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BIOACTIVE PHENOLICS FROM *BUDDLEIA MADAGASCARIENSIS*

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ABSTRACT

Chromatographic investigation of the alcoholic extract of *Buddleia madgascariensis* Lam. resulted in the isolation and identification of 6-O-(3``-O-*p*-coumaroyl-$\alpha$-L-rhamnopyranosyl) catalpol, 6-O-(3``-O-*p*-coumaroyl-α-L- rhamno- pyranosyl) aucubin, acteoside, acaciin, rutin and mimengoside. These compounds were identified on the basis of chemical and spectroscopic analyses. Antibacterial and antioxidant activities of some isolated compounds were tested.

INTRODUCTION

Buddleia madgascariensis Lam. (Buddleiaceae) is indigenous to Madagascar, and cultivated in many subtropical countries for its traditional use to cure asthma, cough and bronchitis as well as a soap substitute[1]. Plants of the genus *Buddleia* are widely used for treatment of liver ailments, diarrhoea, headache and rheumatism as well as for wound healing[2,3]. Many of their chemical constituents were identified and proved to possess antimicrobial[4], antifungal[5], antihepatotoxic[6], skin lightening[7], anti-cataract[8], anti-oxidant[9], anti-inflammatory and analgesic activities[10]. Three flavonoid glycosides[11], in addition to acteoside, aucubin, methyl catalpol[12] and mimengoside A[1,13] were previously reported in *Buddleia madgascariensis*.

It was reported that, active oxygen molecules such as superoxide, (O_2, OOH), hydroxyl (OH) and peroxyl (ROOH) radicals play an important role in oxidative stress related to the pathogenesis of different diseases such as Alzheimer, cataracts, liver toxicity, inflammation processes and DNA damage that leads to carcinogenesis[14]. It was decided also to evaluate the free radical scavenging activity of some of isolated compounds that are expected to have antioxidant potential in comparison with that of ascorbic acid as a standard.

The present study illustrates the isolation and identification of the phenolic compounds and other active constituents.

In addition, the antibacterial activity of some isolated compounds were studied with the aim of discovering additional new natural compound with non-toxic, potent anti-bacterial activity. Owing to the potential role of antioxidants in protection against some heart and liver diseases, cancer (colon, breast, skin) as well as age-related degenerative conditions[15].

MATERIAL AND METHODS

Plant material:

Buddleia madgascariensis Lam. was collected from the Medicinal Plants Garden, Faculty of Pharmacy, Mansoura University in June, 1998. A voucher specimen is kept at the Deparment of Pharmacognosy at the same faculty.

Material and equipment:

Thin layer chromatography (TLC): Precoated silica gel 60 F_{254} (E. Merck, Germany); column chromatography (CC): Kieselgel 70-230 mesh (E. Merck); Rotary flash evaporator (Wheaton, USA); Melting point apparatus: (Fischer-Hohn Scientific Co., USA); UV-spectrophotometer (Schimadzu,1601 PC. Japan); IR-spectrophotometer (Buck 500, USA); ^1H and ^{13}C-NMR spectra were recorded on Varian VXR 400 FT spectrometer operating at 400 and 100 MHz respectively ; Phosphate buffer (0.1 M, pH 7.4) was prepared by mixing 50 ml of $NaH_2PO_4.H_2O$ (0.1 M) with 93.5 ml of NaOH (0.1 M) and volume was completed to 200 ml with distilled water; Nitro blue tetrazolium (NBT) (Sigma Chemical Co.): one tablet (containing 10 mg of NBT) was dissolved in 2.325 ml of phoshate buffer to give 5 mM solution; Phenazine methosulphate (PMS) (Sigma Chemical Co.): 1.5 mg of PMS was dissolved in 2.5 ml of phosphate buffer to give 2 mM solution (freshly prepared); Reduced form of β–nicotinamide adenine dinucleotide phoshate disodium salt (β-NADPH-2Na) (Sigma Chemical Co.): 2.6 mg of β-NADPH-2Na was dissolved in 1 ml buffer to give 3.12 mM solution; Ascorbic acid (Sigma Chemical Co.): as a standard and test compounds each 2 mM methanolic solution; Nutrient agar: prepared using peptone (5 g, Sigma Chemical Co.), beef extract (2 g, Sigma Chemical Co.), sodium chloride (2 g, Sigma Chemical Co.), Agar (20 g, Sigma Chemical Co.), distilled water to 1000 ml.; autoclave (Sigma Chemical Co.); Staphylococcus *aureus* IFO3060, *Pseudomonas aeruginosa* IFO3448 and *E. coli* IFO3301 (The strains were obtained from Departement of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt); gentamicin ampoule 10 mg (E. Merck).

Extraction and isolation:

The aerial parts of *Buddleia madgascariensis* were shade dried, reduced to fine powder (3.5 kg) and exhaustively extracted with methanol. The methanolic extract was evaporated under vacuum to a syrupy liquid. The extract was suspended in water and, successively extracted with n-hexane, $CHCl_3$, ethyl acetate and n-butanol. The ethyl acetate extract (109.9 g) was gradiently chromatographed on silica gel column using ethyl acetate containing increased proportions of MeOH/water. The fractions were monitored using precoated silica gel plates, ethyl acetate/MeOH/water (100:4:3) as solvent system and vanillin/H_2SO_4 as a spray reagent to afford materials **1** (R_f:0.61, 600 mg), **2** (R_f:0.57, 15 mg) and **4** (R_f:0.3, 50 mg). The butanol extract (16.5 g) was chromatographed on silica gel column and eluted with ethyl acetate-MeOH-water (100:12.5:7.5), fractions monitored using formentioned solvent. Three compounds were obtained: **3** (R_f 0.59, 150 mg), **5** (R_f 0.40, 25 mg) and **6** (R_f 0.20, 40 mg).

Strong acid hydrolysis:

Five mg of the tested compound were dissolved in 5 ml methanol, mixed with equal volume of 5% HCl (w/v) and then refluxed for two hrs. The solvent was distilled off, and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and the solvent was distilled off. The residue was dissolved in spectroscopic methanol and kept for identification of the aglycone. The acid mother liquor was neutralised with sodium bicarbonate. The filtrate was evaporated to dryness. The residue was dissolved in pyridine and used for identification of the sugar moiety.

Alkaline hydrolysis (after acid hydrolysis):

The pyridine solution (from acid hydrolysis) was mixed with 2 ml methanol and 5 ml 2M NaOH in glass syringe (10 ml). Air was expelled and the mixture was left for 2 hrs. in dark. The mixture was transeferred into a flask containing 5 ml HCl (2M). The solution was evaporated to dryness and the liberated acid was extracted with ether. The residue containig the deacylated compound was used for detection of the sugar moiety[16].

The antioxidant activity of the isolated compounds:

Principle

The superoxide scavenging activity of the isolated compounds was assessed using a spectrophotometric method[17]. In this method, superoxide anion was generated by reacting phenazine methosulfate (PMS) with reduced form of β-nicotinamide adenine dinucleotide phosphate disodium salt (β-NADPH-2Na). The produced superoxide reacted with the yellow-colored nitro blue tetrazolium (NBT) to produce a blue-colored product with a maximum absorbance at λ_{max} 580 nm. Superoxide scavengers reduce the intensity of the blue color in a potency/concentration-dependant manner.

Procedure:

The assay was carried out according to the method described by Yen and Duh 1994[18]. Table (1) illustrates the content of the different reaction mixtures. The reaction mixture contained (as final concentration): the sample to be tested (50 µM), PMS (20 µM), β-NADPH-2Na (156 µM) and NBT (50 µM) in phosphate buffer (0.1 M, pH 7.4). The final volume of the reaction mixture was 2 ml. The blank sample did not contain NBT, while both blank and control mixtures contained pure methanol instead of the test compound. The samples were incubated at room temperature for 5 minutes and the developed color was measured at 580 nm. All tests were run in triplicate and averaged. The control mixture should give the maximum absorbance against blank solution. The percent inhibition of superoxide generation was calculated for each compound as follows:

$$\%\text{Inhibition of superoxide production} = \frac{(\text{Control absorbance} - \text{test absorbance})}{\text{Control absorbance}} \times 100$$

Table (1): Composition of the reaction mixtures of free radical scavenging bioassay:

Tube	NBT (20 µl)	PMS (20 µl)	Buffer (ml)	Test (50 µl)	NADPH (100 µl)
Blank	-------	+	1.830	methanol	+
Control	+	+	1.810	methanol	+
Test	+	+	1.810	+	+
Standard (Vit. C)	+	+	1.810	+	+

2) The antimicrobial activity of some compounds:

Agar diffusion method was adopted using nutrient agar seeded with either gram-positive (*Staphylococcus aureus)* or gram-negative (*Pseudomonas aeruginosa* or *Escherichia coli*) bacteria. For screening, each tested compound (10 mg/ml in DMF) and DMF (as negative control) and gentamicin (as positive control, 5 mg/ml, in sterile water) were added into the corresponding cups in different seeded strain agar, allowed to diffuse and incubated at 37°C for 24 h. Zones of inhibition were measured using a caliper to the nearest 0.5 mm.

For assay, four concentrations of 6-O-(3``-O-*p*-coumaroyl-α–L-rhamnopyranosyl) catalpol were prepared by two-fold serial dilution using DMF (50000 µg/ml, 25000 µg/ml, 12500 µg/ml and 6250µg/ml), also four concentrations of gentamicin were prepared by the same method using sterile distilled water (5000 µg/ml, 2500 µg/ml, 1250 µg/ml and 625 µg/ml).

100 µl of each concentration was added into the corresponding cups in *E. coli* and *Pseudomonas aeruginosa* seeded agar and 100 µl DMF was added in the fifth cup as a control, allowed to diffuse and incubated at 37°C for 24 h. The inhibition zones were measured to the nearest 0.5 mm. A standard curve of both gentamicin and 6-O-(3``-O-*p*-coumaroyl- α–L-rhamnopyranosyl) catalpol were drawn by plotting log concentration against square of inhibition distance.

6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol (1), white amorphous powder; UV spectrum λ_{max}: 317 and 227.2 nm; IR(cm^{-1}): 3475 (OH), 2934 (CH), 1691 (CO of ester), 1604 and 1514 (aromatic), 1406, 1287, 1136, 834 and 658 cm^{-1}; ^{1}H-NMR (DMSO-d_6) signals at δ ppm :5.01 (1H, *d*, *J* = 9.9 Hz, H-1), 6.43 (1H, *d*, *J* = 6.7 Hz, H-3), 5.02 (H, *dd*, *J*=6, 4.5 Hz, H-4), 2.31 (1H, *m*, H-5), 3.92 (1H, *m*, H-6), 3.68 (1H, *m*, H-7), 2.4 (1H, *t*, *J*=9.9 Hz, H-9), 3.8 (2H, *s*, H-10), 4.62 (1H, d, *J* = 7.8 Hz, H-1`), 3.06 (1H, *m*, H-2`), 3.16-3.24 (1H, *m*, H-3`), 3.16-3.24 (1H, *m*, H-4`), 3.44 (1H, *m*, H-5`), 3.91-3.95 (1H, *m*, $H_a$-6`), 3.66-3.74 (1H, *m*, H_b-6`), 4.91 (1H, *d*, *J* = 1.2 Hz, H-1``), 3.91-3.95 (1H, *m*, H-2``), 4.9 (1H, *dd*, *J* = 9.6 Hz, 2.8, H-3``), 3.55 (1H, *m*, H-4``), 3.63-3.73 (1H, *m*, H-5``), 1.21 (3H, *d*, *J* = 6, H-6``), 6.39 (1H, *d*, *J*=15.8, H-α```) and 7.59 (1H, *d*, *J*=15.8 Hz, H-β```), 7.59 (2H, *d*, *J* = 8.4 Hz, H-2``` and H-6```), 6.81 (2H, *d*, *J*=8.4 Hz, H –3``` and H-5```); ^{13}C-NMR: Table 2.

6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) aucubin (2), white amorphous powder; ^{1}H-NMR (CD_3OD) signals at δ ppm :5.06 (1H, *d*, *J* = 9.5 Hz, H-1), 6.42 (1H, *d*, *J* = 5.8 Hz, H-3), 5.17-5.12 (H, *dd*, H-4), 2.8-2.7 (1H, *dd*, H-5), 3.87 (1H, *m*, H-6), 5.89 (1H, *br.s*, H-7), 2.9-2.8 (1H, *m*, H-9), 3.6 (2H, *s*, H-10), 4.77 (1H, *d*, *J* = 7.9, H-1`), 3.25-3.21 (1H, *m*, H-3`), 3.25-3.21 (1H, *m*, H-4`), 3.49 (1H, *dd*, H-5`), 4.9 (1H, d, *J* = 1.2 Hz, H-1``), 4.73 (1H, *dd*, *J* = 7.8, 2.3, Hz, H-3``), 1.32 (3H, *d*, *J* = 6, H-6``), 6.38 (1H, *d*, *J*=15.6 Hz, H-α```) and 7.68 (1H, *d*, *J*=15.6 Hz, H-β```), 7.46 (2H, *d*, *J* = 8.4 Hz, H-2``` and H-6```), 6.81 (2H, *d*, *J*=8.4 Hz, H –3``` and H-5```); ^{13}C-NMR: Table 2.

Acteoside (3), orange-brown amorphous powder, UV: λ_{max} 332 and 217 nm.; IR(cm^{-1}): 3470 (OH), 2929 (CH), 1694 (CO of ester), 1602 and 1520 (aromatic), 1446, 1155, 810 and 648 cm^{-1}; ^{1}H-NMR (CD_3OD) signals at δ ppm : 6.67 (1H, *d*, *J* = 2 Hz, H-2), 6.69 (1H, *d*, *J* = 8 Hz, H-5), 6.56 (1H, *dd*, *J* = 2, 8 Hz, H-6), 4.04 (1H, m, α-CH_{2a}), 3.72 (1H, *m*, α-CH_{2b}), 2.79 (1H, *m*, β-CH_2) 4.38 (1H, *d*, *J* =7.8 Hz, H-1`), 3.4 (1H, *dd*, *J* = 7.8, 9 Hz, H-2`), 3.82 (1H, *t*, *J* = 9 Hz, H-3`), 4.93 (1H, *m*, 4`), 3.61- 351 (2H, *m*, H-5`, H-6`), 4.93 (1H, *d*, *J* = 1.2 Hz, H-1```), 3.93 (1H, *m*, H-2```), 3.61- 351 (2H, *m*, H-3``, H-5``), 3.31 (1H, *m*, H-4``) 1.1 (3H, *d*, *J* = 6.12 Hz, H-6```), 7.07 (1H, *d*, *J* = 1.5 Hz, H-2```), 6.79 (1H, *d*, *J* = 8 Hz, H-5```), 6.96 (1H, *dd*, *J* = 8, 1.5 Hz, H-6```), 6.28 (1H, *d*, *J* = 15.8 Hz, α-C<u>H</u>), 7.6 (1H, *d*, *J* = 15.8, β-C<u>H</u>); ^{13}C-NMR: Table 3.

Acaciin (4), pale yellow crystals, IR(cm^{-1}): 3480 (OH), 2812 (CH), 1657 (CO), 1612 and 1500 (aromatic), 1439, 1250, 1022, 825 and 766 cm^{-1}; UV: λ_{max}^{MeOH} 325, 270 nm, λ_{max}^{NaOMe} 360, 286, $\lambda_{max}^{AlCl_3}$ 382, 340, 301, 278; $\lambda_{max}^{AlCl_3/HCl}$ 378, 336, 300, 277; $\lambda_{max}^{sod\ acetate}$ 324, 272; $\lambda_{max}^{sod\ acetate/Boric\ acid}$ 324, 271. Acid hydrolysis afforded the aglycone, whose UV: λ_{max}^{MeOH} 327 and 270 nm, λ_{max}^{NaOMe} 364, 276, $\lambda_{max}^{AlCl_3}$ 377, 340, 301, 276; $\lambda_{max}^{AlCl_3/HCl}$ 372, 337, 299, 276; $\lambda_{max}^{sod\ acetate}$ 349, 276; $\lambda_{max}^{sod\ acetate/Boric\ acid}$ 320, 270. ^{1}H-NMR (DMSO-d_6) signals at δ ppm :6.94 (1H, *s*, H-3), 6.45 (1H, *d*, *J* = 2 Hz, H-6), 6.78 (1H, *d*, *J* = 2 Hz, H-8), 8.05 (2H, d, *J* = 9 Hz, H-2`, H-6`),7.14 (2H, *d*, *J* = 9 Hz, H-3`, H-5`), 3.86 (3H, *s*, OCH_3), 5.06 (1H, *d*, *J* = 7.3 Hz, H-1``), 4.55 (1H, *d*, *J* = 1.5 Hz, H-1```), 1.07 (3H, *d*, *J* = 6 Hz, H-6```); ^{13}C-NMR: Table 4.

Rutin (5), pale yellow crystals mp. 190-195°C, UV (nm): λ_{max}^{MeOH} 358, 295sh, 265sh, 258 nm, λ_{max}^{NaOMe} 410, 331sh, 273, $\lambda_{max}^{AlCl_3}$ 427, 336sh, 302sh, 274 $\lambda_{max}^{AlCl_3/HCl}$ 396, 360, 299sh, 269; $\lambda_{max}^{sod\ acetate}$ 412, 316, 270, $\lambda_{max}^{sod\ acetate/Boric\ acid}$ 380, 263; ^{1}H-NMR (DMSO-d_6) signals at δ ppm : 6.17 (1H, *d*, H-6), 6.4 (1H, *d*, H-8), 7.51 (1H, *d*, *J* = 2, H-2`),6.83 (1H, d, *J* = 8, H-5`), 7.52 (1H, *dd*, *J* =2, 8, H-6`), 5.3 (1H, *d*, *J* = 6.32, H-1``), 4.35 (1H, *d*, *J* = 1.5, H-1```), 0.97 (3H, *d*, *J* = 5.8, H-6```), ^{13}C-NMR: Table 4.

Mimengoside A (6), white amorphous powder; IRν_{max} : 3400 (OH), 2933 (aliphatic CH), 1640 (CO), 1383, 1064, 905, 812, 636 cm^{-1}; ^{1}H-NMR (DMSO-d_6) signals at δ ppm : 4.29 (1H, *d*, *J* = 7.3 Hz, H-1`), 4.49 (1H, *d*, *J* = 7.6 Hz, H-1``), 4.64 (1H, *d*, *J* = 7.8 Hz, H-1```), 4.67 (1H, *d*, *J* = 1.2 Hz, H-1````), 1.08 (3H, *d*, *J* = 5.8 Hz, H-6````), 1.09 (3H, *d*, *J* = 6.01 Hz, H-6`); ^{13}C-NMR: Table 5.

Table (2): ^{13}C-NMR (100 MHz) for compound **1** (in DMSO) and compound **2** (in CD_3OD).

Atom	1	2	Atom	1	2
Aglycone			**Rhamnose**		
1	93.3	101.1	1``	98.9	100.1
3	141.1	142.0	2``	69.1	71.5
4	102.5	105.7	3``	74.1	74.9
5	35.7	43.4	4``	69.3	71.7
6	81.9	89.1	5``	68.3	70.5
7	57.7	127.3	6``	18.0	18.2
8	65.5	149.8	**Coumaroyl moiety.**		
9	42.0	48.2	**C=O**	166.6	168.9
10	59.0	64.4	α	114.9	115.5
Glucose			β	144.7	146.9
1`	97.9	98.2	1```	125.3	127.3
2`	73.6	75.0	2```	130.4	131.6
3`	76.5	77.9	3```	116.0	117.0
4`	70.4	74.5	4```	159.9	161.3
5`	77.5	78.3	5```	116.0	117.0
6`	61.5	62.7	6```	130.4	131.2

Table (3): ^{13}C-NMR (75 MHz) data of compound 3 in CD_3OD.

Atom		Atom	
Aglycone		Rhamnose	
1	131.5	1''	102.9
2	116.5	2''	72.3
3	144.6	3''	72.1
4	146.1	4''	73.7
5	117.1	5''	70.4
6	121.3	6''	18.4
α-CH_2	72.2	Caffeic acid	
β- CH_2	36.5	1'''	127.6
Glucose		2'''	116.3
1`	104.1	3'''	146.8
2`	75.9	4'''	149.7
3`	81.7	5'''	114.7
4`	70.5	6'''	123.2
5`	76.1	α-CH	115.2
6`	62.3	β- CH	148.0
		C = O	168.3

Table (4): ^{13}C-NMR (100 MHz) data of compounds 4 and 5 in DMSO.

Carbon No	4	5	Carbon No	4	5
2	162.9	156.4	Glucose		
3	103.8	133.3	1''	100.5	101.2
4	181.9	177.3	2''	73.1	74.1
5	161.1	161.2	3''	76.2	76.5
6	99.6	98.7	4''	70.7	70.6
7	164.0	164.3	5''	75.6	75.9
8	94.8	93.6	6''	66.1	67.1
9	156.9	156.5	Rhamnose		
10	105.4	103.8	1''	99.9	100.8
1`	122.7	121.6	2''	70.3	70.4
2`	128.4	115.2	3''	69.6	70.1
3`	114.7	144.7	4''	72.0	71.9
4`	162.4	148.4	5''	68.3	68.3
5`	114.7	116.3	6''	17.8	17.6
6`	128.4	121.2			
OCH_3	55.6	-----			

Table 5: ^{13}C-NMR (100 MHz) data of Mimengoside A in DMSO

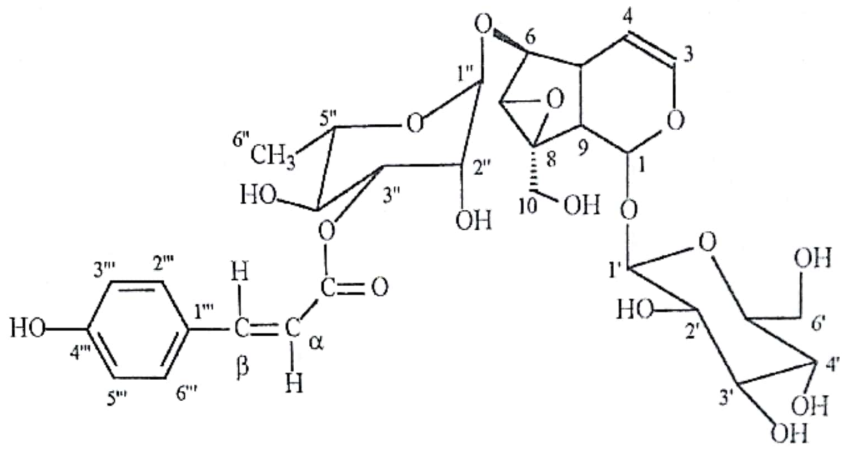
C	Mimengoside A	C	Mimengoside A
C-1	38.4	FUCOSE	
C-2	25.5	C-1'	103.9
C-3	82.4	C-2'	77.0
C-4	43.6	C-3'	84.5
C-5	47.6	C-4'	72.0
C-6	17.5	C-5'	70.3
C-7	31.3	C-6'	17.1
C-8	41.8	GLUCOSE	
C-9	53.5	C-1''	104.8
C-10	36.3	C-2''	75.3
C-11	131.9	C-3''	78.6
C-12	131.6	C-4''	71.9
C-13	84.7	C-5''	77.0
C-14	43.9	C-6''	61.1
C-15	30.9	GLUCOSE	
C-16	25.7	C-1'''	103.8
C-17	41.5	C-2'''	76.1
C-18	51.3	C-3'''	77.3
C-19	37.2	C-4'''	78.1
C-20	31.6	C-5'''	76.2
C-21	31.6	C-6'''	62.9
C-22	25.8	RHAMNOSE	
C-23	12.6	C-1''''	102.6
C-24	64.4	C-2''''	72.6
C-25	18.4	C-3''''	72.4
C-26	19.4	C-4''''	73.7
C-27	19.7	C-5''''	70.2
C-28	76.9	C-6''''	18.5
C-29	33.5		
C-30	23.5		

RESULTS AND DISCUSSION

From the alcoholic extract of the aerial parts of *Buddleia madgascariensis* Lam. (Buddleiaceae), six known compounds were isolated and characterized. Their structures were elucidated based on comparing their physical, chemical and spectral data with the published data.

Compound 1 and 2 are iridoid glycosides (positive Trim and Hill[19] and molisch's tests). Strong acid hydrolysis, of both, afforded glucose and non sugar part but acid hydrolysis followed by alkaline hydrolysis afforded glucose and rhamnose indicating

Zagazig J. Pharm. Sci., December 2002
Vol.11, No. 2, pp. 54-63



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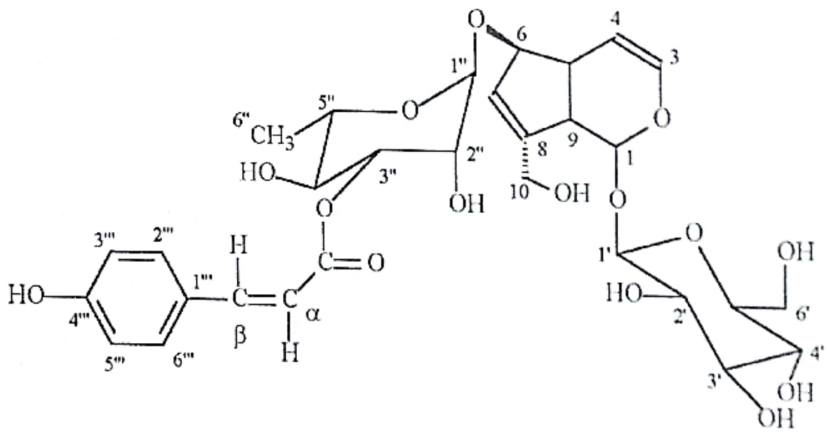

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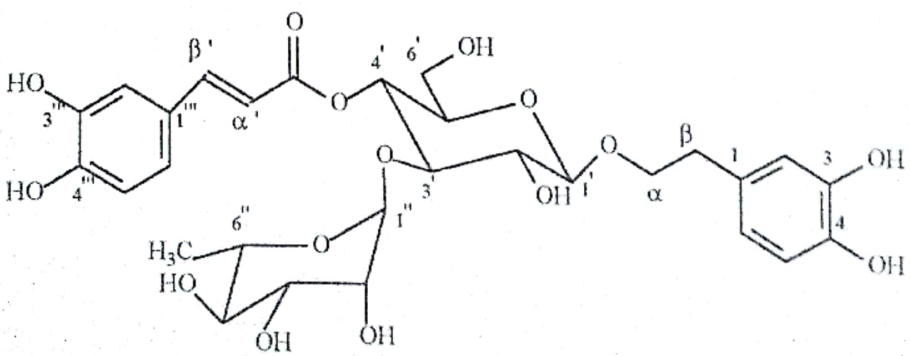

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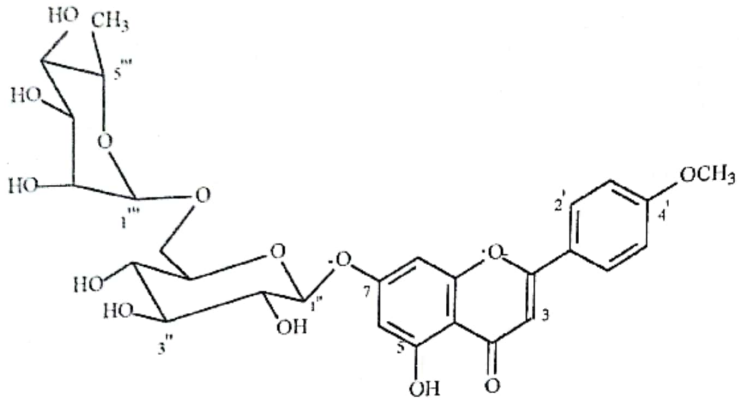

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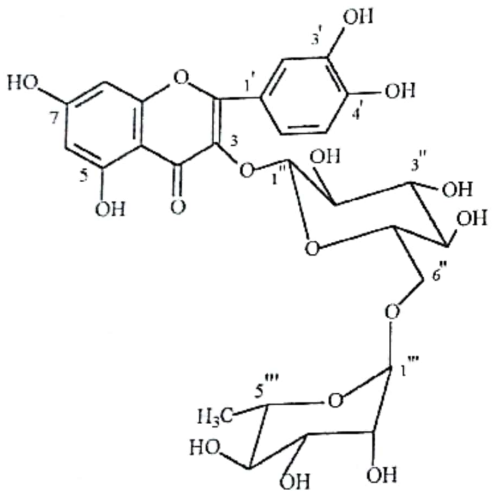

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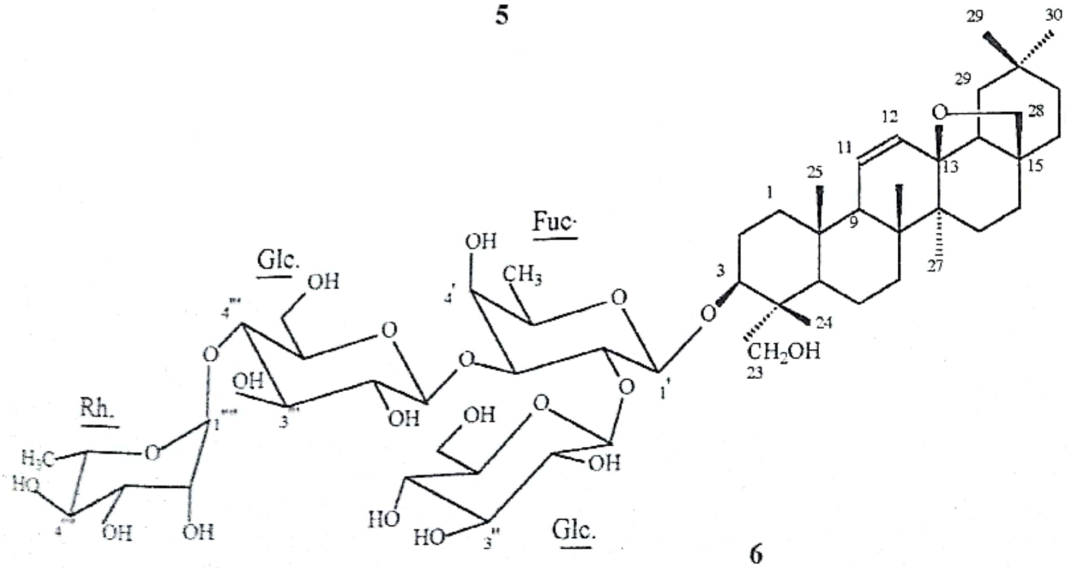

6

that, rhamnose is the acylated part of these coupounds. The sugar moiety of compound 1 was confirmed from the ^{1}H-NMR and ^{13}C-NMR spectra (Table 2). Signals at δ 4.62 ppm (1H, d, J = 7.8, H-1`), 4.85 ppm (1H, d, J = 1.2, H-1``), corresponding to δ 97.9 ppm (C-1`) and 98.9 ppm (C-1``), assigned to the two anomerics of glucose and rhamnose respectively as well as the signal at δ 1.21 (3H, d, J=6.01Hz) and 17.9 assigned to H-6`` and C-6`` of rhamnose. The configuration of β-D-glucose and α-L-rhamnose were established on the basis of coupling constant considerations. The other signals of the sugars are cited in table (2) and could be assigned with the aid of DEPT, COSY and HMQC spectra.

The acyl moiety was identified as p-coumaroyl based on the assignment of the ^{1}H-NMR and ^{13}C-NMR signals (Table 2). It is attached to C-3`` of α-L-rhamnose as deduced from the HMBC correlation between CO of para coumaroyl and H-3`` of rhamnose as well as from the down-field shifting of the proton and carbon signals of position 3 of rhamnose to δ4.90 and 74.1 ppm rather than *ca* 3.5 and 69.0 in the unsubstituted rhamnose. This was confirmed by COSY, DEPT and HMQC experiments. The trans configuration of the p-coumaroyl moiety was established from the high coupling constant $J_{H\alpha, H\beta}$ = 15.8 Hz.

The aglycone of 1 is catalpol as indicated from the assignment of the rest of the ^{1}H-NMR and ^{13}C-NMR signal and confirmed by DEPT, COSY, HMQC and HMBC spectra. The points of attachment of the sugar and acyl moieties could be determined from the HMBC spectrum.

Thus, compound 1 was identified as 6-O-(3``-O-p-coumaroyl-α-L-rhamnopyranosyl) catalpol.

Compound 2 showed almost identical ^{1}H-NMR and ^{13}C-NMR signals except for those of the aglycone. It was identified as aucubin and hence compound 2 is 6-O-(3``-O-p-coumaroyl-α-L-rhamnosyl) aucubin.

Compound 3 occurred as orange-brown amorphous powder soluble in methanol and ethyl acetate and insoluble in chloroform, ether and petroleum ether. It gave positive Molisch's test and bluish color with ferric chloride indicating its glycosidic and phenolic nature. It was identified as acteoside based on spectral data and comparing these data with the published ones of acteoside[20].

Compounds 4 and 5 gave a yellow color with NH_4OH and NaOH and positive molisch's test indicating their flavonoid glycoside nature.

The UV spectra of compound 4 with the different shift reagents indicated that, it is acaciin, 5,7-dihydrohy 4`-methoxy-flavone-7-rutionside, λ_{max} in methanol = 326 nm, bathochromic shift in band I with decreased intensity after the addition of sodium methoxide = +35 absence of significant shift in band II after the addition of sodium acetate, bathochromic shift in band I (+55.2 nm) after the addition of $AlCl_3$, which persisted after the addition of HCl and absence of a clear shift after the addition of $NaOAc/H_3BO_3$. Strong acid hydrolysis and TLC of the hydrolysate afforded glucose and rhamnose. The sugar part is attached to position 7 as proved by the acid hydrolysis, which afforded the aglycone, whose UV spectrum gave a bathochromic shift in band 2 (+6nm) on addition of sodium acetate indicating that position 7 of the aglycone became free. Further confirmation was done by comparing its ^{1}H-NMR and ^{13}C-NMR spectral data with the reported ones[21,22].

Compound 5 obtained as pale yellow crystals, m.p. 190-195^{0}C. UV spectra (in MeOH) with the different shift reagents displayed an absorption band I and II at λ_{max} 358 and 258 nm indicating that it could be a flavonol. It exhibited a bathochromic shift in band I (with increase in intensity after the addition of sodium methoxide) and no decomposition occurred within 5 minutes indicating that, 4` position is unsubstituted and that, position 3 could be blocked. A bathochromic shift in band I (69 nm) after the addition of $AlCl_3$, which decreased to (37.8 nm) after the addition of HCl indicated the presence of a free OH group at C-5 and the presence of O-dihydroxy groups in ring B. This was confirmed by the presence of a bathochromic shift in band I (21.6 nm) after the addition of $NaOAc/H_3BO_3$. Meanwhile, the absence of a significant bathochromic shifts in band II indicated the absence of O-dihydroxy groups in ring A. The bathochromic shift in band II (12.6 nm) after the addition of NaOAc suggested the presence of a free OH group at position 7. The above data indicated that, compound 5 is a flavonol with free hydroxyl groups at positions 5, 7, 3` and 4` and blocked 3 position.

The flavonol nucleus was confirmed from the ^{13}C-NMR signal at δ 177.3 ppm assigned to C-4. The two signal at δ 93.6 and 98.8 assigned to C-8 and C-6 indicated that, these positions are unsubstituted. ^{1}H-NMR signals at δ 7.52 (1H, dd, *J ca* 2 & 8), 7.51 (1H, d, *J ca* 2) and 6.84 (1H, d, *J ca* 8) could be assigned for positions 6`, 2` and 5` positions of disubstituted ring B.

Strong acid hydrolysis and TLC of the hydrolysate afforded glucose and rhamnose. This was confirmed from the ^{13}C-NMR signals at δ 101.2 and 100.8 ppm and ^{1}H-NMR doublets at δ 5.3 (J=6.3 Hz) and 4.5 (J=1.2) ppm assigned for the anomerics of β-D-glucose and α-L-rhamnose respectively. β-configuration of glucose and α-configuration of rhamnose were established from the coupling constants of both. Rhamnose is assigned as the terminal sugar attached to C-6 of glucose as ^{13}C-NMR signal of C-6 appears deshielded to δ 67.0 ppm and H-1 of glucose is deshielded to 5.3 ppm in the ^{1}H-NMR.

The assignment of ^{1}H-NMR and ^{13}C-NMR (Table 4) signals indicated that, compound 5 is rutin. This was confirmed by comparing the spectral data with those reported for rutin[2,3] as well as by TLC comparison with authentic sample.

Compound 6 was obtained as amorphous powder. It gave positive test with Liebermann-Burchard's test indicating its triterpenoid nature and it gave positive molisch's test indicating its glycosidic nature. Strong acid hydrolysis afforded glucose, rhamnose and fucose sugar moieties, which were detected by using TLC against authentic sugar samples using ethyl acetate: methanol: water (100:16.5:13.5) as solvent system on silica gel-GF254. The sugar part consists of four sugar moieties as indicated from the ^{1}H-NMR. Compound 6 was identified by comparing its data with the reported ones of Mimengoside A, which have been previously isolated from *Buddleia officinalis* and *Buddleia madagascariensis*.

Free radical scavenging bioassay:

The results of free radical scavenging activity are illustrated in table (6). All the tested compounds showed a variable antioxidant activities. The activity decreased in the order: acteoside > ascorbic acid> acaaciin > 6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol. Acteoside showed an antioxidant activity higher than that of ascorbic acid.

Table (6): Result of antioxidant activity:

Sample	Absorbance	% Inhibition
Blank	--	--
Control	0.49	0
Ascorbic acid	0.2	59.18
Acteoside	0.16	67.34
Acaacetin-7-O-rutinoside	0.33	32.56
6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol	0.42	14.28

Antimicrobial activities:

The anti-microbial screening of the tested compounds showed a variable antibacterial activity (Table 7). The results can be summarized as follows:

A) Out of the tested compounds. Only acteoside showed activity against *Staphylococcus aureus.*

B) All the tested compounds have activity against *Pseudomonas aeruginosa* and *E.coli.*

C) The activity against *Pseudomonas aeruginosa* decreased in the following order: acteoside > mimengoside A > 6-O-(3``-O-*p*-coumaroyl–α-L-rhamnopyranosyl) catalpol > acaaciin.

D) The activity against *E.coli* decreased in the following order:

acteoside = 6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol > mimengoside A > acaaciin.

Table (7): Results of antimicrobial screening:

Compound	*Pseudomonas aeruginosa*	*Escherichia coli.*	*Staphylococcus aureus*
6-O-(3``-O-*p*-Coumaroyl-□-L-Rhamnopyranosyl) Catalpol	1.5 mm	5.0 mm	-
Acteoside	5.0 mm	5.0 mm	2.5 mm
Mimengoside A	3.5 mm	4.0 mm	-
Acaacetin-7-O-rutinoside	1.0 mm	3.0 mm	-
Gentamycin	9.5 mm	7.5 mm	-

The results of the anti-microbial assay of the 6-O-(3``-O-*p*- coumaroyl-α-L-rhamnopyranosyl) catalpol are cited in table 8. It showed that the minimum inhibitory concentration (M.I.C.) against *Pseudomonas aeruginosa* equal 35.9 µg/ml and that against *E. Coli* equal 24 µg/ml. On the other hand the M.I.C of gentamicin against *Pseudomonas aeruginosa* equal 24 µg/ml and that against *E-Coli* equal 16.8 µg/ml. 6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranos-yl) catalpol in concentration of 50 mg/ml seems to have almost the same activity as gentamicin in concentration of 0.625 mg/ml against *Pseudomonas aeruginosa* (Table 8). While in concentration of 25 mg/ml it has activity against *E.Coli* comparable to that of gentamicin in concentration of 1.25 mg/ml.

Table (8): Results of antimicrobial assay of 6-O-(3``-O-*p*-coumaroyl -α- L-rhamnopyranosyl) catalpol:

Pseudomonas aeruginosa				*Escherichia coli.*			
Conc. µg/ml	LogC.	X mm	X^2 mm^2	Conc. µg/ml	LogC.	X mm	X^2 mm^2
50000	4.7	4	16	50000	4.7	6	36
25000	4.4	3.5	12.25	25000	4.4	5	25
12500	4.1	2.5	6.25	12500	4.1	4	16
6250	3.8	1.5	2.25	6250	3.8	2.5	6.25

Table (9): Results of antimicrobial assay of gentamicin:

Pseudomonas aeruginosa				*Escherichia coli.*			
Conc. µg/ml	Log C.	X mm	X^2 mm^2	Conc. µg/ml	Log C.	X mm	X^2 mm^2
5000	3.7	9.50	90.25	5000	3.7	7.5	56.25
2500	3.4	8.00	64.00	2500	3.4	6.5	42.25
1250	3.1	6.25	39.00	1250	3.1	5.0	25.00
625	2.8	4.00	16.00	625	2.8	3.5	12.25

The genetic events most often responsible for drug resistance are transferable genetic element from resistant species to a sensitive one. Gram-negative bacteria are more resistant than Gram-positive bacteria due to the difference in structure of the cell wall and due to the presence of high lipid contents in the structure of Gram-negative bacteria[27]. Amino-glycoside antibiotics act on the 30-S sub-unit and

interfere with protein synthesis by preventing attachment of the m-RNA or t-RNAs or by blocking the movement of ribosomes along the m-RNA while β-lactam antibiotics act on the cell wall by inhibition of peptido-glycan synthesis[27].

The result of this study showed that 6-O-(3``-O-*p*-coumaroyl- α-L-rhamnopyranosyl) catalpol exhibits activity on gram-negative bacteria rather than gram-positive bacteria so, it may act by the same mechanism of aminoglycosides on the protein synthesis. In addition, the M.I.C of 6-O-(3``-O-*p*-coumaroyl-α-L- rhamnopyranosyl) catalpol against *Ps. aeruginosa* and *E. coli* are greater than that of gentamicin. Hence, several pharmacological and toxicological studies should be done on 6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol to determine the effective non-lethal dose to be used in systemic infections caused by gram-negative bacteria. On the other hand, these studies will determine the chance of using 6-O-(3``-O-*p*-coumaroyl-α-L-rhamnopyranosyl) catalpol in topical preparations especially in nosocomial infections, burns and septic wounds.

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مواد فينولية ذات فعالية بيولوجية من نبات *بدليا مدغشقرينسيس*

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أسفرت الدراسة الكروماتوجرافية للخلاصة الكحولية لنبات *بدليا مدغشقرينسيس* عن فصل ٦-أ- (٣"-أ-بارا كومارويل-ألفا-ل-رامنوبيرانوزيل) كاتالبول، ٦-أ- (٣"-أ-بارا كومارويل-ألفا-ل-رامنوبيرانوزيل) أوكيوبين، أكتيوزايد، أكاسيين، روتين و ميمينجوزايد. و قد تم التعرف على البنية الكيميائيى لهذه المركبات باستخدام طرق التحليل الكيميائي و الطيفي خاصة الرنين النووي المغناطيسي.أحادى و ثنائى الأبعاد.

و كذلك تمت دراسة تأثير بعض هذه المركبات كمضادات للأكسدة و مضادات للبكتيريا. و قد وجد أن أكتيوزايد له تأثير مضاد للأكسدة بينما ٦-أ- (٣"-أ-بارا كومارويل-ألفا-ل-رامنوبيرانوزيل) كاتالبول له تأثير قوي ضد البكتيريا السالبة الجرام