Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 4 pp. 467–471, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

NATURAL DRUGS

POLYPHENOLIC COMPOUNDS FROM *ABUTILON GRANDIFLORUM* LEAVES

MARIA SIKORSKA and IRENA MATŁAWSKA*

Department of Pharmacognosy, Poznań Medical University of Medical Sciences,
4 Święcickiego Str., 61-771 Poznań, Poland

Abstract: The flavonol glycosides: kaempferol 3-*O*-β-(6"-*p*-coumaroyl)-glucopyranoside, kaempferol and quercetin 3-*O*-β-glucopyranosides and 3-*O*-β-rutinosides were isolated and identified from the leaves of *Abutilon grandiflorum*. Their structures were established by chemical and spectroscopic methods. The presence of phenolic acids: *p*-hydroxybenzoic, *p*-coumaric, syringic, and vanillic was confirmed by TLC.

Keywords: *Abutilon grandiflorum* leaves, flavonoids, phenolic acids, isolation, identification

The *Abutilon* L. genus of the *Malvaceae* family comprises about 150 annual or perennial herbs, shrubs or even small trees widely distributed in the tropical and subtropical countries of America, Africa, Asia and Australia. *Abutilon grandiflorum* G. Don. (equal amounts of dried and pulverized leaves and root bark, administered as a tea) is used traditionally in the Tanzania for treating malaria, infectious venereal diseases and "mental disorders" (a common local synonym for severe forms of malaria). The *Abutiolon* extracts showed in *in vivo* and *in vitro* studies anti-malarial effects, but compounds responsible for this activity have not been identified yet. So far, only the presence of N-*cis* and N-*trans*-feruoyltyramine, palmitic and stearic acids together with their 1-and 2-mono acyl glycerols as well as coniferyl alcohol was confirmed in the extracts (1). These classes of compounds, however, seems to be of a little importance for the observed anti-malarial activity. To the best of our knowledge, the leaves of *Abutilon grandiflorum* have not been investigated yet on the phenolics content.

EXPERIMENTAL

Plant material

The leaves of *Abutilon grandiflorum* G. Don were collected in the 2004 year from the plants cultivated in the garden of the Department of Medicinal Plants, Poznań University of Medical Sciences. A voucher specimen has been deposited in the author's laboratory.

Extraction and isolation

The dried leaves (63 g) were extracted 4× with boiling MeOH. The extract was concentrated to a syrupy mass, treated with hot H_2O and filtered. The filtrate was extracted successively with $CHCl_3$, Et_2O and n-BuOH. The Et_2O extract (2.4 g) was chromatographed (CC) on a Sephadex LH-20 column with MeOH as an eluent, yielding fraction **I,** containing free phenolic acids, and fraction **II** containing compound **1**.

The n-BuOH extract (5.6 g) was separated by preparative paper chromatography (PC, Whatman No.3, S_1) to yield a mixture of flavonoids **2**, **3, 4**, and **5.** The individual compounds were finally separated by preparative paper chromatography (PC, Whatman No.3, S_2) to yield compounds **2, 3, 4, 5**. Final clean-up of all compounds was achieved by column chromatography (CC) on Sephadex LH-20 (S_6).

Identification

Chromatography, solvent systems

PC: Whatman No.1 or 3: S_1- HOAc – H_2O (15 : 85, v/v); S_2 – EtOAc -HCO_2H – H_2O (10 : 2 : 3, v/v/v) organic phase, S_3 _ n-BuOH – HOAc – H_2O (4 : 1 : 2, v/v/v).

TLC: Silica gel 60G, Merck: S_4 _ n-PrOH – EtOAc – H_2O (7 : 2 : 1, v/v/v).

2D-TLC: DC-Alufolien, cellulose, Merck: 1st direction S_5 _ C_6H_5Me – HOAc – H_2O (6 : 7 : 3, v/v/v) organic phase; 2nd direction S_1.

CC: Sephadex LH-20 (Pharmacia, Uppsala): S_6 – MeOH.

* Corresponding author: e-mail: irenamatlawska@poczta.onet.pl

Visualization, reagents

Flavonoids: 1% methanolic $AlCl_3$, 0.1% methanolic NA reagent, UV_{365nm}.
Sugars: aniline phthalate and heating at 105°C, vis.
Phenolic acids: UV_{365nm}, diazotized sulfanilic acid and 20% Na_2CO_3 (1:1), vis.

Partial acid hydrolysis

1 mg of compounds **4** and **5** was heated in 1 mL of 0.5% HCl. After 15 min the process was stopped. The obtained hydrolyzates were extracted with ethyl acetate and the organic phases were separated by preparative chromatography (PC, Whatman No.1 S_1). The bands corresponding to the intermediate products were eluted and subjected to UV spectral analysis and complete hydrolysis.

Enzymatic hydrolysis

2 mg of compounds **2** and **3**, were treated with 1 mg of β-glucosidase (Koch-Light) in 1 mL of H_2O at room temp. until the process was completed (the progress of hydrolysis was monitored by PC in S_1). The hydrolyzates were extracted with ethyl acetate to obtain aglycons, whereas the sugars remained in the water layers.

Total acid hydrolysis

1 mg of compounds **2**, **3**, **4** and **5** and the intermediate products obtained during the partial acid hydrolysis**,** were heated in 1 mL of 1% HCl for 1 h. Then the hydrolyzates were extracted with ethyl acetate. The aglycones present in the organic phases were identified by co-chromatography (PC S_2, S_3), whereas the water layers were analyzed for sugars (TLC, S_4).

Alkaline hydrolysis

2 mg of compound **1**, were treated with 0.1 M NaOH at room temp. for 1 h. Then the mixture was acidificated to pH = 4 with 5% HCl. The hydrolyzate was extracted with diethyl ether and *p*-coumaric acid was identified by PC in S_1.

Standards

Flavonoids: compounds previously isolated and identified from different species of the *Malvaceae* family (2-5), sugars: Merck, phenolic acids: Sigma.

Spectral analysis

The UV spectra were recorded on a Perking Elmer Lambda 35 UV/VIS Spectrometer, in methanol before and after addition of the shifts reagents according to Mabry (6). The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in DMSO-d_6 on a Varian Unity 300 MHz Spectrometer with TMS as an internal standard. The chemical shifts are given in ppm.

Kaempferol 3-*O*-β-(6"-*cis*/*trans*-*p*-coumaroyl)-glucopyranoside [1]

PC: R_f S_1 = 0.36 *cis*-, 0.27 *trans*-, yellow needles (10 mg). UV_{lmax} MeOH: 269, 301, 319, 359; + NaOAc 276, 317, 371; +NaOAc/H_3BO_3 267, 315,

Table 1. ^{1}H NMR data of **1** (CD_3OD); **2**, **3**, **4**, **5** (DMSO-d_6 + D_2O d, ppm, *J* = Hz)

Proton	**1**		**2**	**3**	**4**	**5**
	cis	*trans*				
aglycone						
6	6.1d (1.8)	6.1d (1.8)	6.2d (2.0)	6.2d (2.0)	6.2d (2.0)	6.3d (2.0)
8	6.1d (2.0)	6.2d (2.0)	6.5d (2.0)	6.4d (2.0)	6,4d (1.9)	6.6d (2.0)
2'	7.9d (8.8)	7.9d (8.2)	7.5d (1.9)	8.0dd (8.6; 2.0)	7.5d (2.0)	8.2dd (8.6; 2.0)
3'	6.8d (8.8)	6.8d (8.8)	–	6.8dd (8.9; 2.0)	–	7.0dd (8.8; 2.0)
5'	6.8d (8.8)	6.8d (8.8)	6.9d (8.5)	6.8dd (8.9; 2.0)	6.8d (8.4)	7.0dd (8.8; 2.0)
6'	7.9d (8.8)	7.9d (8.2)	7.5dd (1.9; 8.4)	8.0dd (8.6; 2.0)	7.5dd (2.0; 10)	8.2dd (8.6; 2.0)
p-coumaric acid						
2''' 6'''	7.5d (8.6)	7.3d (8.6)				
3''' 5'''	6.6d (8.7)	6.7d (8.4)				
7'''	6.7d (12.5)	7.4d (16.0)				
8'''	5.5d (12.6)	6.0d (15.7)				
sugar						
glucose1''	5.2d (7.6)	5.2d (7.6)	5.3d (7.5)	5.4d (9.1)	5.3d (7.6)	5.2d (8.0)
rhamnose 1'''					4.39s	4.5s
CH_3 of rhamnose					1.0d (5.0)	1.0d (5.0)

Table 2. ^{13}C NMR data of **1** (CD_3OD) and **2**, **3**, **4**, **5** (DMSO-d_6, δ ppm)

Carbon	**1**	**2**	**3**	**4**	**5**
aglycone					156.3
2	159.5	156.3	156.3	156.4	132.9
3	133.8	133.2	133.1	133.0	177.2
4	179.4	178.9	177.5	177.0	161.1
5	163.0	161.5	161.2	163.0	98.1
6	99.9	98.9	98.7	98.5	163.8
7	165.9	164.2	164.0	163.9	93.5
8	94.9	93.4	93.7	93.4	155.9
9	158.3	156.5	156.2	156.3	104.2
10	105.7	104.2	103.4	104.0	120.7
1’	122.7	121.0	120.8	121.1	129.7
2’	131.1	115.5	129.9	115.1	115.0
3’	116.8	144.6	114.9	144.6	159.6
4’	161.4	148.3	159.9	148.1	115.0
5’	116.8	116.6	114.9	116.0	130.2
6’	131.0	121.4	130.0	121.0	
glucose					101.5
1”	104.1	100.5	101.0	100.6	74.2
2”	75. 8	73.9	74.0	73.9	76.0
3”	78.0	77.8	76.5	75.9	70.5
4”	71.6	70.9	70.2	70.7	74.8
5”	74.9	77.2	77.1	76.8	66.7
6”	64.2	61.4	61.0	66.8	
p-coumaric acid					
1”’	127.5				
2”’, 6”’	132.2				
3”’, 5”’	116.0				
4”’	159.8				
7”’	145.0				
8”’	115.6				
9”’	167.7				
rhamnose					
1”’				101.0	100.1
2”’				70.3	71.0
3”’				69.9	70.0
4”’				71.8	71.5
5”’				68.2	68.3
6”’				17.6	17.5

359; + NaOMe 273, 314, 370: + $AlCl_3$ 275, 306, 396; + $AlCl_3$ /HCl 275, 308, 396 nm. Total acid hydrolysis: kaempferol, *p*-coumaric acid, and glucose. Alkaline hydrolysis: kaempferol 3-glucoside, *trans*- and *cis*-*p*-coumaric acid. ^1H and ^{13}C NMR (Tables 1 and 2).

Quercetin 3-*O*-b-glucopyranoside [2]

PC: R_f S_1 = 0.38, yellow needles (10 mg). UV_{lmax} MeOH: 252, 267 (285), 348; + NaOAc 277, 326, 384; + NaOAc/H_3BO_3 267, 297, 370; + NaOMe 275, 326, 406; + $AlCl_3$ 277, 300, 436; +$AlCl_3$ /HCl 274, 297, 360, 400 nm. Total acid and enzymatic hydrolysis: quercetin and glucose. ^1H and ^{13}C NMR (Tables 1 and 2).

Kaempferol 3-*O*-b-glucopyranoside [3]

PC: R_f S_1 = 0.45, yellow needles (20 mg). UV_{lmax} MeOH: 269, 355; + NaOAc 277,306, 379; + NaOAc/H_3BO_3 269, 358; + NaOMe 270, 327, 408; + $AlCl_3$ 270, 305, 352, 400; + $AlCl_3$ /HCl 278, 305, 350, 400 nm. Total acid and enzymatic hydrolysis: kaempferol and glucose. ^1H and ^{13}C NMR (Tables 1 and 2).

Quercetin 3-*O*-α-rhamnopyranosyl (1→6) – β-glucopyranoside [4]

PC: R_f S_1 = 0.49, yellow amorphous powder (35 mg). UV_{lmax} MeOH**:** 260, 270, 366; + NaOAc 276, 386; + NaOAc/H_3BO_3 254, 386; + NaOMe 275, 418; + $AlCl_3$ 277, 310, 441; + $AlCl_3$ /HCl 273, 312, 370, 412 nm. Total acid hydrolysis: quercetin, glucose, rhamnose.

Partial acid hydrolysis: quercetin 3-*O*-glucoside as a secondary heteroside, chromatographically identical with compound **2**. ^1H and ^{13}C NMR (Tables 1 and 2).

Table 3. Chromatographic analysis of free phenolic acids from fraction **I**

Phenolic acid	R_f in solvent system		Fluorescence	color
	I[st] direction S_5	II[nd] direction S_1	$UV_{365\ nm}$	daylight
p-hydroxybenzoic	0.40	0.65	brown	yellow
p-coumaric	0.41	0.53 (*trans*-); 0.77 (*cis*-)	blue	red-violet
vanillic	0.56	060	brown	orange
syringic	0.95	0.60	brown- violet	red

Kaempferol 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranoside [5]

PC: R_f S_1 = 0.52, yellow needles, (9 mg). UV_{lmax} MeOH: 267, 352; + NaOAc 279, 378; + NaOAc/H_3BO_3 270, 350; + NaOMe 270, 400; + $AlCl_3$ 277, 303, 348, 400; + $AlCl_3$ /HCl 277, 300, 345, 400 nm. Total acid hydrolysis: kaempferol, glucose, rhamnose. Partial acid hydrolysis: kaempferol 3-*O*-glucoside as a secondary heteroside, chromatographically identical with compound **3**. ^{1}H and ^{13}C NMR (Tables 1 and 2).

RESULTS AND DISCUSSION

From the methanolic extract of the leaves of *Abutilon grandiflorum,* preliminary purified with chloroform, Et_2O and n-butanol extracts were obtained. Separation of the Et_2O extract on a Sephadex LH-20 column eluted with methanol yielded fractions **I** and **II**. Comparison of the studied fraction with standards (i.e. R_f values, fluorescence under UV_{365nm} and spot colors before and after visualization with mixture of diazotized sulfanilic acid and Na_2CO_3) showed that fraction **I** contained *p*-hydroxybenzoic, *p*-coumaric, vanillic and syringic acids (Table 3). From fraction **II** compound **1**, chromatographically identical with tiliroside was obtained, as a mixture of *cis*/*trans*- isomers. Its structure was confirmed by ^{1}H and ^{13}C NMR spectra (Table 1 and 2). The presence of both isomers was deduced from the location of H-7''' and H-8''' signals as well as their coupling constants: *cis*-isomer (H-7''' at 6.7 ppm, *J* = 12.5 Hz; H-8''' at 5.5 ppm, *J* = 12.6 Hz) and *trans* isomer (H-7''' at 7.4 ppm, *J* = 16.0 Hz; H-8''' at 6.0 ppm, *J* = 15.7 Hz) (Table 1).

The n-butanol extract containing a complex of flavonoid compounds was separated by preparative chromatography on Whatman No. 3 paper with S_1 and S_2 solvent systems and finally purified on Sephadex LH-20 column eluted with methanol. As a result, four known flavonoids **2**, **3**, **4, 5** were isolated. Identification of those compounds was carried out by chromatographic analysis of their hydrolysis products, co-chromatography with standards, as well as by UV, ^{1}H and ^{13}C NMR spectroscopy (2-5). The obtained results for (**1**) were in agreement with the data recorded for the *cis*/*trans*-tiliroside, previously isolated from the flowers of *Lavatera thuringiaca* (2), whereas those for quercetin 3-*O*-β-glucopyranoside [**2**], kaempferol 3-*O*-β-glucopyranoside [**3**], quercetin 3-*O*-α-rhamnopyranosyl (1→6)-β- glucopyranoside [**4**] and kaempferol 3-*O*-α-rhamnopyranosyl(1→6)-β-glucopyranoside [**5**] were in accord with literature data (2-5).

Quercetin and kaempferol analogues are popular in plants, including those from the *Malvaceae* family. Kaempferol 3-O-β-(6"-*p*-coumaroyl) glucoside (tiliroside), was isolated for the first time from *Tilia argentea* by Hörhammer (10). Since then, it has been reported to occur in plants from different families, including the *Abutilon* genus: *Abutilon theophrasti* and *A. indicum* leaves and flowers (3, 4), as well as the other species of the *Malvaceae* family: *Althaea officinalis* L. and *A. rosea* (L.) *Cav* var. *nigra* (11, 5), *Lavatera thuringiaca* L. (2) with *trans*-form as a major component. *Trans*- and *cis*-*p*-coumaroyl esters of kaempferol and quercetin diglucosides from *Strychnos variabilis* were separated as individual compounds (12).

The present paper is a continuing work on flavonoids in the *Malvacae* family plants. During our earlier search on the *Abutilon* species, we found that quercetin and kaempferol 3-*O*-β-glucopyranosides as well as their 3-*O*-rutinosides predominated in the *Abutilon theophrasti* flowers and leaves as well as *A. indicum* leaves. Additionally, quercetin 7-*O*-β-glucopyranoside, together with quercetin and kaempferol 7-*O*-diglucosides and myricetin 3-*O*-β-glucuronopyranoside were isolated and identificated from the *Abutilon theophrasti* flowers.

The most interesting flavonoids, which structures were elucidated by means of acid hydrolysis and spectroscopic methods (UV, HPLC/ESI-MS, ^{1}H, ^{13}C NMR, COSY, HMQC, HSQC and HMBC experiments) were hypolaetin and isoscutellarein 8-*O*-β-glucuronopyranoside 3"-*O*-sulfates, together with hypolaetin 8-*O*-β-glucuronopyranoside found in *A. indicum* leaves. The antimicrobial activity of

hypolaetin and isoscutellarein 8-*O*-β-glucuronopyranoside 3"-*O*-sulfates against *Staphylococcus aureus* was confirmed (13). Flavones: luteolin, chrysoeriol together with luteolin, chrysoeriol, apigenin 7-*O*-β-glucopyranosides were found only in the flowers of *A. indicum* (4). In this species gossypetin 8-glucoside and gossypetin 7-glucoside and cyanidin 3-rutinoside were confirmed by Subramanian (14).

This is the first report on flavonoids and phenolic acids in the leaves of *Abutilon grandiflorum* G. Don.

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Received : 12. 07. 2007