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Phytochemical analysis and anti-diabetic, anti-inflammatory and antioxidant activities of *Loranthus acaciae* Zucc. Grown in Saudi Arabia

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ABSTRACT

The *Loranthus* genus has been demonstrated to be used in the treatment of wide range of diseases e.g. diabetes, inflammations and cancers. Many species of *Loranthus* represent a major source of biologically active constituents. Therefore, our study was carried out to investigate the anti-diabetic, anti-inflammatory and antioxidant effects of *Loranthus acaciae* Zucc. (Loranthaceae) grown in Saudi Arabia. Moreover, our research concerned the guided-fractionation and isolation of possible active compounds from this species. The crude ethanolic extract and its *n*-hexane, chloroform and *n*-butanol fractions were investigated for antidiabetic activity utilizing two methods namely, in alloxan-induced diabetic rats and glucose tolerance test in normal rats. Additionally, the anti-inflammatory activity was studied by the carrageenan-induced rat paw oedema method while DPPH free radical scavenging and *β*-carotene bleaching assays were utilized to determine the antioxidant activity. Various chromatographic and spectroscopic techniques were utilized for the isolation and characterization of the active compounds. Our results exhibited that the crude extract and chloroform fraction has the greatest hypoglycemic and antidiabetic effects. The chloroform fraction and crude extract produced at a dose of 500 mg/kg a significant hypoglycemic effect in diabetic rats with 47.0 and 33.6% reduction in blood sugar levels and in normoglycemic rats 35.6 and 35.4% respectively. A potent anti-inflammatory effect (67.2% at 500 mg/kg) was detected for the chloroform fraction. In addition, the chloroform fraction exhibited a high antioxidative and DPPH-radical inhibitory activity (85.4 and 88.3% respectively). The phytochemical analysis of *L. acaciae* led to the isolation and characterization of four compounds namely, quercetin 3-*O*-*β*-D-glucopyranoside (compound **1**), quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside (compound **2**), (-) catechin (compound **3**), and catechin 7-*O*-gallate (compound **4**). Among these compounds quercetin 3-*O*-*β*-D- glucopyranoside, quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside and catechin 7-*O*-gallate, are isolated for the first time from this plant.

1. Introduction

Loranthus acaciae Zucc. is a species of Loranthaceae family. Loranthaceae is one of the mistletoe families which has about 75 genera and approximately 1000 species of parasitic flowering trees or shrubs (Calvin and Wilson, 2006). The genus *Loranthus*, represents the largest genus in this family with around 500 species. The distribution of Loranthaceae family is ranging from tropical rain forests and mangroves to arid shrublands regions. The plants run in size from little herbs to trees up to 10 m high; the little species are generally woody parasites on tree limbs, while the bigger species are root parasites. The flowers are normally bisexual, and the fruits are quite often one-seeded berries. The berries are eaten by birds, which spread the seeds by putting them on trees (Sheila, 1985; Shaw et al., 2004). In Saudi Arabia and Yemen, Loranthaceae include four genera namely, *Phragmanthera, Oncocalyx, Tapinanthus* and *Plicosepalus*. Six species of these genera are distributed in the South, North and West and of Saudi Arabia (Waly et al., 2012). *Loranthus acaciae* Zucc., (syn: *Plicosepalus acaciae* (Zucc.) Wiens &

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Polhill) is a perennial green semiparasitic mistletoe that directly attaches to another plants e.g. *Acacia* trees via a haustorium (Vaknin et al., 1996). Leaves are mostly alternate, solitary or in clusters, oblong or oblanceolate up to 4 cm long. Flowers are 2–3, in solitary or clustered, pedunculated umbels. The corolla is dark red and often warted below (Vaknin et al., 1996).

Centuries ago, plants of *Loranthus* have been utilized in Chinese folk medicine to treat several ailments, including hypertension, cancer, obesity, gastrointestinal tract damage and rheumatoid disorders (Wang et al., 2008). Furthermore, previous investigations on *Loranthus* plants have demonstrated to possess antioxidant anti-inflammatory and analgesic activities (Liu et al., 2015). In addition, some *Loranthus* species indicated further important pharmacological effects e.g. antimicrobial and potential antitumor activities (Sadik et al., 2003). It is additionally detailed that few species of the genera *Plicosepalus* and *Phragmanthera* were demonstrated to prevent fatty acid synthesis with consequent decrease of body weight in animal (Wang et al., 2008). Other prior studies on *Loranthus* plants additionally indicated diverse pharmacological activities, for example, antihepatotoxic [9] (Yang et al., 1987), antidiabetic (Osadebe et al., 2004), antiviral (Lohézic-Le Dévéhat et al., 2002), antimicrobial (Daud et al., 2005) and cytotoxic activities (Kim et al., 2004). Al-Taweel et al., (2012) studied the hypoglycemic effect of *Plicosepalus curviflorus* and demonstrated that the alcoholic extract of the leaves of *P. curviflorus* possessed an interesting hypoglycemic effect. In addition, Mothana et al., (2012) explored the anti-inflammatory and antioxidative effects of *Loranthus regularis* and demonstrated that the ethyl acetate fraction of *L. regularis* has a great activities (Vaknin et al., 1996).

The information detailed in previously mentioned investigations justifies the conventional utilization of *L. regularis* and *P. curviflorus* in treating of diabetes and inflammations in Saudi Arabia and Yemen (Mossa, 1985; Elshanawani, 1996; Al-Fatimi et al., 2007). *L. acaciae* is quite spread in Saudi Arabia however, the current information about this species is restricted. Based on these data, our work aimed to examine the anti-inflammatory, antidiabetic, and antioxidative activities of *L. acaciae* grown in Saudi Arabia and to isolate the major active compounds.

2. Experimental

2.1. Plant collection and authentication

The collection of *L. acaciae* leaves and stems grown on *Acacia* trees was done during March 2012 from the South of Saudi Arabia. The plant was authenticated by Dr. Mohammed Yusuf at department of Pharmacognosy, College of Pharmacy, King Saud University. Voucher specimen (15809) was deposited in the herbarium of Pharmacognosy Department, College of pharmacy, KSU, Riyadh, SA.

2.2. Preparation of the extracts and fractions

The oven-dried and grinded aerial part (1.5 kg) was extracted with 3000 ml ethanol (CH_3CH_2OH) for 4 h utilizing a Soxhlet apparatus. The obtained ethanolic extract was filtered and concentrated under reduced pressure to yield a 4.1% (w/w) (61 g) of the crude alcoholic extract. 50 g of the extract was then suspended in distilled water and successively partitioned with *n*-hexane, chloroform ($CHCl_3$) and *n*-butanol (BtOH) to give 6.5 g of hexane-, 11.0 g of chloroform- and 20.5 g of butanol-fractions.

2.3. Compound isolation and identification

Chloroform fraction (2 g) was separated using silica gel by column chromatography (72 g, 80 × 3 cm). Elution started with 3% methanol: chloroform and polarity was increased with methanol. The collected fractions (20 ml each) were pooled depending on their TLC behavior to give 11 fractions. Fraction **E** (97 mg), eluted with 5% methanol: chloroform was rechromatographed on a reversed-phase column (50 g, 60 × 1 cm). The column was eluted gradually starting from 40% water: methanol. The collected fractions were merged into 10 sub-fractions according to their TLC. Sub-fraction 4, eluted with 30% water: methanol afford compounds **1** (7 mg) and **2** (5 mg). Furthermore, fraction **I** (78.4 mg), eluted with 10% methanol: chloroform, was rechromatographed on a sephadex column (10 g, 60 × 1 cm). The eluting was gradually starting from 20% methanol. The collected fractions were merged into 15 sub-fractions according to their TLC. Sub-fraction 5 afforded compounds **3** (13 mg) and **4** (9 mg). Structure elucidation was performed using Spectroscopic techniques. The UV and IR experiments were performed on Hitachi-UV-3200 and JASCO 320-A spectrometers. The 1D and 2D-NMR analysis was carried out on a Bruker AMX-500 spectrometer with tetramethylsilane (TMS) as internal standard. For mass analysis, Jeol JMS-700 High Resolution Mass Spectrophotometer was utilized.

Compound 1 (quercetin 3-O-*β*-D-glucopyranoside) (see Fig. 1): yellow amorphous powder; $C_{21}H_{20}O_{12}$; MP: 239–241 °C; UV λ_{max} (MeOH): 257, 272, 360 nm; IR ν_{max} (KBr): 3452, 1661, 1620, 1580, 1520, 1074, 1025, 1008 cm^{-1}; NMR data are demonstrated in Table 4 and similar to the data in the literature (Harborne and Mabry, 1982; Şöhretoğlu et al., 2009).

Compound 2 (quercetin 3-O-*β*-(6-O-galloyl)-glucopyranoside) (see Fig. 1): yellow powder; $C_{28}H_{24}O_{16}$; MP: 192–195 °C; UV λ_{max} (MeOH): 256, 350 nm; IR ν_{max} (KBr): 3366, 1705, 1660, 1616, 1572, 1510, 1454, 1370, 1280 cm^{-1}; NMR data are demonstrated in Table 4 and comparable with those reported in the literature (Şöhretoğlu et al., 2009).

Compound 3 (catechin) (see Fig. 1): yellowish white crystalline solid; $C_{15}H_{14}O_6$; MP: 174–176 °C; UV λ_{max} (MeOH): 275 nm; IR ν_{max} (KBr): 3402, 2852, 1625, 1470, 1276, 1016 cm^{-1}; NMR data are demonstrated in Table 4 and similar to data in the literature (Shen et al., 1993; Davis et al., 1996; Afsar et al., 2018).

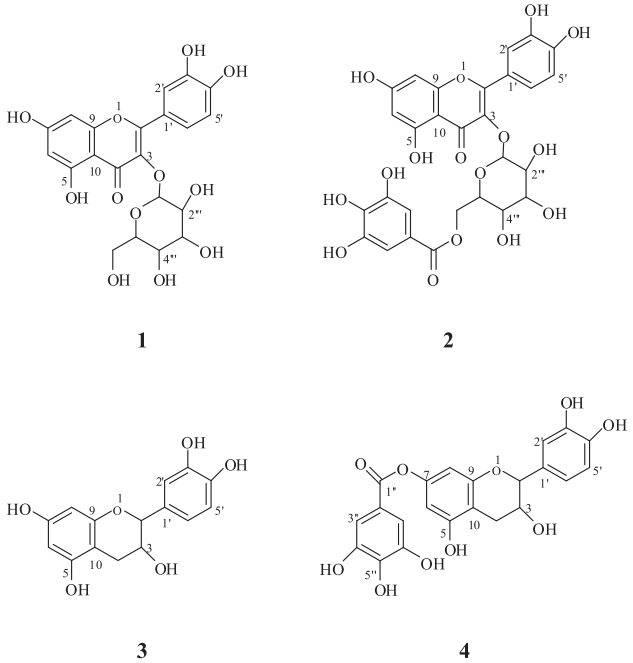

Fig. 1. Isolated compounds from *L. acacia*.

Compound 4 (Catechin 7-O-gallate) (see Fig. 1): yellowish white crystalline solid; $C_{22}H_{18}O_{10}$; MP: 164–166 °C; UV λ_{max} (MeOH): 218, 270 nm; IR ν_{max} (KBr): 3452, 3002, 2858, 1718, 1621, 1572, 1518, 1451, 1070, 1026 cm^{-1}; NMR data are demonstrated in Table 4 and comparable with data in the literature (Tanaka et al., 1983; Tayyaba et al., 2018).

2.4. Experimental animals

Male Wister rats weighing 150–180 g were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University and used for this investigation. The animals were housed under standard controlled conditions (24 °C and a 12 h light/dark cycle). The rats were provided with standard pellet diet and tap water. The investigation was assured by Research Ethics Committee (CBR 4537) of College of Pharmacy, King Saud University.

2.5. Acute toxicity study

The acute Toxicity experiment was carried out according to the test guideline of Organization for Economic Cooperation and Development (OECD guideline 423 for testing of chemicals) (OECD, 2001). Twenty rats were used for the acute toxicity study (five for each group). Different doses of the extract (0.5, 1, 2, and 5 g/kg) were suspended in 0.5% aqueous Tween 80 and administered orally. The animals were continuously observed for signs of toxicity e.g. autonomic, central nervous system, somatomotor and behavioral changes (tremors, convulsions, salivation, diarrhea, lethargy, sleep, etc..) for 12 h and the mortality was observed for 24 h.

2.6. Determination of the anti-inflammatory activity

The assessment of the anti-inflammatory effect was carried out by utilizing the carrageenan-induced rat paw oedema method. Thirty rats were divided into six equal groups ($n = 5$). Group 1 received vehicle control 0.9% NaCl (5 mg/kg). Groups 2–5 received as previously used (Mothana et al., 2012) a dose of 500 mg/kg crude extract, *n*-hexane, chloroform, and *n*-butanol fractions. Rats in group 6 were given indomethacin (10 mg/kg). The samples (extracts and drug) were administered 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub planar of each rat. The oedematous paw volume was measured initially and at every 1 h intervals for 4 h by using plethysmometer (Winter and Porter, 1957).

2.7. Determination of hypoglycemic and anti-diabetic activity

2.7.1. Hypoglycemic test in normal rats

The normal animals were divided into seven equal groups ($n = 5$). All rats were overnight fasted before starting the assay. Group 1 received normal saline. Group 2 was treated with 1 ml tween 80. Groups 3–6 were orally given 500 mg/kg of crude extract, *n*-hexane, chloroform, and *n*-butanol fractions. Animals in group 7 were treated with 10 mg/kg of glibenclamide as a positive control (Pareek et al., 2009). At the end, the blood samples were taken from tail vein just prior to drug administration and at every 30 min intervals for 2 h. The glucose level in serum was controlled immediately utilizing glucose estimation kit Refretron (Roche, Germany) to monitor the hypoglycemic effect of the investigated extract and fractions comparative to negative and positive control groups.

2.7.2. Alloxan induced diabetic rats

Diabetes was induced in the overnight fasted rats by intraperitoneally injection of 150 mg/kg of alloxan in distilled water. Rats were tested for diabetes after one week and those with blood glucose ranging 300–400 mg/dl were chosen for the experimentation. The investigation was carried out as described by EL Tahir et al. (2007). The diabetic animals were divided into seven equal groups ($n = 5$). Group 1 was treated with normal saline and group 2 received tween 80 (vehicle). Groups 3–6 were orally treated with a dose of 500 mg/kg of crude extract, *n*-hexane, chloroform, and *n*-butanol fractions. Group 7 was considered as a positive control and treated orally with 10 mg/kg of glibenclamide. The samples (extract/fractions and drug) were administered to the animals following 24 h of fasting. The measurement of glucose blood level was performed before giving the samples (0 h) and 2 h after.

2.8. Determination of the antioxidant activity

2.8.1. Scavenging activity of DPPH radical

For the determination of the free radical scavenging, DPPH (2, 2-diphenyl-1-picrylhydrazyl) was utilized. The assay was completed as depicted by Brand et al. (1995). This test estimates the free radical scavenging ability of the examined extract and fractions. Various concentrations (10, 50, 100, 500 and 1000 μg/ml) of the extract and fractions were used. Briefly, to obtain a total volume of 1 ml of the test mixture, 500 μl of the extract or fraction was mixed with 375 μl methanol and added 125 μl of 0.04% DPPH ethanolic solution. Ascorbic acid was utilized as positive control. After 30 min of incubation at room temperature in the dark, the reduction in absorbance was estimated at $\lambda = 517$ nm. The radical scavenging ability was determined from the equation:

$$\%\ \text{of radical scavenging activity} = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100$$

2.8.2. β-Carotene–linoleic acid assay

The antioxidant activity of the extracts was assessed utilizing the β-carotene bleaching assay reported by Velioglu et al. (1998) with modifications. To flasks containing 0.02 ml of linoleic acid and 0.2 ml of Tween-20, 1 ml of a β-carotene solution (0.2 mg/ml in chloroform) was added. The chloroform was evaporated under reduced pressure at 40 °C. Then residue solution was directly diluted with 100 ml of distilled water and blended for 1–2 min to make an emulsion. A solution prepared likewise but without β-carotene was utilized as a blank. A control containing 0.2 ml of 80% (v/v) methanol instead of extract was additionally made. A 5 ml of the emulsion was given to a tube containing 0.2 ml of the sample extract at 1 mg/ml. Rutin (1 mg/ml) was utilized as a positive control. After that an incubation of the tubes in a water bath at 40 °C for 2 h was carried out. At the end, the Absorbance for test and standard solutions was measured against the blank at 470 nm at 30 min intervals, with a UV–visible spectrophotometer (UV mini-1240, Shimadzu, Japan). The antioxidant activity was determined utilizing the formula:

$$\%\,\text{of antioxidant activity} = (\text{Abs0} - \text{Abst})/(\text{Abs0}^{\circ} - \text{Abst}^{\circ}) \times 100$$

where Abs0 and Abs0° are the absorbance readings at zero time of incubation for samples and control, respectively. Abst and Abst° are the absorbance readings for samples and control, respectively, after incubation for 120 min.

2.9. Statistical analysis

Statistical differences between control and treatment values of two parameters were analyzed with student's *t*-test using excel Microsoft office. Data were expressed as mean ± S.D. and the difference were statistically significant at $P < 0.05$ compared to control.

All statistical charts were conducted using origin Lab software (version 8, Massachusetts, USA) and excel Microsoft office.

3. Results and discussion

Throughout our screening for interesting pharmacological activities for local medicinal plants, the anti-inflammatory, anti-diabetic and antioxidant activities of the ethanolic extract of *Loranthus acaciae* and its fractions were investigated.

3.1. Acute toxicity

The treated rats did not show any symptoms of toxic reactions or behavioral changes during the 24 h period of investigation. No mortality was found up to the highest dose of 5000 mg/Kg after the completion of 24 h. The LD_{50} of the extract and its fractions were considered more than 5 g/kg body weight orally.

3.2. Anti-inflammatory activity

The results of the acute anti-inflammatory effect of the crude extract of *L. acaciae* and its fractions on carrageenan-induced oedema in hind paws of rats are demonstrated in Table 1. Carrageenan-induced paw oedema remained even 3 h after its injection into the subplantar region of rat paw. It was observed that the crude extract and the chloroform fraction at the dose of 500 mg/kg induced significantly inhibition effect on the oedema formation by 57.5 and 67.2% respectively. Our obtained data revealed that the chloroform fraction possessed efficient anti-inflammatory activity in the acute model of inflammation in comparison to the indomethacin (76.9%). Carrageenan-induced hind paw oedema has been generally utilized as an experimental model of acute inflammation. The development of oedema induced by carrageenan is a biphasic event: the early stage (1 h) is due to the liberation of serotonin, histamine and kinins. The later stage (over 1 h) is related with the activation of kinin-like substances and the liberation of prostaglandins, proteases and cyclooxygenase products (Vinegar et al., 1969). The crude extract and chloroform fraction of *L. acaciae* displayed a moderate inhibitory impact at early stage however could adequately suppress the expansion in paw volume during the late stage (3 h after carrageenan injection) of inflammation. In light of this perception, it might be proposed that this pharmacological action most likely may be ascribed to the inhibition of the liberation of pro-inflammatory mediators of acute inflammation, especially the prostaglandins. Of note, the effectiveness and power of the chloroform fraction was superior to others and almost comparable with those of indomethacin. Consequently, the chloroform fraction was picked for additional refining. The interesting anti-inflammatory activity of the extract and fractions of *L. acaciae* is in concurrence with the results revealed by Mothana et al. (2012) on *L. regularis*. It was uncovered that the ethyl acetate fraction at the dose of 500 mg/kg showed the best anti-inflammatory effect comparable to the positive control where it inhibited the oedema development by 67% (Mothana et al., 2012).

3.3. Hypoglycemic and anti-diabetic activity

The results of the effect of oral administration of extract and fractions of the *L. acaciae* on normal and diabetic rats are incorporated in Table 2. Our results exhibited that the crude extract and chloroform fraction has the greatest hypoglycemic and antidiabetic effects (Table 2). In the normoglycemic rats, the chloroform fraction gave a percentage reduction in blood sugar levels of 35.6% at 2 h after administration. Moreover, chloroform fraction produced a pronounced antidiabetic effect in alloxan-induced rats, giving a percentage reduction in blood sugar level of 47.0% at 500 mg/kg 2 h after administration. This effect was statistically significant and in comparison to the positive control glibenclamide (54.7%).

These results further substantiated the claim and use of *L. acaciae* in folklore medicine in Saudi Arabia as anti-diabetic drug (Waly et al., 2012). The observed hypoglycemic activity of *L. acaciae* is in agreement with a previously reported data on another *Loranthus* species, namely *P. curviflorus* (*L. curviflorus*) which showed significant hypoglycemic activity (Al-Taweel et al., 2012).

3.4. Antioxidant activity

In our investigation, the conceivable antioxidant effects of the *L. acaciae* were assessed utilizing two techniques, that are 2, 2-diphenyl-1-picrylhydrazyl- (DPPH) and β-Carotene-linoleic acid techniques. In the DPPH radical scavenging assay, a strong anti-radical activity was displayed by the chloroform extract starting from 50 to 1000 μg/ml (67.2–88.3%) and by the positive control (ascorbic acid) (90.7% at 1000 μg/ml) whereas only a low free radical inhibitory activity of DPPH (40.6%) was observed for the *n*-hexane extract at the highest concentration 1000 μg/ml (Table 3). Table 3 exhibited also a moderate DPPH-radical inhibitory activity the for the *n*-butanol fraction (78.4%) at a concentration of 1000 μg/ml. In the β-carotene-linoleic acid assay, a strong inhibition of the discoloration of β-carotene (85.4%) was noticed with the chloroform extract at a concentration of 1000 μg/mL (Table 3). This antioxidant effect was in comparison with that of the positive control rutin (89.3%). The *n*-hexane extract exhibited the lowest total antioxidant activity with a value of 57.2%.

The antioxidant ability of the crude extract and fractions of *L. acaciae* in this investigation is in agreement with recent report that has shown a potential radical inhibitory activity for Plicosepalus curviflorus (Loranthaceae) growing in Saudi Arabia (Badr et al., 2016).

Generally, reactive oxygen species (ROS) e.g. hydroxyl radical, hydrogen peroxide and superoxide anion radical take part in normal cell function as well as in pathological disorders, like inflammation. Various investigations have revealed a participation of

Table 1
Anti-inflammatory activity of the extract and fractions of *L. acaciae*.

Treatment (n = 5)	Dose mg/kg	Paw volume (Mean ± S.E)			
		Before Carrageenan	After 3 h	Net Increase after 3 h	% Inhibition
Carrageenan	0.05 ml	0.96 ± 0.02	2.09 ± 0.02	1.13 ± 0.004	
Crude extract + Carrageenan	500	0.97 ± 0.03	1.46 ± 0.02	0.48 ± 0.03**	57.52
Hexane Fr. + Carrageenan	500	0.91 ± 0.03	1.82 ± 0.05	0.91 ± 0.05*	19.02
Chloroform Fr. + Carrageenan	500	0.97 ± 0.03	1.34 ± 0.02	0.37 ± 0.01**	67.25
Butanol Fr. + Carrageenan	500	0.91 ± 0.03	1.82 ± 0.05	0.89 ± 0.07*	20.64
Indomethacin + Carrageenan	100	0.99 ± 0.04	1.25 ± 0.03	0.26 ± 0.02**	76.99

* $P < 0.01$.
** $P < 0.001$ student's *t*-test, n = 5.

Table 2
Hypoglycemic study of extract and fractions of *L. acaciae* on normal and diabetic rats.

Treatment	Dose mg/kg Orally	Glucose Levels in normal rats (mg/dl)		% Inhibition	Glucose Levels in diabetic rats (mg/dl)		% Inhibition
		Before Treatments	2 h after Treatments		Before Treatments	2 h after Treatments	
Normal saline		112.00 ± 2.54	107.25 ± 1.84		301.75 ± 6.57	304.25 ± 5.79	
Vehicle(Tween-80)		110.65 ± 4.85	105.75 ± 2.28		326.25 ± 4.80	314.50 ± 10.38	
Crude extract	500	109.75 ± 0.85	70.45 ± 3.61[c]	35.40	320.00 ± 7.73	212.25 ± 10.60[c]	33.67
n-hexane extract	500	108.50 ± 1.70	98.02 ± 2.98[a]	9.654	296.00 ± 4.10	306.25 ± 7.05	3.46
Chloroform extract	500	108.50 ± 7.05	69.85 ± 0.93[c]	35.62	323.50 ± 4.05	171.25 ± 4.13[c]	47.06
n-butanol extract	500	109.25 ± 1.93	90.32 ± 4.40[b]	17.32	329.50 ± 4.83	298.25 ± 5.32[c]	12.15
Glibenclamide	10	112.25 ± 3.09	60.87 ± 3.15[c]	45.76	339.25 ± 22.2	153.50 ± 4.17[c]	54.75

Data are mean of 5 male in each group ± SD.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ student's *t*-test.

Table 3
DPPH scavenging and antioxidant activities of *L. acacia.*

Sample	*Total antioxidant activity in (%)	Radical Scavenging Activity in (%) (DPPH-radical scavenging assay)				
	1000 (μg/ml)	10	50	100 (μg/ml)	500	1000
n-Hexane fraction	57.2 ± 2.8	6.21 ± 4.2	10.2 ± 3.1	19.3 ± 2.4	24.3 ± 4.6	40.6 ± 3.7
Chloroform fraction	85.4 ± 5.3	48.2 ± 2.6	67.2 ± 2.9	80.4 ± 4.9	84.3 ± 2.7	88.3 ± 2.5
n-Butanol fraction	79.8 ± 3.4	33.7 ± 5.1	50.3 ± 4.1	70.3 ± 3.9	76.3 ± 4.1	78.4 ± 3.2
Ascorbic acid	NT	80.7 ± 2.5	85.1 ± 1.3	85 ± 3.2	88.7 ± 2.7	90.7 ± 4.4
Rutin	89.3	NT	NT	NT	NT	NT

* β-carotene bleaching assay, NT not tested. In the columns, means ± SD.

ROS in models of inflammation, e.g. carrageenan-induced pleurisy in rats (Halliwell and Gutteridge, 2015). It has been observed that the production of reactive species occurs at the site of inflammation and contributes to tissue damage (Conner and Grisham, 1996). It is almost thought that the antioxidant activity of plant phenolics (e.g., flavonoids, phenolic acids and tannins,) resides mainly in their ability to donate hydrogen atoms or electrons and thereby scavenge free radicals (Sharififar et al., 2009). The results of the β-carotene/linoleic acid assay corresponded to the free radical-scavenging activity of tested *L. acaciae*. The extract and fractions especially the chloroform fraction exhibited great antioxidant activity. In view of the known support of ROS in inflammation pathways (Conner and Grisham, 1996) we expect that the two effects are presumably related.

3.5. Isolated compounds from L. acaciae

The ^{1}H NMR spectrum of compound **1** displayed three aromatic proton signals at 7.76 (*d, J* = 2 Hz, H-2′), 6.82 (*d, J* = 8.4 Hz,H-5′) and 7.55 (*dd, J* = 2, 8.4 Hz) in the form of an ABC spin-system proposing a flavonol structure with 3′,4′-disubstituted B-ring, and a pair of meta coupling proton signals at 6.16 (*d, J* = 2 Hz, H-6) and 6.35 (*d, J* = 2 Hz, H-8) for the A ring. It also displayed signals for glucose moiety with anomeric proton resonated at 5.23 (*d, J* = 7.6, H-1″) confirming a β-linkage of the glucose. The ^{13}C NMR spectrum confirmed this hypothesis and exhibited 21 signals involving carbonyl signal resonated at 179.48 (C-4). It showed aromatic carbons at δ 135.46 (C-3), 162.92 (C-5), 166.07 (C-7), 145.79 (C-3′), 149.73 (C-4′) confirming a 3,5,7,3′,4′-oxygenated flavone nucleus. The ^{13}C NMR spectrum displayed also signals at 104.13 (C-1″), 75.59 (C-2″), 77.98 (C-3″), 71.06 (C-4″), 78.27 (C-5″), 62.39 (C-6″) assigned for glucose molecule (Table 4). Based on these spectral data and comparison with reported literature (Harborne and Mabry, 1982; Şöhretoğlu et al., 2009), the compound was identified as quercetin 3-*O*-*β*-D-glucopyranoside. This constituent is isolated for the first time from the species *L. acaciae*. However, it was formerly found in other plants, including *L. regularis* (Mothana et al., 2012). Quercetin 3-*O*-*β*-D-glucopyranoside was reported to exhibit antibacterial and antiinflammatory effect (Islam et al., 2012).

^{1}H and ^{13}C NMR spectral data of compound **2** displayed signals assigned to aromatic system and sugar moieties (Table 4). The ^{1}H NMR spectrum displayed 3 aromatic protons at δ_H 7.59 (*dd, J* = 2.0, 8.0, H-2), 7.56 (*d, J* = 2.0), and 6.74 (*d, J* = 8.0), indicating the presence of *O*-disubstituted B ring. In addition, 2 meta coupled signals in the aromatic field at δ_H 6.20 (1H, *d, J* = 2.0, H-6) and 6.36 (1H, *d, J* = 2.0, H-8) were compatible with a 5,7-dihydroxy substituted A ring of flavonoid. A singlet at δ_H 6.96 (2H) was assigned to the H-2‴ and H-6‴ of gallic acid. The configuration of the anomeric proton of sugar moiety was proposed to be *β* according to the coupling constant (7.2 Hz). The sugar was designated to be a β-glucopyranose according to ^{1}H NMR data. In the ^{13}C NMR spectrum 28 carbon signals were shown. Fifteen carbon signals were assigned to quercetin moiety, 6 carbon signals were assigned to the glucose unit, and the remaining 7 carbon signals were assigned to gallic acid moiety. Additionally, the HMBC spectrum enabled us to establish the locations to be connected among the quercetin, glucopyranose, and gallic acid moieties based on the cross peaks: one due to the coupling between H-1″ and C-3 and the other due to the coupling between H_a-6″ and C-7‴. After the complete interpretation of the NMR data on the basis of the COSY, HSQC, and HMBC experiments, and comparing these data with published data, compound **2** was identified as quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside. This constituent is isolated for the first time from the species *L. acaciae*. Quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside has been found to show antioxidant, anti-inflammatory, lipid peroxidation inhibitory effects. The strong inhibitor efficiency was noted to be greater than those of non-galloylated flavonol glycosides (Şöhretoğlu et al., 2009).

The ^{1}H NMR spectrum of compound **4** (Table 4) displayed signals at 7.20 (*s*, galloyl), 7.19 (*d, J* = 1.8 Hz, H-2′), 6.85 (*d, J* = 7.8 Hz, H-5′), 6.84 (*dd, J* = 7.8 Hz, and *J* = 1.8 Hz, H-6′), 6.70 (*d, J* = 2.1 Hz, H-6), 6.19 (*d, J* = 2.1 Hz, H-8), 4.63 (*d, J* = 7.5 Hz, H-2), 3.28 (*m*, H-3), 2.70 (*dd, J* = 14 Hz and *J* = 7 Hz, H-4). The ^{13}C NMR spectrum showed the presence of 22 carbons resonated for a flavanol residue with gallic acid. The interpretation of correlations

Table 4
^{1}H and ^{13}C NMR data for compounds **1**, **2** and **4** in CD_3OD.

Position	Compound 1 δ_H (*J* in Hz) δc ppm		Compound 2 δ_H (*J* in Hz) δc ppm		Compound 4 δ_H (*J* in Hz) δc ppm	
2		158.84		161.4	4.63 (*d*, *J* = 7.5)	81.09
3		135.46		133.8	3.28 (*m*)	68.48
4		179.48		179.9	2.91 (*dd*, *J* = 14,7), 2.58 (*dd*, *J* = 14,7)	28.64
5		162.92		161.48		156.31
6	6.16 (*s*)	99.79	6.15 (*d*, *J* = 2)	98.5	7.20 (*d*, *J* = 2.1)	102.13
7		166.07		164.59		151.89
8	6.35 *s*	94.60	6.31 (*d*, *J* = 2)	93.4	6.18 (*t*, *J* = 2.1)	101.95
9		158.35		157.9		157.73
10		105.51		104.2		107.13
1′		122.93		121.6		131.99
2′	7.76 *s*	117.39	7.52 (*d*, *J* = 2)	115.7	7.19 (*d*, *J* = 1.8)	115.24
3′		145.79		144.8		146.45
4′		149.73		148.3		146.40
5′	6.82 (*d*, *J* = 8.1)	115.86	6.69 (*d*, *J* = 8)	114.5	6.85 (*d*, *J* = 7.8)	116.23
6′	7.55 (*t*, *J* = 2)	123.06	7.53 (*d*, *J* = 2,8)	122.3	6.86 (*dd*, *J* = 7.8, 1.8)	120.06
1″	5.22 (*d*, *J* = 7)	104.13	5.19 (*d*, *J* = 7.5)	102.8		120.80
2″	3.55 (*t*, *J* = 7)	75.59	3.93 *m*	74.2		110.60
3″	3.35*	77.98	3.48*	76.2		146.78
4″	3.45*	71.06	3.43*	70.8		140.57
5″	3.20 (m)	78.27	3.50 (*m*)	74.4		146.78
6″	3.68 (*dd*, *J* = 2, 11.6), 3.56 (*dd*, *J* = 5.2, 11.6)	62.39	3.73 (*dd*, *J* = 2, 11.6), 3.56 (*dd*, *J* = 5.2, 11.6)	62.8		110.60
1‴				119.8		
2‴			6.69 *s*	108.7		
3‴				144.8		
4‴				138.3		
5‴				144.8		
6‴			6.69 *s*	108.7		
7‴				166.76		167.16

* Signal patterns unclear.

in the COSY, HSQC, and HMBC experiments and the comparison with data reported previously (Tanaka et al., 1983; Afsar et al., 2018), compound **4** was concluded to be catechin 7-*O*-gallate. This constituent is isolated for the first time from the species *L. acaciae*.

Polyphenolic compounds particularly flavonoids have pulled a lot of attention in relevancy their wide range of pharmacological activities within the bar of various conditions and diseases including oxidative stress, hyperglycemia, inflammations, cancers and cardiovascular disorders (Garcia-Mediavilla et al., 2007). In fact, flavonoids demonstrating powerful antioxidant activity have been suggested to be useful in the management of diabetes mellitus and inflammations. Additionally, many reports have proposed that this type of natural products might act as inhibitors on biological targets mostly enzymes like α-glycosidase, α-amylase and Dipeptidyl Peptidase IV (DPP-4) that are involved in type 2 diabetes as well as enzymes involved in inflammation such as nitric oxide synthase (iNOS), cyclooxygenase (COX-2) and lipoxygenase (Fan et al., 2013; Li et al., 2009; Morales et al., 2006; Peng et al., 2016; Sarian et al., 2017; Tadera et al., 2006; Tunon et al., 2009). Consequently, all these mechanisms might partly give an explanation for the anti-inflammatory, antidiabetic and antioxidant effects of *L. acaciae* extract and the isolated flavonoid-compounds.

4. Conclusion

In conclusion, our results plainly uncovered that *L. acaciae* extracts particularly the chloroform extract possessed potent anti-inflammatory, antidiabetic and antioxidant effects which underlines its traditional use for various ailments including inflammations and diabetes. Additionally, the phytochemical investigation of the active chloroform fraction led to the isolation and characterization of four compounds namely, quercetin 3-*O*-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside, (-) catechin, and catechin 7-*O*-gallate. Among these compounds, quercetin 3-*O*-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside and catechin 7-*O*-gallate are reported for the first time from *L. acaciae*. More examinations are required to affirm these activities and to clarify the mechanism of action. Besides, further investigations on the isolation and characterization of active entities ought to be proceeded.

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Conflict of interest

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

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