Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(8); 1322-1324

ISSN: 0975-4873

Research Article

Anti-oxidant Activity of Quercetin-3-o-glucuronide from *Nelumbo nucifera*

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Available Online:10th August, 2016

ABSTRACT
The fresh florets of *Nelumbo nucifera* is examined to contain the flavonoid Quercetin-3-O-glucuronide. Modern physical methods like UV, NMR and chemical reactions, PC and hydrolytic studies were used to ascertain the structure. The isolated Glycoside has been found to contain ample anti-oxidant activity as it is compared with a standard.

Keywords: anti-oxidant activity; flavonoids; Quercetin-3-O-glucuronide.

INTRODUCTION

Nelumbo nucifera Gaertn. belonging to Nymphaeceae family revealed the presence of phytochemical constituents like flavonoids, alkaloids, phenols, etc. N-nornuciferine, O-nornuciferine, nuciferine, and roemerine are the 4 main aporphine alkaloids responsible for the pharmacological properties of the plant[1]. Kaempferol 3-O-glucosylglucoside was reported from *Nelumbo nucifera*[2]. Ethanol seed extracts inhibited herpes simplex virus type 1 (HSV-1) multiplication in HeLa cells without cytotoxicity by inhibiting gene expression of HSV-1[3]. An ethanol rhizome extract reduced the blood sugar level of normal rats and glucose-fed hyperglycemic and streptozotocin-induced diabetic rats[4].

EXPERIMENTAL

Extraction and fractionation

Fresh corolla of *Nelumbo nucifera* (2kg) was extracted with 85% methanol (5x500ml). The combined alcoholic extract was concentrated *in vacuo*. The aq. concentrate was fractionated successively with light petrol (60-80ºC) (3x250ml), peroxide free Et_2O (4x250ml) and EtOAc (8x250ml). The EtOAc fraction alone was taken up for the study.

EtOAc fraction: (Flavonol glycoside: Quercetin-3-O-glucuronide)

The EtOAc fraction was concentrated *in vacuo* and left in an ice chest for few days. A yellow solid that separated when subjected to PC revealed the presence of a single glycoside. A yellow solid on recrystallization from methanol was obtained. It developed a green colour with alc. Fe^{3+} and orange red colour with Mg-HCl, yellow colour with NaOH and appeared as purple spot under UV turning yellow on exposure to NH_3. It responded to Wilson's boric acid and Gibb's test but did not answer the Horhammer-Hansel test. It responded to Molisch's test showing that it could be a flavones glycoside. It had λ_{max} MeOH 257, 266sh 330sh, 356; +NaOMe 271sh, 332sh, 412; +$AlCl_3$ 273, 303sh, 433; +$AlCl_3$-HCl 268, 303sh, 364, 401 and +NaOAc 271, 334sh, 401; +NaOAc-H_3BO_3 260, 298sh, 377nm.

Acid hydrolysis of the glycoside

The glucuronide (50mg) dissolved in hot aq. MeOH was hydrolyzed with 2M HCl at 100ºC for about 2h. The excess alcohol was distilled off *in vacuo* and the resulting aq. solution was extracted with Et_2O. The residue from Et_2O fraction was studied as presented below.

Identification of the aglycone (Flavonol-quercetin)

The aglycone recovered from the hydrolytic fraction of the glycoside on recrystallization gave yellow needles (m.p. 305-306ºC). It was soluble in organic solvents and sparingly soluble in hot water. It gave a red colour with Mg-HCl; olive green with alc. Fe^{3+}, golden yellow colour with NH_3 and NaOH and appeared yellow under UV and UV/NH_3. It had λ_{max} MeOH 256, 271sh, 300sh, 370; +NaOMe 245sh, 321, 412(dec); +A1Cl_3 272, 304sh, 332, 457; +$AlCl_3$-HCl 265, 301sh, 328, 427; +NaOAc 272, 330, 389 and +NaOAc-H_3BO_3, 260, 302sh, 338nm.

Identification of aglycone (Flavonol-quercetin)

The aglycone recovered from the hydrolytic fraction of the glycoside on recrystallization gave yellow needles, m.p. 305-306ºC (yield 0.02%). It was soluble in organic

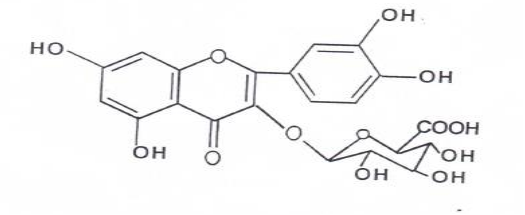

Figure 1: quercetin-3-O-glucuronide

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Table 1: IC50 value of quercetin-3-O-glucuronide

Compound ID	IC_{50} (μ_g)
quercetin-3-O-glucuronide	>523.438

Table 2: OD at 490nm of quercetin-3-O-glucuronide

CON µg/mL	BHT		quercetin-3-O-glucuronide	
500	0.112	0.116	0.194	0.194
250	0.141	0.148	0.198	0.203
125	0.116	0.146	0.184	0.181
62.5	0.171	0.176	0.199	0.207
31.25	0.188	0.199	0.204	0.211
CONTROL	0.188	0.2	0.192	0.207

Table 3: Percentage inhibition of quercetin-3-O-glucuronide

CON µg/mL	BHT		quercetin-3-O-glucuronide	
500	42.282	39.959	-0.052	-0.207
250	27.001	23.748	-1.962	-4.75
125	39.907	24.522	5.266	6.402
62.5	11.668	9.138	-2.943	-6.918
31.25	2.839	-2.478	-5.369	-8.828
Control H	3.149	-3.149	1.033	-6.711

solvents and sparingly soluble in hot water. It gave a red colour with Mg-HCl, olive green with alc. Fe^{3+}, golden yellow colour with NH_3 and NaOH. It appeared yellow under UV and UV/NH_3 and it answered Horhammer-Hansel test and Wilson's boric acid tests. It had λ_{max} MeOH values of 254, 271sh, 300sh, 368; +NaOMe 245sh, 321, 412(dec); +A1Cl_3 272, 304sh, 332, 457; +$AlCl_3$-HCl 265, 301sh, 328, 427; +NaOAc 272, 330, 389(dec); and +NaOAc-H_3BO_3, 260, 302sh, 338nm. Aglycone part of the compound was identified as quercetin.

Identification of sugar moiety

The aqueous solution from the above was neutralized with $PbCO_3$ and filtered. The concentrated filtrate on PC when examined by paper chromatography agreed with those of glucuronic acid. Thus, the sugar moiety was identified as glucuronic acid.

RESULTS AND DISCUSSION

The fresh corolla of *N.nucifera* has been found to contain quercetin-3-O-glucuronide. The UV spectral values of the glycoside and the aglycone are respectively 359nm (band I) and 1257nm (band II) and 370nm (band I) and 256nm (band II) respectively, indicating the presence of flavonol skeleton in both and also revealing the presence of glycosylation at C-3. This is further evidenced by the aglycone responding to the Horhammer-Hansel test, while the glycoside did not. A bathochromic shift of 56nm in band I observed in its NaOMe spectrum indicated the presence of free -OH at C-4'. A bathochromic shift of 45nm in the glycoside and 57nm for aglycone in $AlCl_3$-HCl spectra was noticed. This confirms the presence of free OH at C-5. The NaOAc spectra of both glycoside and aglycone indicated the presence of free OH at C-7, as evidenced by the bathochromic shift of 14nm and 16nm, respectively. A bathochromic shift of 18nm in band I for glycoside and aglycone on the addition of H_3BO_3 indicated the presence of an O-dihydroxyl grouping in the B-ring (at C-3' and C-4') in both, a shift of 32nm in the glycoside and 30nm in the aglycone (band I) with respect to $AlCl_3$-HCl spectra. In the ^{1}H NMR spectrum (400MHz, DMSOd6, TMS) of the glycoside quercetin-3-O-glucuronide, the signal appearing at δ12.3ppm corresponds to the -OH at C-5. The signal at δ9.8-10.5ppm is due to the hydroxyl proton at C-7. The doublet appearing in the region of δ7.4ppm (d, J=8Hz) and δ7. lppm corresponds to the proton at C-2' and C-6', while the proton of C-3' appears at δ8.05ppm. The signal appearing at δ6.8ppm (d, J=8Hz) corresponds to C-5' proton. C-8 proton due to meta coupling with C-6 proton appears as a doublet at δ6.6ppm (d, J=2.2Hz). C-6 proton, due to meta coupling with C-8 proton appears as a supporting evidence for the structure of the flavonol glycoside quercetin-3-O-glucuronide is provided by the ^{13}C-NMR (100MHz, DMSO –d_6, TMS) 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 glycosylation, the signal of C-3 is shifted upfield by δ2.6ppm. The down field shift of 'ortho related' C-2 signal by δ12.54ppm also confirms this. The large shift in C-2 /resonance also reflects the semi-olefinic character of the flavonol C-2, C-3 double bond. The signal at δ0.82ppm of C-10 is less intense due to the longer relaxation time of the quaternary carbon. The signal C-6" at δ9.7ppm confirms the glycoside as 3-O-glucuronide and the identity was confirmed by CO and PC with an authentic sample of quercetin-3-O-glucuronide from *Frankenialyerulenta.* The structure of the glycoside quercetin-3-O-glucuronide was further evidenced by mass spectrum. The mass spectrum of the glycoside quercetin-3-O-glucuronide shows prominent peaks at m\z 479 and is in agreement with the identification of the glycoside as quercetin3-O-glucuronide. The fragments at m\z 154 and m\z 136 illustrate the substitution pattern in A and B-rings. Other peaks of quercetin-3-O-glucuronide also in favour of the structure of the compound. Based on the above evidences, the glycoside quercetin-3-O-glucuronide has been characterized as quercetin-3-O-glucuronide. (Misquelianin).

Anti-Oxidant Activity

Objective

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species than enzymatic anti-oxidants and non-enzymatic anti-oxidants. This imbalance leads to cell damage[5] and health problems. A lack of anti-oxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease[6] and inflammatory diseases. so The purpose of this study was to assess the anti-oxidant property of quercetin-3-O-glucuronide.

Materials and methods

Multimode microplate reader-BioTek, USA

Refrigerated Centrifuge-Eppendorf Germany

DPPH (1,1-Diphenyl-2-picrylhydrazyl)
DMSO

Cell culture and MTT assay procedure

To 150µl of 0.1mM DPPH in methanol, a volume of 50µl of the flavonoid glycoside (range 20 to 1000µg/ml) was mixed and kept in the dark room temperature for 60 minutes.

After incubation, the absorbance was recorded at 490nm.

The results were compared with control.

The positive control was butyl hydroxyl toluene in concentration range 50 to 250g/ml.µ

The anti-oxidant activity was expressed as IC50 value.

From the optical densities the percentage growths were calculated using the following formula:

Percentage Inhibition=100-[(T)/(C)] ×100, Where T is the optical density of test, C is the optical density of control.

From the percentage growths a dose response curve was generated and GI_{50} values were interpolated from the growth curves.

Result and discussion

DPPH inhibition

quercetin-3-O-glucuronide exhibited potent inhibition in DPPH assay The flavonoid glycoside of each concentration was performed in duplicate and cumulative variation were maintained less than 20% between the data points. The assay was performed in a 96-well plate as described in the below 96-well format. The results and raw data have been illustrated in Tables 1 to 3.

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