

Secondary metabolites and bioactivities of *Albizia anthelmintica*

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ABSTRACT

Background: *Albizia* species are rich in phenolics and terpenes in the different plant organs. They are widely used in traditional Chinese medicine. So this study investigated the phytochemical and biological activities of *Albizia Anthelmintica*. **Materials and Methods:** Column chromatography has been performed for the isolation of compounds. Bioactivity studies of *A. anthelmintica* leaves were carried out on aqueous ethanol extract and some pure compounds were tested for their antioxidant activities. **Results:** Eight compounds have been isolated for the first time from *A. anthelmintica*. The aqueous ethanol extract of *A. anthelmintica* showed moderate anti-inflammatory activity and significant for both analgesic and antioxidant activities. Quercetin-3-O- β -D-glucopyranoside, kaempferol-3-O- β -D-glucopyranoside, kaempferol-3-O-(6 β -O-galloyl- β -D-glucopyranoside and quercetin-3-O-(6 β -O-galloyl- β -D-glucopyranoside) exhibited potent antioxidant scavenging activity towards diphenyl-picrylhydrazine.

Key words: *Albizia anthelmintica*, analgesic, anti-inflammatory, antioxidant, diphenyl-picrylhydrazine, flavonoids

INTRODUCTION

The use of natural products with therapeutic properties is an ancient as human civilization and for a long time. Recently, herbal medicines have increasingly been used to treat many human diseases.^[1] Natural antioxidants, especially phenolics and flavonoids are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods.^[2] Numerous studies were carried out on plants with antioxidant properties.^[3,4] However, there is still great interest in finding new antioxidants from natural sources. In Egypt, many plants are used today in folk medicine and are sold at herbal vendors and shops.^[5] The ancient Egyptians were familiar with many medicinal herbs and aware of their usefulness in the treatment of various diseases. They used the plant organs such as roots, rhizomes, flowers, leaves, fruits, seeds, and oils. They applied their medicaments in the form of powders, pills,

suppositories, creams, pastes, and ointments.^[6,7] However, scientific evidence for the medicinal properties of such plants is not always demonstrated. *Albizia* is a large genus belonging to family fabaceae, which comprises about 150 species,^[8] widely distributed in the tropics, with the great diversity in Africa and Central South America, of mostly fast-growing sub-tropical and tropical trees, and shrubs. *Albizia* species were reported as rich in phenolic compounds^[9-11] saponins, and triterpenoidal saponins.^[12,13] Some *Albizia* species were found to be used in traditional Chinese medicine for treatment of insomnia, irritability and wounds,^[14] the bark extract of *Albizia julibrissin* is a sedative drug and an anti-inflammatory for treating swelling, and pain of the lungs, skin ulcers, wounds, bruises, abscesses, boils, haemorrhoids, and fractures, and has displayed cytotoxic.^[15] The *Albizia* members in Africa are used in folk medicine for the treatment of rheumatism, cough, diarrhea and injuries.^[16] Plants have played a major role in the introduction of new therapeutic agents. It is our opinion that instead of random search of plants, a selective search based on traditional knowledge would be focused and productive and certainly more economic. The present study, deals with the isolation and identification of phenolic compounds from

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A. anthelmintica Brongn. cultivated in Egypt, and evaluation of the biological activity of its alcoholic extract and the isolated compounds.

MATERIALS AND METHODS

All instruments were used found at Instituto de Productos Naturales y, Agrobiología, Tenerife, Spain.

General

Column chromatography was carried out on Polyamide 6 and Sephadex LH-20. NMR experiments were performed on a Bruker AMX 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in ^1H NMR and 100, 125 MHz in ^{13}C NMR. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard and DMSO d_6 as solvent at room temperature.

Chemicals and kits

Etodine and acetylsalicylic acid (El Nasr Co., Egypt), Carrageenan were used for the induction of acute inflammation in rats, Tween 80, and diphenyl-picrylhydrazine (DPPH) (Sigma Co.). Doses of the tested materials and drugs for biology were administered orally by gastric tube.^[17]

Plant materials

The leaves of *A. anthelmintica* Brongn. were collected from El Zohria garden. The plant materials were identified by Dr. M. El-Gibaly, Lecturer of Taxonomy and Consultant for Central Administration of Plantation and Environment. The collected samples were air dried, powdered, and kept for chemical analysis. Voucher specimens were kept in herbarium, Egypt, National Research Center, El-Tahrir St., Dokki.

Extraction and isolation

The air dried powder leaves of *A. anthelmintica* (1.5 kg) were crushed and extracted with aqueous ethanol by soaking at room temperature then the aqueous ethanol extract was evaporated under reduced pressure. Twenty grams of the dry residue was used for pharmacological studies. Weigh samples of the leaves of *A. anthelmintica* were used to prepare the solutions, which were diluted with distilled water to the appropriate concentration of the experiment. The rest of the extract was defatted using successive extraction by petroleum ether and chloroform, the residue was extracted with n-butanol, affording a dry extract (105 g), which was fractionated by chromatography on Polyamide 6 CC. The column was eluted with water and with water-methanol step gradient. The obtained fractions (500 ml of each fraction) were subjected to paper chromatography using BAW (n-butanol : acetic acid : water; 4:1:5; the upper layer) and 15% acetic acid as developing solvents, and the similar fractions were collected together to give three

major fractions (I-III), which were examined by 2D paper chromatography. Fraction I was applied to a Sephadex LH-20 column using saturated butanol for elution to give two compounds 1 (19 mg) and 2 (23 mg). Fraction II Purified on a polyamide CC using MeOH : benzene : water (60:38:2) as solvent to give three subfractions (1-3). Subfraction 1 was applied on a polyamide column using MeOH : benzene : water (60:38:2) as solvent to give a pure compound 3 (35 mg), subfraction 2 has been chromatographed on preparative paper chromatography using BAW for elution and gave one compound 4 (21 mg). Subfraction 3 was applied on Sephadex LH-20 CC using saturated butanol to give two subfractions which then purified on Sephadex LH-20 CC using MeOH-H₂O (1:1) to give a pure samples of compounds 5 (33 mg) and 6 (26 mg). Fraction III was subjected to a Sephadex LH-20 CC using MeOH-H₂O (1:1) to give two pure compounds 7 (13 mg) and 8 (16 mg). All the isolated compounds were further purified on Sephadex LH-20 CC using MeOH-H₂O (1:1) to give pure samples.

Animals

Mature Swiss female albino rates weighing 150-200 g and Mature Swiss female albino mice (20-25 g) were used in this study. Animals were obtained from the Animals House Colony of the National Research Center, Cairo, Egypt. The animals were kept under the same hygienic conditions, and on a standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), casein 95% pure (10.5%) and starch (54.3%).

Pharmacological screening

Evaluation of anti-inflammatory activity

The anti-inflammatory testing was performed according to the method of Winter.^[18] For this purpose, 24 rats weighing 150-200 g b.wt were used. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 ml of 1% (w/v) carrageenin in distilled water into their footpads. Rats were divided into 4 groups of 6 rats each. The 1st group was kept as control, and was given the respective volume of the solvent (few drops of Tween 80 in distilled water). The 2nd and 3rd groups were orally administered the aqueous ethanolic extract of *A. anthelmintica* in doses of 200 mg/kg and 400 mg/kg b.wt. respectively, 1 h before carrageenin injection. The last group was administered Etodine in a dose of 5 mg/kg b.wt. orally as a standard reference. The paw volume of each rat was measured using Plethysmometer; before carrageenin injection and then hourly for 4 h post administration of the plant extracts.

The edema rate and inhibition rate of each group were calculated as follows:

$$\text{Edema rate (E) \%} = \frac{V_t - V_o}{V_o}$$

$$\text{Inhibition rate (I) \%} = E_c - E_t / E_c$$

Where:

V_o is the volume before carrageenin injection (ml),
 V_t is the volume at 't' hour after carrageenin injection (ml),
 E_c is the edema rate of control group,
 E_t is the edema rate of treated group.

Evaluation of analgesic activity using writhing test

Experimental models used in this study were selected to investigate both centrally and peripherally mediated analgesic effects of the tested extracts. For this purpose, the acetic acid abdominal constriction method was used to elucidate the peripheral effect. An acetic acid-induced abdominal constriction in mice (Writhing effect) was determined by the method described by Collier.^[19] Twenty four mice were divided into four equal groups and pretreated as follows: Group I, which served as a control, was orally received distilled water in appropriate volumes. Groups II and III were received the extract of *A. anthelmintica* leaves at oral doses of 200 mg/kg and 400 mg/kg b.wt. respectively. Group IV was orally received acetyl salicylic acid in a dose of 100 mg/kg b.wt. After 30 min, each mouse was administered 0.7% of an aqueous solution of acetic acid (10 ml/kg b.wt.) and the mice were then placed in transparent boxes for observation. The number of writhes was counted for 20 min. after acetic acid injection. The number of writhes in each treated group was compared to that of a control untreated group. The number of writhing's and stretching's was recorded and the percentage protection was calculated using the following ratio:

Percentage of protection

$$= \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

Antioxidant activity

The DPPH assay was performed as described by Shirwaikar.^[20] This method depends on the reduction of the purple DPPH radicals to a yellow colored DPPH and the remaining DPPH radicals which showed maximum absorption at 517 nm were measured, 2 ml of various concentrations of each compound were added to 2 ml solution 0.1 mM DPPH. An equal amount of methanol and DPPH served as control. After 20 min of incubation at 37°C in the dark, the absorbance was recorded at 517 nm, the experiment was performed in triplicates. Decreasing of the DPPH solution absorbance indicated as increase of the DPPH radicals scavenging activity. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity \%} = 1 - [A_{\text{sample}} / A_{\text{control}}] \times 100$$

Where A_{sample} and A_{control} are absorbance of sample and control.

RESULTS AND DISCUSSION

Eight compounds have been isolated from *A. anthelmintica* for the first time gallic acid,^[21] (1) quercetin-3-O-β-D-glucopyranoside,^[22] (2) kaempferol-3-O-(6''-O-galloyl-β-D-glucopyranoside,^[23] (3) kaempferol-3-O-β-D-glucopyranoside,^[24] (4) quercetin-3-O-(6''-O-galloyl-β-D-glucopyranoside,^[23] (5) quercetin-3-O-β-galactopyranoside,^[25, 26] (6) quercetin,^[27] (7) and kaempferol.^[28] (8). Identification of the isolated compounds was achieved by chemical and spectroscopic analysis.

Compound 3 was isolated as yellow amorphous powder with R_f values: 0.30 (HOAc-15%), 0.52 (BAW), it has a purple color under ultraviolet (UV) light turning to bright yellow when fumed with ammonia vapors, and gives yellow color when sprayed with Naturstoff reagent. Complete acid hydrolysis yielded kaempferol, gallic acid and glucose as sugar moiety in which all of them were co-chromatographed with authentic samples. ¹H NMR spectrum showed four proton signals in the aromatic region two appeared at δ 7.93 (*d*, *J* = 8.9 Hz, H-2',6') and 6.75 (*d*, *J* = 8.9 Hz, H-3',5') characteristic for ring B, also two signals appeared at δ 6.38 (*d*, *J* = 2 Hz, H-8), and 6.18 (*d*, *J* = 2 Hz, H-6) for the ring A, which together characteristic for kaempferol moiety. In the aliphatic region a doublet signal of the anomeric proton appeared at δ 5.44 (*d*, *J* = 7.4 Hz) for the sugar moiety whose β-configuration was estimated from its value. A singlet signal appeared at δ 6.91 was assignable to the two equivalent protons of the gallic acid,^[21] which was determined to be adjacent to the 6'' position in the sugar moiety by the downfield shift for H-6'' at δ 4.26 and 4.16. The ¹³C NMR spectrum showed twenty five signals, six of them attributed to the sugar moiety, thirteen to the kaempferol aglycones and the remaining six signals for gallic acid. The C-2 down field shift at 157.10 confirmed the glycosylation at C-3 position.^[29] Furthermore, the downfield of the C-6'' at δ 63.16 was evidence for the site of attachment of gallic acid moiety at glucose unit. From these all data compound 3 was identified as kaempferol-3-O-(6''-O-galloyl-β-D-glucopyranoside), which was identical with those reported in literature.^[23]

Compound 5 was isolated as a pale yellow powder with R_f values: 0.26 (HOAc-15%) and 0.33 (BAW), it has a purple color under UV light turning to yellow when fumed with ammonia vapors, and orange color when sprayed with Naturstoff reagent. Complete acid hydrolysis yielded quercetin, gallic acid, and glucose as sugar moiety in which

all of them were co-chromatographed with authentic samples.

^1H NMR spectrum of this compound was similar to those belonging to quercetin moiety appeared at δ 7.68 ($d, J = 2.1$ Hz, H-2'), 7.70 ($d, J = 8.5$ and 2.1 Hz, H-6'), 6.83 ($d, J = 8.5$ Hz, H-5'), for ring B and at δ 6.47 ($d, J = 2.1$ Hz, H-8), and 6.28 ($d, J = 2.1$ Hz, H-6), which are characteristic for ring A, the spectrum also indicated the presence of a signal at δ 5.55, which belonging to the anomeric proton of the sugar moiety with a coupling constant 7.4 indicating that the anomeric carbon is β -configuration. A singlet signal appeared at δ 7.01 belonging to gallic acid.^[21] The galloyl moiety was determined to be adjacent to the 6-position in the sugar moiety by the downfield shift for H-6' at δ 4.35 and 4.30. ^{13}C NMR spectrum showed 26 signals, six of them attributed to the sugar moiety, 15 to the quercetin aglycones,

and the remaining 5 signals for gallic acid. The C-2 down field shift at 156.79 confirmed the glycosylation at C-3 position.^[29] Furthermore, the downfield of the C-6' at δ 63.55 was evidence for the site of attachment of gallic acid moiety at glucose unit. Comparison of the ^1H and ^{13}C NMR data of compound 5 with those of published in literature revealed the structure of Quercetin-3-O-(6''-O-galloyl- β -D-glucopyranoside).^[23] The spectral data for the two compounds 3 and 5 are reported in Table 1. The structure of the two compounds is drawn in Figure 1.

Bioassay

The results of Anti-inflammatory activity of *A. anthelmintica* [Table 2], exhibited a significant anti-inflammatory activity. Moreover, the results revealed that the aqueous ethanol extract of *A. anthelmintica* possessed the highest activity at a dose level of 400 mg/kg

Table 1: Spectral data for compounds 3 and 5

Atom no.	3		5	
	^1H	^{13}C	^1H	^{13}C
2	–	157.10	–	156.79
3	–	133.54	–	133.74
4	–	177.64	–	177.64
5	–	161.51	–	161.54
6	6.18 ($d, J = 2$)	99.20	6.17 ($d, J = 2.1$ Hz)	99.08
7	–	164.81	–	164.53
8	6.38 ($d, J = 2$)	94.13	6.34 ($d, J = 2.1$ Hz)	93.90
9	–	156.80	–	156.67
10	–	104.24	–	104.26
1'	–	121.02	–	121.32
2'	7.93 ($d, J = 8.9$ Hz)	131.12	7.43 ($d, J = 2.2$ Hz)	115.64
3'	6.75 ($d, J = 8.9$ Hz)	115.44	–	145.14
4'	–	160.29	–	148.80
5'	6.75 ($d, J = 8.9$ Hz)	115.44	6.72 ($d, J = 8.4$ Hz)	116.14
6'	7.93 ($d, J = 8.9$ Hz)	131.12	7.57 ($dd, J = 8.5$ and 2.2 Hz)	122.23
1''	5.44 ($d, J = 7.4$ Hz)	101.88	5.44 ($d, J = 7.4$ Hz)	101.73
2''	–	74.46	–	74.42
3''	–	74.46	–	74.62
4''	–	69.71	–	69.90
5''	–	76.54	–	76.66
6''	–	63.16	–	63.55
C-1'''	–	119.70	6.89	119.69
C-2''', 6'''	6.91	108.95	–	108.96
C-3''', 5'''	–	145.83	–	145.79
C-4'''	–	138.79	–	138.76
C=O	–	166.01	–	166.05

Table 2: Anti-inflammatory activity of alcoholic extract of *Albizia anthelmintica*

Drugs	% change 1 h	% inhibition	% change 2 h	% inhibition	% change 3 h	% inhibition	% change 4 h	% inhibition
Control	96.67±9.838	–	107.6±15.84	–	111.5±12.03	–	116.2±11.50	–
Etodine 5 mg/kg	69.5±3.463	-28.11	57.5±5.458*	-46.56	52.9±3.075*	-52.56	52.52±6.292*	-54.80
<i>A. anthelmintica</i> 400 mg/kg	55.86±2.541	-42.22	63.29±7.128	41.18	66.95±5.254*	-39.96	64.67±7.185*	-44.35
<i>A. anthelmintica</i> 200 mg/kg	62.05±6.954	-35.81	72.34±7.274	-32.77	88.33±8.225	-20.78	72.19±7.091*	-37.87

Values represent the mean±SE of six animals for each groups; * $P < 0.05$: Statistically significant from the control using one way ANOVA (using Tukey as post hoc test)

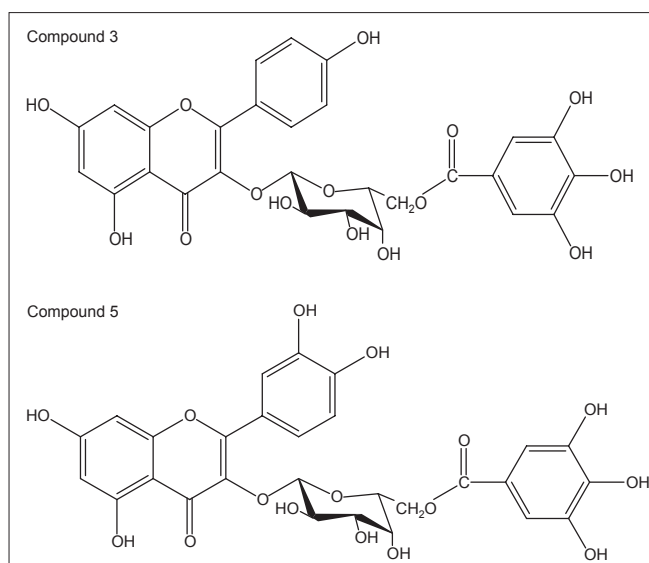


Figure 1: Structures of compounds 3 and 5

Table 3: Analgesic activity of alcoholic extract *Albizia anthelmintica*

Group	No. of writhes/20 min	Protection (%)
Control	49.2±5.877	-
Acetylsalicylic acid 5 mg/kg	28±2.429*	-43.09
<i>A. anthelmintica</i> 400 mg/kg	11.4±1.778*	-76.83
<i>A. anthelmintica</i> 200 mg/kg	23.4±2.619*	-52.44

Values represent the mean±SE of six animals for each groups; * $P < 0.05$; Statistically significant from the control using one way ANOVA (using Tukey as Post hoc test)

Table 4: Diphenyl-picrylhydrazine radical scavenging activity of the total extract of *Albizia anthelmintica* and some isolated compounds

Compound	DPPH free radical scavenging activity IC ₅₀ (µg/ml) ^a
Alcoholic extract	29.49±0.98
kaempferol-3-O-β-D-glucopyranoside	25.56±3.38
Quercetin 3-O-β-D-glucopyranoside	12.41±1.19
kaempferol-3-O-(6"-O-galloyl-β-D-glucopyranoside)	17.26±3.12
quercetin-3-O-(6"-O-galloyl-β-D-glucopyranoside)	20.82±2.76
Ascorbic acid	7.90±0.2

^a Values of IC₅₀ were calculated as mean of triplicate determination±standard deviation, concentrations in µg/ml required scavenging the DPPH radical (100 µg/ml) by 50%; DPPH=Diphenyl-picrylhydrazine; IC=Inhibition concentration

after 4 h, as it significantly decreased the weight of edema induced by carrageenin in rat paw by 44.35% inhibition compared to 54.80% inhibition of Etodine (5 mg/kg). Where the inhibition was 39.96% at the same dose level relative to the reference standard (52.56%) after 3 h. At lower dose level 200 mg/kg, the aqueous ethanolic extract *A. anthelmintica* displayed 37.87% inhibition after 4 h. The

results of the analgesic activity of the aqueous ethanolic extract of *A. anthelmintica* is compiled in [Table 3], the results showed that doses *A. anthelmintica* 200 mg/kg and 400 mg/kg displayed significant analgesic activity compared to the control and the reference standard aspirin at 5 mg/kg. Furthermore, 76.83% protection was exhibited by the aqueous ethanolic extract *A. anthelmintica* at 400 mg/kg, which was reduced to 52.44% protection at lower dose level (200 mg/kg). The analgesic activity of the extract can be attributed to their content of phenolic constituents. The total alcoholic extracts of *A. anthelmintica* and some of the isolated pure compounds [Table 4], were tested for their free radicals scavenging activity on DPPH. The alcoholic extracts exhibited antioxidant scavenging activity towards DPPH, with IC₅₀ value 29.49 µg/ml. Quercetin-3-O-β-D-glucopyranoside exhibited potent antioxidant scavenging activity towards DPPH, with IC₅₀ value of 12.41 µg/ml, Ascorbic acid was used as a positive control.

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