STRUCTURE-ACTIVITY RELATIONSHIPS OF FLAVONOIDS ISOLATED FROM SOME MEDICINAL PLANTS: ANTIOXIDANT AND ANTIDIABETIC PROPERTIES*

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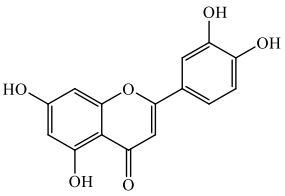
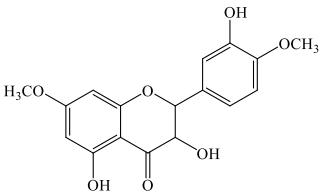
Abstract

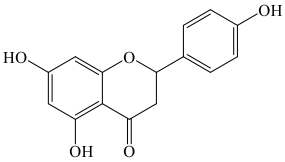
The present research deals with chemical structure and some biological activities relationship of isolated flavonoids from some medicinal plants. Three flavonoids, namely luteolin **1**, dihydroquercetin 7,4′-dimethyl ether **2** have been isolated from the peel of *Citrus maxima* (Burm.) Marr; naringenin **3** is obtained from *Peltophorum pterocarpum* (DC.) K. Heyne flower. Their structures were comparatively confirmed by UV, chemical test and co-TLC with their respective standards. Their biological activities were evaluated for antioxidant properties using the DPPH assay, α-amylase and α-glucosidase inhibition *in vitro*, and *in vivo* hypoglycemic effects in an alloxan-induced diabetic mice model. The antioxidant effects of flavonoids **1**, **2,** and **3** by DPPH assay were IC_{50} values 1.5 μM, 9.4 μM, and 27.7 μM, respectively. Luteolin (**1**) exhibited the most potent antioxidant, α-amylase, and α-glucosidase inhibitory activities. *In vivo*, luteolin (**1**) also demonstrated significant hypoglycemic effects. This study suggests that flavonoids with hydroxyl groups at C-5 and C-7 in ring A, a carbonyl oxygen at C-4, a double bond between C-2 and C-3 in ring C, and hydroxyl groups at C-3' and C-4' in ring B are likely to possess potent antioxidant and antidiabetic activities.



Luteolin (**1**)



Dihydroquercetin-7,4′-dimethyl ether (**2**)



Naringenin (**3**)

Keywords: luteolin, dihydroquercetin-7,4′-naringenin, antioxidant, antidiabetic

Introduction

Flavonoids, a class of polyphenolic compounds widely distributed in the plant kingdom, encompass a diverse array of chemical structures and biological activities. They are characterized by their C_6-C_3-C_6 carbon skeleton, which forms the basis for several subclasses including flavonols, flavones, flavanones, flavanols (catechins), anthocyanidins, and isoflavones (Havsteen, 2002). They are considered as active principles in various medicinal plants (Wollenweber, 1988) and have been reported to possess anticancer, antioxidant, antimicrobial, antihypertensive, antiulcer, antipyretic and antidiabetic properties etc. *Citrus maxima* (Rutaceae), locally known as Kyaw-gaw, is reported to be used for cough, fever, asthma, diarrhea, ulcer, and diabetes and as a sedative. The plant possesses significant bioactivities like antioxidant, antimicrobial, anti-inflammatory and antidiabetic (Sapkota *et al*., 2022). The plant *Peltophorum pterocarpum* (Fabaceae) is locally known as Thin-baw-mazeli in Hpa-an Township, Kyin State and also distributed in tropical and subtropical regions including Myanmar (Kree *et al*., 2003). *It* is a rich source for phenols and the wood, leaves, and flowers of the plant are used as medicinal agents in traditional medicine. In the present study, exploration of some biological activities and evaluation

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of structure-activity relationship of three flavonoids isolated from two medicine plants: *C. maxima*, and *P. pterocarpum* were reported.

(a) **(b)**

Figure 1. Photographs of (a) Kyaw-gaw-peel and (b) Thin-baw-mazeli flower

Materials and Methods

Plant Material

Kyaw-gaw-thi from fruit garden, Hpa-an Township and Thin-baw-mazeli flower from Hpa-an University campus, Kayin State were collected in May 2020. The former was identified as *Citrus maxima* (Burr.) Merr and the latter as *Peltophorum pterocarpum* (DC.) K. Heyne by the authorized botanist at the Department of Botany, Mawlamyine University and Hpa-an University. The samples: Kyaw-gaw peel and Thin-baw-mazeli flower were dried in the shade for a week, cut into very small pieces, and then ground into a purely fine powder using an electric motor grinder. The powdered samples were stored in airtight containers to prevent moisture changes and contamination.

Extraction and Isolation of Compounds

The dried [*Citrus maxima* (Kyaw-gaw)] peel sample (300 g) was crushed to coarse powder and extracted exhaustively with 95 % ethanol in an air-tight bottle. The alcoholic extract was concentrated and dried under reduced pressure to get a viscous mass (62.5 g), which was extracted with petroleum ether followed by partitioned between ethyl acetate and water. The ethyl acetate extract of *C. maxima* peel (9.5 g) was subjected to column chromatography on a normal-phase silica gel GF_{254} adsorbent, increasing the polarity of the eluent solvent system (PE: EA), and five main fractions (F I to F V) were obtained. Fraction F III was subjected to column chromatography with a PE-EtOAc gradient system; compounds **1** (0.13 % yield as white crystal) and **2** (0.005% yield as pale yellow crystal) were afforded. The dried flower [*P. pterocarpum* (Thin-baw-mazeli)] powder (300 g) was also extracted as usual with alcohol to get a viscous mass (72.8 g), which was extracted with petroleum ether followed by partitioning between ethyl acetate and water. The former (ethyl acetate extract: 11.5 g) was on column chromatography on a normal-phase silica gel GF_{254} adsorbent, increasing the polarity of the eluent solvent system (PE: EA), and seven main fractions (F I to F V) were obtained. Fraction F IV was subjected to column chromatography with a PE-EtOAc gradient system, and compound **3** (0.009 % yield as white amorphous) was obtained. They were purified and confirmed by chemical tests, melting point, UV and co-TLC with respective authentic

compounds and assigned as **1** for luteolin, 2 for dihydroquercetin-7,4′-dimethyl ether, and 3 for naringenin.

Screening of Antioxidant Activity of Isolated Flavonoids

The effect of isolated flavonoid compounds (luteolin **1**, dihydroquercetin7,4'-dimethylether **2,** and naringenin **3)** on the DPPH radical was determined by using the method of Marinova and Batchvarov (2011). The control solution was prepared by mixing 1.5 mL of 120 µM DPPH solution and 1.5 mL of 95% ethanol using a shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 120 µM DPPH solution (Absorbance = 0.8941 used for control) and 1.5 mL of each flavonoid: **1**, **2,** and **3** solution (40-0.625 µg/mL). The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm by using a UV-7504 spectrophotometer. Absorbance measurements were made in triplicate for each concentration, and then the mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation:

$$\text{Oxidative inhibition (\%)} = \frac{A_{DPPH-}(A_{smaple-}A_{blank})}{A_{DPPH}} \times 100$$

where, A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample and DPPH solution

A_{blank} = absorbance of sample and EtOH solution

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and 50 % inhibition concentration (IC_{50}) values were calculated by the linear regression programme.

Determination of *In Vitro α*-Amylase Inhibition Activity

The α-amylase enzyme inhibition potential was assessed by using a 3,5-dinitrosalicylic acid (DNS) assay (Kajaria *et al*., 2013). The reaction mixture contained 1 mL of phosphate buffer (0.04 M, pH = 6.9), 1 mL of α-amylase: 1 U/mL, and 1 mL of varying concentrations of isolated flavonoid compounds (luteolin **1**, dihydroquercetin-7,4'-dimethylether **2,** and naringenin **3)** that were preincubated at 37 ºC for 20 min. Next, 0.4 mL of 1% soluble starch (0.004 M phosphate buffer pH 6.9) was added as a substrate and incubated at 37 ºC for 30 min; 0.6 mL of the 3,5-dinitrosalicylic acid (DNS) reagent was then added and boiled for 20 min. The absorbance of the resulting mixture was assessed at 540 nm using a UV spectrophotometer. Metformin, a well-known α-amylase inhibitor, is an excellence standard in a wide range of concentrations. Solution without test samples were used as controls and the blank solution (without amylase), and each test was carried out in triplicate. The outcome was expressed as percentage inhibition, which was calculated using the following equation.

$$\text{\% Inhibition} = \frac{C-(A-B)}{C} \times 100$$

Where A= absorbance of the sample

B = absorbance of blank (without amylase)

C = absorbance of the control (without test sample)

Standard deviation (SD) and 50 % inhibition concentration (IC_{50}) values were calculated by the linear regression programme.

Determination of *In Vitro* α-Glucosidase Inhibitory Assay

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al*., 2011. The substrate solution, p-nitrophenyl glucopyranoside (PNPG), was prepared in 0.04 M phosphate buffer, at pH 6.9. 1 mL of α-glucosidase (0.5 U/mL) was preincubated at 37 ºC for 20 min with 1 mL of isolated flavonoid compounds (luteolin **1**, dihydroquercetin-7,4′-dimethyl ether **2** and naringenin **3**). Then 0.5 mL of 0.003 M (PNPG) as a substrate dissolved in 0.04M phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was then incubated at 37 ºC for 20 min and stopped by adding 1 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-coloured p-nitrophenol released from PNPG at 405 nm using a UV spectrophotometer. Samples without plant extracts was used as control and the blank solution (without amylase) and each test was carried out in triplicate. The outcome was expressed as percentage inhibition, which was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{C-(A-B)}{C} \times 100$$

Where, A = absorbance of the sample

B = absorbance of blank (without glucosidase)

C = absorbance of the control (without test sample)

Standard deviation (SD) and 50 % inhibition concentration (IC_{50}) values were calculated by the linear regression programme.

Screening of Hypoglycemic Effect of Isolated Flavonoids

Eighteen albino mice were obtained from Animals Services Division, DMR (Lower Myanmar), were allowing free access to water and rodent diet, and surrounding temperature maintained at 22 ± 0.5 °C daily life cycle from 6:00 pm to 6:00 am. In order to determine the normal blood sugar level, the blood sample was taken by cutting 1 mm on the tip of the tail of overnight-fasted mice. One drop of blood was collected on the test strip, and blood glucose concentration was read by glucometer in Figure 2. The result was expressed in mg/dL and taken as baseline glucose level. After overnight fasting, the mice were divided into 6 groups of three animals each. Group II-VI were made diabetic (hyperglycemia) by intraperitoneal administration with 150 mg/kg body weight of alloxan (Yanarday and Colak, 1998). Alloxan was dissolved in normal saline just before use. The blood glucose level was checked at 72 h after alloxan injection. The mice that increased blood glucose levels were used in the experiment.

Group I : Normal control mice

Group II : Alloxan induced diabetic mice with 150 mg/kg body dose, diabetic control

Group III : Alloxan induced diabetic mice treated with 15 mg/kg body weight dose of antidiabetic control drug metformin

Group IV : Alloxan induced diabetic mice treated with 15 mg/kg body weight dose of luteolin

Group V : Alloxan induced diabetic mice treated with 15 mg/kg body weight dose of dihydroquercetin7,4′-dimethyl ether

Group VI : Alloxan induced diabetic mice treated with 15 mg/kg body weight dose of naringenin

After administration of samples and a standard drug, the blood samples were collected from the tips of the tails of mice. The blood samples from each mouse were analyzed for blood glucose content at 1 h, 2 h, 3 h, and 4 h, subsequently using a glucometer kit, and the percent reduction in blood glucose level was calculated by the following equation.

$$\text{Reduction (\%)} = \frac{\text{FBG-blood glucose level}}{\text{FBG}} \times 100$$

where, FBG = fasting blood glucose level

Figure 4. A photograph showing measurement of blood glucose content with glucometer

Results and Discussion

Phytoconstituents Present in Selected Medicinal Plants

Primary phytochemical screening of selected medicinal plants was performed using the appropriate methods. The phytochemical tests revealed the presence of metabolites such as alkaloids, carbohydrates, glycosides, saponins, organic acids, phenolic compounds, flavonoids, tannins, steroids, and terpenoids. Cyanogenic glycosides and reducing sugar were absent in the sample. These results are summarized in Table 1.

Table 1 Preliminary of Phytochemical analysis on the selected samples

No.	Tests	Extract	Test reagents	Observation	Remark	
					I	**II**
1.	alkaloid	1% HCl	dragendroff's reagent	orange ppt	+	+
2.	flavonoids	EtOH	EtOH, Mg turning and conc. HCl	pink colour solution	+	+
3.	glycosides	H_2O	10 % lead acetate	white ppt	+	+
4.	carbohydrates	H_2O	10 % α-naphthol and conc. H_2SO_4	red ring	+	+
5.	cyanogenic glycosides	H_2O	sodium picrate	no brick red colour	-	-
6.	organic acids	H_2O	bromocresol green	green colour	+	+
7.	phenolic compounds	H_2O	10 % $FeCl_3$	dark green colour	+	+
9.	reducing sugars	H_2O	benedict's solution	brick red ppt	-	-
10.	saponins	H_2O	distilled water	frothing	+	+
11.	tannins	H_2O	1% gelatin	white ppt	+	+

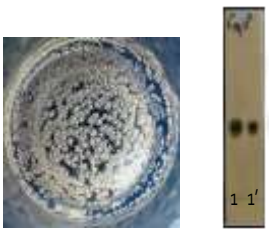
No.	Tests	Extract	Test reagents	Observation	Remark	
					I	**II**
12.	steroids	pet-ether	Ac_2O and conc.H_2SO_4	green colour	+	+
13.	terpenoids	$CHCl_3$	Ac_2O and conc.H_2SO_4	pink colour	+	+

(+) = presence (-) = absence ppt = precipitation

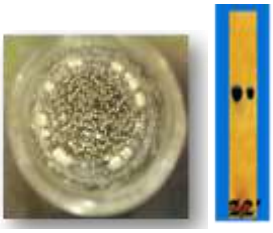
I = Kyaw-gaw peel
II = *P. pterocarpum* flower

Confirmation of Isolated Compounds

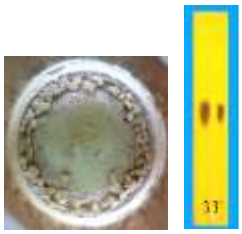
Compounds **1** (0.13 % yield as white crystal) and **2** (0.005 % yield as pale yellow crystal) were obtained from the EtOAc extract of *C. maxima* peel. Their melting points were 329 ºC and 165 ºC, which were consistent with those of luteolin (329 ºC) (O'Neil *et al*., 2001) and dihydrquercetin-7,4'-dimethyl ether (165 ºC) (O'Neil *et al*., 2001). Moreover, their R_f values were 0.4 and 0.57 comparably with the authentic compounds luteolin and dihydrquecetin7,4'-dimethyl ether on co-TLC chromatograms with PE: EtOAc (3:1 and 2:1, v/v) in Figure 3. Compounds **1** and **2** were UV active and contained a carbonyl group for giving a yellow precipitate with 24-DNP solution. The two isolated compounds **1** and **2** were classified as flavonoid due to the appearance of pink coloration when these compounds were dissolved in ethanol and then treated with concentrated HCl and Mg ribbon. So isolated compounds **1** and **2** were confirmed as luteolin and dihydroquercetin-7,4'-dimethyl ether. Compound **3** (0.009 % yield as white amorphous) was isolated from the EtOAc extract of [*P. pterocarpum* (Thin-baw-mazeli)] flower. The melting point of compound **3** (251 °C) was found to be similar to that of naringenin (251 °C) (O'Neil *et al*., 2001). The R_f value was 0.49 comparably with the authentic compound, naringenin, on the co-TLC chromatogram with PE: EtOAc (2:1, v/v). It was classified as flavonoid due to the appearance of pink coloration when the compound was dissolved in ethanol and then treated with concentrated HCl and Mg ribbon. It was observed that a carbonyl group was present due to a positive 2,4-DNP test. It gave a brown spot on the TLC chromatogram while spraying with 10% $FeCl_3$. Therefore, compound **3** was confirmed as a flavonoid, naringenin.



Compound **1**(White crystal)
1′ (Authentic luteolin)
Solvent system -PE:EA(3:1)
Spraying reagent - 5%$FeCl_3$
R_f - 0.4
mpt - 329 °C



Compound **2.** (Pale yellow crystal)
2′ (Authentic dihydroquercetin-7,4'-dimethyl ether)
Solvent system- PE: EA (2:1)
Spraying reagent -10% $FeCl_3$
R_f - 0.57
mpt - 165 ºC



Compound **3.** (White amorphous)
3′ (Authentic naringenin)
Solvent system- PE: EA (2:1)
Spraying reagent -10% $FeCl_3$
R_f - 0.49
mpt -251 ºC

Figure 3 Crystal appearance and co-thin layer chromatogram of the isolated compounds 1, 2 and 3

Antioxidant Capacity of Isolated Compounds

The free radical scavenging capacity of the extract was determined using the stable free radical containing DPPH. It can accept an electron or hydrogen radical, the odd electron in it makes the solution to appear deep violet in color. The absorption vanishes when DPPH accepts an electron, resulting in decolorization. An antioxidant's DPPH radical scavenging ability is supposed to be due to its hydrogen donating property (Soares *et al*., 1997). Free radical scavenging activity of the extracts on DPPH radicals increased with an increase in concentration. At 0.625-40 μM, the scavenging activity of compounds **1 (luteolin)**, **2 (dihydroquercetin-7,4'-dimethyl ether)** and **3 (naringenin)** on DPPH radical ranged from 23.7 to 74.77 %, 15.32 to 68.65%, and 9.45 to 52.65%, and IC_{50} values of these extracts were 5.91, 16.06, and 27.21 μg/mL, respectively. Similarly, the standard ascorbic acid (0.625-40 μM) showed significant free radical scavenging activities dose-dependently ranging from 26.09 to 100 %, and IC_{50} value was 1.18 μM. The radial scavenging (antioxidant) capacity of luteolin was significantly shown. The detailed results are shown in Table 2 and Figure 3.

Table 2 Oxidative Inhibition % for Different Concentrations and IC_{50} Values of Isolated Flavonoid Compounds

Extracts	Oxidative Inhibition % of different concentrations (μM)							IC_{50}(μM)
	0.625	**1.25**	**2.5**	**5**	**10**	**20**	**40**	
Luteolin	23.63± 0.033	35.41± 0.002	52.82± 0.046	68.91± 0.002	76.43± 0.003	84.7±0. 022	94.7± 0.022	1.91
DHQ-DME	15.52± 0.015	21.81± 0.024	34.49± 0.032	39.25± 0.021	45.91± 0.002	52.6±0. 041	68.6±0 .041	16.06
Naringenin	9.54± 0.015	15.81± 0.024	21.46± 0.032	29.20± 0.021	35.93± 0.002	42.6± 0.041	52.6± 0.041	27.21
Ascorbic acid*	26.06± 0.037	52.41± 0.013	70.53± 0.006	88.37± 0.006	94.69± 0.001	100	100	1.18

*standard antioxidant agent, DHQ-DME = dihydroquercetin-7,4′-dimethyl ether

Data are expressed as mean ± SD (n=3).

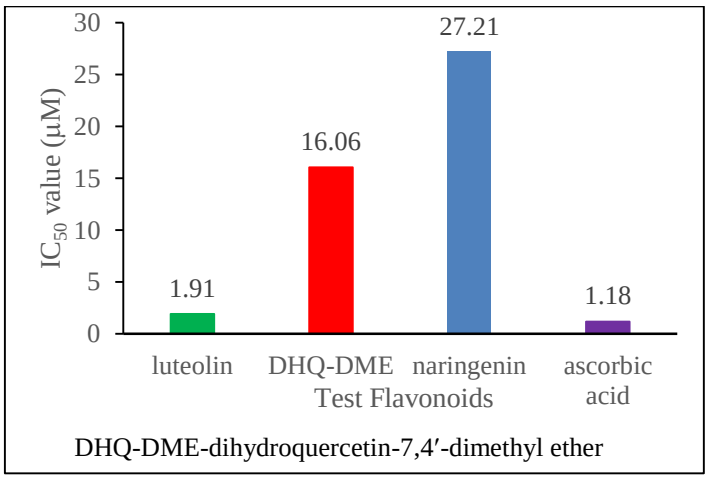


Figure 3. A bar graph of IC_{50} value of oxidative inhibition of isolated flavonoids

In vitro Alpha-amylase Inhibitory Effects of Isolated Flavonoid Compounds

α-Amylase is one of the main enzymes involved in the breakdown of dietary starch, giving rise to oligosaccharides that can be further broken down to absorbable monosaccharides in the brush border of the intestine. Inhibition of this enzyme is therefore considered an active strategy for managing diabetes. The inhibitory activity of the polyphenolic compounds increased with increasing concentration, indicating a dose-dependent effect (Sachan *et al*., 2019). Among three isolated compounds, luteolin (96.82% at 400 μM) had the highest inhibition, followed by dihyroquercetin-7,4′-dimethyl ether (77.35 %) and naringenin (71.22 %) at 400 μM in Table 3 and Figure 4.

Table 3 α-Amylase Inhibitory Activities of Isolated Flavonoid Compounds

Sample	Inhibition % of different concentrations (μM)					IC_{50} (μM)
	25	50	100	200	400	
Luteolin	35.13± 1.40	65.58 ± 1.04	74.53± 1.13	87.72 ± 0.59	96.82 ± 0.78	37.20
DHQ-DME	23.96± 1.09	54.33 ± 1.48	61.98± 1.84	68.98 ± 1.47	77.41 ± 1.37	47.75
Naringenin	21.35± 0.42	47.02 ± 0.18	58.51± 1.11	63.12 ± 0.64	71.22 ± 0.34	63.96
*Metformin	21.85± 0.73	45.30 ± 1.01	66.76± 1.07	75.09 ± 0.85	86.61 ± 0.90	60.00

*Standard antioxidant agent, DHQ-DME = dihydroquercetin-7,4′-dimethyl ether

Data are expressed as mean ± SD (n=3).

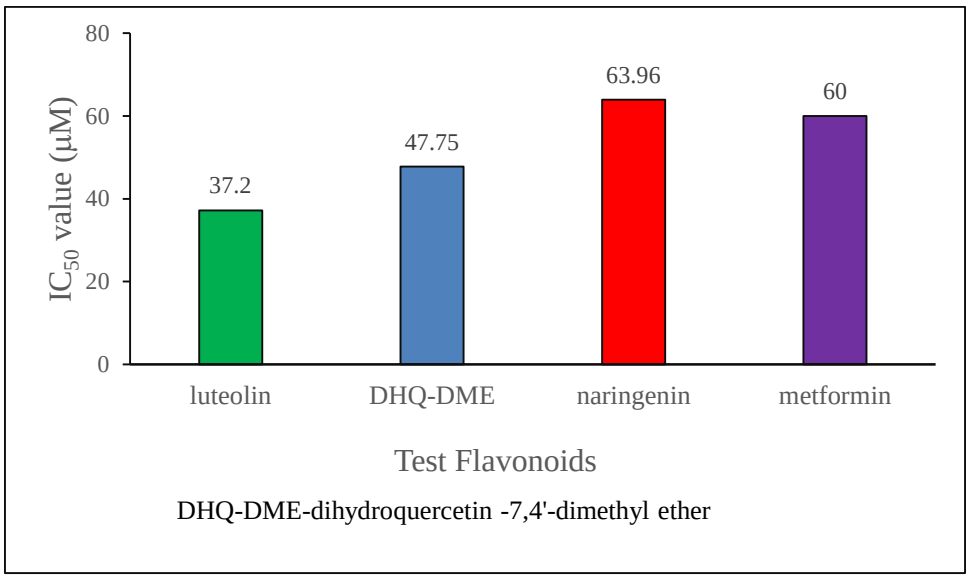


Figure 4. A bar graph of IC_{50} value of α-amylase inhibitory effect of isolated flavonoids

In vitro α-glucosidase Inhibitory Effects of Isolated Flavonoid Compounds

Luteolin. Dihydroquercetin-7,4'-dimethyl ether and naringenin from *C. maxima* peel and *P. pterocarpum* flower were evaluated for their α-glucosidase inhibitory effect compared to the positive control metformin (400 μM final concentration). All test compounds: luteolin, dihyroquercetin-7,4'-dimethyl ether demonstrated a significantly higher α-glucosidase inhibitory effect than metformin in Table 4 and Figure 5. Luteolin showed the highest inhibitory effect (44.30 % - 93.32 %) inhibition and (IC_{50} value = 28.00 μM) against α-glucosidase enzyme action. Dihyroquercetin-7,4'-dimethyl ether and naringenin displayed a significantly α-glucosidase enzyme inhibitory effect when compared to the positive control metformin. Based on these results, it is evident that luteolin results in significantly enhanced activity against α-glucosidase.

Table 4. α-Glucosidase Inhibitory Activities of Isolated Flavonoid Compounds

Sample	Inhibition % of different concentrations (μM)					IC_{50} (μM)
	25	50	100	200	400	
Luteolin	44.30± 1.40	72.87± 0.98	85.33± 1.13	89.22± 0.59	93.32± 0.78	28.00
DHQ-DME	41.35± 0.42	66.02± 0.18	72.51± 1.11	78.12± 0.64	86.22± 0.34	32.50
Naringenin	36.08± 0.72	68.20± 0.89	78.36± 0.63	82.85± 0.86	87.07± 0.57	35.05
*Metformin	34.04± 1.09	58.33± 1.08	68.44± 0.84	75.18± 1.07	81.41± 1.30	40.50

*Positive control antidiabetic drug, DHQ-DME = dihydroquercetin-7,4′-dimethyl ether
Data are expressed as mean ± SD (n=3).

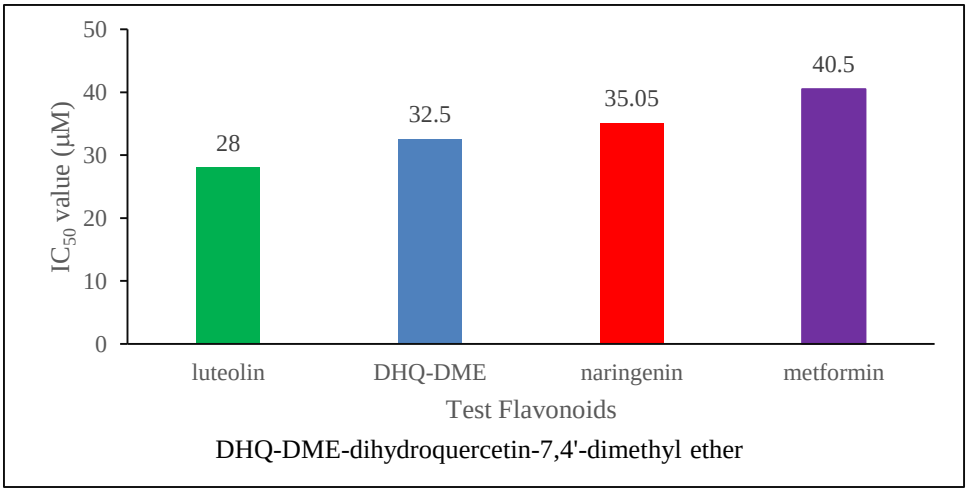


Figure 5 A bar graph of IC_{50} value of α-glucosidase inhibition effect of isolated flavonoids

In vivo Hypoglycemic Effect of Isolated Flavonoid Compounds on Alloxan-induced Albino Mice

In vivo hypoglycemic effect of isolated flavonoids compounds: luteolin, dihydroquercetin-7,4'-dimethyl ether, and naringenin and positive control metformin was evaluated on an alloxan-induced diabetic mice model. The optimum hypoglycemic effect of three isolated compounds and positive control (15 mg/kg b. wt dose) was clear after 4 h of administration, where $p < 0.05$. The % reducing ability of blood glucose levels were found to be 41.62 % for luteolin, 38.97 % for dihydroquercetin-7,4'-dimethyl ether, and 32.91% for naringenin in Table 5 and Figure 6. So, the hypoglycemic effect of luteolin was similar to that of positive control metformin (41.87 % reduction of blood glucose level).

Table 5. Blood Glucose Levels in Alloxan-Induced Diabetic Mice Treating with Isolated Flavonoids after 4 h of administration

No.	Treatment	Blood Glucose Concentration (mg/dL)				
		0 h	1 h	2 h	3 h	4 h
I	Normal	100.00± 3.39	101.00± 7.35	100.00± 5.00	100.67± 8.37	95.67± 4.03
II	Alloxan (150 mg/kg)	253.00± 21.25	279.00± 22.25	275.00± 29.25	275.00± 24.50	269.00± 22.50
III	*Metformin (15 mg/kg)	192.67± 11.61 (0%R)	159.67± 11.18 (16.78%R)	135.33± 16.95 (29.76%R)	123.33± 15.67 (35.08%R)	112.00± 14.14 **(41.87%R)
IV	Luteolin (15 mg/kg)	205.00± 21.60 (0%R)	187.67± 17.80 (8.45%R)	168.00± 26.90 (18.05%R)	145.33± 17.80 (29.10%R)	119.67± 24.70 **(41.62%R)
V	DHQ-DME (15 mg/kg)	207.00± 28.50 (0%R)	171.69± 25.60 (17.06%R)	156.67± 24.30 (24.31%R)	142.67± 27.10 (31.78%R)	126.33± 26.90 **(38.97%R)
VI	Naringenin (15 mg/kg)	207.67± 24.36 (0%R)	187.00± 27.32 (9.95%R)	173.67± 28.23 (16.37%R)	162.67± 23.60 (21.67%R)	139.33± 29.42 **(32.91%R)

*positive control, **P<0.05, R= reduction of blood glucose level of treated diabetic mice

DHQ-DME – dihydroquercetin-7,4′-dimethyl ether

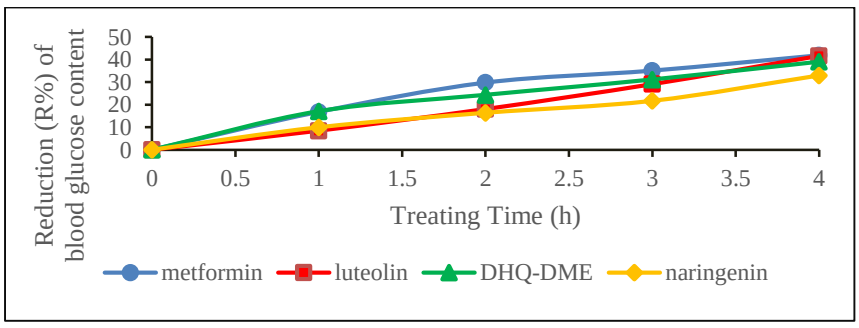


Figure 6. A line graph of hypoglycemic effect of Alloxan-induced diabetic mice treating with isolated flavonoids after 4h of administration

Structure-Activity Relationship of Isolated Flavonoid Compounds

Luteolin has two hydroxyl groups at the C-3' and C-4' positions on the B ring, as well as at the 5 and 7 positions on the A ring. It features a carbonyl oxygen at C-4 and a double bond between C-2 and C-3 on ring C. Dihydroquercetin-7,4'-dimethyl ether, a quercetin derivative, has methoxy groups at C-7 on ring A and C-4' on ring B but lacks the double bond between C-2 and C-3. Naringenin contains hydroxyl groups at the 5 and 7 positions of the A ring, at C-3 of the C ring, and at C-4' of the B ring, along with a carbonyl group at C-4. Like dihydroquercetin-7,4'-dimethyl ether, naringenin also does not have a double bond between C-2 and C-3 on ring C.

Luteolin possesses two hydroxyl groups at the 3' and 4' positions on the B ring, enhancing its antioxidant activity by donating electrons to free radicals. Dihydroquercetin-7,4'-dimethyl ether, a quercetin derivative, has a methoxy group at the 4' position, increasing its lipophilicity and bioavailability, while its 3' hydroxyl group aids in radical scavenging. Naringenin features hydroxyl groups at the 5 and 7 positions of the A ring and a carbonyl group at the C-4 position of the C ring, allowing it to interact with metal ions and improve its radical-quenching ability. In terms of antioxidant potency, luteolin is the most effective (IC_{50} = 1.91 µM), followed by dihydroquercetin-7,4'-dimethyl ether (IC_{50} = 16.06 µM) and naringenin (IC_{50} = 27.21 µM).

The presence of hydroxyl groups is crucial for flavonoids to inhibit alpha-amylase and alpha-glucosidase. Luteolin's multiple hydroxyl groups enhance its ability to interact with the enzymes' active sites, resulting in lower IC_{50} values. Dihydroquercetin-7,4'-dimethyl ether has a methoxy group at the 4' position of the B ring, increasing lipophilicity and potentially improving binding affinity, although its IC_{50} is higher than luteolin's. Naringenin contains hydroxyl groups at the C-5 and C-7 positions of the A ring and a carbonyl group at the C-4 position of the C ring, allowing it to interact with enzyme active sites, but its overall configuration leads to higher IC_{50} values compared to luteolin and dihydroquercetin-7, 4’-dimethyl ether in both enzyme assays.

Conclusion

The preliminary phytochemical analysis revealed the presence of bioactive secondary metabolites in the plant sample. In this study, three flavonoids: two from the peel of *C. maxima* (luteolin and dihydroquercetin-7,4'-dimethyl ether) and one from the flower of *P. pterocarpum* (naringenin) were isolated. These compounds were extracted from the ethyl acetate fraction and confirmed through chemical tests, melting point analysis, UV and co-thin layer chromatography (co-TLC) with their respective authentic compounds. Regarding their biological activities, luteolin demonstrated the most significant effects in terms of oxidative inhibition, as well as α-amylase and α-glucosidase enzyme inhibition, leading to a reduction in blood glucose levels. Luteolin was followed by dihydroquercetin-7,4'-dimethyl ether, and naringenin. From the structure-activity relationship studies, we can conclude that flavonoid compounds exhibiting antioxidant activity, along with inhibition of alpha-amylase and alpha-glucosidase enzymes and a reduction in blood glucose levels, typically contain specific structural features. These include hydroxyl groups at the C-5 and C-7 positions on ring A, a carbonyl oxygen at the C-4 position, a double bond between C-2 and C-3 on ring C, and hydroxyl groups at the C-3' and C-4' positions in ring B. These key structural components are suggested to enhance their biological activities.

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