, anti-virus and adjusting immunity[2].

Inflammation is a part of the immune response against infection and has been implicated in a broad range of diseases like as including diabetes, cancer, hypertension and atherosclerosis[3, 4]. It is the body's immediate response to damage its tissues and cells by pathogens, noxious substances, or physical injury[5]. These instigators induce activation of inflammatory mediators such as kinins, cyclo-oxygenase products and cytokines, which have become key targets for therapeutic intervention in a range of diseases including pain[6].

Ipomoea sepiaria (Convolvulaceae) is a source of the classical Ayurvedic medicinalplant *Lakshmana*[7]. It is a glabrous or occasionally pubescent, slender twinning climber with a slightlythickened or tuberous perennial root.The root system consists of afairly long, somewhat thickened taproot andseveral slightly thinner or slender branches, arising from its base with very few wiryrootlets[8]. Leaves are simplealternate, entire, blotched with brownishpatches towards the middle[9]. Flowers are delicate purple or whitewith a purple eye, along with short to longpeduncles and short pedicels[10].

In folklore practice this herb is known as agood antidote to arsenic poisoning, uterinetonic, aphrodisiac and anti-ulcer drug[11]. It is reported tobe used in burning sensation, strangury, generaldebility and sterility in women[12]. The literatures further specify theuse of root in case of diabetes[13] and constipation[14]. As *I. sepiaria* plant is used in the treatment of leucorrhoea, itshould possess some antimicrobial property. The aim of this study is to screen the anti-inflammatory activity of 3′- methoxy quercetin-3-O-rutinoside isolated from the flowers of *Ipomoea sepiaria*.

2. Materials and Methods

Collection of plant material

The fresh flowers of *Ipomoea sepiaria* were collected in the month of September - October from the area of Cauvery basin, Thiruvarur, Tamilnadu, India. These Plants were identified and authenticated by Dr. S. Dharmarajan, Assistant Professor & Head, Department of Botany, Thiru.Vi.Ka. Government Arts College, Bharathidasan University, Trichirappalli, Tamilnadu, India. The voucher specimen (TGACBOT-134) was maintained in our research laboratory for future reference. The collected fresh flower materials were washed properly and dried in shade. Dried plant material was subjected to reduction to coarse powdered and stored in airtight container for further use.

Isolation and Identification

The important stage in the experimental work includes first the isolation of chemical substances from the chosen plant and secondly, the characterization of those isolated compounds. The flowers *Ipomoea sepiaria* (2.5 Kg) were extracted with 90% methanol (MeOH) (6 X 500ml) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with peroxide free ether (3 x 250 ml) and ethyl acetate (5 x 250 ml) (Sigma Aldrich Co., India). The ether fraction was concentrated *in vacuo* and left in an ice chest for about a week. A yellow solid that separated was filtered. The pale yellow needles were obtained (m.p. 306-308˚C) on crystallization from Ethanol. It was soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, Olive green colour with alcoholic Fe^{3+}, golden yellow colour with NH_3 and NaOH, yellow solution with a pale green fluorescence with con. H_2SO_4 appeared yellow under UV and NH_3. It answered Wilson's boric acid, Horhammer-Hansal and

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Gibb's tests but did not respond to Molisch's test. It had λ_{max}^{MeOH} nm 255, 269sh, 370; +NaOMe 247, 306, 420; +$AlCl_3$ 272, 304 sh, 333, 460; + ($AlCl_3$ / HCl) 264, 303 sh, 358, 426; +NaOAc 276, 329sh, 390; and + (NaOAc/ H_3BO_3) 261, 303 sh, 388 and had R_f values as depicted in Table 1. It was identified as 3′- methoxy quercetin and the same was confirmed by paper chromatography (p.c) and melting point (m.p) with an authentic sample of 3′- methoxy quercetin from *Rhododendron petal*[15].

The ethyl acetate fraction was concentrated *in vacuo* and left in an ice-chest for few days. A yellow solid (m.p. 312-314˚C) that separated was filtered and studied. It developed a green colour with alcoholic Fe^{3+} and a pink colour with Mg-HCl. It appeared deep purple under UV that turned yellow on exposure to NH_3. It responded to Wilson's boric acid, Molisch and Gibb's tests, but did not answer the Horhammer-Hansal tests. It had λ_{max}^{MeOH} nm 254, 268sh, 299sh, 358; +NaOMe 272, 330, 410; +$AlCl_3$ 275, 302 sh, 433; + ($AlCl_3$ / HCl) 268, 303 sh, 362 sh, 400; +NaOAc 277, 334, 393; and + (NaOAc/ H_3BO_3) 267, 299 sh, 387. It was identified as 3′- methoxy quercetin-3-O-rutinoside and the same was confirmed by paper chromatography (p.c) and melting point (m.p) with an authentic sample from *Wrightia tinctoria*[16].

Supporting evidence for the structure of the flavonol glycoside is provided by the HPLC (Shimadzu, Columbia), UV (Perkin Elmer Spectrophotometer), IR (Perkin - Elmer spectrometer) and NMR (400 MHz, DMSO-d_6 and TMS) spectral data were recorded on a Bruker AMX 400 NMR spectrometer. Chemical shifts were reference to the respective residual solvent peaks and the values were recorded in δ.

3′- methoxyquercetin-3-O-rutinoside

Yellow solid; m.p. 312-314˚C; RT 26.7 min; UV λ_{max}^{MeOH} 254, 358 nm; IR (KBr): ν_{max} 3288, 2982, 2945, 2834, 1646, 1481, 1405, 1230, 1107, 1082, 1060, 1015 and 920cm^{-1};^{1}H-NMR spectrum δ (400 MHz, DMSO-d_6, TMS): δ ppm 6.397 (H-6), 6.204 (H-8), 7.566 (H-2′), 6.843 (H-5′), 6.866 (H-6′), 5.14 (H-1″), 4.13 (H-1‴), 1.31 (- OCH_3), 1.01 (Rha- CH_3), 12.6 (5-OH), 9.18 (7-OH), 3.0 ~ 3.84 (Rest of sugar protons); ^{13}C- NMR(400 MHz, DMSO-d_6 and TMS): δ ppm 177.3 (C-4); 164.22 (C-7); 161.31 (C-5); 160.0 (C-9); 156.3 (C-2); 151.3 (C-3′); 148.3 (C-4′); 133.2 (C-3); 125.5 (C-1′); 121.4 (C-6′); 116.3 (C-5′); 115.8 (C-2′); 104.1 (C-10, 1‴);101.8 (C-1″); 98.7 (C-6); 93.7 (C-8); 77.5 (C-5″, 4‴); 75.8 (C-2″, 2‴); 73.4 (C-3″); 74.1 (C-3‴); 69.9 (C-5‴); 67.9 (C-6″); 67.3(C-4″); 54.71 (3′-OCH_3); 17.5 (C-6‴).

Hydrolysis of the glycoside

The glycoside dissolved in hot aqueous methanol was hydrolyzed with H_2SO_4 (5%) at 100˚C for about 2 hrs. The excess of alcohol was distilled off *in vacuo* and the resulting aqueous solution was extracted with ether. The residue from ether fraction was isolatedas described below. The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resulting solution extracted with ethyl acetate.

Phytochemical screening of plant extract

A small amount of the dry extract was used for the phytochemical tests[17] for compounds which include alkaloids, flavonoids, tannins, saponins, glycosides, phenol and terpenoids while steroids, coumarin and cardiac glycosides are absent in all the crude extracts.

Anti-inflammatory activity by Carrageenan induced rat paw oedema

The anti-inflammatory activity of the test compounds were evaluated in albino rats employing the method[18]. Male albino rats (200 - 250 g) of Wistar strain were procured from the College animal house. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of six animals. The different test concentration at the dose of 100 and 200 mg / kg of isolated compounds, 300 mg methanolic extracts and Diclofenac sodium (98 %, purchased from Sigma-Aldrich, India) at dose of 10 mg/kg were administrated to the animals by oral route. Control group animals were received 1 % DMSO at the dose of 10 mL/kg body weight. They housed in cages and maintained under standard conditions at 26 ± 2 °C and relative humidity 60 - 65 % and 12 hours light and 14 hours dark cycles each day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet and water adlibitum. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

The acute inflammation was induced by the sub-plantar administration of 0.1 mL of 1 % carrageenan in the right paw. Paw volume was measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, 3, 4 and 5 hours intervals[19]. The efficacy of different drug was tested on its ability to inhibit paw oedema as compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

The Percentage inhibition of paw oedema was calculated by the formula as below.
% Inhibition of Paw oedema = [(VC – VT) / VC] x 100
Where, VC = Paw oedema of control group and VT = Paw oedema of treated group

Acute toxicity studies

Acute toxicity studies were carried out according to the literature[20]. Animals were fasted for eighteen hours and used. A dose of 200 mg/kg of isolated compound and 300 mg/kg methanolic extracts of *Ipomoea sepiaria* were administrated orally to 12 rats, additionally three rats were kept as control. The control group received distilled water. Then they were observed for 72 hours. Since no mortality was observed and the behavioral pattern was unaffected. No depth was observed at the end of the study.

Statistical analysis

The experimental results were expressed as multiple comparisons of Mean ± SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple

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Comparisons Test and statistical significance was defined as $P < 0.05$.

3. Results and Discussion

Chemical Constituents

3′-methoxy quercetin-3-O-rutinoside has been isolated from the fresh flowers of *Ipomoea sepiaria*. The aglycone had λ_{max}^{MeOH} 254, 358 nm suggesting the presence of a flavonol skeleton. No bathochromic shift was observed (band I) and its NaOMe spectrum (band I) indicated the absence of free OH group at C-3′. The presence of free -OH group at C-5 was evident from the bathochromic shift of +56 nm in $AlCl_3$-HCl spectrum (band I). A shift of 7 nm towards the longer wavelength (band II) in the NaOAc spectrum indicated the presence of a free OH group at C-7.

In both glycoside and the aglycone no additive bathochromic shift was noticed in $AlCl_3$ spectrum (band I) with respect to $AlCl_3$-HCl spectrum, which confirms the absence of O-dihydroxyl grouping in the B-ring. No characteristic change was noticed in band I of the glycoside and aglycone on the addition of NaOAc-H_3BO_3 thereby revealing the absence of O-dihydroxyl grouping in the B-ring. A bathochromic shift observed in MeOH spectrum (band I) of the aglycone obtained on hydrolysis of the glycoside as compared to the glycoside suggested the site of glycosylation to be at C-3. It was also supported by the fact that the glycoside did not respond to Horhammer-Hansel test.

The IR spectrum indicated the presence of (IR) ν_{max} 3288, 2982, 2945, 2834, 1646, 1481, 1405, 1230, 1107, 1082, 1060, 1015and 920 cm^{-1}. HPLC analysis showed the presence of a flavonol glycoside and its identified peak with the retention time of 26.7min.^{1}H NMR spectrum (400 MHz, DMSO-d_6, TMS) of the glycoside, the signal appearing at δ 12.6 ppm corresponds to -OH at C-5 and downfield region signal at 9.18 ppm is due to the hydroxyl proton at C-7. The doublet appearing in the region of δ 7.566 ppm and δ 6.866 ppm correspond to the protons at C-2′ and C-6′. The methyl protons of -OCH_3 unit can be seen at δ 1.316 ppm. The signal appearing at δ 6.84 ppm corresponds to C-5′ proton. C-8 proton due to meta coupling with C-6 proton appears as a doublet at δ 6.39 ppm. C-6 proton due to meta coupling with C-8 proton appears as a doublet at δ 6.20 ppm. H-1″ of glucose resonates at δ 5.14 ppm while the H-1‴ of rhamnose at δ 4.13 ppm. The methyl protons of the rhamnose appear at δ1.01 ppm, the remaining sugar protons appear in the region of δ 3.0 to 3.84 ppm.

Supporting evidence for the structure of the flavonol glycoside is provided by the ^{13}C-NMR spectral data. The ^{13}C-NMR spectral data for the corresponding aglycone taken out from the literature are also tested for easy comparison. Due to the glycosylation, the signal of C-3 is shifted up field by δ 1.51 ppm. The downfield shift of Ortho-related C-2 signal by δ 10.6 ppm also confirms the same. The large shift in C-2 resonance also reflects the semi-olefinic characters of the flavonol C-2, C-3 double bond. The signal of OCH_3 unit can be seen at δ 54.7 ppm. The signal at δ 104.1 ppm of C-10 is less intense due to longer relaxation time of the quaternary carbon. The signal of C-6‴ of rhamnose at δ 17.5 and that of C-6″ signal at δ 67.9 ppm clearly show that the glycoside is a 3′-methoxy quercetin-3-O-rutinoside[21].Based on their R_f values, HPLC, UV, IR, ^{1}H-NMR and ^{13}C-NMR data's, the glycoside obtained from EtOAc fraction of the flower from *Ipomoea sepiaria* could be confirmed as 3′-methoxy quercetin-3-O-rutinoside (Figure. 1).

Anti-inflammatory activity

Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents. The paw oedema induced by carrageenan involves several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins[22]. The intraperitoneal injection of the carrageenan produced an inflammatory oedema which increased gradually, reaching its maximum at the 5th hour after injection. In this study, the isolated compound 3′-methoxy quercetin-3-O-rutinosidetested at doses of 100 and 200 mg/kg exhibited significant anti-inflammatory activity in carrageenan induced rat paw oedema model (Table 2). Diclofenac Sodium is a reference drug, a potent non-steroidal anti-inflammatory which acts by inhibiting cyclo-oxygenage. It showed significant inhibitory effects were observed started at first hour after carrageenan administration. The compound 3′-methoxy quercetin-3-O-rutinoside at the dose of 200 mg/kg had the highest percentage inhibition of the paw volume (2.97 ± 0.05) and at the dose of 100 mg/kg caused (3.45 ± 0.03) inhibition of the paw volume at 4 hours a performance well comparable with the standard drug Diclofenac Sodium (2.65 ± 0.04). Also after the administration of carrageenan with the methanol extract of *Ipomoea sepiaria* at the dose of 300 mg/kg exerted considerable inhibitory effect on paw oedema in rats starting from the first hour. The maximum inhibition (3.48 ± 0.04) elicited by the methanol extract was recorded at 5 hours. In the carrageenan-induced rat paw oedema model, 3′-methoxy quercetin-3-O-rutinosideshowed significant inhibitory effect on the oedema formation. This effect started from the first hour and was maintained in all the inflammatory phases, suggesting that the main mechanism of action of the tested compound may involve prostaglandin biosynthesis pathway and may influence other mediators of inflammation.

4. Conclusion

The isolation of active compounds is important to propose medicinal plants *Ipomoea sepiaria* as alternative approaches to resistance management and significant inhibitory effect on the oedema formation. By ways of solvent extraction, hydrolysis and high purity of flavonoid with 3′-methoxy quercetin-3-O-rutinoside structure were acquired in our research. The method is suitable for isolation of flavonoid from the *Ipomoea sepiaria* in industry.

5. Conflict of Interest

The authors declare no conflict of interest.

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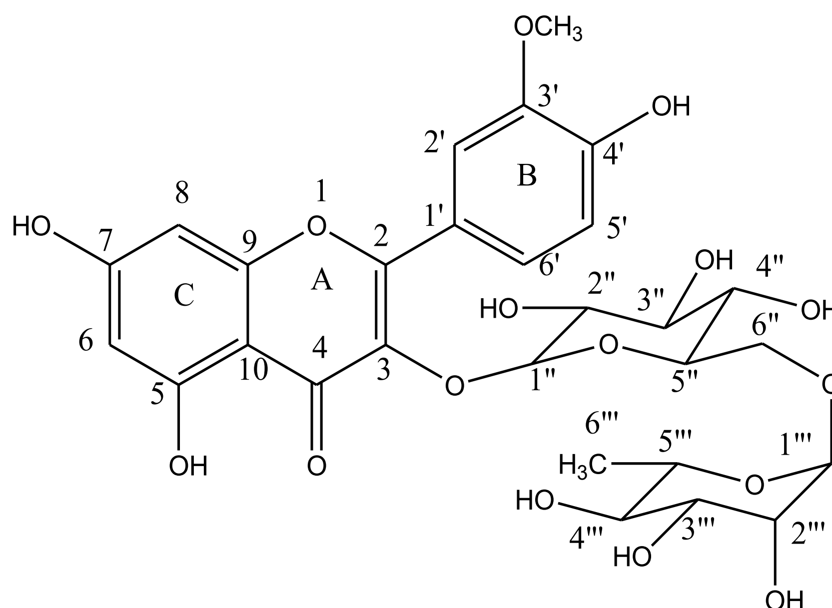

Figure 1: 3'-methoxy quercetin-3-O-rutinoside

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Table 1: R_f (X100) values of the constituents of the flowers of *Ipomoea sepiaria*
(Whatman No.1, Ascending, 30 ± 2˚C)

Compound	*Developing Solvents								
	a	b	c	d	e	f	g	h	i
[1]Glycoside	-	-	03	18	46	74	79	52	81
[2]Aglycone (Complete hydrolysis)	-	-	05	20	48	91	81	61	83
3′- methoxy quercetin(authentic)	-	-	04	19	48	90	80	61	82
Glycoside from partial hydrolysis	28	08	62	33	60	38	64	58	67
3′- methoxy quercetin-3-O-glucoside (authentic)	27	07	62	34	59	38	63	57	67

Compounds
[1]Glycoside - 3′- methoxy quercetin-3-O-rutinoside
[2]Aglycone - 3′- methoxy quercetin
***Solvent key**
a → H_2O
b → 5% aq.HOAc
c → 15% aq.HOAc
d → 30% aq.HOAc
e → 60% aq.HOAc
f → BAW (n-BuOH: HOAc: H_2O=4:1:5, upper phase)
g → phenol saturated with water
h → forestol (HOAc: conc. HCl)
i → TBA (t-butanol-acetic acid-water, 3:1:1)

Table 2: Anti-inflammatory activity of 3′-methoxy quercetin-3-O-rutinosideextracted from *Ipomoea sepiaria*

S. No.	Treatment	Anti-inflammatory activity (Cm) (M±SD)				
		1 h	2 h	3 h	4 h	5 h
1	Normal Control	2.33±0.01	2.33±0.01	2.32±0.01	2.31±0.05	2.31±0.01
2	Inflammatory Control (1% carragenan)	3.81±0.05	3.60±0.06	3.52±0.02	3.51±0.04	3.52±0.02
3	Standard (1% carragenan + diclofenac sodium 10 mg/kg)	3.85±0.08	3.40±0.13	3.19±0.14	2.86±0.06	2.65±0.04
4	1% carragenan + 3′-methoxy quercetin-3-O-rutinoside (100 mg/kg)	3.83±0.04	3.71±0.08	3.62±0.02	3.54±0.03	3.45±0.03
5	1% carragenan + 3′-methoxy quercetin-3-O-rutinoside(200 mg/kg)	3.76±0.05	3.58±0.07	3.24±0.11	3.10±0.06	2.97±0.05
6	1% carragenan + methanol extract (300 mg/kg)	3.82±0.02	3.74±0.13	3.68±0.01	3.56±0.07	3.48±0.04

Data presented above are mean ± standard deviation (M±SD) values of three replicates.

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