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Antihyperglycaemic and Antihyperlipidemic Activities of *Pleiogynium timorense* Seeds and Identification of Bioactive Compounds

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Abstract: The aim of this study is to evaluate antihyperglycaemi cand antihyperlipidemic activities of *Pleiogynium timorense* (DC.) Leenh (Anacardiaceae) seeds as well as to isolate and identify the bioactive compounds. Antihyperglycaemic effect was evaluated by measuring the effect of two dose levels (150 and 300 mg/kg) of 70% methanol extract of *Pleiogynium timorense* seeds on the blood glucose level. In addition, the effect of the plant extract on the lipid profile was determined by measuring serum total lipids (TL), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Furthermore, the bioactive compounds were isolated and identified by chromatographic and spectrometric methods. The results showed that the methanolic extract of the seeds significantly reduced the levels of blood glucose, (TL), (TC), (TG) and (LDL-C) but no significant effect on (HDL-C) by comparing with control group. Furthermore, four phenolic compound were isolated which were identified as; catechin, gallic acid, paramethoxybenzaldehyde and pyrogallol which were isolated for the first time from the seeds of the plant. In addition sulphur -containing compound (sulpholane) was isolated for the first time from the plant and from the family. To our knowledge, this is the first study about antihyperglycaemic and antihyperlipidemic activities of the seeds of *Pleiogynium timorense* and its bioactive compounds. So, the methanolic extract of the seeds of *Pleiogynium timorense* could be a step towards the development of new antihyperglycaemic and antihyperlipidemic drugs.

Keywords: Antihyperglycaemic, Bioactive Compounds, Phenolic, *Pleiogynium timorense*, Seeds

1. Introduction

Diabetes is one of the most common metabolic diseases in the world. It involves great disturbance in glucose and lipid metabolism and it causes many side effects [1, 2]. Herbal plants were used in medicine for treatment of many diseases to avoid the side effects of synthetic drugs [3]. Family Anacardiaceae is known as the sumac or cashew family, it containsabout82 genera and 850 species. The name Anacardiaceae is derived from the Genus *Anacardium*, which is meaning heart- like, due to the nut shape [4]. *Pleiogynium timorense* (DC.) Leenh. family (Anacardiaceae**),** is an evergreen tree and it is cultivated in Egypt as an ornamental plant, it is indigenous to tropical and subtropical areas [5, 6]. The genus *Pleiogynium* contains only one species with different scientific and common names, it is well known as Gambozia [7]. *Pleiogynium timorense* (DC.) Leenh. was reported to have various biological activities, the leaves showed antimicrobial, antioxidant, anti-inflammatory and hypoglycaemic activities [8, 9]. Cyanidin-3-glucoside was found to be one of the constituents of the fruits that showed antioxidant activity [10]. The pericarp and seeds of *Pleiogynium* showed anti-inflammatory, analgesic antioxidant, hepatic and nephro-protective activities, in

addition, quercetrin, quercetin, rutin and catechin were isolated from the pericarp [11]. From the seeds, α–amyrin and 5,24 (28)-cholestadien-24-methylen-3b-ol were isolated and identified [12]. The bark showed antiproliferative activity, in addition, three new bioactive trihydroxyalkylcyclohexenones were isolated and identified [13]. In our recent research, the constituents of the seeds and pericarp of the plant were identified by using HPLC–ESI-MS/MS [14]. Nothing was reported in the available literature concerning the antihyperglycaemic or antihyperlipidemic activities of *Pleiogynium timorense* seeds extract. Therefore, this study aims to evaluate antihyperglycaemic and antihyperlipidemic activities of *Pleiogynium timorense* (DC.) Leenh (Anacardiaceae) seeds as well as to isolate and identify the bioactive compounds. So, the methanolic extract of the seeds of *Pleiogynium timorense* could be a step towards the development of new antihyperglycaemic and antihyperlipidemic drugs.

2. Materials and Methods

2.1. General Experimental Procedures

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). ^{1}H-NMR and ^{13}C-NMR (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063– 0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F_{254} plates. Solvent mixtures; S1:BAW (n-butanol/acetic acid/ water 4:1:5, (v/v/v) upper phase, S2: (15% acetic acid), S3:(30:70 methanol: chloroform) and S4: hexane: ethyl acetate (8.5:1.5 v/v). Paper Chromatography (PC) Whatman No. 1 (Whatman Led. Maid Stone, Kent, England), sheets for qualitative detection of flavonoids and sugars.

2.2. Plant Identification and Collection

Fresh fruits of *Pleiogynium timorense* (DC.) Leenh. (Anacardiaceae) plant was collected from Zoo garden, Giza, Egypt in April 2013. The plant was identified by Dr Mohammed El-Gebaly, Department of Botany, National research centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the ministry of agriculture and director of Orman botanical garden, Giza, Egypt, and a voucher speciemen was kept in our laboratory in National Research Centre, possessing number 2001.

2.3. Animals

Sprague Dawley rats of both sexes (130-150 gm) were used throughout the experiments. Animals were housed under standard environmental conditions (23 ±1°C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues.

2.4. Experimental Design

2.4.1. Antihyperglycaemic

Rats were divided into 4 different groups (6 rats each); group 1 (control) received distilled water, group 2 (positive control) was administered glucose (1g/Kg) and groups 3&4 were administered *Pleiogyniumtimorense* seeds extract at two dose levels (150 – 300 mg /Kg) 45 minutes before glucose loading (1 g/kg). The drug solutions or vehicle were administered orally by gastric intubation by using syringe to assess the experiments. Blood samples were collected from *retro-orbitalVenus plexus* and analyzed at 0, 15, 30, 45 and 60 min after administration of glucose.

2.4.2. Antihyperlipidemic

Rats were divided into 4 different groups (6 rats each); group 1 was control group whichreceived normal diet, groups 2, 3 and 4 were allowed to feed on hyperlipidemic diet to induce hyperlipidemia through the feeding period. One of the last three groups continued feeding on hyperlipidemic diet without any supplementation to save as hyperlipidemic group (positive control) and the other two groups were allowed to feed on hyperlipidemic diet with two dose levels (150 – 300 mg /Kg) groups 3&4. Standard diet composition was described by [15] and hyperlipidemic diet was described by [16]. At the end of experimental period after six weeks, rats were anaesthetized with ether according to the method that was described by [17]. Blood samples were collected from orbital *Venus plexus* in nonheparinized tubes, centrifuged at 3000 rpm for 15 minutes, and blood sera were then stored at -20°C in a freezer before they were analyzed.

2.4.3. Analytical Determination

Serum glucose was estimated by using Trinder method [18]. Total lipid, total cholesterol and LDL-C were determined according to the methods of [19-21], respectively, serum HDL-C was determined according to the methods of [22]. Moreover, triglycerides were estimated enzymatically by [23].

2.4.4. Statistical Analysis

Data were analyzed using one-way ANOVA.

P-value <0.05 was considered statistically significant.

2.5. Extraction and Isolation

The air dried powder of *Pleiogynium timorense* seeds (1Kg.) was defatted with petroleum ether (60-80°C), percolated with methanol 70% till exhaustion, then the extract was evaporated under reduced pressure to yield 50 g dried extract. The fraction was subjected to column chromatography by using polyamide as adsorbent and elution was carried out with water; then the polarity was decreased by adding methanol. One hundred and fifty fractions of 50 ml conical flask were collected. The fractions that showed similar PC in S1 and S2 were combined.

Elution with water: methanol (50:50) afforded one

compound which gave pink color with vaniline sulphoric acid reagent. It was purified on Sephadex LH-20 column by using methanol as eluent to give (compound 1). Elution with water: methanol (30:70) afforded the presence of one compound as a violet band under short UV light. The compound was purified by Sephadex LH-20column and it was eluted with methanol to give (compound 2). Elution with water: methanol (20:80) afforded the presence of one compound as a dark band under short UV light. The compound was purified by Sephadex LH-20column eluted with methanol to give (compound 3). Elution with water: methanol (10:90) afforded the present of one compound as a violet band under short UV light. The compound was purified by Sephadex LH-20column and it was eluted with methanol to give (compound 4). Elution with water: methanol (5:95) afforded the presence of one compound as a yellow color under short UV light. The compound was purified on a Sephadex LH-20 column and it was eluted with methanol to give (compound 5).

3. Results and Discussion

3.1. Antihyperglycaemic and Antihyperlipidemic Activities

This is the first report concerning antihyperglycaemic and antihyperlipidemic activities of *Pleiogynium timorense* seeds extract. Previous report showed that the methanol extract of the leaves revealed a potent hypoglycemic activity [8]. The results showed that the serum glucose level was significantly increased ($p<0.05$) in positive control compared with that of normal control group, while the two dose levels of the seeds (150&300 mg/kg) showed a significant decrease ($p<0.05$) in the serum glucose level compared with that of positive control in dose-dependent manner (table 1). So, the seeds extract revealed a potent antihyperglycemic activity and it can be used as a protective drug against diabetes.

Table 1. *The effect of 70% methanol extract ofPleiogynium timorense (DC.) seeds extracts on blood glucose level.*

Groups	0–time	15 min	30 min	45 min	60 min
Control	2.53 ± 0.012	2.39 ± 0.023	2.62 ± 0.020	2.71 ± 0.010	2.43 ± 0.013
positive Control	2.77 ± 0.09	6.81 ± 0.071*	8.03 ± 0.031*	11.6 ± 0.027*	9.77 ± 0.018*
Seed Ext 150 mg/kg	2.48 ± 0.06	5.70 ± 0.045*#	5.61 ± 0.036*#	5.02 ± 0.041*#	5.16 ± 0.052*#
Seed Ext 300 mg/kg	2.57 ± 0.029	5.42 ± 0.021*#	5.90 ± 0.058*#	5.0 ± 0.038*#	4.96 ± 0.018*#

One - wayANOVA, P <0.05.
** Significantly different from control group.*
#Significantly different from positive control group.

Hyperlipidemia is one of the most common causes for health problems worldwide that leads to coronary heart diseases due to atherosclerosis [24]. The main cause of obesity is eating a high fat diet which leads to increasing the level of cholesterol that in turn leads to obesity [25]. The lipid profile was significantly changed ($p<0.05$) in high fat diet fed rats that indicated the hyperlipidemia, sothe serum lipid profile parameters (TL, TC, TG and LDL-C) were significantly increased ($P<0.05$) in the positive control group compared with those of negative control (table 2). Treatment with seeds extract at the two dose levels for six weeks showed a significant ($p<0.05$) decrease in total lipid, triglyceride and total cholesterol levels in hyperlipidemic rats compared with those of positive control. The high density lipoprotein cholesterol (HDL-C) is an effective lipoprotein that is produced in liver and intestine and plays an important role in the protection against pathogenesis of atherosclerosis [26]. The results showed that the level of HDL-Cwas significantly decreased ($p<0.05$) in hyperlipidemic rats compared with the control group but there was not any significant effect on high density lipoprotein cholesterol in the two dose levels of the seeds (150&300 mg/kg) compared with the positive control group. It was concluded thatthe existence of phenolic contents in methanolic extract of the seeds of *Pleiogynium timorense* could play an important role in these biological activities.

Table 2. *The effect of 70% methanol extract of Pleiogynium timorense (DC.) seeds extracts on lipid profile.*

Groups	TL (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	329.5±8.77	54.43±1.87	56.34±2.3	34.3±0.42	25.6±2.34
Positive control	342.2±3.32*	109.33±2.32*	94.67±1.23*	20.2±1.33*	56.3±6.24*
SE 150 mg/kg	276.1±25.34#	73.45±5.44#	62.74±4.32#	22.4± 3.32	39.3±6.55#
SE 300 mg/kg	239.1±21.34 #	83.32±3.56#	73.31± 3.33#	29.1±1.77	29.2±2.43 #

One - wayANOVA, P <0.05.
** Significantly different from control group*
#Significantly different from positive control group
TL: total lipid, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, SE: seed extract.

3.2. Structure Elucidation of the Isolated Compounds

Compound 1 was isolated as white crystals (15 mg), itgave pink color with vanilinesulphuric acid reagent.

^{1}H-NMR data of compound 1: δ4.00 (H-3, m), 4.56 [H-2, d, J (H-2, H-3a) 7.8 Hz], 2.90 [H-4e, dd, J (H-4e, H-3a) 5.50 Hz, J (H-4e, H-4a) 16.10 Hz], 2.54 [H-4a, dd, J (H-4a, H-3a) 8.50 Hz, J (H-4a, H-4e) 16.10 Hz], 6.01 [H-8, d, J (H-8, H-6) 2.3 Hz], 5.87 [H-6, d, J (H-6, H-8) 2.3 Hz], 6.73 [H-6′, dd, J (H-6′, H-2′) 1.94 Hz, J (H-6′, H-5′) 8.19 Hz], 6.89 [H-2′, d, J

(H-2′, H-6′) 1.95 Hz], 6.79 [H-5′, d, J (H-5′, H-6′) 8.07 Hz].

^{13}*C-NMR*data of compound 1: *δ* 28.7 (C-4), 69 (C-3), 83.1 (C-2), 95.7 (C-6), 96.5 (C-8), 101 (C-2′), 115.5 (C-5′), 116.3 (C-6′) and peaks at *δ* of 132.4, 144.6, 146.4, 157.1, 156.1, 157.8 and 158 for other aromatic carbons.

Compound 2 was isolated as white amorphous powder (10 mg), it gave a single spot with violet color under short UV light with $R_f$values of 0.67cm (S1) and 0.54cm (S2) on PC. It showed a positive color reaction with $FeCl_3$ similar to authentic gallic acid.

^{1}H-NMR data of compound2: *δ*: 7.08 (2H s, H-2, 6)

^{13}C-NMR data of compound 2:δ 169.16 (C=O), δ 144.98 for (C 3, 5), δ 138.15 (C-4), δ 120.74 (C-1) andδ 108.9 (C-2, 6).

Compound 3 was isolated as liquid with strong aroma, it gave a single spot with dark color under short UV light with $R_f$value of 0.25 in hexane: ethyl acetate (8.5:1.5 v/v).

^{1}H-NMR spectral data of compound 3in $CDCl_3$: 3.7 (s, 3H, Me), 6.9 (d, 2H, 3&5), 7.9 (d, 2H, 2&6) and 9.7 (s, 1H, CHO).

^{13}C NMR in $CDCl_3$ spectral data of compound 3:

δ 54.9 (Me), 113.2 (C3, C5), 127.8 (C2, C6), 131.78 (C1), 164.8 (C4) and 191.38 (CHO).

Compound 4 It was isolated as brown needles (20 mg), m.p. (131-134°C), it gave a single spot with violet color under short UV light, with $R_f$values of 0.82cm (BAW) and 0.4cm (15% HOAc) on PC. It showed positive color reaction with $FeCl_3$. EI-MS*m/z*: 126 (100, M+).

^{1}H-NMR spectral data of compound 4 (DMSO-*d6*): δ: 6.45 (t, H-5), 6.28 (d, *J* = 8.0, 2H, H-4 and 6,).

^{13}C-NMR spectral data of compound 4 (DMSO-*d6*):

δ: 107.8 (C-4, C-6), 119.2 (C-5), 133.8 (C-2), 147.05 (C-1, 3).

Compound 5 was isolated as yellow crystals (5 mg) it gave a single spot with yellow color under short UV light with *Rf*value = 0.6 in hexane: ethyl acetate (8.5: 1.5 v/v).

^{1}H-NMR spectral data of compound 5in CDCl3: 2.3 (m, 2H, 3 and 4) &δ 3.4 (t, 2H, 2 and 5)

^{13}C NMR in CDCl3 spectral data of compound 5: δ 22.7 (C 3 and C4) &δ 51.09 (C2, C5).

3.3. Identification of the Isolated Compounds

^{1}H-NMR spectrum of compound 1showed a doublet peak at δ 4.56ppm for H-2, a peak as multiple at δ 4.00 for H-3, a peak at δ 2.54 for H-4a, a peak at δ 2.90 for H-4e, a peak as doublet at δ 5.87 for H-6, with meta coupling with H-8, a peak at δ 6.01 for H-8 as doublet with meta coupling with H-6, peak at δ 6.89 for H-2′as doublet with meta coupling with H-6′, peak at δ 6.79 for H-5′ as doublet with ortho coupling with H-6′, peak at δ 6.73 for proton H-6′ as doublet of doublet with ortho coupling with H-5′ and meta coubling with H-2′. ^{13}C-NMRdata of compound 1showed a peak at *δ* 28.7 for carbon (C-4), peak at *δ* 69 for carbon (C-3), peak at δ 83.1for carbon (C-2), peak at δ 95.7 for carbon (C-6), peak at δ 96.5 for carbon (C-8), peak at δ 101 for carbon (C-2′), peak at δ 115.5 for carbon (C-5′), peak at *δ* 116.3 for carbon (C-6′).

Based on these data and by comparing with authentic sample and with the published data [27], compound 1was identified as catechin. This is the first report for its isolation from the seeds of the plant.

Compound 2:^{1}H-NMR spectrum showed a sharp singlet at *δ* 7.08 for the two protons H-2, 6.^{13}C-NMR spectrum of compound 2 showed that carbon of carbonyl of carboxlic group at *δ* 169.16, *δ* 144.98 for C-3, 5, *δ* 138.15 for C-4, *δ*120.74 for C-1, and *δ*108.96 for C-2, 6. In addition, the comparison with an authentic sample, confirmed the identification of the isolated compound 2 as gallic acid which was previously isolated from the leaves of the plant [9], it is the first time for its isolation from the seeds of the plant.

Compound 3:^{1}H-NMR spectrum showed a singlet peak atδ3.7 for three protons of methyl, peak at δ 6.9 as doublet for two protons H3 & H5, peak at 7.9 as doublet for two protons H2 and H6, singlet peak at 9.7 for CHO.

^{13}C NMR in CDCl3 spectrum showed a peak at δ 54.9 for methyl, peak at 113.2 for C3, C5, peak at 127.8 for C2, C6, peak at 131.78 for C1, peak at 164.8 for C4, peak at 191.38 for C=O of aldehydic group.

These spectral data were inaccordance with those which were reported for paramethoxybenzaldehyde [28]. Thus, compound 3was identified as paramethoxybenzaldehyde. It is the first time for its isolation from the seeds of the plant.

Compound 4:^{1}H-NMR spectrum showed a peak at δ: 6.45 as triplet for H-5, peak at 6.28 as doublet for two protons H-4, 6, 2H. ^{13}C-NMR spectrum showed a peak at δ: 107.8 for C-4, C-6, peak at 119.2 for C-5, peak at 133.8 for C-2, peak at 147.05 for C-1, 3. Based on these data and by comparing with the published data [29], compound 4was identified as pyrogallol. It is the first time for its isolation from the seeds of the plant.

Compound 5: ^{1}H-NMR spectrum showed a peak at δ: 2.3 ppm as mutiple for H-3 and H-4, a peak at δ 3.4ppm as triplet for two protons H-2 and H-5. These protons were shifted due to the presence of electron withdrawing group as sulphone group.

Based on these data and compared with the published data [30], compound 5was identified as tetrahydrothiophene 1,1-dioxide (Sulfolane). It is the first time for its isolation from the plantand from the family. It is used as organic solvent, but it was isolated for the first time as natural product from marine organisms [30], and in our work it is the first isolation from plant origin.

The structures of isolated compounds were illustrated in (Figure 1).

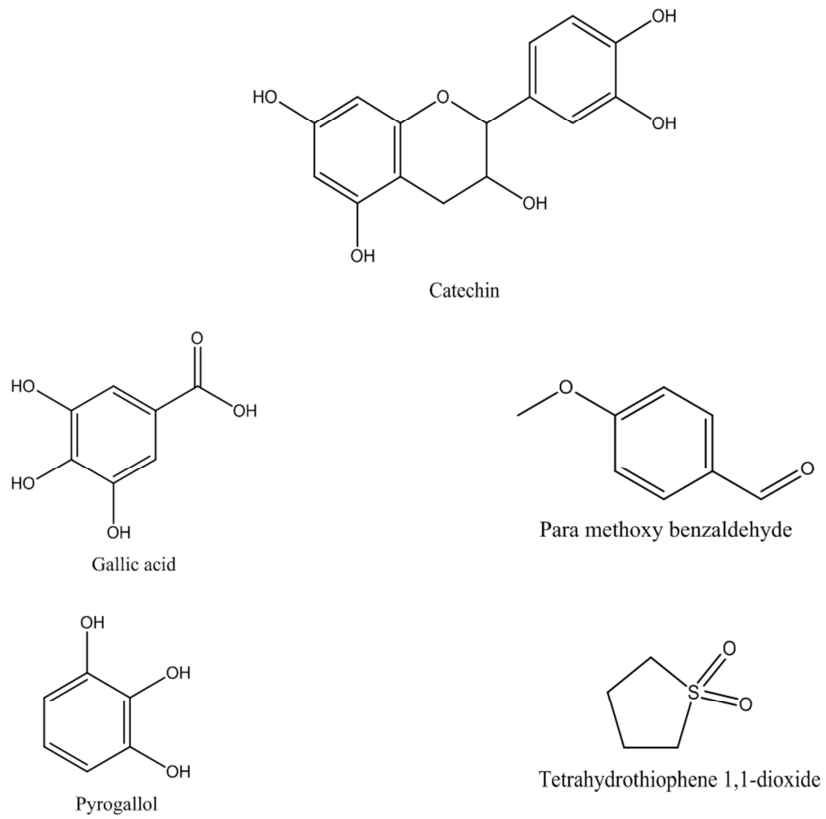

Figure 1. *Chemical structures of the compounds isolated from 70% methanol extract of Pleiogynium timorense seeds.*

4. Conclusion

To our knowledge, this is the first study about antihyperglycaemic and antihyperlipidemic activities of the seeds of *Pleiogynium timorense* and its bioactive compounds. So, the study aims to use the methanol extract of the seeds of *Pleiogynium timorense* as a step towards the development of new antihyperglycaemic and antihyperlipidemic drugs.

Conflicts of Interest

The authors declare that they don't have any conflict of interest.

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