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Pterygota alata (Roxb.) R. Br.: Chemical constituents, Anti-hyperglycemic Effect and Anti-oxidative Stress in Alloxan-induced Diabetic rats

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- ✓ diabetes

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Abstract

Twenty four compounds (4 non alkaloidal nitrogenous bases and 20 flavonoids) were isolated and identified from 70% methanol/water extract of *Pterygota alata* (Roxb.) R. Br. leaves and stems using chromatographic and spectroscopic techniques. The phenolics and flavonoids were qualitatively perceived through HPLC fingerprint analysis and their total contents were estimated quantitatively as 53.036 mg AE/100 g and 10.731 mg GAE/ 100 g dry weights, respectively. Potential activity of the aqueous methanol extract against alloxan inducing oxidative stress and diabetes in male rats was preliminary investigated (4 groups of 10 rats /group). Animal group receiving the extract (500 mg/kg bw i.p.) revealed a significant acute anti-hyperglycemic effect potentiated after 4 and 24 hrs of treatment with blood glucose levels of 202 and 160.16 mg/dl, respectively, compared to alloxan and standard Diamicron (5 mg/kg bw, p.o.) groups, as well as to a normoglycemic control group at $P<0.05$. PAE significantly increased serum GSH and attenuated MDA and NO contents in rats (2.39 mmol/l, 48.16 nmol/l and 1.71 µmol/l, respectively) after 24 hrs of treatment compared to alloxan control 1.01 mmol/l, 4.69 nmol/l, 118.9 µmol/l, respectively and to normoglycemic control at $P<0.05$.

1. Introduction

Pterygota Schott & Endl. (Sterculiaceae) consists of about 15 species distributed in Tropical Asia and Africa. *Pterygota alata* (Roxb) R. Br. is a large deciduous tree known as Budah coconut tree. The plant is naturally distributed in South Asia and Myanmar. For a long time, *P. alata* has been controversial from a systematic point of view. On the bases of various morphological features, *Sterculia alata* Roxb. had been revised to the genus *Pterygota* and hence the name was revised to *P. alata* [1- 4]. The seeds of *P. alata* are consumed, possess a narcotic like effect and utilized as an alternate for opium in India. The bark juice of *Pterygota* species was used traditionally in the management of hemorrhoids, dropsy, swelling oedema, gout, leprosy and pain [2]. The tree heartwood was used in the production of cement-bonded wood for floor boards [2].

The first phytochemical study on the leaves and stems of *P. alata* was carried out by Lin *et al*. [5] yielding four phenylpropanoids (cinnamic acid, *p*-methoxy cinnamic acid, 1,6-*O*-dicinnamoyl glucose and 1-*O*-*p*-coumaroyl 6-*O*-cinnamoyl-*β*-D-galactoside), four triterpenes (taraxeryl acetate, fridelin, epifriedelanol and oleanolic acid), two lignans (epieudesmin and diayangambin), one steroid (stigmasterol), an anthraquinones (thespeson) and a flavonoid (apigenin). Jahan *et al*. [6] reported that the chloroform fraction of the crude ethanol extract of *P. alata* bark showed high concentrations of phenolics and flavonoids as well as free radical scavenging property. Chatterji [7], Qin [8] and Hussain *et al*. [9] found that the leaves of *S. urens, S. lychnophora* and *S. foetida* possessed significant anti-hyperglycemic activity with an improvement of body weight.

Recently, in developing nations and industrialized countries the occurrence of diabetes is escalating. Imbalance between ROS (Reactive oxygen species) and the antioxidant defense system mediated oxidative stress which is a key factor in the mechanism of several diseases including diabetes [10]. Thus, apart from traditional treatment a suitable antioxidant therapy would benefit in diabetes.

Flavonoids and phenolic phytochemicals are thought to promote optimal health partly by protecting cellular components against free radical induced damage, via their antioxidant and free radical scavenging effects [11]. Hence on the above fact, the present study was designed to carry out a qualitative phytochemical investigation of the flavonoids and phenolics of *P. alata* cultivated in Egypt, in addition to quantitative estimation of their total contents using HPLC. No study has been carried out on the biological activities of *P. alata* extract. Therefore, the present study is an attempt to explore the anti-hyperglycemic activity of *P.alata* and its antioxidant effects in alloxan-induced diabetic rats.

2. Material and Methods

2.1. *General*

1D and 2D NMR experiments (^{1}H, ^{13}C, HMQC and HMBC) were recorded on a Jeol EX-500 spectrometer: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR), Bruker-400 spectrometer: 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR). UV spectrophotometer (Shimadzu UV-240), EI-MS: Finnigan-Mat SSQ 7000 spectrometer. CC Polyamide S6 (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) using MeOH/H_2O as eluent. CC Silica gel 60 (Merck, 0.063-0.2mm) using $CHCl_3$/MeOH (9:1). PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems: 1) H_2O, 2) 15% HOAc (H_2O–HOAc 85:15), 3) 50% HOAc (H_2O–HOAc 50: 50), 4) BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer), 5) BBPW (C_6H_6–*n*-BuOH– pyridine–H_2O 1:5:3:3, upper layer). Solvents 4 and 5 were used for sugar analysis. Sephadex LH-20 (Pharmacia). Authentic samples were obtained from the Phytochemistry and Plant Systematics Department, NRC. Acid hydrolysis for *O*-glycosides (2N HCL, 2 hrs, 100 °C) were carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties. The sugar units of *C*-glycoside flavonoids were determined using ferric chloride degradation [12].

2.2. *Plant material*

Fresh leaves and stems of *P. alata* were collected from the Zoological Garden, Giza, Egypt. The plant was kindly identified by Dr. M. El Gibali, former researcher of botany, National Research Centre (NRC), Egypt.

2.3. *Extraction and isolation*

Air-dried, grounded leaves and stems of *P. alata* (2.7 kg) were extracted three times by repeated percolation (40-60°C) with 70% methanol/water till exhaustion then evaporated under reduced pressure affording 135 g residue.The residue (125 g) was defatted with petroleum ether (40-60 ºC) then subjected to a polyamide column (150 × 5 cm) starting with water as eluent then decreasing the polarity by increasing the concentration of methanol. A total of 86 fractions were collected, each of about 100 ml. Similar fractions were combined according to their PC properties using H_2O, 15% HOAc and BAW as eluents to give five main fractions (A-E). Fraction A (100% H_2O) was applied to a Silica gel column (70 × 2.5 cm) using $CHCl_3$: MeOH (9:1) and ending with 100% methanol, followed by PPC using BAW followed by H_2O as eluents yielded compounds **1**-**4**. Fraction B (20% MeOH/H_2O) was rechromatographed on smaller polyamide column (70 × 2.5 cm) using MeOH: Toluene: H_2O (60:38:2) yielded a subfraction (20%) which was applied on Sephadex LH-20 column (35 × 2.5 cm) using H_2O as eluent, followed by PPC using BAW (twice) yielded compounds **5**-**8**. Fraction C (40 % MeOH/H_2O) was rechromatographed on smaller polyamide column (70 × 2.5 cm) using MeOH: Toluene: H_2O (60:38:2) yielded four subfractions. They were applied on Sephadex LH-20 column (35 × 2.5 cm) using MeOH: H_2O (1:1) as eluent followed by PPC using BAW (twice) yielded compounds **9**-**12**. Fraction D (60% MeOH/H_2O) was chromatographed on PPC using BAW and applied on Sephadex LH-20 column (35 × 2.5 cm) using MeOH: H_2O (1:1) yielded compound **13** and **14**. Fraction E (80% MeOH/H_2O) yielded compounds **15**-**20** by separation on PPC using BAW followed by water and rechromatography on Sephadex LH-20 column (35 × 2.5 cm) using MeOH: H_2O (1:1). Fraction F (100% MeOH) was also chromatographed on PPC using BAW then on Sephadex LH-20 column (35× 2.5 cm) using MeOH to yield compounds **21**-**24**.

2.4. *HPLC profiles of phenolics and flavonoids*

Five grams of dried powdered leaves and stems of *P. alata* were mixed separately with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2 mm millipore membrane filter before injection. Chromatographic separations were performed using HPLC Hewlett Packered (series 1050) (Hewlett Packered Inc., Palo Alto, CA) equipped with auto-sampler injector, solvent degasser, ultraviolet (UV) detector set at (280 nm for phenolics and 330 nm for flavonoids determinations), and quaternary HP pump (series 1050). The column temperature was kept at 35°C. Gradient separation was carried out using methanol and acetonitrile (2:1) as a mobile phase at a flow rate of 1 ml/min [13].

Isolated and authentic phenolics and flavonoids were dissolved in the mobile phase and injected into HPLC. The retention time and the peak area were determined for the phenolics and flavonoids by the data analysis of Hewlett Packered software.

2.5. *Biochemical assay*

2.5.1. *Animals*

Albino mice weighing 25-30 g were used for determination of LD_{50}. Male albino rats, 8 weeks old (weighing 120–150 g) obtained from the breeding colonies of the National Research Centre (Dokki, Giza, Egypt), and acclimatised with free access to food (standard laboratory pellets of 20% protein, 5% fats, and 1% vitamins) and tap water for at least 1 week at room temperature at 23-25 °C. The animals were fasted for 24 hrs before induction of hyperglycemia but allowed free access to water.

2.5.2. *Induction of experimental diabetes and Experimental groups*

Diabetes was induced by a single injection of a freshly prepared alloxan monohydrate (Sigma, No. 242-646-8) according to rat weight at dose (150 mg\kg, i.p.) to overnight-fasted rats [14]. After a period of three days the rats which did not develop more than 200 mg/dl glucose levels, were rejected. Control rats (Normoglycemic) received only the saline. The alloxan-induced diabetic rats were classified into three groups (1–3) each of them with 10 rats. Group 1; received saline and served as hyperglycemic group. Group 2; received Diamicron (5 mg\kg, p.o.) and was given as standard group. Group 3; received PAE (500 mg\kg, i.p.).

2.5.3. *Collection of blood and determination of blood glucose level (BGL)*

Blood samples were collected by cutting the tail-tip of the rats at zero, 4 and 24 hrs after injection of PAE. The blood glucose level was determined by the glucose-oxidase principle [15] using the OK glucometer (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl [16].

2.5.4. *Oxidative stress biomarkers*

2.5.4.1. *Determination of serum reduced glutathione content*

Reduced glutathione content of serum was measured according to method of Bulaj *et al.* [17]. In centrifuge tubes containing 0.5 ml of precipitating solution (10% trichloroacetic acid-6 mM Na_2EDTA), 50 ul of serum was added, vortexed and centrifuged at 2000 rpm for 5 min. 1.85 ml of potassium phosphate buffer (100mM, pH 8) and 0.1 ml of Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)] were added to 0.1 ml of the resulting clear supernatant and mixed thoroughly. After 5 minutes, the absorbance was measured at 412 nm using a double beam spectrophotometer (Schimadzu, Japan) against reagent blank. The GSH level in serum was expressed as mmole/l and calculated from the following formula: GSH content (mmole/l)=At/As× Cs× dilution factor , Where: At= absorbance of test sample, As= absorbance of standard and Cs= concentration of standard.

2.5.4.2. *Determination of thiobarbituric acid reactive substances*

The method was employed to determine lipid peroxides formation in serum as thiobarbituric acid reactive substances (TBARS). Lipid peroxidation products were measured as malondialdehyde (MDA); one of the degradation products of lipid peroxides [18]. In centrifuge tubes, 0.5 ml of supernatant of 10% serum homogenate, 3 ml of *Ortho*-phosphoric acid (1%, *v/v*) and 1 ml of thiobarbituric acid (0.67%, *w/v*) were added. After heating for 20 minutes in a boiling water bath, the mixture was cooled and 4 ml of n-butanol were added and mixed vigorously, then separated by centrifugation at 3000 for 10 min. Optical density was measured against reagent blank at 532 nm using a double beam spectrophotometer (Schimadzu, Japan). TBARS concentration in serum was expressed as nmole MDA/l and calculated from the following equation:

TBARS (nmole/l) = ODt/ODs × Cs × dilution factor, Where: OD: optical density, Cs: Concentration of standard malondialdehyde (MDA) solution.

2.5.4.3. *Determination of nitric oxide content*

Nitric oxide (NO), an unstable reactive nitrogen free radical, was determined in the present study using biochemical method of Montgomery and Dymock [19] where the production of NO is expressed by endogenous nitrate/nitrite metabolites. In a test tube, 0.25 ml of zinc sulphate (10%) was added to 0.25 ml serum and left for 15 min to deproteinize it. Samples were then centrifuged at 12000 rpm for 20 min using cooling centrifuge (Hermle, Germany). To 250 µl of the obtained supernatant, 250 µl of NEDD reagent [N-(1-Naphthyl) ethylenediamine dihyrochloride; 0.1% (*w/v*) in distilled water] was added and incubated at 37°C for 10-15 min afterward 250 µl of sulphanilamide solution [2 % (*v/v*) in distilled water] was added and incubated at 37°C for 10-15 min. The mixture was cooled and the absorbance of the pink coloured chromophore was measured at 540 nm using a double beam spectrophotometer (Schimadzu, Japan) against a reagent blank where 250 µl distilled

water was used instead of the sample. The level of total nitrite/nitrate (NO_x) was expressed as µmol/l and was calculated by: NOx (µmol/l)= At/As×Cs×dilution factor, where At: absorbance of the test sample, As: absorbance of the standard sample and Cs: concentration of standard.

2.5.5. *Statistical analysis*

All values are presented as means ± SEM (standard error of the means) for 10 rats in each group. Comparison between groups was carried out using the non-parametric one-way analysis of variance (ANOVA) followed by Tukey-HSD multiple comparisons test to judge the difference between various groups. Difference was considered significant when $P<0.05$. Graph pad software (version 7.00) and Excel 10 were used to carry out these statistical tests and plot graphs.

3. Results

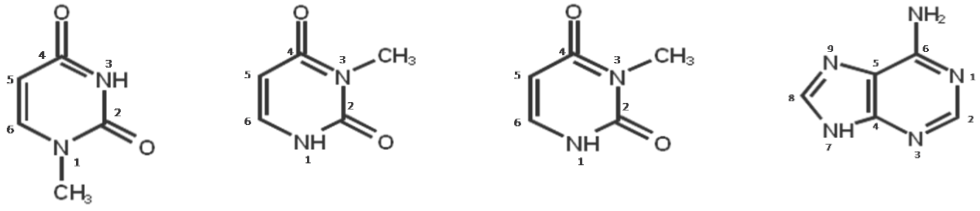
3.1. *Phytochemical investigation*

Twenty four compounds; 4 non alkaloidal nitrogenous bases (Figure 1) and 20 flavonoids (Figure 2) were isolated and identified from PAE. Their structures were elucidated using chemical methods (acid hydrolysis, alkaline hydrolysis and $FeCl_3$ degradation), spectroscopic (UV, 1D and 2D NMR experiments) and spectrometric (EI-MS) analysis. Further confirmation was carried out through comparison of their spectroscopic data with previously reported values [20-37]. They were identified as four non alkaloidal nitrogenous bases: uracil (**1**), 1-methyl uracil (**2**), 3-methyl uracil (**3**) and adenine (**4**) and 20 flavonoids: luteolin 6-*C*-*β*-glucopyranoside-7-*O*-*β*-glucopyranoside (lutonarin; **5**), luteolin 6-*C*-*α*-arabinopyranoside-8-*C*-*β*-glucopyranoside (isocarlinoside; **6**), luteolin 6-*C*-*β*-glucopyranoside-8-*C*-*α*-arabinopyranoside (carlinoside; **7**), apigenin 6,8-di-*C*-*β*-glucopyranoside (vicenin; **8**), apigenin 6-*C*-*α*-arabinopyranoside-8-*C*-*β*-galactopyranoside (corymboside; **9**), apigenin 6-*C*-*α*-rhamnopyranoside-8-*C*-*β*-glucopyranoside (isoviolanthin; **10**), apigenin 6-*C*-*β*-glucopyranoside-8-*C*-*α*-rhamnopyranoside (violanthin; **11**), apigenin 6-*C*-*β*-glucopyranoside-7-*O*-*β*-glucopyranoside (saponarin; **12**), luteolin 7-*O*-*β*-glucuronide (**13**), apigenin 7-*O*-*β*-glucuronide (**14**), luteolin 7-*O*-*β*-glucopyranoside (**15**), apigenin 7-*O*-*β*-glucopyranoside (**16**), kaempferol 3-O-*β*-(4''-*p*-coumaryl)-glucopyranoside (**17**), kaempferol 3-O-*β*-(*p*-coumaryl)-glucopyranoside (**18**), diosmetin (**19**), apigenin 7,4'-dimethyl ether (**20**, apigenin (**21**), acacetin (**22**), luteolin (**23**), and kaempferol (**24**). All compounds were isolated for the first time from *P. alata* with exception of apigenin [5]. The data of the isolated compounds were listed as following:

Uracil (1) [20]: White powder, *Rf*. 0.36 (BAW). UV spectral data, λ_{max} (nm): MeOH: 208, 259. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 11.01 (2NH, bs, H-1, 2), 5.43 (1H, d, *J* = 7.6 Hz, H-5), 7.39 (1H, d, *J* = 7.6 Hz, H-6), ^{13}C-NMR (100 MHz in DMSO-d_6, δ, ppm): 152.1 (C-2), 164.9 (C-4), 100.6 (C-5), 142.9 (C-6). EIMS (*m/z*): 112.16. HMBC showed correlations between H-5 (δ5.43) with C-4 (δ164.9) and C-6 (δ142.9) and H-6 (δ7.39) with C-2 (δ152.1), C-4 (δ164.9) and C-5 (δ100.6).

1-Methyl uracil (**2**) [21]: White powder, *Rf*. 0.36 (BAW). UV spectral data, λ_{max} (nm): MeOH: 208, 250. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 11.01 (1NH, s, H-3), 7.39 (1H, d, *J* = 7.6 Hz, H-5), 5.43 (1H, d, *J* =7.6 Hz, H-6), 3.17 (3H, s, CH_3). ^{13}C-NMR (100 MHz in DMSO-d_6, δ, ppm): δ152.1 (C-2), 165.1 (C-4), 100.7 (C-5), 142.9 (C-6), 30.2 (CH_3). EIMS (*m/z*): 126.11.

3-Methyl uracil (**3**) [21]: White powder, *Rf*. 0.43 (BAW). UV spectral data, λ_{max} (nm): MeOH: 204, 258. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 11.02 (1NH, s, H-1), 7.38 (1H, d, *J* = 7.6 Hz, H-5), 5.43 (1H, d, *J* =7.6 Hz, H-6), 3.21 (3H, s, CH_3). ^{13}C-NMR (100 MHz in DMSO-d_6, δ, ppm): δ152 (C-2), 165.1 (C-4), 100.7 (C-5), 142.8 (C-6), 25.6 (CH_3).



1 **2** **3** **4**

Figure 1: Chemical structures of compounds **1**-**4**.

Adenine (**4**) [22]: White powder, *Rf*. 0.33 (BAW). UV spectral data, λ_{max} (nm): MeOH: 207, 260. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 8.4 (1NH, s), 8.01 (1H, s, H-2), 7.91 (1H, s, H-8), 5.45 (1H, s, NH_2).^{13}C-

NMR (100 MHz in DMSO-d_6, δ, ppm): 154.3 (C-2), 144.8 (C-4), 120.2 (C-5), 156.3 (C-6), 141.5 (C-8). EIMS (*m/z*): 135.16. HMBC showed correlations between H-2 (δ 8.01) with C-4 (δ 144.8) and C-6 (δ 156.3), H-8 (δ 7.91) with C-4 (δ 144.8) and C-5 (δ 120.2).

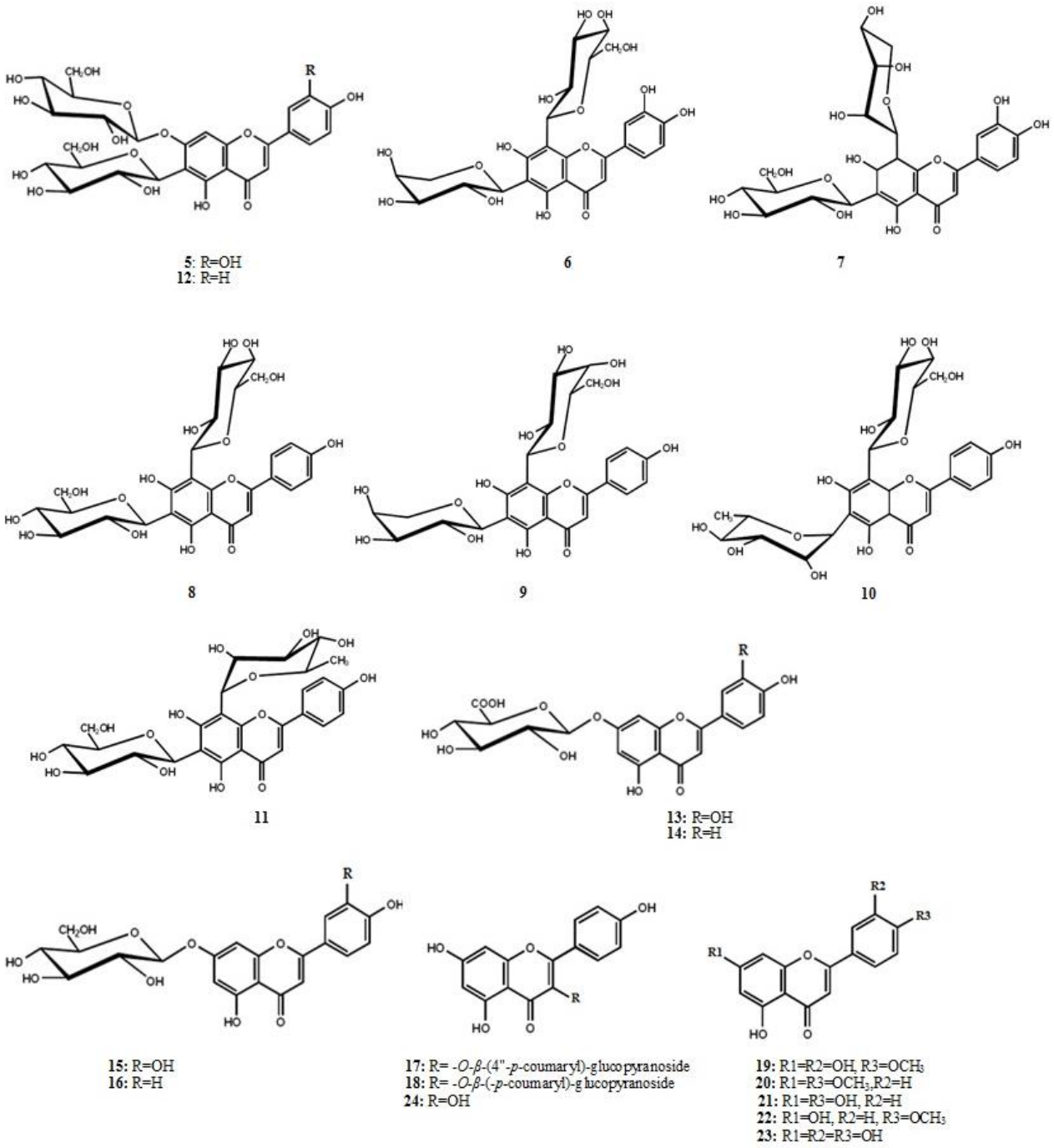


Figure 2: Chemical structures of flavonoid compounds **5-24**.

Luteolin 6-*C*-*β*-glucopyranoside-7-*O*-*β*-glucopyranoside (lutonarin) (**5**) [23]: Yellow powder, *Rf*. 0.08 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 267, 347; MeOH/NaOMe: 262, 415; $AlCl_3$: 267, 298, 345, 416; $AlCl_3$/HCl: 263, 299, 350, 380; NaOAc: 267, 413; NaOAc/ H_3BO_3: 263, 403. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.54 (1H, dd, *J* = 2.1,8.8 Hz, H-6′), 7.43 (1H, d, *J* = 2.1 Hz, H-2′), 6.71 (1H, d, *J* = 8.8 Hz, H-5′), 6.67(1H, s, H-8), 6.57 (1H, s, H-3), 4.95 (1H, d, *J* = 7.0 Hz, H-1‴), 4.85 (1H, d, *J* = 9.5 Hz, H-1″), 3-4 (10H, m, H-2″- H-6″, H-2‴- H-6‴).

Luteolin 6-*C*-*α*-arabinopyranoside-8-*C*-*β*-glucopyranoside (isocarlinoside) (**6**) [24,25]: Yellow powder , *Rf*. 0.31 (BAW). UV spectral data, λ_{max} (nm): MeOH: 272, 349; MeOH/NaOMe: 278,346, 409; $AlCl_3$: 271, 346, 409; $AlCl_3$/HCl: 271, 303, 385; NaOAc: 282, 326, 395; NaOAc/H_3BO_3: 266, 379. ^{1}H-NMR (400 MHz in DMSO-d_6,

δ, ppm, *J*/Hz): 7.54 (1H, d, *J* = 2.1 Hz, H-2′), 7.51 (1H, dd, *J* = 2.0,8.0 Hz, H-6′), 6.86 (1H, d, *J* = 7.8 Hz, H-5′), 6.51(1H, s, H-3), 4.85 (1H, d, *J* = 9.6 Hz, H-1‴), 4.69 (1H, d, *J* = 8.8 Hz, H-1″), 3-4 (9H, m, H-2″- H-5″, H-2‴- H-6‴). ^{13}C-NMR (100 MHz in DMSO-d_6, δ, ppm): 165.8 (C-2), 104.1 (C-3), 183.8 (C-4), 157.3 (C-5), 108.1 (C-6), 164 (C-7), 101.5 (C-8), 154.4 (C-9), 104.5 (C-10), 122.2 (C-1′), 115.4 (C-2′), 146.6 (C-3′), 150.6 (C-4′), 116.79 (C-5′), 119. 5 (C-6′),74.4 (C-1″), 74 (C-2″), 70.9 (C-3″), 70.2 (C-4″), 68.8 (C-5″), 73.3 (C-1‴), 68.2 (C-2‴), 78.6 (C-3‴), 69.8 (C-4‴), 81.72 (C-5‴), 61.4 (C-6‴).

Luteolin 6-*C*-*β*-glucopyranoside-8-*C*-*α*-arabinopyranoside (carlinoside) (**7**) [26,27]: Yellow amorphous powder, *Rf*. 0.39 (BAW). UV spectral data, λ_{max} (nm): MeOH: 259sh, 272, 347; MeOH/NaOMe: 268, 335, 407; $AlCl_3$: 276, 333, 418; $AlCl_3$/HCl: 278, 298, 360, 385; NaOAc: 281, 400; NaOAc/ H_3BO_3: 271, 401. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.4 (1H, dd, *J* = 2.0,8.0 Hz, H-6′), 7.36 (1H, d, *J* = 2.1 Hz, H-2′), 6.85 (1H, d, *J* = 8.5 Hz, H-5′), 6.63 (1H, s, H-3), 5.07 (1H, d, *J* = 8.0 Hz, H-1‴), 4.71 (1H, d, *J* = 10.6 Hz, H-1″), 3-4 (9H, m, H-2″- H-5″, H-2‴- H-6‴).

Apigenin 6,8-di-*C*-*β*-glucopyranoside (vicenin) (**8**) [28]: Yellow amorphous powder, *Rf*. 0.44 (BAW). UV, spectral data, λ_{max}(nm): MeOH: 274, 329; MeOH/NaOMe: 286, 327, 396; $AlCl_3$:278, 305,349; $AlCl_3$/HCl:278, 302,343; NaOAc: 282, 385; NaOAc/ H_3BO_3: 277, 318, 358. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.99 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.86 (2H, d, *J* = 8.8 Hz, H-3',5′), 6.78 (1H, s, H-3), 4.72 (1H, d, *J* = 9.6 Hz, H-1‴),4.67 (1H, d, *J* = 9.6 Hz, H-1″),3-4 (10H, m, H-2″- H-6″, H-2‴- H-6‴).

Apigenin 6-*C*-*α*-arabinopyranoside-8-*C*-*β*-galactopyranoside (corymboside) (**9**) [29]: Yellow powder, *Rf*. 0.44 (BAW). UV, spectral data, λ_{max}(nm): MeOH: 273, 331; MeOH/NaOMe: 283, 334, 398; $AlCl_3$: 275, 305, 343; $AlCl_3$/HCl:278, 305,344; NaOAc: 283, 386; NaOAc/ H_3BO_3: 276, 324, 339. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.87 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.87 (2H, d, *J* = 8.8 Hz, H-3',5′), 6.61 (1H, s, H-3), 4.70 (1H, d, *J* = 8.6 Hz, H-1″),4.64 (1H, d, *J* = 9.6 Hz, H-1‴),3-4 (9H, m, H-2″- H-5″, H-2‴- H-6‴).^{13}C-NMR (125 MHz in DMSO-d_6, δ, ppm): δ163.1 (C-2), 102.7 (C-3), 181.7 (C-4), 155.9 (C-5), 108.9 (C-6), 161.4 (C-7), 105.3 (C-8), 156.3 (C-9), 102.9 (C-10), 122.4 (C-1′), 128.2 (C-2′), 116.4 (C-3′), 159.9 (C-4′), 116.4 (C-5′), 128.8 (C-6′),75.7 (C-1″), 70.7 (C-2″), 79.1 (C-3″), 72.1 (C-4″), 70.1 (C-5″), 74.8 (C-1‴), 71.6 (C-2‴), 79.6 (C-3‴), 71.1 (C-4‴), 81.4 (C-5‴), 60.9 (C-6‴).

Apigenin 6-*C*-*α*-rhamnopyranoside-8-*C*-*β*-glucopyranoside (isoviolanthin) (**10**) [30]: Yellow powder, *Rf*. 0.39 (BAW). UV, spectral data, λ_{max}(nm): MeOH: 272, 334; MeOH/NaOMe: 282, 332, 398; $AlCl_3$: 272, 305, 339; $AlCl_3$/HCl:271, 303,338; NaOAc: 279, 334, 350; NaOAc/ H_3BO_3: 274, 329. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz)): 7.95 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.85 (2H, d, *J* = 8.8 Hz, H-3',5′), 6.68 (1H, s, H-3), 4.93 (1H, d, *J* = 1.8 Hz, H-1″),4.72 (1H, d, *J* = 9.6 Hz, H-1‴), 3-4 (9H, m, H-2″- H-5″, H-2‴- H-6‴), 0.81(1H, d, *J* = 6.0 Hz, H-6″).^{13}C-NMR (125 MHz in DMSO-d_6, δ, ppm): δ163.9 (C-2), 102.7 (C-3), 182.1 (C-4), 158.1 (C-5), 108.8 (C-6), 160.1 (C-7), 105.6 (C-8), 155.8 (C-9), 102.9 (C-10), 122.3 (C-1′), 129.7 (C-2′), 116.3 (C-3′), 161.6 (C-4′), 116.3 (C-5′), 129.7 (C-6′),77.7 (C-1″), 74.7 (C-2″), 74.6 (C-3″), 72.7 (C-4″), 72.8 (C-5″), 18.8 (C-6″), 73.9 (C-1‴), 71.4 (C-2‴), 79.4 (C-3‴), 71.2 (C-4‴), 82.4 (C-5‴), 61.9 (C-6‴).

Apigenin 6-*C*-*β*-glucopyranoside-8-*C*-*α*-rhamnopyranoside (violanthin) (**11**) [30]: Yellow powder, *Rf*. 0.34 (BAW). UV, spectral data, λ_{max}(nm): MeOH: 273, 334; MeOH/NaOMe: 283, 332, 398; $AlCl_3$: 28, 306, 351; $AlCl_3$/HCl: 279, 303, 342, 383; NaOAc: 281, 373; NaOAc/ H_3BO_3: 275, 324, 337. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.74 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.89 (2H, d, *J* = 8.5 Hz, H-3',5′), 6.42 (1H, s, H-3), 4.88 (1H, d, *J* = 2.0 Hz, H-1‴),4.64 (1H, d, *J* = 9.2 Hz, H-1″), 3-4 (9H, m, H-2″- H-6″, H-2‴- H-5‴), 1.18 (1H, d, *J* = 6.0 Hz, H-6‴).^{13}C-NMR (125 MHz in DMSO-d_6, δ, ppm): δ165.8 (C-2), 102.3 (C-3), 181.7 (C-4), 160.3 (C-5), 109.4 (C-6), 162.9 (C-7), 101.9 (C-8), 153.9 (C-9), 103.5 (C-10), 121.3 (C-1′), 128.8 (C-2′), 116.2 (C-3′), 161.4 (C-4′), 116.2 (C-5′), 128.8 (C-6′),73.2 (C-1″), 70.6 (C-2″), 79.2 (C-3″), 70.2 (C-4″), 81.4 (C-5″), 61.6 (C-6″), 77.4 (C-1‴), 75.2 (C-2‴), 75.3 (C-3‴), 72.2 (C-4‴), 72.3 (C-5‴), 18.8 (C-6‴).

Apigenin 6-*C*-*β*-glucopyranoside-7-*O*-*β*-glucopyranoside (saponarin) (**12**) [23]: Yellow powder, *Rf*. 0.34 (BAW). UV spectral data, λ_{max}(nm): MeOH: 271, 334; MeOH/NaOMe: 278, 398; $AlCl_3$: 272, 301, 339, 400; $AlCl_3$/HCl: 273, 302, 343, 398; NaOAc: 271, 343; NaOAc/ H_3BO_3: 272, 337. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 8.08 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.94 (2H, d, *J* = 8.7 Hz, H-3',5′), 6.77(1H, s, H-3), 6. 75 (1H, s, H-8), 5.02 (1H, d, *J* = 7.2 Hz, H-1‴), 4.75 (1H, d, *J* = 9.0 Hz, H-1″), 3-4 (10H, m, H-2″- H-6″, H-2‴- H-6‴).

Luteolin 7-*O*-*β*-glucuronide (**13**) [31]: Yellow powder, *Rf*. 0.13 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 268, 347; MeOH/NaOMe: 264,402; $AlCl_3$: 272, 310, 364, 424; $AlCl_3$/HCl: 275, 296, 359; NaOAc: 268, 410; NaOAc/ H_3BO_3: 268, 413. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 7.43 (1H, d, *J* = 1.8 Hz, H-2′), 7.41 (1H, dd, *J* = 1.8, 8.5 Hz,H-6′),6.9 (1H, d, *J* = 8.5 Hz, H-5′), 6.76 (1H, d, *J*=2.0Hz, H-8), 6.71 (1H, s, H-3), 6.41 (1H, d, *J* = 2.0Hz, H-6), 5.16 (1H, d, *J* = 7.6 Hz, H-1″), 3-4 (5H, m, H-2″- H-6″).^{13}C-NMR (125 MHz, in DMSO-d_6, δ, ppm, *J*/Hz): δ 164.9 (C-2), 104.4 (C-3), 182.3 (C-4), 161.5 (C-5), 99.8 (C-6), 162.8 (C-7), 94.9 (C-8) , 157.3 (C-9), 105.5 (C-10), 121.6 (C-1′), 113.7 (C-2′), 146.4 (C-3′), 150.1 (C-4′), 116.1 (C-5′), 119.2 (C-6′), 99.4 (C-1″), 72.9 (C-2″), 75.4 (C-3″), 71.4 (C-4″), 75.9 (C-5″), 170.5 (C-6″).

Apigenin 7-*O*-*β*-glucuronide (**14**) [32]: Yellow powder, *Rf*. 0.19 (BAW). UV spectral data, λ_{max} (nm): MeOH: 269, 332; MeOH/ NaOMe: 276, 393; $AlCl_3$: 272, 299, 340, 383; $AlCl_3$/HCl: 275, 300, 339, 382; NaOAc: 269, 329; NaOAc/H_3BO_3: 269, 329. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.93 (2H, d, *J* = 8.7 Hz, H-2′,H-6′), 6.91 (2H, d, *J* = 8.7 Hz, H-3′,5′), 6.84 (1H, s, H-3), 6.76 (1H, d, *J* = 2.0 Hz, H-8), 6.41 (1H, d, *J* = 2.0 Hz, H-6), 5.12 (1H, d, *J* = 7.5 Hz H-1″), 3-4 (5H, m, H-2″- H-6″).

Luteolin 7-*O*-*β*-glucopyranoside (**15**) [31]: Yellow powder, *Rf*. 0.34 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 267, 348; MeOH/NaOMe: 267, 401; $AlCl_3$: 268, 295, 350, 398; $AlCl_3$/HCl: 264, 292, 349, 391; NaOAc: 266, 349, 405; NaOAc/H_3BO_3: 259, 371. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.35 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′),7.27 (1H, d, *J* =2.0 Hz, H-2′), 6.72 (1H, d, *J*=2.0Hz, H-8), 6.62 (1H, d, *J* = 8.5 Hz, H-5′), 6.57 (1H, s, H-3), 6.33 (1H, d, *J* = 2.0Hz, H-6), 5.02 (1H, d, *J* = 7.7 Hz, H-1″), 3-4 (5H, m, H-2″- H-6″).

Apigenin 7-*O*-*β*-glucopyranoside (**16**) [31]: Yellow powder, *Rf*. 0.51 (BAW). UV spectral data, λ_{max} (nm): MeOH: 267, 332; MeOH/NaOMe: 268, 388; $AlCl_3$: 275,301, 345, 385; $AlCl_3$/HCl: 275, 300, 340, 395; NaOAc: 267, 347, 389; NaOAc/H_3BO_3: 268, 295, 331. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.82 (2H, d, *J* = 8.7 Hz, H-2′,H-6′), 6.85 (2H, d, *J* = 8.7 Hz, H-3′,5′), 6.75 (1H, s, H-3), 6.72 (1H, d, *J* = 2.0 Hz, H-8), 6.36 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 7.7 Hz H-1″), 3-4 (5H, m, H-2″- H-6″).

Kaempferol 3-O-*β*-(4"-*p*-coumaryl)-glucopyranoside (**17**) [33]: Yellow powder, *Rf*. 0.4 (BAW). UV spectral data, λ_{max} (nm): MeOH: 267, 299, 315; MeOH/NaOMe: 275, 311, 370; $AlCl_3$: 275, 306, 322, 393; $AlCl_3$/HCl: 276, 304, 321, 390; NaOAc: 275, 311, 378; NaOAc/H_3BO_3: 268, 315, 371. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.94 (2H, d, *J* = 8.5 Hz, H-2′,H-6′), 7.33 (2H, d, *J* = 8.0 Hz, H-2‴,6‴, coumaryl), 7.29 (1H, d, *J* = 16.3 Hz, H-7‴, coumaryl),6.81 (2H, d, *J* = 8.5 Hz, H-3′,5′), 6.77 (2H, d, *J* = 8.0 Hz, H-3‴,5‴, coumaryl), 6.26 (1H, d, *J* = 2.0 Hz, H-8), 6.1 (1H, d, *J* = 16.3 Hz, H-8‴, coumaryl), 6.05 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 7.0 Hz H-1″), 3-4 (5H, m, H-2″- H-6″). 13C-NMR (125 MHz in DMSO-d6, δ, ppm): δ156.43 (C-2), 133.15 (C-3), 177.40 (C-4), 161.19 (C-5), 98.77 (C-6), 164.19 (C-7), 93.62 (C-8), 156.34 (C-9), 103.94 (C-10), 120.82 (C-1′), 130.01 (C-2′), 115.76 (C-3′), 159.91 (C-4′), 115.76 (C-5′), 130.01 (C-6′), 101.11 (C-1″), 74.26 (C-2″), 75.21 (C-3″), 72.13 (C-4″), 74.58 (C-5″), 59.92 (C-6″), 124.92 (C-1‴), 130.73 (C-2‴), 115.02 (C-3‴), 159.77 (C-4‴), 115.02 (C-5‴), 130.73 (C-6‴), 144.53 (C-7‴), 113.71 (C-8‴), 166.01 (C-9‴).

Kaempferol 3-O-*β*-(*p*-coumaryl)-glucopyranoside (**18**) [34]: Yellow powder, *Rf*. 0.4 (BAW). UV spectral data, λ_{max} (nm): MeOH: 268, 313; MeOH/NaOMe: 275, 330, 370; $AlCl_3$: 274, 305, 402; $AlCl_3$/HCl: 275, 304, 402; NaOAc: 277, 309, 376; NaOAc/H_3BO_3: 268, 313, 365. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.97 (2H, d, *J* = 8.5 Hz, H-2′,H-6′), 7.38 (2H, d, *J* = 8.5 Hz, H-2‴,6‴; coumaryl), 7.33 (1H, d, *J* = 16.3 Hz, H-7‴, coumaryl), 6.81 (2H, d, *J* = 8.5 Hz, H-3‴,5‴; coumaryl), 6.84 (2H, d, *J* = 8.5 Hz, H-3′,5′), 6.2 (1H, d, *J* = 2.0 Hz, H-8), 6.15 (1H, d, *J* = 16.3 Hz, H-8‴; coumaryl), 6.00 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 7.0 Hz H-1″), 3-4 (5H, m, H-2″- H-6″).

Diosmetin (**19**) [35]: Yellow powder, *Rf*. 0.83 (BAW). UV spectral data, λ_{max} (nm): MeOH: 269, 347; MeOH/NaOMe: 275, 330, 387; $AlCl_3$: 273, 301, 347; $AlCl_3$/HCl: 268, 272, 306, 339; NaOAc: 275, 310, 383; NaOAc/H_3BO_3: 268, 350. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ7.45 (2H, m, H-2′, H-6′), 7.14 (H, d, *J* = 8.5 Hz H-5′), 6.83 (1H, s, H-3), 6.47 (1H, d, *J* = 1.5Hz; H-8), 6.15 (1H, d, *J* = 1.5Hz; H-6), 3.83 (3H, s, OCH_3).

Apigenin 7,4'-dimethyl ether (**20**) [36]: Yellow powder, *Rf*. 0.82 (BAW). UV spectral data, λ_{max}(nm): MeOH: 269, 334; NaOMe: 270, 398; $AlCl_3$: 269,299, 350, 378; $AlCl_3$/HCl: 268, 300, 351, 394; NaOAc: 268, 349; NaOAc/H_3BO_3: 269, 347. ^{1}H NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 8.04 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 7.11 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.94 (1H, s, H-3), 6.85 (1H, d, *J* = 2.0 Hz, H-8), 6.46 (1H, d, *J* = 2.0 Hz, H-6), 3.83 (1H, s, OCH_3), 3.62 (1H, s, OCH_3).

Apigenin (**21**) [31]: Yellow amorphous powder, *Rf*. 0.72 (BAW). UV spectral data, λ_{max} (nm): MeOH: 272, 331; NaOMe: 268, 332sh, 400; $AlCl_3$: 279,306, 351, 387; $AlCl_3$/HCl: 279, 304, 346, 386; NaOAc: 281, 306, 387; NaOAc/ H_3BO_3: 268,342. ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): δ7.92 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.91 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.75 (1H, s, H-3), 6.44 (1H, d, *J* = 2.5 Hz, H-8), 6.14 (1H, d, *J* = 2.5 Hz, H-6). EIMS (*m/z*): 270.2

Acacetin (**22**) [37]: Yellow amorphous powder, *Rf*. 0.7 (BAW). UV spectral data, λ_{max}(nm): MeOH: 269, 303, 326; NaOMe: 276, 295sh, 375; $AlCl_3$: 276,303, 346, 378; $AlCl_3$/HCl: 278, 301, 338, 377; NaOAc: 276, 300, 359; NaOAc/H_3BO_3: 272, 297, 333. ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): δ8.0 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 7.09 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.8 (1H, s, H-3), 6.4 (1H, d, *J* = 2.0 Hz, H-8), 6.11 (1H, d, *J* = 2.0 Hz, H-6), 3.85 (1H, s, OCH_3).

Luteolin (**23**) [31]: Yellow amorphous powder, *Rf*. 0.66 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 266, 344; MeOH/NaOMe: 273, 320sh, 408; $AlCl_3$: 271, 299, 360,398; $AlCl_3$/HCl: 271, 254, 387; NaOAc: 272, 342, 400; NaOAc/H_3BO_3: 259, 370. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 7.25 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′),7.22 (1H, d, *J* =2.0 Hz, H-2′), 6.54 (1H, d, *J* = 8.5 Hz, H-5′), 6.37 (1H, s, H-3), 6.15 (1H, d, *J*=2.0Hz, H-8), 5.91 (1H, d, *J* = 2.0Hz, H-6).

Kaempferol (**24**) [37]: Yellow amorphous powder, *Rf* = 0.74 (BAW). UV spectral data, λ_{max}(nm): MeOH: 266,292sh, 319sh, 366; NaOMe: 276, 320sh, 411; $AlCl_3$: 269, 305, 350, 423; $AlCl_3$/HCl: 267, 305, 350, 424; NaOAc: 274, 306, 378; NaOAc/H_3BO_3: 265, 294, 319, 369.^{1}H NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 8.04 (2H, d, *J*= 8.5, H-2',6'); 6.93 (2H,d, *J*= 8.5, H-3',5'); 6.41 (1H, d, *J*= 2.0, H-8); 6.16(1H,d, *J* = 2.0, H-6). EIMS (*m/z*): 286.1

3.2. *HPLC profile of phenolics and flavonoids and their total quantitative estimation*

Total phenolic and total flavonoid contents of PAE were estimated as 10.731 mg/g calculated as gallic acid equivalent (GAE) and 53.036 mg/g calculated as apigenin equivalent (AE), respectively. Phenolic acids and flavonoid compounds are among important classes of bioactive molecules in plants, which are known because of their antidiabetic and antioxidant potential [38].

According to HPLC analysis of phenolics and flavonoids profile of PAE (Table 1), eighteen phenolic compounds were detected at 280 nm, in addition to twenty isolated flavonoids were detected at 330 nm based on the used reference phenolics and flavonoids.

Table 1: HPLC qualitative estimation of the phenolic and flavonoid profiles of PAE.

Comp.	**Phenolic profile**			**Flavonoid profile**		
No.	Rt	Phenolic compounds	% Area	Rt	Flavonoid compounds	% Area
1	3.52	Syringic acid	0.4197	9.09	Luteolin 6-*C*-*β*-glucoside 7-*O*-*β*-glucoside	0.7965
2	6.87	Gallic acid	0.2134	9.33	Luteolin 6-*C*-*α*-arabinoside-8-*C*-*β*-glucoside	1.5930
3	8.35	Protocatechuic acid	0.4432	10.09	Apigenin 8-*C*-*β*-glucoside	5.7984
4	8.52	Catechein	1.2603	10.56	Luteolin 6-*C*-*β*-glucoside-8-*C*-*α*-arabinoside	4.2155
5	9.02	Chlorogenic acid	1.1503	11.19	Apigenin 6, 8-di-*C*-*β*-glucoside	2.3814
6	9.41	Catechol	1.4706	11.55	Apigenin 6-*C*-*α*-arabinoside-8-*C*-*β*-galactoside	3.4061
7	9.67	Epi-catechin	1.3214	11.97	Apigenin 6-*C*-*α*-rhamnoside-8-*C*-*β*-glucoside	2.0243
8	9.86	*p*-OH benzoic acid	0.4261	12.13	Apigenin 6-*C*-*β*-glucoside-8-*C*-*α*-rhamnoside	1.4494
9	10.17	Caffeic acid	0.9099	12.20	Luteolin 7-*O*-*β*-glucoside	2.4445
10	10.25	Vanillic acid	0.7081	12.37	Luteolin 7-*O*-*β*-glucuronide	1.8948
11	11.73	*p*-Coumaric acid	1.8760	13.28	Apigenin 7-*O*-*β*-glucopyranoside	10.8701
12	11.95	Ferulic acid	3.6503	13.45	Apigenin 7-*O*-*β*-glucuronide	0.9044
13	12.36	Isoferulic acid	2.1148	13.78	Kaempferol 3-*O*-*β*- glucopyranoside	1.0235
14	12.95	Reversetrol	1.2423	15.05	Kaempferol 3-*O*-*β*-(4''-*p*-coumaryl)-glucoside	1.1918
15	13.08	Ellagic acid	3.2001	15.28	Kaempferol 3-*O*-*β*-(*p*-coumaryl)-glucoside	0.9336
16	13.37	Oleuropein	1.4774	15.63	Luteolin	8.3473
17	13.58	*O*-Coumaric acid	4.2999	16.36	Kaempferol	0.4502
18	13.78	Salicylic acid	0.9067	16.55	Apigenin	0.8835
19	-	-	-	18.34	Apigenin 7,4'-dimethyl ether	0.1992
20	-	-	-	19.01	Acacetin	0.1200

The main phenolic compounds in the PAE were detected as *O*-coumaric, ferulic and ellagic acids with % Area 4.299, 3.651 and 3.201, respectively, while apigenin 7-*O*-*β*-glucopyranoside and luteolin were the major flavonoids with % Area 10.871 and 8.347, respectively (Table 1).

3.3. *Biological investigation*

3.3.1. *Acute toxicity estimation of PAE*

PAE showed 50% mortality of rats and no adverse effects up to 6.250 g/kg. The median lethal dose (LD_{50}) of PAE was estimated according to the Lorke method [39].Therefore, a dose of 500 mg/kg bw of the PAE was selected as an average dosing schedule for the biochemical studies.

3.3.2. *Anti-hyperglycemic effect of PAE*

3.3.2.1. *Effect of PAE on BGL and BW*

The results of the acute effect of PAE (500 mg/kg bw, i.p.) on the blood glucose level (BGL) and body weight (BW) of diabetic rats at zero time, after 4 and 24 hrs are presented in Table (2). Drastic changes were observed upon alloxan injection (150 mg\kg bw, i.p.); the diabetic animals showed rapid swift in the BGL and decrease in BW. The observed changes in diabetic group were found to be reverted in animals treated with PAE. In the PAE fed rats, BGL were significantly attenuated after 4 hrs to 202 mg/dl and 24 hrs to 160.16 mg/dl at $P<0.05$ compared to hyperglycemic control; after 4 hrs (488.33 mg/dl) and 24 hrs (448.50 mg/dl). In addition, a restoration of BW was observed after 24 hours of PAE treatment (7.22% increase of BW) compared to normoglycemic goups (14.39%) and to a decrease in BW in the hyperglycemic control (-15.07%). The effects of PAE (500 mg/kg bw, i.p.) and Diamacron (5 mg\kg, p.o.) (9.97%) treatments on BGL and BW of diabetic rats after 24 hrs are nearly the same.

Table 2: Effect of PAE on BGL and BW of Alloxan-induced diabetic rats

Treatments	BGL (mg\dl)			BW (g)		
	0 hrs	4 hrs	24 hrs	0 hrs	24 hrs	% changes
Normoglycemic	97.8±4.49	94.60±8.16	95.00±6.78	128.33±3.15	147±2.23	14.39±2.09
Hyperglycemic	551.10±22.90$^{@}$	488.33±46.12$^{@}$	448.50±43.07$^{@}$	127.5±0.92	108.33±5.57*#	15.07±4.15
Standard control (Diamicron)	502±20.08$^{@}$	405.42±24.08$^{@}$	161.42±11.15*	126.33±3.62	138.925±1.7$^{@}$	9.97±3.70
PAE	436.50±33.29$^{@*}$	202.00±30.30$^{@*\#}$	160.16±23.2$^{@}$*	113.66±4.14	121.67±3.3	7.22±1.11

Values are expressed as mean ± SEM, n=10.Values are statistically significant at $P<0.05$, *Significant from saline normoglycemic control, $^{@}$ Significant from Alloxan hyperglycemic control, $^{\#}$ Significant from diamicron standard control

3.3.2.2. *Effect of PAE on the oxidative stress biomarkers (GSH, MDA and NO)*

The potential effect of PAE (500 mg/kg bw, i.p.) on the oxidative stress biomarkers (GSH, MDA and NO) in serum of alloxan-induced diabetic rats after 24 hrs were represented in Table (3).

Table 3: Effect of PAE on serum reduced glutathione content (GSH), melanodialdehyde level (MDA) and nitric oxide content (NO) of Alloxan-induced diabetic rats

Treatments	Parameters		
	GSH (mmol/l)	MDA (nmol/l)	NO (µmol/l)
Normal saline	2.44±0.02	70.35±1.05	2.47±0.17
PAE	2.39±0.04$^{@}$	48.16±2.52$^{@}$	1.71±0.12$^{@}$
Alloxan	1.01±0.12*	118.9±5.29*	4.69±0.48*

Values are expressed as mean ± SEM, n=10.Values are statistically significant at $P<0.05$, * Significant from saline normoglycemic control, $^{@}$ Significant from Alloxan hyperglycemic control

Our results revealed that diabetes inducing oxidative stress in rats caused significant increase in serum MDA, NO concentrations and decrease in GSH compared to normoglycemic control at ($P<0.05$) (Table 4). Regarding the effect of PAE treated diabetic group, the data reported a significant increase in serum concentration of GSH (2.39 mmol/l) and a significant decrease in MDA (48.16 nmol/l) and NO (1.71 µmol/l) contents compared to hyperglycemic control (1.01, 118.9 and 4.69, respectively) at $P<0.05$.

4. Discussion

In order to create new drugs, *in vivo* laboratory experiments are among the best strategies for studying the pathophysiology of syndromes. Investigation of PAE acute toxicity in rats revealed a large margin of safety.

Hyperglycemia is the primary symptom of diabetes and is held responsible for some complications because high glucose level directly injures cells and induces lipid peroxidation [40]. Alloxan-induced hyperglycemia animal model is characterized by rigorous increase of blood glucose level (BGL) and decrease of body weight (BW) attributed to various metabolic alteration arising from catabolism of fats and proteins, gluconeogenesis, loss of tissue and structural proteins and increased muscle wasting [41].
Alloxan induction resulted in an elevated BGL (hyperglycemia) and significant drop of BW. The i.p. administration of PAE was halting the sustained hyperglycemia and reverting back the body weight values to that of control animals. This anti-hyperglycemic property of *P. alata* could be ascertained to its antioxidant components. Natural antioxidants can counterbalance the oxidative stress, and cellular oxidation reaction under proper conditions [10]. Oxidative stress, leading to an increased production of ROS (reactive oxygen species), as well as lipid peroxidation in diabetes [42]. However, TBA (thiobarbituric acid) test measuring the malondialdehyde formation is still the reliable method for assay. GSH (an important part of the non-enzymatic antioxidant system) is a major non-protein thiol in living organisms, which plays a central role in co-ordinating the body's antioxidant defense processes. Moreover, nitrate and nitrite are indicators (markers) of endogenous nitric oxide (NO) production, possesses both antioxidant and pro-oxidant properties. In free radical-mediated LPOs (Lipid peroxides), NO reacts rapidly with peroxyl radicals as an effective chain-terminating (breaking) antioxidant. On administration of PAE extract to diabetic rats the serum MDA and NO levels have decreased and the GSH levels have increased. This indicates that PAE offers significant protection against oxidative stress. Moreover, MDA, NO and GSH levels in serum may thus be used as a parameter to assess oxidative stress in treated diabetics [43].

Conclusions

The leafy branches of *P. alata* are evaluated for the first time quantitatively and qualitatively for its total phenolic and flavonoid constituents. Twenty flavonoids were isolated and identified in addition to 18 phenolic compounds which were detected through HPLC analysis. Biological investigation of PAE (500 mg/kg bw i.p) in diabetic rats elucidated a consistent acute anti-hyperglycemic activity and a prominent antioxidative stress activity in serum. PAE effectively reversed the effect of alloxan- induced oxidative stress and changes in blood glucose levels as well as revealing an improvement of body weight, in diabetic rats after 24 hours of treatment. This study implies that PAE may be effective in controlling hyperglycemia and oxidative damage and this activity could be attributed to its phenolic and flavonoid contents. However, further deep *in vivo* studies are necessary to develop PAE as potential hypoglycemic nature products.

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