European Journal of Chemistry 2 (4) (2011) 535-538

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Phytochemical studies on *Diplotaxis harra* growing in Sinai

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ARTICLE INFORMATION

Received: 16 April 2010
Received in revised form: 05 May 2010
Accepted: 11 June 2010
Online: 31 December 2011

KEYWORDS

Cruciferae
Diplotaxis harra
Flavonoid
Antimicrobial activity
Antibacterial activity
Ethanolic extract

ABSTRACT

Five main flavonoid glycosides were isolated from the ethanolic extract of *Diplotaxis harra* (Cruciferae), and were identified as quercetin, isorhamnetin 3-rhamnoside, isorhamnetin 3-*o*-rutinoside, isorhamnetin 3-glucosyl-4`-rhamnoside and isorhamnetin 3-o-*β*-glucoside. These compounds were identified according to their R_f values, partial and complete acid hydrolysis, UV, ^{1}H NMR, ^{13}C NMR and 2D NMR (HSQC, HMBC) spectroscopy. The alcoholic extract of plant was evaluated against some bacterial strains which showed moderate antibacterial activity, while petroleum ether extract doesn't show any activity.

1. Introduction

Nature has always been man's first reliance in satisfying his needs throughout the ages, use of natural resources for the treatment of ailments still remains one of the important targets. Many plants have formed the basis of traditional medicine in countries such as India and Pakistan [1]. Natural products, derivatives and analogues represent over 50% of all drugs in clinical use and about 25% are derived from higher plants [2]. So, investigation of the pharmacological activities and the active agents from medicinal plants reported in traditional medicine is of interest.

Family Cruciferae is one of the largest families in the plant kingdom which is rich in medicinal species. It includes 338 genera and 3350 species which are distributed worldwide. In Egypt, it represents about 53 genera and 107 species [3,4]. Plants of this family were used as anti-diabetic, anti-bacterial, anti-fungal, anti-cancer, and anti-rheumatic, also it showed a potent insecticidal activity [5].

Our medicinal plant *Diplotaxis harra* is a desert perennial herb with a 20-30 cm height, the stem is multiple branched and often woody at base and locally known as Harra [5]. It has been reported to have analgesic effect, and has moderate toxicity [6], the qualitative composition and quantitative contents of glycosides in the flowers of *Diplotaxis tenuifolia* were studied and found that, the most diverse group are the flavonoids representing mainly derivatives of isorhamnetin and quercetin were observed in sepals, anther and stigma representing about 4.2 % of air-dry row weight of the flower. The highest values were observed in petals and the lowest values were in peduncles [7]. The phytochemical analysis of aerial parts has shown the presence of flavonoids, tannins, glucosinolates and sterols [8].

2. Experimental

2.1. Plant material

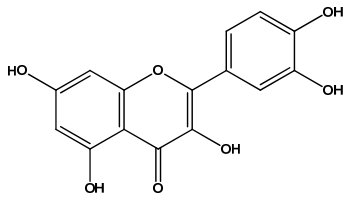
The aerial parts of *Diplotaxis harra* were collected from two regions South and North Sinai during the year 2006. The plant samples were kindly identified by Prof. Dr. Ahmed Morsy, Botany Department, Desert Research Center, Mathef El-Mataria, Egypt. A voucher specimen of the plant materials were kept in the Herbarium of Desert Research Center (No. drcc20/774). Plant samples were air-dried in shade, grounded to fine powder, packed in tightly closed containers and stored for phytochemical studies.

2.2. Adsorbents and solvent systems

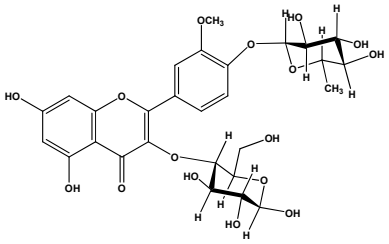
TLC plates: Silica gel G-60 F_{254} Merck. Silica gel 60 (70-230 mesh) was used for column chromatography. Preparative paper chromatography: Whattman No. 3.

Solvent systems: (a) Chloroform:Methanol (9:1), (b) Ethyl acetate:Methanol:Water (30:5:4), (c) Ethyl acetate:Methanol: Acetic acid:Water (65:15:10:10), (d) Butanol:Acetic acid:Water (4:1:5 upper layer) were used for developing the chromatograms. Visualization of chromatograms was achieved under UV before and after exposure to ammonia vapor or by spraying with aluminum chloride [9], all solvents used were of analytical grade.

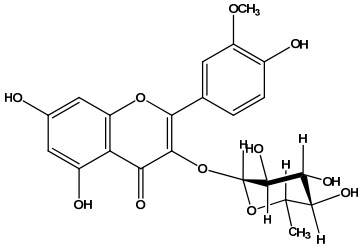
ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.2.4.535-538.62



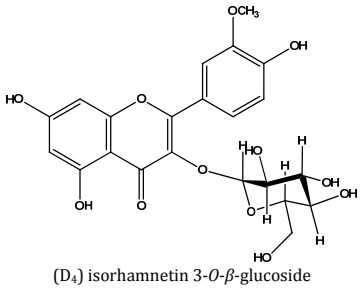
(D_1) quercetin



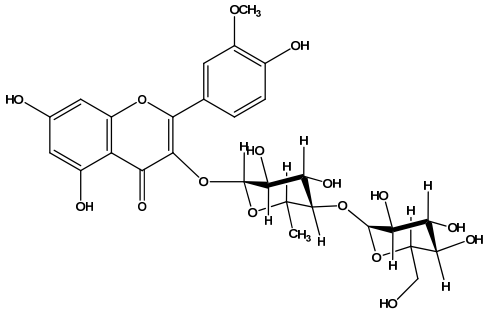
(D_2) isorhamnetin 3-glucoside-4`-rhamnoside



(D_3) isorhamnetin-3-rhamnoside



(D_4) isorhamnetin 3-*O*-*β*-glucoside



(D_5) isorhamnetin 3-*O*-rutinoside

Scheme 1

2.3. Instrumentation

NMR (Jeol ECA-600 spectrometer at 600.17 MHz for ^1H and 150.91 MHz for ^{13}C), ^1H - ^{13}C correlations were established by using HMQC and HMBC pulse sequences respectively. ^1H-^1H correlations were performed using double quantum filtered COSY technique.

2.4. Extraction and isolation

1 kg of the dried powdered aerial parts of the plant materials was defatted followed by extraction in a Soxhlet apparatus with 95% ethanol. The ethanolic extract of plant was concentrated under reduced pressure to afford 150 g. TLC examination of extract using solving systems a & b revealed the presence of the spots of phenolic nature. Extract diluted with water (300 mL), filtered over a piece of cotton then successively extracted with chloroform, ethyl acetate and *n*-butanol. Each sub-extract was dried over anhydrous sodium sulphate, and concentrated to yield 10, 18.5 and 30 g dry extracts, respectively.

TLC examination of all extracts using systems a & b revealed the presence of the same spots in ethyl acetate and chloroform extracts. Accordingly both extracts were combined together (20 g), and applied to the top of column chromatography packed with silica gel (410 g) and eluted gradually with chloroform-methanol, 100 fractions of 150 mL each were collected after TLC examinations and reduced to gave four sub-fractions, each fraction was concentrated under reduced pressure to yield 5.2, 6, 2 and 0.8 g, respectively. The 6 g fraction was subjected to silica gel column which led to the isolation of compounds D_1 & D_2 [10] (Scheme 1).

The *n*-butanol fraction (30 g) was found to have different chromatograms on TLC using system (b), then subjected to a preparative TLC using system (b) followed by a repeatedly preparative paper chromatography (PPC) using system (d). Bands corresponding to the flavonoids were separately extracted with methanol, concentrated to yield 8 g and 10 g respectively, the sub-fractions were injected in preparative HPLC (Agilant 1200 series) equipped with Diode Array Detector (DAD adjusted at 254 and 350 nm) using C_{18} RP-column, the eluting system started with water and gradually increased with acetonitrile, the elution gradient was linear, the run time was 45 min, giving compound D_3, D_4, and D_5 (Scheme 1).

2.5. Antimicrobial activity determination

The antimicrobial activity was determined by the agar overlay method [11]. Melted soft nutrient agar (3 mL, 0.7 %) at 50 ^oC with 50-100 mL of the test bacteria grown in nutrient broth was poured over 20 mL (1.5 %) nutrient agar plates. Samples of the extracts and pure compounds (5 mL) were deposited over solidified agar, and after 18 h of incubation at 37 ^oC, inhibition zone diameter was measured. The strains were obtained from Microbiology Department, Faculty of Science, Menoufia University. A liquid of known antibiotic (Chloramphenicol) and methanol as the solvent control were also analyzed.

Table 1. ^{1}H-NMR spectral data of the isolated flavonoids.

Compound	H6	H8	H6`	H2`	H5`	H1``	H1```	R.S.P.*	OCH_3	CH_3
D_1	6.37, *d*, 2.0Hz	6.4, *d*, 2.0Hz	7.49, *d*, 0.5Hz	7.69, *d*, 8.5Hz	6.85, *d*, 8.5Hz	-	-	-		-
D_2	6.1, *d*, 2.5 Hz	6.30, *d*, 2.5 Hz	8.08, *d*, 7.8Hz	8.08, *dd*, 8.5, 2.5Hz	6.80, *d*, 8.5Hz	5.50, *d*, 7.2 Hz	5.00, *d*, 2.0 Hz	3.10-3.30, *m*	3.70, s	1.1, *d*, 6Hz
D_3	6.1, *d*, 2.5Hz	6.30, *d*, 2.5 Hz	7.53, *d*, 8.5Hz	7.80, *dd*, 2.5, 8.5Hz	6.90, *d*, 8.5 Hz	5.50, *d*, 2.5 Hz	-	3.00-4.00, *m*	3.70, s	1.14, *d*, 6Hz
D_4	6.1, *d*, 2.0Hz	6.30, *d*, 2.0Hz	7.50, *d*, 8.5 Hz	7.80, *d*, 2.5Hz	6.83, *d*, 8.5 Hz	5.50, *d*, 7.4 Hz	-	3.00-4.00, *m*		-
D_5	6.13, *d* 2.0 Hz	6.33, *d*, 2.0 Hz	7.40, *d* , 8.0 Hz	7.40, *dd* 8.0, 2.2Hz	6.80, *d*, 8.4 Hz	5.20, *d*, 7.6 Hz	4.50, *d*, 1.6Hz	3.00-4.01 *m*		0.91, *d*, 6Hz

*R.S.P.: remaining sugar protons.

The minimum inhibitory concentrations (MICs) were determined by applying to the agar plates 5 mL of methanolic solutions of the samples, starting with a maximum concentration of 1000 μg/mL (1024 μg/mL in case of the control antibiotics), then reduced by successive two-fold dilutions of that stock solution. MIC determinations were carried out in five independent experiments, and MICs were expressed as the lowest concentration inhibiting bacteria growth samples that showed no antimicrobial activity at concentration of 1000 µg/mL were considered inactive.

3. Results and discussion

3.1. Phytochemical studies

The investigation of the methanolic extract of *Diplotaxis harra* revealed the presence of five flavonol compounds identified as (D_1) quercetin, (D_2) isorhamnetin 3-glucoside-4`-rhamnoside, (D_3) isorhamnetin-3-rhamnoside, (D_4) isorham netin 3-*O*-*β*-glucoside and (D_5) isorhamnetin 3-*O*-rutinoside according to R_f values, UV, ^{1}H, ^{13}C and 2D-NMR (Tables 1 and 2; Scheme 1) and by comparing with the published data [12-17].

Table 2. ^{13}C NMR of the isolated compounds.

Carbon No*	D_1	D_2	D_4
2	156.6	156.77	156.6
3	136.4	136.08	136.2
4	176.3	180.44	176.3
5	161.2	160.767	161.2
6	98.8	99.34	98.8
7	164.5	164.5	164.0
8	93.7	94.34	93.7
9	148.2	157.983	148.2
10	103.4	104.241	103.4
1`	120.4	121.803	120.4
2`	116.1	`113.30	116.1
3`	145.5	116.636	145.5
4`	147.2	147.48	147.2
5`	115.5	115.86	115.5
6`	122.4	122.61	122.4
Rh.1``	-	102	-
Gl 1`	-	102.2	101.3
R.S.P.	-	63-78	74.0
CH_3	-	19.44	-
OCH_3	-	56	56

* Rh.: Rhamnose; R.S.P.: Remaining sugar protons; Gl.: Glucose.

3.2. Anti-microbial activity determination

10 g of the aerial parts of the plants were extracted using 70% ethanol. The crude ethanol extracts of the investigated plants were emulsified in water in three concentrations (250, 500 and 1000 ppm) and tested for antimicrobial activity (Table 3). The crude ethanol extracts (1000 ppm) of the investigated plant showed maximum inhibition zones against the tested strains as follow; the plant show maximum inhibition against *Klebsiella pneumonia* followed by *Bacillus subtilis* and *Staphylococcus aureus*. LD_{50} of the ethanol extracts is less than 500 ppm for most tested strains.

Table 3. Antimicrobial activities of methanol extract of *Diplotaxis harra* (Inhibition zoon mm) on some bacteria.

Microorganism	*Diplotaxis harra* Inhibition zoon (mm)	Chloramphenicol Inhibition zoon (mm)
B. subtilis	18.0	20
S. aureus	17.0	16
P. auregenosa	14.0	12
K. pneumonia	22.0	18
E. coli	7.0	5
Enterobacter sp	9.0	8
S. typhi	8.0	12

4. Conclusion

Diplotaxis harra is an important plant, where it has analgesic effect [5] and antimicrobial effect against *Staphlcoccus aureus* and *Bacillus subtilis* [8] and wasn't subjected to any previous studies regarding its chemical constituents, so we isolated some flavonoids compound which varied in activities. Quercetin was reported to possess anti-tumor activity and is capable of interacting with carcinogens in gastero intestinal tract; isorhamnetin 3-*O*-*β*-*D*-glucoside has protective effect against oxidation-induced cell damage. [18], isorhamnetin 3-*O*-*β*-rutinoside has antioxidant and anti-inflammatory activity [19]. Methanol extract has moderate activity against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas auregenosa*, *Echerichia coli*, *Enterobacter sp*, *Salmonella typhi*), so we can show the antibacterial activity of the plant was mainly due to the presence of isolated compounds.

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