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ORIGINAL ARTICLES

Flavonoid constituents from *Morettia philaena* (Del.) DC. and their antimicrobial activity

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ABSTRACT

Phytochemical investigation of the whole *Morettia philaena* (Del.) DC**.** plant led to the identification of nine flavonoids isolated using chromatographic techniques. These are kaempferol, kaempferol 3-*O*-β-glucopyranoside, kaempferol3, 7di-*O*-β-glucopyranoside,kaempferol3-*O*-β-sophoroside-7-*O*-β-glucopyranoside, quercetin, quercetin 3-*O*-β-glucopyranoside, quercetin 3-*O*-β-gentobioside, orientin and isoorientin. Their structures were established through chemical and spectral analysis. The antimicrobial activity of the isolated flavonoids and the aqueous methanol extract against six bacteria and one fungus species was studied. Among them, the isolated aglycones, mono-*O*- glycosides and C- glycosides exhibited interesting high activity against most of the tested organisms.

Key words: Flavonoids, *Morettia philaena* (Del.) DC., antimicrobial activity.

Introduction

Flavonoids are a class of natural products possessing a diverse range of pharmacological properties (Havsteen, 2002). Antimicrobial activity is one of the well-known actions of flavonoids (Tim Cushnie & Lamb, 2005). *Morettia philaena* (Del.) DC. belonging to family Brassicaceae is one of two *Morettia* species which occur in Egypt (Täckholm, 1974 & Boulos, 1999). It is used by the Sudanese local people to nourish the sheep and chicken, it has also been used as an ingredient in local medicine for several ailments (El-Egami *et al*., 2011).

Previous phytochemical studies conducted on the flowering aerial parts of *M. philaena* reported the isolation and identification of six flavonoids (Singab *et al*., 2000). These were kaempferol, quercetin, kaempferol 3-*O*-β-glucopyranoside, quercetin 3-*O*-β-glucopyranoside, quercetin 3-O-[2"-(6"'-p-coumaroyl-*O*-β-glucopyranosyl)-α-L-arabinopyranosyl]-7-O-glucopyranoside and kaempferol 3-O-[2"-(6"'-p-coumaroyl-*O*-β-glucopyranosyl)-α-L-arabinopyranosyl]-7-O-glucopyranoside. The qualitative and quantitative analysis of the essential oils were also reported (El-Egami *et al*., 2011). The present work revealed the isolation and identification of nine flavonoids, five of them were reported for the first time from the species under study. Their antimicrobial activity was also discussed.

Material and Methods

*General experimental procedur*e:

NMR experiments were recorded on a Jeol EX-500 spectrometer: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR). UV absorption spectra were recorded on Shimadzu model-2401 CP spectrophotometer, EIMS on Finnigan-Mat SSQ 7000 spectrometer, while ESIMS on LCQ Advantage Thermo Finnigan spectrometer. Column chromatography was carried on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany) using MeOH/H_2O mixtures of decreasing polarities as eluent and Sephadex LH-20 (Pharmazia) using MeOH as eluent. The paper chromatography (descending) Whatman No. 1 and 3 MM papers, using solvent systems (1) H_2O, (2) 15% HOAc (H_2O–HOAc 85:15), (3) BAW (n-BuOH–HOAc–H_2O 4:1:5, upper layer), (4) BBPW (Benzene–n-BuOH–pyridine–H_2O 1:5:3:3, upper layer); solvents 3 and 4 were used for sugar identification. Complete acid hydrolysis for *O*-glycosides (2 N HCl, 2 h, 100° C) were carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties. The sugar units of *C*-glycoside flavonoids were determined using ferric chloride degradation (Mabry *et al*., 1970). Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC.

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Plant Material:

The whole plant of *M. philaenae* was collected from Cairo- Suez desert road, Egypt in March 2007. The sample was identified by Prof. Dr. Salwa A. Kawashty and Dr. Sameh R. Hussein. A Voucher specimen (no. 968) was deposited in the herbarium of the National Research Centre (CAIRC).

Microorganism strains:

Microorganisms included in this study were obtained from the culture collections of microbiology Department, faculty of science, Ain Shams University and identified by Dr. Madeha Ghobashy. They included three Gram-negative bacteria strains (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*), three Gram-positive bacteria strains (*Streptococcus pyogens, Staphylococcus aureus* and *Bacillus cereus*) and one fungus species (*Candida albicans*). The cultures of bacteria and yeast were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Culture media and antibiotic Controls:

Mueller-Hinton agar, Mueller-Hinton broth, potato dextrose broth and potato dextrose agar media were purchased from Diffco company. Penicillin (1,000,000 IU) and Fluconazole (150 µg) antibiotics were purchased from Pfizer company.

Extraction and isolation:

Fine powdered air-dried whole plant of *M. philaenae* (450 g) was extracted under reflux two times with 70% methanol/water, and then evaporated under reduced pressure and temperature. The residue (59 g) was successively extracted with ethyl acetate and methanol (three times for each solvent). The ethyl acetate fraction (15 g) was chromatographed on PC using BAW as eluent to yield compounds **1** (17 mg), **2** (12 mg), **5** (22 mg) and **6** (25 mg). The methanol fraction (23 g) was subjected to a polyamide column (85 × 3 cm) and eluted with MeOH/H_2O mixtures of decreasing polarities to yield five main fractions (I-V). Fraction I (20-30 % MeOH/H_2O) yielded compounds **3** (18 mg) and **4** (14 mg). Fractions II-V afforded compounds **7** (18 mg), **8** (15 mg) and **9** (21 mg). The isolation and purification was achieved by a combination of Sephadex column (35 × 1.5 cm) using MeOH: H_2O (1:1) and PPC using H_2O, 15% HOAc and BAW as eluents, then finally purified on Sephadex column using methanol as eluent.

Determination of antimicrobial assay:

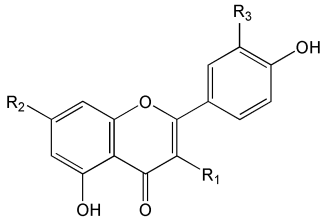
The aqueous methanol extract as well as the nine isolated compounds were *in vitro* evaluated for their antimicrobial activity against six species of bacteria and one fungus species (*Candida albicans*) by well diffusion method (Mitscher *et al*., 1972; Pepeljnjak *et al*, 2005). The experiment was performed using a culture at 37 °C for 24 hours on 10 ml of Mueller Hinton Broth for bacteria and 48 hours on potato dextrose broth for yeast. Agar (20 ml) was poured into sterile petri-dishes and allowed for solidification. Wells were made in agar plates using sterile cork pore of 10 mm diameter. The cultures were adjusted to approximately 10^6 CFU/ml with sterile saline solution. One hundred and fifty micro liters of the suspensions were spread over the plates containing agar media using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. Preliminary antimicrobial screening of the isolated flavonoids was performed on 8 mg dry weight (according to the available amount of the isolated flavonoids). Each tested sample was dissolved in 1 ml of DMSO and sterilized by filtration through a 0.22 µm membrane filter (by using Millopore memrane filter apparatus). 150 µL of each sample (mg/ml) were added separately to the appropriate wells in the petridishes. The antimicrobial activity was recorded by measuring the diameter of the clear inhibition zone at the end of incubation period (mm). In these tests, Penicillin (1,000,000 IU) and Fluconazole (150 µg) were used as experimental positive controls for microorganism strains (bacterial&yeast respectivily) and DMSO as a negative control for which no inhibitory effect could be observed. Experiments were carried out in triplicate for each strain of microorganism evaluated. The reported results are the average value with standard deviation.

Results and Discussion

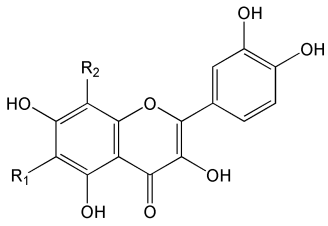
Identification of the isolated compounds:

Nine flavonoids including kaempferol (**1**), kaempferol 3-*O*-β-glucoside (**2**), kaempferol 3,7 di-*O*-β-glucopranoside (**3**), kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucopyranoside (**4**), quercetin (**5**), quercetin 3-*O*-β-

glucoside (**6**), quercetin 3-*O*-β-gentobioside (**7**), orientin (**8**) and isoorientin (**9**) were isolated from the whole plant of *M. philaena*. Compounds **3**, **4** and compounds **7-9** were isolated for the first time from this species (Figure 1).



Comp.	R_1	R_2	R_3
(1)	OH	OH	H
(2)	*O*-glucopyranoside	OH	H
(3)	*O*-glucopyranoside	*O*-glucopyranoside	H
(4)	*O*-sophroside	*O*-glucopyranoside	H
(5)	OH	OH	OH
(6)	*O*-glucopyranoside	OH	OH
(7)	*O*-gentobioside	OH	OH



Comp.	R_1	R_2
(8)	H	*C*-glucopyranoside
(9)	*C*-glucopyranoside	H

Fig. 1: Chemical structures of flavonoid compounds isolated from *M. philaenae*

The chemical structures of the isolated compounds were elucidated by extensive UV analysis, ^{1}H &^{13}C NMR and MS spectral data (Mabry *et al*, 1970; Markham, 1982; Agrawal & Bansal, 1989; Markham & Geiger, 1994). Spectral data of the isolated compounds were in a good accordance with those previously reported (Mabry *et al*, 1982; Agrawal & Bansal, 1989; Markham & Geiger, 1994; Marzouk, 2008; 2011). Details of the isolated compounds are listed below:

*Kaempferol (**1**):*

Yellow amorphous powder, R_f 0.77 (BAW). ESIMS; *m/z* 286[M] $^{+}$.

*Kaempferol 3-O-β-glucopyranoside (**2**):*

Yellow amorphous powder, R_f 0.64 (BAW).Negative ESIMS; *m/z* 447[M-H] $^{-}$.

*Kaempferol 3,7 di-O-β-glucopyranoside (**3**):*

Yellow amorphous powder, R_f 0.38 (BAW). UV/Vis (λmax) nm: (MeOH) 266, 345; (+NaOMe) 266, 387; (+$AlCl_3$) 274, 300, 354, 399; (+$AlCl_3$/HC1) 274, 299, 348, 399; (+NaOAc) 265, 354, 398; (+NaOAc/H_3BO_3) 266, 314, 352. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.76 (2H, d, *J* = 9.0, H-2′,6′); 6.87 (2H, d, *J* = 9.0, H-3′,5′); 6.66 (1H, d, *J* =1.8, H-8); 6.35 (1H, d, *J* = 1.8, H-6); 5.45 (1H, d, *J* =7.5, H-1″); 5.07 (1H, d, *J* = 7.5, H-1‴); 3–4 (m, sugar protons overlapped with -OH proton signals). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.6 (C-4), 163 (C-7), 161.1 (C-5), 159.7 (C-4′), 157.2 (C-2), 156.9 (C-9), 133.7 (C-3), 130.9 (C-2′), 130.9 (C-6′), 121.0 (C-1′), 116.1 (C-3′), 116.1 (C-5′),105.9 (C-10), 101.2 (C-1″), 100.2 (C-1‴), 98.9 (C-6), 94.6 (C-8), 77.4 (C-5‴), 77.3 (C-5″), 76.6 (C-3″), 76.4 (C-3‴),74.5 (C-2″), 73.2 (C-2‴), 70.2 (C-4″), 69.9 (C-4‴), 61.1 (C-6‴), 61.0 (C-6″). Negative ESIMS; *m/z* 609[M-H] $^{-}$.

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*Kaempferol-3-O-β-sophoroside-7-O-β-glucopyranoside (**4**):*

Yellow amorphous powder, *Rf* 0.21 (BAW). UV/vis (λ_{max}) nm: (MeOH) 265, 344 (+NaOMe), 266, 389, (+AlCl3) 275, 299, 344, 394; (+$AlCl_3$ HCl) 275, 300, 344, 393; (+NaOAc) 265, 380; (NaOAc/H_3BO_3) 265, 345.^{1}H NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz: 7.8 (2H, d, *J* = 9.0, H-2',6'); 6.95 (2H,d, *J* = 9.0, H-3',5'); 6.7 (1H, d, *J* = 2.0, H-8); 6.4 (1H,d, *J* = 2.0, H-6); 5.65 (1H, d, *J* =7.2, H-1″); 5.07 (1H, d, *J* = 7.2, H-1‴), 4.46 (1H, d, *J* =7.0, H-1⁗); 3–4 (m, sugar protons overlapped with -OH proton signals). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.5 (C-4), 164 (C-7), 161.4 (C-5), 159.7 (C-4′), 158.2 (C-2), 156.8 (C-9), 133.1 (C-3), 130.6 (C-2′), 130.6 (C-6′), 121.1 (C-1′), 116.2 (C-3′), 116.2 (C-5′),105.2 (C-10), 104.1 (C-1⁗), 99.6 (C-1‴), 98.8 (C-6), 98.7 (C-1″), 94.5 (C-8), 82.5 (C-2″), 77.4 (C-5″), 77.1 (C-3⁗), 76.9 (C-5⁗), 76.6 (C-3″), 76.2 (C-3‴), 74.2 (C-2⁗), 73.9 (C-5‴), 73.1 (C-2‴), 70.1 (C-4‴), 69.7 (C-4⁗), 69.6 (C-4″), 60.9 (C-6‴), 60.8 (C-6⁗), 60.5 (C-6″), Negative ESIMS; *m/z* 771.2 $[M-H]^-$.

*Quercetin (**5**):*

Yellow amorphous powder, R_f 0.59 (BAW). EIMS; *m/z* 302$[M]^+$.

*Quercetin 3-O-β-glucopyranoside (**6**):*

Yellow amorphous powder, R_f 0.5 (BAW). Negative ESIMS; *m/z* 463$[M-H]^-$.

*Quercetin 3-O-β-gentobioside (**7**):*

Yellow crystals, R_f 0.32 (BAW). UV/Vis (λmax) nm: (MeOH) 257, 269sh, 299sh 358; (+NaOMe) 272, 327, 410; (+$AlCl_3$) 274, 303sh, 433; (+$AlCl_3$/HC1) 271, 299, 364sh, 403; (+NaOAc) 271, 325, 393; (+NaOAc/H_3BO_3) 262, 299, 387. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.7 (1H, dd, *J* = 2.0, 8.5, H-6′); 7.52 (1H, d, *J* = 2.0, H-2′); 6.87 (1H, d, *J* = 8.5, H,5′); 6.38 (1H, d, *J* =2.0, H-8); 6.15 (1H, d, *J* =2.0, H-6); 5.56 (1H, d, *J* =7.5, H-1″); 4.63 (1H, d, *J* = 7.5, H-1‴); 3–4 (m, sugar protons overlapped with -OH proton signals). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.8 (C-4), 164.3 (C-7), 161.3 (C-5), 156.2 (C-9), 155.6 (C-2), 148.4 (C-4′),144.8 (C-3′), 133.2 (C-3), 122.3 (C-6′), 121.2 (C-1′), 116.1 (C-5′), 115.6 (C-2′), 103.9 (C-10), 102.9 (C-1‴), 100.9 (C-1″), 98.6 (C-6), 94.2 (C-8), 76.5 (C-5‴), 76.4 (C-5″), 76.4 (C-3″), 76.3 (C-3‴),74.1 (C-2‴), 73.3 (C-2″), 69.9 (C-4‴), 69.7 (C-4″), 67.9 (C-6″), 60.6 (C-6‴). Negative ESIMS; *m/z* 625$[M-H]^-$.

*Orientein (Luteolin 8-C-β-glucoside) (**8**) :*

Yellow amorphous powder, R_f 0.33 (BAW). Negative ESIMS; *m/z* 447$[M-H]^-$

*Isoorientein (Luteolin 6-C-β-glucoside) (**9**) :*

Yellow amorphous powder, R_f 0.35 (BAW). Negative ESIMS; *m/z* 447$[M-H]^-$

Antimicrobial assay:

Based on the results of inhibition zones, aqueous methanol extract showed the greatest activity against the three tested Gram–*ve* bacterial strains, in regard with the inhibitory effect of control (penicillin antibiotic). This activity may be related to the ability of the tested extract to affect permeability of the Gram –ve bacteria cell wall which unlike the Gram +ve one, where the former contains an outer membrane composed of phospholipids and lipopolysaccharides leads to increase the negative charge of the cell membrane and helps to stabilize the overall membrane structure (Wang and Quinn, 2010).

The isolated flavonoids were also screened for their antimicrobial activities (Table 1). Among the tested flavonoids; quercetin was the most active compound against all tested bacterial strains. The mono- *O* & *C*-glycosides (compounds **2**, **6**, **8** and **9**) showed a relatively strong antimicrobial activity against the most of the studied microorganisms, while the di- and tri-O-glycosides exhibited a relatively weak activity. It is noteworthy that quercetin 3-*O*-β-glucoside had its most inhibitory activity against *Candida albicans;* it was as potent as Fluconazole antibiotic, along with its activity against the most of tested organism. The relationship between the structure and the antimicrobial activity of the nine flavonoids isolated in this study seem to suggest that the most bioactive flavonoids are those that have a free hydroxyl group at C-7, ortho dihydroxyl groups at 3` and 4` positions and the unsubstitution of C-6. It was also observed that the aglycones have stronger antimicrobial activity than their glycosides and low or no activity was observed when C-3 and C-7 positions are glycosylated.

Table 1: Antimicrobial activity of the aqueous methanol extract and the pure isolated flavonoids from *M. philaenae,* in comparison with Penicillin (antibacterial control), Fluconazole (antifungal agent).

Strains / Sample		Inhibition zone diameter (mm/mg sample)						
		Gram -ve			Gram +ve			Yeast
		E. coli	*Pseudo. aeroginosa*	*Klebseilla pneumonia*	*Strepto. pyogens*	*Staph. aureus*	*Bacillus Cereus*	*Candida albicans*
Aqueous methanol extract		28.4±0.14	21.3±0.35	16.7±0.14	-	-	-	-
1	Kaempferol	-	12.2±0.14	-	-	11.1±0.28	14.1±0.47	11.2±0.28
2	K 3-*O*-β-gluco-pyranoside	-	9.95±0.28	-	-	15.0±0.42	12.1±0.14	
3	K 3, 7 di-*O*-β-glucopyranoside	-	-	-	-	16.0±0.42	12.0±0.28	-
4	K 3-*O*-β-sophro-side-7-*O*-β-gluco-pyranoside	-	13.0±0.28	-	-	-	15.0±0.71	-
5	Quercetin	25.0±0.42	28.0±0.28	27.0±0.14	14.0±0.42	16.0±0.14	12.0±0.28	12.0±0.71
6	Quercetin 3-*O*-β-glucopyranoside	14.0±0.14	12.0±0.28	-	-	11.0±0.42	12.1±0.14	16.0±0.57
7	Q 3-*O*-β-gento-bioside	13.0±0.14	21.0±0.42	-	-	-	12.0±0.85	-
8	Orientin	12.0±0.28	9.95±0.21	-	-	14.0±0.14	-	13.0±0.42
9	Isoorientin	12.0±0.28	9.0±0.57	-	-	16.0±0.14	-	11.0±0.28
Positive control	Penicillin	39.0±0.14	17.0±0.71	16.0±0.57	35.05±0.07	16.0±0.14	30.0±0.85	-
	Fluconazole	-	-	-	-	-	-	14.0±0.57

Conclusion:

In this study, we reported the isolation of nine flavonoids from *M. philaenae*; five of them were isolated for the first time from this species. The results of the preliminary antimicrobial assay revealed that the crude extract and the flavonoids from *M. philaenae* could be used for future development of naturally occurring antimicrobials.

Acknowledgements

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