

Phenolic contents and bioactivities of pericarp and seeds of *Pleiogynium solandri* (Benth.) Engl. (Anacardiaceae)

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ABSTRACT

Objective(s): This study aimed to develop drugs from natural sources to overcome the side effects of many of synthetic drugs. Methanol extracts of both pericarp and seeds of *Pleiogynium solandri* were used to investigate antioxidant, hepatoprotective, and renal function protective, analgesic, and anti-inflammatory effects and to determine the chemical composition of the extract responsible for bioactivity.

Materials and Methods: Methanol (70%) extracts of the seeds and pericarps of *P. solandri* were prepared. Hot plate method was used to test analgesic activity, carrageenan-induced paw inflammation method was used to test anti-inflammatory activity, and colorimetric methods were used to test antioxidant, hepatoprotective (by determination of serum alanine and aspartate aminotransferase activities), and renal function protective effects (by measuring uric acid and creatinine levels). Chromatographic methods and means of ¹H-NMR, ¹³C-NMR, and UV spectra were used for isolation and identification of the responsible compounds.

Results: In this study for the first time, four phenolic compounds were isolated from the pericarp of *P. solandri* which were identified as catechin, quercetin, quercitrin and rutin. Methanolic extract of both seeds and pericarp of *P. solandri* showed strong antioxidant effect, hepatoprotective, renal function protective, analgesic, and anti-inflammatory effects. However, seed extract had lower effect than pericarp in a dose dependent manner.

Conclusion: This study showed that methanol extract of pericarp of *P. solandri* is more powerful than that of the seed regarding its antioxidant, hepato-protective; renal function protective, analgesic, and anti-inflammatory effects. The phenolic compounds isolated from the methanol extract of pericarp were responsible for bioactivity.

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Introduction

Nowadays, the development of drugs from natural sources is recommended to overcome the side effects of many of the synthetic drugs. Recent research on medicinal plants has generated a great deal of information about the biologically active chemical components that are responsible for the claimed medicinal effects.

Pleiogynium solandri (Benth.) Engl. (Anacardiaceae), is an evergreen tree indigenous to tropical and subtropical regions; it is known in Arabic as Gambozia and is cultivated in Egypt as an ornamental plant, which has many synonyms as *Pleiogynium timorense* and *Pleiogynium cerasiferum* (1, 2). Plant family of Anacardiaceae contains plants with edible fruits and seeds (3) e.g. mango, red mombin, and other species of Spondias genus. *P. solandri* is among plants which have edible fruits used in preparation of jellies, jams, and

preserves (4). Previous phytochemical studies that resulted in the isolation of quercetin, myricetin, rutin, quercitrin, hyperin, lupeol, β -sitosterol from the leaves, and aqueous and alcoholic extracts of the leaves showed good antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* (5). The ethanolic extract of the leaves showed significant hypoglycemic, antioxidant and anti-inflammatory properties, additionally, twelve phenolic compounds were isolated from the plants leaves including kaempferol, gallic acid, kaempferol-3-O- β -D-galactopyranoside, kaempferol-3-O- β -D-glucopyranoside, quercetin 3-O- β -D-galactopyranoside, quercetin 3-O- β -D-glucopyranoside, kaempferol 3-O- β -D-6"-methylglucuronopyranoside, kaempferol 3-O- β -D-glucuronopyranoside, myricetin-3-O- α -L-rhamnopyranoside, 3,5-di-O-galloylquinic acid, 1,4,6-tri-O-galloyl- β -D-glucopyranose, and 1,3,4,6-tetra-O-

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galloyl- β -D-glucopyranose (6). The fruits have been reported to have antioxidant activity, additionally; cyaniding 3-glucoside was isolated from the fruits (7). Nothing could be traced in the available literature that deals with the chemistry of seeds and other constituents of fruits of this plant. The present study was undertaken to investigate the use of methanol extracts of both fruits and seeds of *P. solandri* as antioxidant, hepatoprotective, renal function protective, analgesic and anti-inflammatory, and to determine the chemical composition of the extract responsible for bioactivity with the aim of developing a natural drug.

Materials and Methods

Plant

Fruits of *P. solandri* plant were collected from Zoo garden, Giza, Egypt in April 2010. The plant was identified by Dr Kamal El Batanony, professor of Taxonomy and Botany, Faculty of Science, Cairo University. Methanol (70%) extracts of the seeds and pericarp of *P. solandri* were used.

Animal

Sprague Dawley rats of both sexes weighing 130-150 g were used throughout the experiments. Animals were housed under standard environmental conditions ($23 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, 12:12 hr light-dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. Animal care and the experimental protocols were approved by Institutional Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain.

Acute toxicity study

The LD₅₀ of methanol (70%) extracts of the *P. solandri* (seeds and pericarp) was determined using rats. Male and female albino rats (10 rats in each group) were orally administered the tested extracts at doses ranging from 1.0 to 5.0 g/kg. LD₅₀ was calculated using Karber method (8). Two dose levels of *P. solandri* were chosen (150 and 300 mg/kg) to determine the most effective dose at exerting physiological activity. In a preliminary study, the tested doses of 50 and 75 mg/kg induced insignificant changes in case group compared to control.

Antioxidant, hepatoprotective, and renal- function protective effects

Rats were assigned into two main groups: normal and damaged liver groups that administrated CCl₄ (carbon tetra chloride). Each group classified into 6 different groups (6 rats in each): group 1: was control received distilled water, group 2 was administered silymarin (50 mg/kg), and groups 3-6 were administered *P. solandri* tested extracts at two

dose levels for each extract (150 and 300 mg/kg) for 15 days. The drug solutions or vehicle were administered orally by gastric intubation using syringe to assess the experiments. At the end of experimental period, rats were anaesthetized with ether according to the method described by Cocchetto and Bjornsson (9). Blood samples were collected from orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 15 min, and blood sera were collected and stored at -20°C before they were analyzed.

Determination of serum total antioxidant capacity (TAC) level

Serum total antioxidant capacity (TAC) level was assessed colorimetrically using a test reagent kit according to the method described by Koracevic *et al* (10).

Principle

Determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H₂O₂); antioxidants in the sample eliminate a certain amount of the provided H₂O₂. The residual H₂O₂ is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro-2-hydroxybenzene sulfonate to a colored product. The absorbance of blank (Ab) and sample (As) was read at 505 nm against distilled water.

Determination of serum alanine aminotransferase (ALT) activity

Serum alanine aminotransferase (ALT) activity was assessed colorimetrically using a test reagent kit according to the method described by Reitman and Frankel (11).

Principle

The method depends on the reaction between L-alanine and α -keoglutaric acid in the presence of ALT to form pyruvic acid and L-glutamic acid. The pyruvate formed was measured in its derivative form (2,4-dinitrophenylhydrazone) colorimetrically at 505 nm.

Determination of serum aspartate aminotransferase (AST) activity

Serum AST activity was assessed colorimetrically using a test reagent kit according to the method described by Reitman and Frankel (11).

Principle

The method depends on the reaction between L-aspartic acid and α -ketoglutaric acid in the presence of AST to form oxaloacetate, which in turn reacts with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone, which can be detected colorimetrically at 505 nm.

Uric acid assay

Serum uric acid is the end product of purine metabolism, and is cleared through the kidney by glomerular filtration.

Kit provides a convenient means for detecting uric acid in biological samples such as serum and urine. Pretreatment of samples are not required. Uric acid level can be measured using colorimetric method (at 570 nm) according to the method of Barham and Trinder (12).

Creatinine assay (Bartles et al, 1972)

Creatinine is a breakdown product of creatine phosphate. Creatinine is produced and excreted at a constant rate, and blood creatinine is used to determine glomerular filtration rate.

Creatinine is measured in biological fluids (serum and urine) according to the method of Bartles and Bohmer (13). In the assay, creatinine is converted to creatine by creatininase, it is then converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color ($\lambda_{\max} = 570 \text{ nm}$).

Determination of analgesic activity

The hot plate-induced thermal pain method used to test analgesic activity according to the method of Roszkowski et al (14); the temperature is controlled for $53^{\circ}\text{C} (\pm 0.1^{\circ}\text{C})$. The reaction time was considered as the time elapsed between placing of the mouse on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, forepaw or jumping in an attempt to escape from the pain. The mice showing initial reaction time of 10 sec or less were selected for this study. Increase in reaction time in drug-treated groups was compared with that of the control group. The mean reaction time was calculated 30 min post-drug administration during 4 hr with a cut-off time of 30 sec. Animals were grouped into the following groups:

1. Group 1: the control group given distilled water (10 ml/kg b.wt).
2. Group 2: administered 50 mg/kg paracetamol.
3. Groups 3 and 4: administered 150 and 300 mg/kg of *P. solandri*, seeds extract respectively.
4. Groups 5 and 6: administered 150 and 300 mg/kg *P. solandri* pericarp extract, respectively.

Determination of anti-inflammatory activity

The carrageenan-induced rat paw edema method was employed to test anti-inflammatory activity according to the method of Winter et al (15) using plethysmometer system. Plethysmometer is a volume meter and the standard instrument for measurement of rodent paw volume (16). This is a test to screen potential anti-inflammatory or anti-edema agents. The paw measured was inserted into water in a clear acrylic cell, up to the wrist joint.

After 30 min of drugs administration till the end of experimental duration (4 hr), the volume of water displaced was measured by a transducer. Six groups of rats (six rats in each) were treated as previously mentioned in analgesic experimental design as follows:

1. Group 1: is the control group given distilled water (10 ml/kg b.wt).
2. Group 2: administered 5 mg/kg indomethacin.
3. Groups 3 and 4: administered 150 and 300 mg/kg from seeds extract of *P. solandri*, respectively.
4. Groups 5 and 6: were administered 150 and 300 mg/kg of pericarp extract of *P. solandri*, respectively.

Statistical analysis

Data were analyzed using one-way ANOVA. *P*-value < 0.05 was considered statistically significant.

UV-visible spectrophotometer

Beckman DU7 and Shimadzu UV 240 (PIN 204-5800) were used for recording UV spectra and measuring the absorbance in UV and visible range.

NMR Spectrometers

Varian Unity Inova 400 (297) 5 mm tubes, Austria, were appropriate.

Extraction and isolation

The air dried powder of *P. solandri* pericarp (2kg) was defatted with petroleum ether ($60-80^{\circ}\text{C}$) (3l), percolated with methanol 70% till exhaustion, then the extract was evaporated under reduced pressure to yield 150 g dried extract.

The fraction was subjected to column chromatography using polyamide as adsorbent and elution was carried out with water; then decreasing the polarity by adding methanol. Fractions (50 ml each) were collected and separately concentrated to a small volume. All fractions were screened by PC (Whatman No. 1) using n-butanol: acetic acid: water (4:1:5,v/v/v) and acetic acid: water (15:85,v/v) as solvent systems; similar fractions were pooled and the solvents were separately evaporated under reduced pressure.

Elution with water: methanol (90:10) afforded the presence of flavonoids. The fraction was applied to subcolumn on Sephadex LH-20; methanol as eluent was used to collect fractions (50 ml each) and separately concentrate to small volume. All fractions were screened by PC (Whatman No. 1) using n-butanol: acetic acid: water (4:1:5,v/v/v) and acetic acid: water (15:85,v/v) as solvent systems; similar fraction were pooled and the solvents were separately, evaporated under reduced pressure. Fractions 5-7 afforded one compound which gave dark purple color under UV light changing to yellow on exposure to ammonia vapor and yellow color with AlCl_3 . It was purified on Sephadex LH-20 column using methanol as eluent to yield compound 1.

Table 1. (A-on normal rats). Effect of *Pleiogynium solandri* (Benth.) methanol water (70%) extracts of seed and pericarp on total antioxidant capacity, liver enzymes marker, and kidney function in serum of normal rats

Groups	TAC (mmol/l)	ALT (IU/l)	AST (IU/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	1.24 ± 0.023	26.3 ± 1.43	42 ± 1.08	0.43 ± 0.011	1.27 ± 0.012
Silymarin 50	1.56 ± 0.025*	21.4 ± 1.66*	36 ± 2.13*	0.38 ± 0.02*	1.25 ± 0.07
Seed Ext 150	1.31 ± 0.019*#	25.6 ± 0.94#	40.43 ± 2.3#	0.41 ± 0.013#	1.23 ± 0.015
Seed Ext 300	1.38 ± 0.020*#	23.7 ± 0.54*#	38.35 ± 2.81*#	0.44 ± 0.032#	1.18 ± 0.03*#
Fruit Ext. 150	1.44 ± 0.031*#	22.3 ± 1.21*	37.5 ± 1.18*	0.31 ± 0.021*#	1.20 ± 0.05
Fruit Ext. 300	1.49 ± 0.022*#	20.1 ± 1.10*	34.8 ± 2.04*#	0.34 ± 0.019*#	1.16 ± 0.012*#

One-way ANOVA, significant at P -value ≤ 0.05 ; * Significantly different from control group; # Compared to silymarin treatment group; TAC: total antioxidant capacity; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CCL₄: carbon tetra chloride

Elution with water: methanol (70:30) afforded the presence of flavonoids. The fraction was applied to subcolumn on Sephadex LH-20 using methanol as eluent. Fractions (50 ml each) were collected and separately concentrated to a small volume. All fractions were screened by PC (Whatmann No. 1) using *n*-butanol-acetic acid-water (4:1:5,v/v/v) and acetic acid:water(15:85,v/v) as solvent systems; where similar fraction were pooled and the solvents were, separately, evaporated under reduced pressure.

(Fractions 2-6) afforded one compound which gave dark purple color under UV light changing to yellow on exposure to ammonia vapor and yellow color with AlCl₃. It was purified on Sephadex LH-20 column using methanol as eluent to give compound 2.

Elution with water: methanol (40:60) afforded the presence of flavonoids. The fraction was applied to subcolumn on Sephadex LH-20; using methanol as eluent. Fractions (50 ml each) were collected and separately concentrated to a small volume. All fractions were screened by PC (Whatmann No. 1) using *n*-butanol-acetic acid-water (4:1:5,v/v/v) and acetic acid: water(15:85,v/v) as solvent systems; where similar fraction were pooled and the solvents were, separately, evaporated under reduced pressure.

(Fractions 4-8) afforded one compound which gave yellow color under UV light, on exposure to ammonia vapor and with AlCl₃. It was purified on Sephadex LH-20 column using methanol as eluent to give compound 3.

Elution with water: methanol (30:70) afforded one compound which gave pink color with vanillin sulfuric acid reagent. It was purified on Sephadex LH-20 column using methanol as eluent to give compound 4.

Results

LD₅₀ determination revealed that the investigated extracts were nontoxic up to 5g/kg which was the maximum soluble dose and also it is the maximum dose can be given according to the typical protocol for an acute study with rats (16).

TAC showed significant increase in all treatment groups with two studied extracts compared with the control values. The two dose levels of the seeds and pericarp extracts resulted in significant reduction of TAC value when compared with silymarin treated group. Liver enzymes (ALT and AST) significantly decreased in all treatment groups except in group with lower dose of seed extract that did not differ with control value. As well as, ALT and AST significantly decreased in groups treated with silymarin while compared with seed extract (at both doses) (Table 1). Creatinine serum concentration was decreased significantly in groups treated with the two doses of pericarp extract comparing with the control or silymarin groups while creatinine value did not changed in groups treated with seed extract. Uric acid level was not significantly changed compared with control in groups treated with seed extract (150 mg/kg) and silymarin, while in other treated groups uric acid values significantly decreased in comparison to control and silymarin treatment groups (Table 1).

Table 2 demonstrates that CCL₄ treatment caused significant depletion in TAC serum content. The lower dose of seed extract (150 mg/kg) increased TAC level in CCL₄ hepatic damaged rats compared with CCL₄-treatment alone; while the higher dose of seed extract had similar effect on TAC with that of silymarin-treatment group. However, pericarp

Table 2. (B-On injured rat). Hepatoprotective effect of *Pleiogynium solandri* (Benth.) methanol water (70%) extracts of seed and pericarp on total antioxidant capacity, liver enzymes marker, and kidney function in serum of carbon tetrachloride-induced hepatic damage rats

Groups	TAC (mmol/l)	ALT (IU/l)	AST (IU/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
CCL ₄	0.89 ± 0.014	46.5 ± 1.12	88.2 ± 2.3	1.32 ± 0.07	2.43 ± 0.04
Silymarin 50+CCL ₄	1.21 ± 0.018*	25.8 ± 1.33*	67.3 ± 0.94*	0.76 ± 0.06*	1.72 ± 0.05*
Seed Ext 150+CCL ₄	0.95 ± 0.024*#	36.6 ± 1.09*#	74.8 ± 1.12*#	0.89 ± 0.04*#	2.1 ± 0.03#
Seed Ext 300+ CCL ₄	1.20 ± 0.017*	32.1 ± 1.34*#	70.2 ± 2.00*#	0.85 ± 0.05*#	1.97 ± 0.05*
Fruit Ext. 150+CCL ₄	1.31 ± 0.012*#	37.2 ± 1.21*#	65.2 ± 1.62*	0.74 ± 0.03	1.67 ± 0.05*
Fruit Ext. 300+CCL ₄	1.39 ± 0.022*#	33.6 ± 0.87*#	61.6 ± 1.33*#	0.78 ± 0.04	1.60 ± 0.06*

One-way ANOVA test, significant at P -value ≤ 0.05 ; * compared to CCL₄- treatment group; # compared to Silymarin treatment group; TAC: total antioxidant capacity; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CCL₄: carbon tetra chloride

Table 3. Analgesic effect of *Pleio gynium solandri* (Benth.) methanol water (70%) extracts of seed and pericarp compared to control and paracetamol- treated groups on hot plate- induced pain in rats

Groups	30 min	60 min	120 min	180 min	240 min
Control	5.81 ± 0.22	5.62 ± 0.33	5.53 ± 0.17	5.48 ± 0.14	5.40 ± 0.34
Paracetamol 50 mg/kg	11.52 ± 0.25*	10.22 ± 0.45*	8.42 ± 0.66*	7.81 ± 0.24*	7.44 ± 0.21*
Seed Ext 150 mg/kg	5.92 ± 0.32#	6.42 ± 0.54#	7.16 ± 0.22*#	7.62 ± 0.30*	7.55 ± 0.43*
Seed Ext 300 mg/kg	6.73 ± 0.33*#	7.50 ± 0.24*#	7.80 ± 0.36*#	8.21 ± 0.44*	7.78 ± 0.21*
Fruit Ext. 150 mg/kg	6.55 ± 0.27#	7.81 ± 0.23*#	8.11 ± 0.41*	7.86 ± 0.54*	7.31 ± 0.18*
Fruit Ext. 300 mg/kg	7.35 ± 0.22*#	7.93 ± 0.34*	8.45 ± 0.50*	8.90 ± 0.29*#	8.15 ± 0.31*

One-way ANOVA, P -value ≤ 0.05 ; * Significantly different from control group; # Significantly different from paracetamol group

extract showed significant increase in TAC value more than silymarin effect in a dose dependent manner in hepatotoxic groups. Liver enzymes values significantly elevated in CCL₄-toxicated rats than the other CCL₄ toxicated treated groups. All tested extracts significantly inhibited the elevation of ALT and AST caused by CCL₄ in a dose dependent manner. Serum creatinine and uric acid significantly increased by CCL₄ intoxication. Pericarp extract was significantly different from silymarin treatment group in decreasing creatinine value. Although seed extract treatment significantly reduced creatinine value, it was still lower than the reduction due to silymarin treatment (Table 2).

CCL₄-treatment group showed significant elevation in uric acid, however silymarin pretreating significantly decreased uric acid elevation caused by CCL₄. The lower dose of seed extract did not change the uric acid value when compared with CCL₄ alone. The higher dose of seed extract and the two dose levels of pericarp extract reduced uric acid value in a dose dependent manner compared with CCL₄ or silymarin. Both seed and pericarp extracts at their two dose levels showed significant increase in the reaction time compared with the control group (Table 3).

Table 4 shows the effect of different tested materials on rat paw edema versus indomethacine (stander drug). Pericarp extract exerted its powerful anti-inflammatory effect after 120 and 180 min of treatment with 300 and 150 mg/kg doses, respectively. However, seed extract had lower anti-inflammatory effect in a dose dependent manner.

Isolated compounds

Compound 1 was isolated as yellow powder, 15 mg, single spot; R_f values = 32 and 44 in solvent

systems: *n*-butanol:acetic acid:water (4:1:5 v/v/v) and 15% acetic acid, respectively. It gave dark purple color under UV changing to yellow on exposure to ammonia vapor and yellow color with AlCl₃.

UV spectral data of compound 1

λ_{\max} nm (MeOH): 258, 359 flavonol, (NaOMe): 272, 410 (free OH at 4'), (AlCl₃): 275, 433 (free OH on ring A and B), (AlCl₃/HCl): 271, 400 (free OH at 5 and ortho OH at ring B), (NaOAc): 271, 393 (free OH at 7), (NaOAc/H₃BO₃): 262, 387 (ortho OH at ring B).

¹H-NMR spectrum (DMSO-d₆)

7.5 (1 H, d, J = 2.1 Hz, H-2'), 7.49 (1 H, dd, J = 2.1, 8.4 Hz, H-6'), 6.7 (1 H, d, J = 8.4 Hz, 5'), 6.4 (1 H, d, J = 2.1 H-8), 6.2 (1 H, d, J = 2.1 H-6), 5.2 (1 H, d, J = 7.2 Hz, H-1''), 4.3 (1H, d, J = 1.5 Hz, H-1'''), 1.1 (3 H, d, J = 6.6 Hz, Me).

Thus compound 1 is identified as quercetin 3-O- α -rhamnosyl (1''' \rightarrow 6'')- β -glucoside (Rutin)

Compound 2 was isolated as yellow amorphous powder (4 mg) with R_f values 0.58 (BAW) and 0.66 (15% HOAc) on PC. It appeared as deep purple spot under UV light changed to yellow with ammonia vapor indicated that compound 2 is flavonol with free 5- and 4'-OH. Complete acid hydrolysis yielded quercetin as an aglycone and rhamnose as the sugar moiety in which both of them were co-chromatographed with authentic samples.

UV spectral data of compound 2

λ_{\max} nm (MeOH): 256, 350 (flavonol), (NaOMe): 270, 393 (free OH at 4'), (AlCl₃): 276, 430 (free OH on ring A and B), (AlCl₃/HCl): 272, 401 (free OH at 5 and ortho OH at ring B), (NaOAc): 272, 372 (free OH at 7), (NaOAc/H₃BO₃): 260, 367 (ortho OH at ring B).

Table 4. Anti-inflammatory effect of *Pleio gynium solandri* (Benth.) methanol water (70%) extracts of seed and pericarp on rat paw edema, compared to control and indomethacine- treated groups

Groups	30 min	60 min	120 min	180 min	240 min
Control	0.381±0.013	0.378 ±0.010	0.370±0.009	0.373±0.004	0.365±0.008
Indomethacine 5 mg/kg	0.192±0.006*	0.182±0.004*	0.180±0.008*	0.272±0.011*	0.310±0.005*#
Seed Ext 150 mg/kg	0.241±0.014*#	0.230±0.016*#	0.226±0.003*#	0.219±0.11*#	0.221±0.007*#
Seed Ext 300 mg/kg	0.220±0.005*#	0.216±0.006*#	0.205±0.008*	0.198±0.001*#	0.208±0.006*#
Fruit Ext. 150 mg/kg	0.231±0.007*#	0.211±0.012*#	0.200±0.014*#	0.196±0.005*#	0.188±0.007*#
Fruit Ext. 300 mg/kg	0.202±0.007*	0.194±0.003*#	0.186±0.011*	0.176±0.004*#	0.170±0.006*#

One-way ANOVA, P -value ≤ 0.05 ; * Significantly different from control group; # Significantly different from indomethacine group

¹H-NMR spectrum (DMSO-d₆)

7.26 (2H, m, H-2' and H-6'), 6.83 (1H, d, J = 9 Hz, 5'), 6.4 (1H, d, J = 2.5, H-8), 6.14 (1H, d, J = 2.5, H-6), 5.2 (1H, d, J = 2 Hz, H-1''), 0.78 (3H, d, J = 6 Hz, Me).

Thus compound 2 is identified as quercetin 3-O- α -rhamnoside.

Compound 3 was isolated as yellow powder (5 mg) with R_f values 0.91 (BAW) and 0.12 (15% HOAc) on PC and these values were within the range of flavonoid aglycone. It gave yellow spot under UV light changed to yellow fluorescence with ammonia vapor indicated that compound P₃ is flavonol with free 3- and 5-OH groups.

UV spectral data of compound 3

λ_{\max} nm (MeOH): 260, 368 (flavonol), (NaOMe): 272, 406 (free OH at 4'), (AlCl₃): 272, 446 (free OH on ring A and B), (AlCl₃/HCl): 266, 430 (free OH at 3,5, and ortho OH at ring B), (NaOAc): 272, 404 (free OH at 7'), (NaOAc/H₃BO₃): 260, 384 (ortho OH at ring B)

¹H-NMR spectrum (MeOD)

7.74 (1 H, d, J = 2 Hz, H-2'), 7.55 (1 H, d, J = 2.1, 8.4 Hz, H-6'), 6.92 (1H, d, J=Hz, 5'), 6.4 (1 H, d, J = 2, H-8), 6.15 (1 H, d, J = 2, H-6).

Thus compound 3 is identified as quercetin.

Compound 4 isolated as white crystals (15 mg) with R_f 0.6 (in 30% methanol:chloroform) give pink color with vanillin sulphuric acid reagent.

¹H-NMR spectral data of compound 4

δ 4.56 [H-2, d, J(H-2, H-3a) 7.8 Hz], 4.00 (H-3, m), 2.54 [H-4a, dd, J(H-4a, H-3a) 8.50 Hz, J(H-4a, H-4e) 16.10 Hz], 2.90 [H-4e, dd, J(H-4e, H-3a) 5.50 Hz, J(H-4e, H-4a) 16.10 Hz], 5.87 [H-6, d, J(H-6, H-8) 2.3 Hz], 6.01 [H-8, d, J(H-8, H-6) 2.3 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], 6.73 [H-6', dd, J(H-6', H-2') 1.94 Hz, J(H-6', H-5') 8.19 Hz], and 8.00 (phenolic protons, m).

¹³C-NMR spectral data of compound 4

Carbon atoms showed peaks at δ 27.7 (C-4), 66.3 (C-3), 80.9 (C-2), 93.9 (C-6), 95.1 (C-8), 114.5 (C-2), 115.1 (C-5), 18.4 (C-6). Other aromatic carbons showed peaks at δ of 99.1, 130.6, 144.6, 144.8, 155.3, 156.1, and 156.4.

Thus compound 4 is identified as catechin.

Discussion

Recently, the ethanolic extract of the leaves showed significant hypoglycemic, antioxidant and anti-inflammatory properties (6). The fruits have been reported to have antioxidant activity (7). Reviewing the current literature, nothing was traced concerning the biological activities of seeds and pericarp of this plant.

In this study, antioxidant, hepatoprotective, renal function protective, analgesic and anti-inflammatory

effects of both seeds and pericarp of *P. solandri* have been studied. The phenolic compounds isolated from the methanol extract of pericarp were responsible for bioactivity.

According to the results, the two dose levels of the seeds and pericarp extracts resulted significant reduction in TAC value when compared with silymarin treated group. All treatment groups had hepatoprotective effect in both normal and hepatotoxic groups except the lower dose of seed extract that did not differ than control value. The two doses of pericarp extract had a powerful renal function protective effects comparing with the control or silymarin groups while seeds extracts have lower effect than that of pericarp (Tables 1, 2).

Both seed and pericarp extracts with their two dose levels showed powerful analgesic effect by increasing the reaction time compared with the control group (Table 3). Table 4 showed the effect of different tested materials on rat paw edema versus indomethacin (stander drug). Pericarp extract exerted its powerful anti-inflammatory effect at two estimated doses. However, seed extract had lower anti-inflammatory effect in a dose dependent manner.

These biological activities are due to the chemical composition of the extract since catechin has been reported to have antioxidant activity (18-20).

Isolated compounds

UV spectral data of compound 1 in MeOH with few drops of NaOMe showed a bathochromic shift of 50 nm relative to band I in MeOH with increase in intensity; which indicated the presence of free 4'-OH. The bathochromic shift of 16 nm in band II in the presence of NaOAc indicated the presence of a free 7-OH. Bathochromic shift of 42 nm in band Ia in the presence of AlCl₃/HCl relative to band I in methanol indicated the presence of free 5-OH. The presence of orthodihydroxyl pattern at ring-B was confirmed due to 21 nm bathochromic shift in band I with NaOAc/H₃BO₃ relative to band I in MeOH.

¹H-NMR spectrum of compound 1 was in consistence with the presence of quercetin, glucose, and rhamnose. The spectrum showed a broad signal at δ 12.7 for 5-OH. The aromatic protons of B-ring appeared as multiplet at δ 7.55 assigned to H₂'-6' and doublet for H-5' at δ 6.85 with J= 8.5 Hz due to ortho coupling with H-6'. Two doublets of ring A at δ 6.35 and δ 6.15 with J= 2 Hz due to meta-coupling assigned to H-8 and H-6, respectively. The doublet signal of the anomeric protons appeared at δ 5.35 with J= 7.5 Hz indicating the β -configuration of glucose, and at δ 4.4 with J= 2 Hz indicating the α -configuration of rhamnose. The chemical shift values of the anomeric protons and methyl group of rhamnose moiety demonstrated interglycosidic linkage in compound 1 at C-1''' of rhamnose sugar (21). Complete acid hydrolysis yielded quercetin as a

glycone, glucose, and rhamnose as the sugar moieties in which both of them were co-chromatographed with authentic samples.

Thus compound 1 is identified as quercetin 3-O- α -rhamnosyl (1'' \rightarrow 6'')- β -glucoside (Rutin) previously isolated from *P. solandri* leaves (5).

UV spectral data of compound 2 in MeOH with few drops of NaOMe showed a bathochromic shift of 47 nm relative to band I in MeOH with the increase in intensity confirming the presence of free 4'-hydroxyl group. The bathochromic shift of 13 nm in band II in the presence of NaOAc suggests the presence of a free 7-OH group. Bathochromic shift of 47 nm in band Ia in the presence of AlCl₃/HCl relative to band I in methanol indicates the presence of free 5-OH group. The presence of orthodihydroxyl pattern at ring-B was confirmed by 27 nm bathochromic shift in band I with NaOAc/H₃BO₃ relative to band I in MeOH.

¹H-NMR spectrum of compound 2 showed the consistence of the presence of quercetin and rhamnose where the aromatic protons of the B-ring appeared as multiplet at δ 7.26 assigned to H-2' and H-6', and doublet for H-5' at δ 6.83 with J = 9 Hz due to ortho-coupling with H-6'. Two doublets of ring A at δ 6.49 and δ 6.14 with J = 2.5 Hz due to meta-coupling were assigned for H-8 and H-6, respectively. Doublet signal of the anomeric proton of the rhamnose moiety at δ 5.2 with J = 2 Hz indicated the α -configuration of rhamnose moiety and a doublet of methyl group of rhamnose at 0.78 with J = 6 Hz. Complete acid hydrolysis yielded quercetin as aglycone, and rhamnose as the sugar moiety in which both of them were co-chromatographed with authentic samples. Thus compound 2 is identified as quercetin 3-O- α -rhamnoside which was previously isolated from *P. solandri* leaves (5).

UV spectral data of compound 3 in MeOH with few drops of NaOMe showed a bathochromic shift of 39 nm relative to band I in MeOH with increase in intensity confirming the presence of free 4'-OH group. The bathochromic shift of 7 nm in band II in the presence of NaOAc confirms a free 7-OH group. Bathochromic shift of 57 nm of band Ia in the presence of AlCl₃/HCl relative to band I in methanol indicating the presence of free 5-OH group. The presence of ortho-dihydroxyl pattern at ring-B was confirmed from 16 nm bathochromic shift in band I with NaOAc/H₃BO₃ relative to band I in MeOH.

¹H-NMR spectrum of compound 3 showed a broad signal at δ 12.5 ppm for 5-OH. The aromatic protons of the B-ring appeared as doublet at δ 7.74 assigned to H-2' with J = 2 Hz due to meta-coupling with the proton H-6' at δ 7.55 and a doublet for H-5' at δ 6.92 with J = 8 Hz due to ortho-coupling with H-6'. The two doublets of ring A at δ 6.42 and δ 6.15 with J = 2 Hz due to meta-coupling were assigned to H-8 and H-6, respectively (21). Thus compound 3 is

identified as quercetin previously isolated from *P. solandri* leaves (5).

¹H-NMR spectrum of compound 4 showed peaks at δ 4.56 as doublet for one proton H-2, at δ 4.00 for one proton H-3 as multiple, at δ 2.54 for one proton H-4a, at δ 2.90 for one proton H-4e, at δ 5.87 as doublet for proton H-6, with meta-coupling with H-8, at δ 6.01 for proton H-8 as doublet with meta-coupling with H-6, at δ 6.89 for proton H-2' as doublet with meta-coupling with H-6', at δ 6.79 for proton H-5' as doublet with ortho-coupling with H-6', at δ 6.73 for proton H-6' as doublet of doublet with meta-coupling with H-2' and ortho-coupling with H-5' and 8.00 (phenolic protons, m).

¹³C-NMR spectrum of compound 4 showed peaks at δ 27.7 for carbon (C-4), at δ 66.3 for carbon (C-3), at δ 80.9 for carbon (C-2), at δ 93.9 for carbon (C-6), at δ 95.1 for carbon (C-8), at δ 114.5 for carbon (C-2), at δ 115.1 for carbon (C-5'), at δ 18.4 for carbon (C-6') and other aromatic carbons showed peaks at δ of 99.1, 130.6, 144.6, 144.8, 155.3, 156.1 and 156.4.

Thus compound 4 is identified as catechin, which was isolated, for the first time in this article from the plant.

Conclusion

This study showed that methanol extract of pericarp of *P. solandri* is more powerful than that the extract of the seed regarding its antioxidant, hepatoprotective, renal function protective, analgesic, and anti-inflammatory effects. The phenolic compounds isolated from the methanol extract of pericarp are responsible for its bioactivity.

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