

Flavonoid glycosides and pharmacological activity of *Amphilophium paniculatum*

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ABSTRACT

Background: Nothing is reported on *Amphilophium paniculatum* (L.) Kunth. This study aimed at investigation of chemical constituents of the leaves of *Amphilophium paniculatum*, grown in Egypt, in addition to pharmacological evaluation. **Materials and Methods:** Isolation of a new compound, along with 5 known flavonoids. Pharmacological activities were carried out on different extracts of *A. paniculatum* leaves. **Results:** Identification of a new flavone glycoside, acacetin 8-C-β-D- glucopyranosyl 1-(1→2)-α-L-rhamnopyranoside (1) in addition to 5 known flavonoids. The 70% ethanol crude extract and its successive chloroform, ethyl acetate, and 100% ethanol extracts showed significant anti-inflammatory activity, analgesic effect, antipyretic activity, antioxidant activity, and anti-hyperglycemic activity. Determination of the median lethal dose (LD₅₀) revealed that the different extracts were safe.

Key words: *Amphilophium paniculatum*, bignoniaceae, flavonoids, pharmacology

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INTRODUCTION

Bignoniaceae is a family of flowering plants, which comprises about 800 species in 120 genera, some species are cultivated as ornamentals, distributed in tropical and subtropical of South America, Africa, and India.^[1-3] Nearly all members of this family have woody stems. The genus *Amphilophium*^[4-5] has woody liana, leaves 2-3 foliolate, and fruits are smooth, flat, and somewhat woody. Nothing is reported on *Amphilophium paniculatum* (L.) Kunth. This study aimed at investigation of chemical constituents and pharmacological activity of the leaves of *Amphilophium paniculatum*, grown in Egypt, in order to support the possibility of its uses as a natural resource in therapeutics and to demonstrate the correlation between chemical composition and bioactivity.

MATERIALS AND METHODS

General

NMR spectra were recorded on a JEOL EX-500 MHz NMR spectrometer in DMSO-*d*₆ using TMS as internal

standard, mass spectra (±) ESI-MS: LCQ Advantage Thermo Finnigan spectrometer.

Plant material

Amphilophium paniculatum (L.) Kunth leaves (Family Bignoniaceae) were collected from El-Orman garden, Giza, Egypt. The plant samples were kindly identified by Mm. Tressa Labib, Taxonomist, El-Orman garden, Giza, Egypt.

Extraction and isolation

The air-dried powder of *Amphilophium paniculatum* leaves (1 kg) was extracted by 70% ethanol. The aliquot of ethanol extract was evaporated under reduced pressure to give 150 g extract, which was suspended in water (1L) and then extracted successively with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The successive extracts were separately evaporated under reduced pressure to yield 20 g, 25 g, 30 g, and 20 g respectively. The *n*-butanol fraction (20 g) was subjected to column chromatography on polyamide 6S. The column was eluted with distilled water followed by water/ethanol step-gradient. Finally, the column was eluted with absolute ethanol to ensure a perfect elution process. The separated fractions resulting from the column were examined by PC using solvent systems (BAW and 15% ACOH) under UV light. The similar fractions were collected together to give 6 major fractions, which were subjected to Sephadex

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LH-20 columns to give a new flavonoid, acacetin 8-C- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranoside (1), in addition to 5 known flavonoid compounds.

Animals

Albino mice of 25-30 gm body weight and adult male Albino rats of Sprague Dawley Strain of 130-150 g body weight were used in this study, obtained from the animal house colony of National Research Centre, Egypt. All animals were kept under the same hygienic conditions and on a standard laboratory diet.

Chemicals and kits

Metformin (Chemical Industries Development, Giza, ARE), alloxan (Sigma Co: Cairo, Egypt). Biodiagnostic kit for assessment of blood glucose and glutathione levels, Glutathione kit (Wak Company-Germany) for the assessment of antioxidant activity. Indomethacin (Epico, Egyptian Int. Pharmaceutical Industries Co.). Carrageenan (Sigma Co.) Tramadol (October Pharma, Egypt). Acetic acid (Sigma Co.). Vitamin E (Pharco Pharmaceutical Co.). Paracetamol (Misr Co., Egypt, Cairo).

Doses of the tested materials were administered orally by gastric tube.^[6]

Pharmacological screening

The air-dried powder of *Amphilophium paniculatum* leaves (600 g) was extracted by 70% ethanol. The aliquot of ethanol extract was evaporated under reduced pressure to give 65 g extract. The residue of extract was suspended in water 1000 ml and then extracted successively with chloroform, ethyl acetate, and 100% ethanol. The extracts were separately evaporated under reduced pressure to yield 14 g, 12 g, and 14 g, respectively. The extracts were kept in tightly sealed sample tubes for the biological study.

Median Lethal Dose (LD₅₀)

Determination of the LD₅₀ of extracts of *A. paniculatum* leaves was estimated where all doses were expressed in terms of extract weight/animal weight.^[7] Preliminary experiments were done to determine the minimal dose that kills all animals (LD₁₀₀) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected in a group of 6 animals by subcutaneous injection. The mice were then observed for 24 hrs., and symptoms of toxicity and mortality rates in each group were recorded, and the LD₅₀ was calculated.

Anti-inflammatory activity

This effect was determined according to the method described by Winter *et al.*^[8] Sixty male albino rats, weighing 130-150 g were divided into 10 groups, each of 6 animals;

first group: Rats that received 1 ml of saline serving as control, second group: Rats that received 50 mg/Kg of 100% ethanol of plant extract, third group: Rats that received 100 mg/Kg of 100% ethanol of plant extract, fourth group: Rats that received 50 mg/Kg of 70% ethanol of plant extract, fifth group: Rats that received 100 mg/Kg of 70% ethanol of plant extract, sixth group: Rats that received 50 mg/Kg of chloroform of plant extract, seventh group: Rats that received 100 mg/Kg of chloroform of plant extract, eighth group: Rats that received 50 mg/Kg of ethyl acetate of plant extract, ninth group: Rats that received 100 mg/Kg of ethyl acetate of plant extract, tenth group: Rats that received 20 mg/Kg of the reference drug, indomethacin.

One hour later, all the animals received a sub-plantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Four hours after drug administration, the rats were sacrificed; both hind paw excised and weighed separately.

$$\% \text{Edema} = \frac{\text{Weight of right paw} - \text{weight of left paw}}{\text{weight of left paw}} \times 100$$

Analgesic activity

Animals were acclimatized to the laboratory conditions for at least 1 hr. before testing and were used once during the experiment.

Acetic acid induced writhing test: Sixty Swiss male albino mice (20-25 g) were divided into 10 groups, each of 6 animals were used. The first group: Received 1 ml of saline serving as control, second group: Received 50 mg/kg of 100% ethanol of plant extract, third group: Received 100 mg/kg of 100% ethanol of plant extract, fourth group: Received 50 mg/kg of 70% ethanol of plant extract, fifth group: Received 100 mg/kg of 70% ethanol of plant extract, sixth group: Received 50 mg/kg chloroform of plant extract, seventh group: Received 100 mg/kg chloroform of plant extract, eighth group: Received 50 mg/kg of ethyl acetate of plant extract, ninth group: Received 100 mg/kg of ethyl acetate of plant extract, tenth group: Received the reference drug, tramadol 20 mg/kg. Thirty minutes later, 0.6% acetic acid was injected intraperitoneal (0.2 ml /mice). Each mouse was then placed in an individual clear plastic observe chamber, and the total no of writhes/30 min. was counted for each mouse.^[9]

Antipyretic activity

This effect was carried out following the method of Buch *et al.*^[10] Thirty-six male albino rats of average body weight 100 g were divided into 6 groups; each group of 6 animals: First group: Rats that received 1 ml of saline serving as control, second group: Rats that received 100 mg/Kg of 100% ethanol of plant extract, third group:

Rats that received 100 mg/Kg of 70% ethanol of plant extract, fourth group: Rats that received 100 mg/ Kg of chloroform of plant extract, fifth group: Rats that received 100 mg/Kg of ethyl acetate of plant extract, sixth group: Rats that received 20 mg/kg of the reference drug, paracetamol. The normal rectal temperature was recorded before the start of the experiment. Pyrexia was induced by intramuscular injection of 1 mg/100 g body weight of 44% yeast suspension. The site of injection was then massaged to spread the suspension beneath the skin. After 18 hrs, the rectal temperature was recorded for all groups to serve as the base line of elevated body temperature, to which the anti-pyretic effect will be compared. One and two hrs later, other records of rectal temperature were determined.

Antioxidant activity

Male albino rats of the Sprague Dawley Strain (130 g-140 g) were injected intraperitoneally with alloxan (150 mg/kg body weight) to induce diabetes mellitus.^[11] Sixty-six rats were divided into 11 groups each of 6 animals: First group: Normal rats served as negative control received 1 ml saline, second group: Diabetic rats served as positive control received 1 ml saline, third group: Diabetic rats that received 7.5 mg/kg b.wt. of vitamin E as reference drug, fourth group: Diabetic rats that received 50 mg/Kg of 100% ethanol plant extract, fifth group: Diabetic rats that received 100 mg/Kg of 100% ethanol plant extract, sixth group: Diabetic rats that received 50 mg/Kg of 70% ethanol plant extract, seventh group: Diabetic rats that received 100 mg/Kg of 70% ethanol plant extract, eighth group: Diabetic rats that received 50 mg/Kg of chloroform plant extract, ninth group: Diabetic rats that received 100 mg/Kg of chloroform plant extract, tenth group: Diabetic rats that received 50 mg/Kg of ethyl acetate plant extract, eleventh group: Diabetic rats that received 100 mg/Kg of ethyl acetate plant extract. After 7 days, blood samples were collected from the rats.

Determination of blood glutathione

Glutathione in blood was determined according to the method of Beutler *et al.*^[12]

Glutathione (GSH) concentration in blood = $A_{\text{sample}} \times 66.66 \text{ mg/dl}$

Antihyperglycemic activity

Male albino rats of the Sprague Dawley Strain (130 g-140 g) were injected intraperitoneally with alloxan (150 mg/kg body weight) to induce diabetes mellitus. Hyperglycemia was assessed after 72 hrs by measuring blood glucose and after 2 and 4 weeks intervals. Animals were divided into 6 groups: First group: Diabetic rats that served as positive control, second group: Diabetic rats that received 100 mg/ Kg of 100% ethanol of plant extract, third group: Diabetic rats

that received 100 mg/Kg of 70% ethanol of plant extract, fourth group: Diabetic rats that received 100 mg/Kg of chloroform of plant extract, fifth group: Diabetic rats that received 100 mg/Kg of ethyl acetate of plant extract, sixth group: Diabetic rats that received 100 mg/Kg of cidophage drug as reference drug. At the end of each study period, blood samples were collected from the retro-orbital venous plexus through the eye canthus of anesthetized rats after an overnight fast. Serum was isolated by centrifugation, and the blood glucose level was measured.^[13]

Statistical analysis

The obtained data were analyzed by using the Student's *t* test.^[14]

RESULTS AND DISCUSSION

Chromatographic separation of the *n*-butanol led to the isolation of a new flavonoid glycoside (1) in addition to 5 known flavonoids. The isolated flavonoid compounds showed chromatographic properties and ultraviolet absorption spectra with shift reagents characteristic of flavones.

ESI-MS analysis of compound **1** showed molecular ion peak at m/z 593.181[M+1]⁺ corresponding to C₂₈H₃₂O₁₄. The ¹H-NMR spectrum [Table 1] displayed AA'BB' system of a disubstituted benzene ring at δ 7.98 (d, J=8.45) and 7.12 (d, J=8.45). The spectrum showed also a singlet at δ 6.69 for H-3, a singlet at δ 6.15 assigned for H-6, and a methoxy singlet at δ 3.86. For the glycosyl moiety, the spectrum showed two anomeric protons at δ 4.87 (d, J=7.68) for glucose and at δ 4.66 (d, J=1.5) together with the doublet at δ 1.1 (d, J=5.4) indicated a rhamnosyl group in the molecule. The downfield shift of the anomeric proton of glucose rather than the anomeric proton of rhamnose confirmed that the glucose is the terminal sugar and this was supported by comparison with spectral data of the isomeric compound acacetin 8-*C*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside,^[15] in which the anomeric proton of rhamnosyl was down field shifted than that of glucose, where the rhamnose is terminal sugar. Further confirmation was achieved by prolonged acid hydrolysis of compound **1** to give glucose

Table 1: ¹H-NMR spectral data of compound 1

H-position	Multiplicity	δ -Values
2',6'	doublet	7.98 (J=8.45)
3',5'	doublet	7.12 (J=8.45)
3	singlet	6.69
6	singlet	6.15
1''	doublet	4.66 (J=1.5)
1'''	doublet	4.87 (J=7.68)
OCH ₃	singlet	3.86
CH ₃ -Rh	doublet	1.1 (J=5.4)

in the sugar portion, indicating that the glucose was the terminal sugar. From these data, compound 1 [Figure 1] was identified as acacetin 8-C- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranoside.

The spectral data of the known compounds were in accordance with the published data. They were identified as apigenin 6,8-di-C- β -D-glucopyranoside (vicenin 2),^[16] apigenin-7 methyl ether 4'-glucoside,^[17] apigenin-4'-methyl ether 7-O-rutino-pyranoside,^[18] apigenin 7-O- β -D-glucopyranoside,^[19] and apigenin.^[19]

Bioassay

The 100% ethanol, 70% ethanol, chloroform, and ethyl acetate extracts of *A. paniculatum* were tested for their different pharmacological activity. Study of the acute toxicity of 70% ethanol extract of *A. paniculatum* leaves was the most safe up to 5 gm/kg. The anti-inflammatory activity results showed that the ethyl acetate (100 mg/ kg.b.wt.) is the most potent extract as it reduced the edema by 61.3%. These results nearly similar to the reference drug, indomethacin (64.6% change), followed by 100% ethanol (100 mg/ kg.b.wt.), ethyl acetate (50 mg/ kg.b.wt.), 70% ethanol (100 mg/ kg.b.wt.), 100% ethanol (50 mg/ kg.b.wt.), 70% ethanol (50 mg/ kg.b.wt.), then chloroform(100 mg/ kg.b.wt.) extracts with percent of change were 59.7%, 54.4%, 54.2%, 47.6%, 41.9%, and 27.8%, respectively. The least effective extract was the chloroform(50 mg/kg.b.wt.), 21.8% [Table 2]. The analgesic activity showed that the percent of change for the reference drug, tramadol, is 59.8%, and the results indicated that the 100% ethanol extract (100 mg/ kg.b.wt.) possess reasonable analgesic activity followed by 70% ethanol (100 mg/ kg.b.wt.), ethyl acetate (100 mg/ kg.b.wt.), 100% ethanol (50 mg/kg.b.wt.), 70% ethanol (50 mg/ kg.b.wt.), ethyl acetate (50 mg/kg.b.wt.) then chloroform (100 mg/kg.b.wt.) extracts with percent of change were 44.3%, 39.1%, 35.4%, 35.4%, 27.7%, 24.0%, and 18.0%, respectively. The least effective extract was the chloroform (50 mg/kg.b.wt.); the percent of change was 12.4%. Anti-pyretic activity of 100% ethanol extract was the most effective, the percent inhibition was 5.6% after 2 hrs, while it was 6.1% for the reference drug, subsequently by 70% ethanol then ethyl acetate extracts with percent of change 3.1% and 2.6% respectively, while the least anti-pyretic effective extract was the chloroform; the percent of change was [Table 3] 1.6%. Antioxidant activity results showed that the 100% ethanol (100 mg/kg) extract possess highly antioxidant activity; producing a percent of change 3.0% was nearly similar to thereference drug, vitamin E, producing a percent of change of 1.1%, followed by ethyl acetate (100 mg/kg), 100% ethanol (50 mg/kg) and 70% ethanol (100 mg/kg) extract with percent of change were 3.0%, 4.4%, 8.3%, and 10.5%, respectively, followed by the ethyl acetate (50 mg/kg), 70% ethanol (50 mg/ kg),

and chloroform (100 mg/kg) with percent change 12.7%, 15.2%, 21.1%, respectively. The least effective extract was the chloroform (50 mg/kg.b.wt.); the percent of change was 27.3% [Table 4]. The anti-hyperglycemic activity of 100%

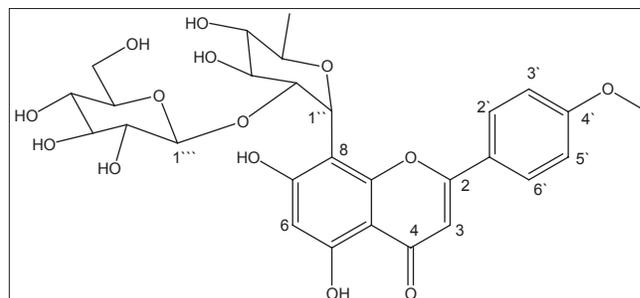


Figure 1: Structure of compound 1

Table 2: Acute Anti-inflammatory activity of 100% ethanol, 70% ethanol, chloroform and ethyl acetate extracts of *A. paniculatum* leaves

Group	Dose in mg/ kg.b.wt	% edema	
		Mean \pm S.E	% of Change
Control	1 ml Saline	61.8 \pm 1.7	-----
100% ethanol	50	32.4 \pm 1.1*	47.6
100% ethanol	100	24.9 \pm 0.6*	59.7
70% ethanol	50	35.9 \pm 1.2*	41.9
70% ethanol	100	28.3 \pm 0.9*	54.2
Chloroform	50	48.3 \pm 2.4*	21.8
Chloroform	100	44.6 \pm 2.1*	27.8
Ethyl acetate	50	28.2 \pm 1.3*	54.4
Ethyl acetate	100	23.9 \pm 0.8*	61.3
Indomethacin	20	21.9 \pm 0.3*	64.6

*Statistically significant at $P < 0.01$; Percentage of change calculated with reference to the control group (healthy rats that received 1 ml saline and kept under the same hygienic conditions)

Table 3: Analgesic effect of 100% ethanol, 70% ethanol, chloroform and ethyl acetate extracts of *A. paniculatum* leaves

Group	Dose in mg/kg.b.wt	No. of abdominal constrictions	% Inhibition
Control	1 ml Saline	48.3 \pm 1.2	-----
100% ethanol	50	31.2 \pm 0.7*	35.4
100% ethanol	100	26.9 \pm 0.3*	44.3
70% ethanol	50	34.9 \pm 0.8*	27.7
70% ethanol	100	29.4 \pm 1.1*	39.1
Chloroform	50	42.3 \pm 1.4	12.4
Chloroform	100	39.6 \pm 0.9*	18.0
Ethyl acetate	50	36.7 \pm 1.1*	24.0
Ethyl acetate	100	31.2 \pm 1.3*	35.4
Tramadol	20	19.4 \pm 0.6*	59.8

Percentage of inhibition calculated with reference to the control group (healthy rats that received 1 ml saline and kept under the same hygienic conditions)

Table 4: Antioxidant activity of 100% ethanol, 70% ethanol, chloroform and ethyl acetate extracts of *A. paniculatum* leaves in comparison with vitamin E

Group	Blood glutathione (mg%)	% change from control
Control (1 ml saline)	36.2±1.4	-----
Diabetic	21.7±0.6*	40.1
Diabetic+Vitamin E (7.5 mg/kg)	35.8±1.3	1.1
Diabetic+100% ethanol (50 mg/kg)	33.2±1.31	8.3
Diabetic+100% ethanol (100 mg/kg)	35.1±1.2	3.0
Diabetic+70% ethanol (50 mg/kg)	30.7±1.1*	15.2
Diabetic+70% ethanol (100 mg/kg)	32.4±1.1*	10.5
Diabetic+chloroform (50 mg/kg)	6.3±0.7*	27.3
Diabetic+chloroform (100 mg/kg)	28.6±0.9*	21.1
Diabetic+ethyl acetate (50 mg/kg)	31.6±1.1*	12.7
Diabetic+ethyl acetate (100 mg/kg)	34.6±0.7 *	4.4

* Statistically significant different from control group at $P < 0$

ethanol (100 mg/kg) extract showed a percent change in serum glucose level of 20.4% and 45.1% after 2 and 4 weeks, respectively. The metformin (100 mg/kg) rats group, the reference drug, shows a decrease in serum glucose level by 29.6% and 66.9% after 2 and 4 weeks, respectively. The percent change of 100% ethanol extract after 4 weeks exceeds that the reference drug after 2 weeks. It is evident from the presented results that the 100% ethanol extract possess significantly anti-hyperglycemic activity followed by 70% ethanol (100 mg/kg) then ethyl acetate (100 mg/kg) extract with percent of change 22.3%, 38.0%, and 26.3%, 37.5% after 2 and 4 weeks, respectively. The least effective extract was the chloroform extract with percent of change 13.4% and 24.3% after 2 and 4 weeks.

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