Natural Product Sciences
21(3) : 170-175 (2015)

Isolation of Quercetin and Isorhamnetin Derivatives and Evaluation of Anti-microbial and Anti-inflammatory Activities of *Persicaria glabra*

R. Manivannan* and R. Shopna

Department of Chemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu-612001, India

Abstract – The present study aims to detect the rare flavonoids isolated from the leaves of *Persicaria glabra.* The known flavonoids: quercetin (**1**), isorhamnetin (**2**), avicularin (**3**) and new one isorhamnetin-3-*O*-α-L-(6"-*E*-*p*-coumaroyl)-rhamnoside (**4**) were identified by HPLC, UV, IR and NMR. *P. glabra* has used traditionally for its analgesic, anti-inflammatory and anti-rheumatic properties. To find out the ingredients responsible for the efficiency of this plant, we have used to study the anti-microbial and anti-inflammatory activities of different extracts.
Keywords – *Persicaria glabra,* Flavonoids, Anti-microbial, Anti-inflammatory

Introduction

Phytochemicals show a potential for modulating human metabolism in a manner useful to prevent acute and degenerative diseases.[1] Flavonoids, the most generous group of secondary metabolites in plant kingdom which pharmacological effects such as anti-inflammatory, anti-microbial, anti-oxidative, radical-scavenging, enzyme inhibiting as well as enzyme-inducing properties.[2,3] Many flavonoids such as quercetin and rutin have revealed to be inhibitors of the cycloxygenase and lipoxygenase. However, a prostaglandin-independent was also proposed.[4] Based on the literatures we have chosen *Persicaria glabra* (Willd.) (Polygonacea) based on its availability, and therapeutic value which has not done mostly in earlier. The present study aims to detect the rare flavonoids has isolated from the leaves of *P. glabra* and the results were reported here. The known flavonoids: quercetin (**1**), isorhamnetin (**2**), avicularin (**3**), and a new one isorhamnetin 3-O-α-L-(6"-E-*p*-coumaroyl)-rhamnoside (**4**) were identified (Fig. 1) using HPLC, UV, IR and NMR by comparison with the published data. Phytochemicals may help slow the aging process and reduce the risk of many diseases such as pain, asthma, cancers, heart diseases, cataracts, and osteoporosis and urinary tract infections etc., so we decided to find out anti-microbial and anti-inflammatory activities of flavonoids isolated from plant extracts.

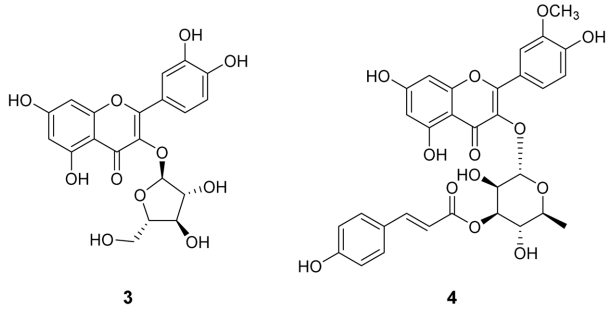

Fig. 1. Structure of isolated flavonoids avicularin (**3**) and isorhamnetin-3-*O*-α-L-(6"-*E*-*p*-coumaroyl)-rhamnoside (**4**).

Experimental

General experimental procedures – Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. Optical rotation was measured on a JASCO P-2000 polarimeter (Tokyo, Japan). A UV spectrum has recorded on Ultraviolet spectrophotometer (UV2550, shimadzu, Japan). An IR spectrum has measured on FT-IR spectrograph (Perkin Elmer Spectrophotometer, USA) with KBr tablets from 4000 to 400 cm^{-1} with resolution 2 cm^{-1}. NMR experiments have performed on a Bruker AMX 400 instrument (Bruker Company, Faelladen, Switzerland) standard pulse sequences running at 400 MHz for ^{1}H NMR and ^{13}C NMR. Chemical shifts gave in δ (ppm) about TMS as internal standard material and the coupling constants (*J*) are in Hz. Column chromatography (CC) has performed on silica gel 60 as stationary phase (particle size 0.04 - 0.036 mm, 230 - 400 mesh,

*Author for correspondence
R. Manivannan, Department of Chemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu-612001, India
Tel: +91- 9095715550; E-mail: manickam_mani@yahoo.co.in

ASTM E. Merck, Germany) and activated by heating at 110 ºC for one hour before to use. Analytical TLC was carried out on 0.25 mm Brinkman precoated Silica Gel F254 plates (silica gel 60 / 230 - 400 mesh, Merck, Germany). The different solvent systems in volumetric ratios were employed (vol. ratios): Compound **1**: EtOAc/MeOH/H_2O (98:1:1); Compound **2**: *n*-BuOH/AcOH/H_2O (4:1:5, organic phase); Compound **3**: $CHCl_3$/EtOAC/MeOH (14:3:3). Flavonoids were visualized by UV light 365 nm, with NH_3 vapors and by spraying with 1% $AlCl_3$ in MeOH. A sugar was detected by spraying with aniline phthalate solution in *n*-BuOH and heating at 105 ºC. A Shimadzu HPLC system (Columbia, MD), was used with UV detection at 280 - 350 nm. A chromatographic system comprising a Spectra Physics P-200 series gradient pump (Fremont, CA, USA), a rheodyne injector fitted with a 20-FL loop, the C18 column (250 × 4.6 mm) (phenomenex, Torrance, CA, USA) was used.

Plant materials – The leaves 2.30 kg of *P. glabra* was collected during April from the river basin of Cauvery in Thanjavur District, Tamilnadu (India) and authenticated by Prof. N. Ramakrishnan, (Department of Botany) and voucher specimens (GACBOT-160) was deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India. The leaves of *P. glabra* were extracted with 90% methanol (MeOH) (4 × 500 mL) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with peroxide free ether (3 × 250 mL) and ethyl acetate (4 × 250 mL).

Extraction and isolation – Traditional methods, such as silica gel column chromatography and thin-layer chromatography (TLC) has used to separate and purification of bioactive compounds from *P. glabra*. The air dried powdered leaves of *P. glabra* (2.30 kg) was extracted with 95% MeOH and evaporated under reduced pressure to give a MeOH extract (75.6 g), which was dissolved in water (1000 mL) and then successively partitioned with benzene, petroleum ether and EtOAc yielding 10.8 and 19.4 g of residue, respectively. The petroleum ether fraction (10.8 g) was separated over a silica gel column to yield compound **1** (5.2 g) [n-BuOH/AcOH/H_2O (4:1:5, organic phase)] and to yield compound **2** (3.1 g) [$CHCl_3$/EtOAc/MeOH (14:3:3)]. Similarly, the EtOAc fraction (19.6 g) was subjected to column chromatographic methods with EtOAc/MeOH/H_2O (98:1:1) and EtOAc/HCOOH/H_2O (10:2:3) as eluents to afford compounds **3** (8.3 g) and **4** (6.2 g). There was no sign of realizing any crystalline material from the benzene fraction.

Acid hydrolysis – 10 mg of the crude leaf EtOAc extract was refluxed separately with 5% H_2SO_4 (5.0 mL) for 2 h. The hydrolysates extracted with EtOAc and held extracts washed with water, evaporated to dryness and resolved in MeOH. Identification of the aglycones was done by TLC (on silica gel 60) with standards of quercetin (R_f: 0.78) isolated from *Prunus spinosa*[5], isorhamnetin (R_f: 0.34) existed from *Pyruscommunis.*[6] The sugars in the aqueous layer has identified by TLC (on silica gel 60) with reliable samples using different solvent system: L-arabinose (n-BuOH/AcOH/H_2O (4:1:5, organic phase) (R_f: 0.20), L-rhamnose (EtOAc/HCOOH/H_2O, 10:2:3; R_f: 0.33) and *p*-coumaric acid (toluene/ethyl acetate/formic acid, 36:12:5; R_f: 0.56).

Anti-microbial activity – Anti-microbial activity test was carried out in the following variation of the method originally described by Bauer *et al*.[7] Muller Hinton ager was prepared and autoclaved at 15 lbs pressure for 20 min and cooled to 45 ºC.The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The plant extracts were prepared at different dose individually placed on the each petriplates discs and placed control and standard (streptomycin and amphotericin) discs. The plates were incubated at 37 ºC for 24 h. After incubation period, the zone of inhibition surrounding the discs was measured using a transparent ruler and the diameter recorded in mm.

Anti-inflammatory activity by Carrageenan induced paw edema – The anti-inflammatory activities of the test compounds were evaluated in male albino rats (200 - 250 g). Animals have fasted overnight and divided into control, standard and different test groups each consisting of six animals at the dose of 300 mg/kg of petroleum ether, ethyl acetate and methanolic extracts and Diclofenac sodium (100 mg/kg) was administrated to the animals by oral route. Control group animals have received 1% DMSO at the dose of 10 ml/kg body weight. They housed in cages under standard conditions at 26 ± 2 ºC and relative humidity 60 - 65% and 12 h light and 14 h dark cycles each day for one week before and during the experiments. All animals have fed with standard rodent pellet diet, and water adlibitum. Before starting the experiment on animals, the experimental protocol has 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 has induced by the sub-plantar administration of 0.2 ml of 2% carrageenan in the right paw. Paw volume has measured by using digital plethys-

mometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2 3 and 4 h intervals. The efficiency of different treated groups has tested on its ability to inhibit paw edema compared to control group.

Volume of edema = Final Paw Volume – Initial Paw Volume

The Percentage inhibition of paw edema has calculated by the formula as below.

% Inhibition of Paw edema = [(VC – VT) / VC] × 100

Where, VC = Paw edema of control group and VT = Paw edema of treated group

Statistical analysis – The experimental results expressed as statistical comparisons of Mean ± SEM was carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 has considered as statistically significant.

Quercetin (1) – Yellow powder; mp 183 - 185 ºC; RT: 30.7; UV λ_{max}^{MeOH} (log ε) 255 - 370 nm; IR ν_{max} (KBr) : 3390, 2950, 2830, 1652, 1614, 1520, 1455, 1026, 827 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm, *J*, Hz): 6.21 (1H, d, *J* = 2 Hz, H-6), 6.44 (1H, d, *J* = 2 Hz, H-8), 7.64 (1H, d, *J* = 2.2 Hz, H-2'), 6.92 (1H, d, *J* = 8.5 Hz, H-5'), 7.52 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 9.43 (1H, s, 4'-OH), 9.30 (1H, s, 3'-OH), 9.66 (1H, s, 3-OH), 12.59 (1H, s, 5-OH), 10.42 (1H, s, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, *J*, Hz): δ 179.4 (C-4), 165.2 (C-7), 160.2 (C-5), 158.1 (C-9), 148.6 (C-4'), 147.2 (C-2), 145.6 (C-3'), 134.6 (C-3), 123.2 (C-1'), 121.5 (C-6'), 116.8 (C-5'), 117.4 (C-2'), 104.2 (C-10), 99.6 (C-6), 94.8 (C-8).

Isorhamnetin (2) – Yellow needle; m.p. 302 -304C; RT: 11.20; UV λ_{max}^{MeOH} (log ε) 260 - 280 nm; IR ν_{max} (KBr) : 3310, 2930, 2840, 1670, 1628, 1510, 1462, 1034, 590 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm, *J*, Hz): 6.21 (1H, d, *J* = 2.1 Hz, H-6), 6.42 (1H, d, *J* = 21 Hz, H-8),7.68 (1H, d, *J* = 2.1 Hz, H-2'), 6.92 (1H, d, *J* = 8.5 Hz, H-5'), 7.58 (1H, dd, *J* = 8.5, 2.1 Hz, H-6'), 9.74 (1H, s, 4'-OH), 3.76 (1H, s, 3'-OMe), 9.48 (1H, s, 3-OH), 12.52 (1H, s, 5-OH), 10.76 (1H, s, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, *J*, Hz): δ 176.8 (C-4), 165.6 (C-7), 160.4 (C-5), 157.4 (C-9), 148.8 (C-4'), 156.8 (C-2), 145.9 (C-3'), 134.8 (C-3), 121.5 (C-1'), 121.4 (C-6'), 116.1(C-5'), 115.4 (C-2'), 104.1 (C-10), 99.0 (C-6), 93.8 (C-8).

Quercetin 3-*O*-α-L-arabinofuranoside (Avicularin) (3) – Yellow needle crystals; m.p. 180 - 183 ºC; RT: 13.06; UV λ_{max}^{MeOH} (log ε) 255 - 358 nm; IR ν_{max} (KBr): 3252, 1645, 1612, 1516, 1442, 1360, 1310, 1024, 828 cm^{-1}; ^{1}H NMR and ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm, *J*, Hz) data were presented in Table 1.

Isorhamnetin-3-*O*-α-L-(6''-*E*-*p*-coumaroyl)-rhamnoside (4) – Yellow amorphous powder; m.p. 200 - 202 ºC; RT 26.2 min; UV λ_{max}^{MeOH} (log ε) 265 - 342 nm; IR ν_{max} (KBr): 3272, 2918, 2844, 1695, 1640, 1624, 1515, 1236, 1060 and 593 cm^{-1}; ^{1}H NMR and ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm, *J*, Hz) data was presented in Table 1.

Results and Discussion

Chemical constituents – Spectral data of compounds **1 - 3** are consistent with those of the compounds reported in previous studies.[5,6,8-10] Compound **4** as yellow amorphous solid; mp: 200 - 202 ºC. This compound also gave positive color reactions for a hydroxyl flavone with several reagents.[11] The UV spectrum showed in the presence of diagnostic shift reagents[12] pointed to the presence of free hydroxyl groups at C5, C7 and C4' of a 3-substituted flavonoid framework. However, the complete assignments of ^{1}H and ^{13}C NMR data of compound (**4**) pointed to 5, 7, 4'-trihydroxy-3'-methoxyflavonol derivative. The ^{1}H NMR spectrum showed five aromatic protons signals at (δ 6.19, d, *J* = 2.1 Hz, H-6; δ 6.47, d, *J* = 2.1 Hz, H-8; δ 7.75, d, *J* = 2.1 Hz, H-2'; δ 6.94, d, *J* = 8.5 Hz, H-5'; δ 7.68, dd, *J* = 8.5 / 2.1 Hz, H-6') are typical of an AX system in B ring and their corresponding carbon signals appear at δ 98.3, 93.7, 111.8, 115.6, 121.8, respectively. Also methoxy protons signal was present at δ 3.84 ppm (3H, s) which showed with the carbon resonance at δ 147.4 (C-3'). These data clearly confirmed the characteristic pattern of isorhamnetin as aglycone.[13] In addition, an anomeric α-rhamnose proton was recognized in this spectrum as a doublet at δ 5.48 ppm. With respect to the question of α- or β-linkage of the sugar moieties, it has been found the coupling constant *J* = 1.58 Hz. corresponded to the anomeric proton of α-linked rhamnose.[14] The methyl protons of the sugar rhamnose appear at δ 1.16 ppm which is therefore assigned to a 6-deoxy sugar (rhamnose) and rest of the sugar protons appear in the range δ 3.37 - 3.56 ppm.[15] The ^{1}H-NMR spectrum of **4** also showed signals ascribed to sugar moieties and a *p*-coumaroyl residue (Table 1). The arrangements of the sugar units were assigned after hydrolysis of **4** compared to those of reliable sugar samples. The lower field shifts of H2-6''' (δ 7.38, *J* = 8.2 Hz) of one glycosyl unit suggested the substitution site of the *p*-coumaroyl unit. Also the signals at 6.36 and 7.62 ppm (both *d*, J_{AB} = 15.8 Hz) assigned to *trans* olefinic protons suggested the presence of *p*-coumaric acid as the acyl moiety. In the ^{13}C NMR spectrum, the signal at 167.7 ppm (s, C=O) supported this proposal.

The ^{1}H NMR is suitable method to distinguish between

Table 1. ^{1}H and ^{13}C NMR data of 3 & 4 (DMSO-d_6, 400 MHz)

Position	δ_C (3)	δ_H [*J* (Hz)] (3)	δ_C (4)	δ_H [*J* (Hz)] (4)
2	160.2		157.6	
3	133.8		134.2	
4	181.2		177.5	
5	162.6		160.8	
6	100.2	6.22 (1H, d, *J* = 2.0)	98.5	6.23 (d, *J* = 2.1)
7	167.1		164.4	
8	94.4	6.38 (1H, d, *J* = 2.0)	93.2	6.56 (d, *J* = 2.1)
9	159.4		156.7	
10	104.9		103.8	
1'	123.6		121.9	
2'	117.1	7.61 (1H, d, *J* = 2.0, s)	114.6	7.80 (d, *J* = 2.1)
3'	146.0		145.1	
4'	150.5		148.4	
5'	116.2	6.94 (1H, d, *J* = 8.0)	115.4	6.90 (d, *J* = 8.5)
6'	122.5	7.56 (1H, dd, *J* = 8.0, 2.0)	121.7	7.62 (dd, *J* = 2.1, 8.5)
1"	110.8	5.52 (1H, d, *J* = 1.56)	100.0	5.48 (brd, *J* = 1.6)
2"	82.7	4.40 (br, d, *J* = 1.3)	70.2	3.48 (dd, *J* = 3.3, 1.6)
3"	78.1	3.85 (m)	70.7	3.56 (dd, *J* = 9.5, 3.3)
4"	89.3	3.88 (dd, *J* = 3.8, 8.3)	72.3	3.37 (d, *J* = 9.5)
5"	63.4	3.60 (m, 2H)	68.8	3.47 (dq, *J* = 9.5, 6.2)
6"			17.9	1.16 (s)
3-OH				9.38 (s)
5-OH		12.57 (1H, s)		12.56 (s)
7-OH				10.78 (s)
4'-OH				9.72 (s)
3'-OMe			55.4	3.80 (s)
1'''			126.9	
2'''			131.1	7.38 (d, *J* = 8.2)
3'''			116.7	6.80 (d, *J* = 8.7)
4'''			161.3	
5'''			116.7	6.80 (d, *J* = 8.7)
6'''			131.1	7.38 (d, *J* = 8.2)
7'''			146.9	7.62 (d, *J* = 15.8)
8'''			114.3	6.36 (d, *J* = 15.8)
9'''			167.7	

both *Z*, *E*- isomerism of cinnamic acid. The *E*-cinnamoyl residue is detectable by a pair of doublets with shift values of 6.15-6.64 ppm for C8-H and 7.43 - 7.81 ppm for C7-H, the corresponding large coupling constant is about 16 Hz, which agrees well with findings of this study.[16] The ^{13}C NMR spectrum contained 31 carbon signals, 15 of them has assigned to the flavonol aglycone and one was methoxy carbon signal verified the isorhamnetin, and remaining 15 signals has attributed to sugar rhamnose with addition of *p*-coumaroyl unit. The sugar moiety has proved to be acylated at C- to achieve 3 of the aglycone as deduced from the anomeric proton at δ_H 5.48 and δ_C 135.9 ppm, which were in close agreement compound **4** as isorhamnetin-3-*O*-α-L-(6"-E-*p*-coumaroyl)-rhamnoside, which represents, to the best of our knowledge, a new ingredient from natural resources.

Anti-microbial activity – The methanol, petroleum ether and ethyl acetate extracts of *P. glabra* was evaluated for anti-microbial activity against clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Candida albicans*. The small amount of the extract was carried over with

Table 2. Anti-microbial activity of different extracts of *Persicaria glabra* (50 μg/mL) and antibiotic (10 μg/mL) against bacterial species

Micro organisms	Zone of inhibition (mm)				
	Methanol extract	Pet. ether extract	Ethyl acetate extra	Streptomycin	Amphotericin
Staphylococcus aureus	19 ± 1.10	24 ± 0.56	26 ± 0.52	25 ± 0.34	–
Pseudomonas aeruginosa	18 ± 0.53	21 ± 0.61	24 ± 0.47	23 ± 0.37	–
Klebsiella pneumonia	15 ± 0.44	18 ± 1.18	20 ± 1.19	21 ± 0.29	–
Escherichia coli	6 ± 0.92	9 ± 1.24	11 ± 0.78	14 ± 0.26	–
Candida albicans	7 ± 0.57	10 ± 0.28	12 ± 0.73	–	13 ± 0.33

Values are mean inhibition zone (mm) ± S.D of three replicates

Table 3. Determination of paw volume of rats for *Persicaria glabra* extracts

Groups	Initial paw volume	Paw volume at different time interval (in mL)			
		1 h	2 h	3 h	4 h
Control (1% DMSO)	1.28 ± 0.19	1.82 ± 0.12	1.98 ± 0.27	2.19 ± 0.34	2.08 ± 0.16
Diclofenac Sodium (100 mg/kg)	1.26 ± 0.24	1.56 ± 0.18	1.64 ± 0.22	1.81 ± 0.25	1.78 ± 0.19
Methanol extract	1.27 ± 0.18*	1.79 ± 0.32*	1.97 ± 0.24*	2.17 ± 0.28*	2.06 ± 0.12*
	1.29 ± 0.15**	1.67 ± 0.30**	1.91 ± 0.28**	2.09 ± 0.26**	2.00 ± 0.14**
Petroleum ether extract	1.27 ± 0.20*	1.66 ± 0.28*	1.80 ± 0.30*	2.01 ± 0.24*	1.88 ± 0.16*
	1.28 ± 0.19**	1.58 ± 0.29**	1.69 ± 0.24**	1.97 ± 0.10**	1.80 ± 0.25**
Ethyl acetate extract	1.25 ± 0.27*	1.62 ± 0.26*	1.70 ± 0.13*	1.93 ± 0.11*	1.81 ± 0.06*
	1.26 ± 0.14**	1.57 ± 0.14**	1.68 ± 0.22**	1.84 ± 0.18**	1.75 ± 0.03**

All values are expressed in Mean ± SEM
* 200 mg/kg; ** 300 mg/kg

these inoculums easily removed by diffusion into the agar. Ethyl acetate extract showed better inhibitory activity against most of the pathogens. The maximum zone of inhibition against *S. aureus* (26 ± 0.52 mm) followed by *P. aeruginosa* (24 ± 0.47 mm), *K. pneumonia* (20 ± 1.19 mm) and *E. coli* (11 ± 0.78 mm). Petroleum ether and methanol extract showed high anti-microbial activity against *S. aureus* with 24 ± 0.56 and 19 ± 1.10 mm and lesser active against *E. coli* with 9 ± 1.24 and 6 ± 0.92 mm (Table 2). Also both extracts showed extended antifungal activity against *C. albicans*. The inhibition zone was viewed against *C. albicans* with ethyl acetate extract 12 ± 0.73 mm, petroleum ether extract 10 ± 0.28 mm and methanol extract 7 ± 0.57 mm. Thus the results held *P. glabra* extracts revealed better control of the pathogens than the commercial antibiotics showed with *S. aureus* 25 ± 0.34, *P. aeruginosa* 23 ± 0.37, *K. pneumonia* 21 ± 0.29, *E. coli* 14 ± 0.26 *and C. albicans* 13 ± 0.33 mm.

Anti-inflammatory activity studies – To evaluate the anti-inflammatory activity of petroleum ether, ethyl acetate and methanol extract of *P. glabra* leaves. Carrageenan-induced paw edema was tested on male albino rats weighing (200 - 250 g) is suitable experimental animal model for evaluation of anti- edematous effect on natural products.[17] Carrageenan (2%) was injected into five groups (six animals each). Group I served as control, group II petroleum ether extract 300 mg, group III ethyl acetate extract 300 mg, group IV methanol extract 300 mg, group V standard diclofenac sodium 100 mg. Carrageenan induced edema is a biphasic response. The first phase was mediated through the release of histamine, serotonin, and kinins whereas the second phase was related to the release of prostaglandin and slow reacting substances which peak at 4h.[18] All tested groups decreased the thickness of edema of the hind paw compared to the control group as showed in Table 3. The percentage decrease in the paw volume in the group of animals treated with *P. glabra* methanolic extract 300 mg was 2.06 ± 0.12 and for the petroleum ether 300 mg was 1.88 ± 0.16 and ethyl acetate 300 mg/kg was 1.75 ± 0.03 at 4 h. It shows the plant extract has significant anti-inflammatory effect and the results have compared with Diclofenac sodium 100 mg/kg and show percentage paw volume decrease of 1.78 ± 0.19. The group II & III showed pronounced anti-inflammatory effects after three hours of injection. This effect may be due to quercetin and isorhamnetin glycosides composition in the plant extracts. Since quercetin as an aglycone is important for the pharmacological effects of quercetin

glycosides for the animal host,[4] dosage recommendations for quercetin and its glycosides was converted into quercetin aglycone equivalents. Group IV has shown to inhibit the induced inflammatory response to carrageenan to a lesser extent than petroleum ether and ethyl acetate fractions. It may be attributed to the fact; the plant methanol extract being in crude form contains a smaller concentration of bioactive compounds.

In conclusion, the presence of isorhamnetin 3-O-α-L-(6"-*E*-*p*-coumaroyl)-rhamnoside (**4**) in ethyl acetate extract of *P. glabra* was reported for the first time. Bioactive substances from this plant can therefore employ to develop antimicrobial drugs for treatment of various bacterial and fungal infections. The petroleum ether and ethyl acetate extracts showed pronounced anti-inflammatory effects after three hours of injection. This effect may be due to its flavonoids quercetin, isorhamnetin and their glycosides composition.

References

(1) Tripoli, E.; Guardia, M.L.; Giammanco, S.; Majo, D.D.; Giammanco, M. *Food Chem*. **2007**, *104*, 466-479.

(2) Nijveldt, R. J.; Van Nood, E.; Van Hoorn, D. E.; Boelens, P. G.; Van Norren, K.; Van Leeuwen, P.A. *Am. J. Clin. Nutr*. **2001**, *74*, 418-425.

(3) Cos, P.; Calomme, M.; Sindambiwe, J. B.; De Bruyne, T.; Cimanga, K.; Pieters, L.; Vlietinck, A. J.; Vanden Berghe, D. *Planta med*. **2001**, *67*, 515-519.

(4) Gálvez, J.; Crespo, M. E.; Jiménez, J.; Suárez, A.; Zarzuelo, A. *J. Pharm. Pharmacol*. **1993**, *45*, 157-159.

(5) Olszewska, M.; Wolbiś, M. *Acta. Pol. Pharm*. **2001**, *58*, 367-372.

(6) Rychlińska, I.; Gudej, J. *Acta. Pol. Pharm*. **2002**, *59*, 53-56.

(7) Bauer, A. W.; Kirby, W. M. M.; Sherries, J. C.; Thuck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 426-493.

(8) Saleh, N. A. M.; El-Negoumy, S.I.; El-Hadidi, M. N.; Hosni, H. A. *Phytochemistry*. **1983**, *22*, 1417-1420.

(9) Kim, G. B.; Shin, K. S.; Kim, C. M.; Kwon, Y. S. *Kor. J. Pharmacogn*. **2006**, *37*, 177-183.

(10) Park, B. J.; Matsuta, T.; Kanazawa, T.; Park, C. H.; Chang, K. J.; Onjo, M. *Chem. Nat. Comp*. **2012**, *48*, 477-479

(11) Harborne, J. B.; Mabry, T. J.; Mabry, H. The flavonoids; Academic press: New York, **1975**.

(12) Mabry, T. J.; Markham, K. R.; Thomas, M. B. The Systematic Identification of Flavonoids; Springer-Verlag: Berlin, **1970**.

(13) Kumari, G. N.; Rao, L. J. M.; Rao, N. S. P. *Proc. Indian Acad. Sci*. (Chem. Sci). 1986, *97*, 171-176.

(14) Markham K.R.; Geiger, H. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeutero dimethyl sulfoxide: Advances in Research since 1986, ed; J.B. Harborne. Chapman and Hall; London, **1994**, pp 441-497.

(15) Manivannan, R.; Prabakaran, K.; Ilayaraja, S. *Int. J. Pharm. Sci. Rev. Res*. **2013**, *22*, 264-268.

(16) Ibrahim, R.; Barron, D. Phenylpropanoids In: Methods in Plant Biochemistry, Vol.1, Plant Phenolics, series ed; P.M. Dey and J.B. Harborne, ed; J.B. Harborne, Academic Press, London, **1989**, pp 75-111.

(17) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med*. **1962**, *111*, 544-547.

(18) Vinegar, R.; Schreiber, W.; Hugo, R. *J. Pharmacol. Exp. Ther*. **1969**, *166*, 96-103.

Received November 27, 2014
Revised February 23, 2015
Accepted March 6, 2015