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Vol. 8(14), pp. 550-557, 10 April, 2014
DOI: 10.5897/JMPR2014.5370
ISSN 1996-0875
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Journal of Medicinal Plant Research

Full Length Research Paper

Polyphenol derivatives from bioactive butanol phase of the Tunisian narrow-leaved asphodel (*Asphodelus tenuifolius* Cav., Asphodelaceae)

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Received 20 January, 2014; Accepted 31 March, 2014

Dichloromethane, ethyl acetate and butanol phases of the organic extract obtained from *Asphodelus tenuifolius* Cav., a plant growing spontaneously in Tunisia were assessed for antifungal and antibacterial effects using disc diffusion and dilution methods, respectively. The butanol phase exhibited significant antifungal activity against *Candida albicans*, *Candida parapsilosis* and *Candida krusei* and a considerable level of antibacterial activity towards *Escherichia coli* (minimum inhibitory concentration (MIC) = 729 µg/ml) and *Pseudomonas aeruginosa* (MIC = 156 µg/ml). Column chromatography and normal phase preparative high performance liquid chromatography (HPLC) techniques were used to isolate *trans*-N-feruloyltyramine (1), Luteolin (2), Luteolin-7-*O*-*β*-D-glycopyranoside (3), Apigenin (4) and Chrysoeriol (5) from the bioactive butanol extract. The structures of these polyphenols were established on the basis of comparison of complete spectroscopic data with those present in the literature. Some of these compounds have been found for the first time in *A. tenuifolius* or even in plants of genus *Asphodelus*. Their possible involvement in the antimicrobial activity of the extract has been discussed.

Key words: *Asphodelus tenuifolius* Cav., narrow-leaved asphodel, antimicrobial effects, polyphenol derivatives, structure elucidation.

INTRODUCTION

The use of medicinal plants to treat illness and to preserve human health presumably predates the first recorded history. In the modern era, chemists and biologists are highly interested in studying the medicinal

potential of natural extracts aiming at the discovery of useful drugs.

As a contribution to the chemical and biological studies of Medicinal plants growing in Tunisia, the present work deals with the investigation of the narrow-leaved asphodel, *Asphodelus tenuifolius* Cav. (Asphodelaceae), one of the seven species within the *Asphodelus* L. genus grown in Tunisia (Cuénod et al., 1954). Some authors judged *A. tenuifolius* Cav. to be either a variety or a sub-species of the fistulosus asphodel (*Asphodelus fistulosus* var. *tenuifolius* (Cav.) Baker (Le Floc'h et al., 2010) or *A. fistulosus* subsp *tenuifolius* (Cav.) Trab). Later, it has been shown, on the basis of biometric and genetic criteria, that *A. tenuifolius* and *A. fistulosus* L. are clearly two independent species (Ruíz Rejón et al., 1990; Díaz Lifante, 1991).

A. tenuifolius Cav. is an annual or a biennial plant with fibrous roots, a low stem, all leaves radical, fistulous and narrow with a length of 3 to 7 cm. Flowers are clearly bell-shaped and fructiferous pedicels are articulated (Cuénod et al., 1954). This small plant is widely used for various culinary purposes.

The leaves are either boiled or cooked in oil, the seeds are crushed and mixed with flour to make bread and the young shoots are added raw to food to enhance the taste. This plant is little appreciated as pasture. In Egypt, the seeds are reported to be diuretic and are eaten with yogurth (A guide to Medicinal Plants in North Africa, 2005). *In vitro* antimicrobial activities of crude extracts from Indian-herbal medicinal *A. tenuifolius* have been studied. Benzene extract exhibited good antibacterial activity against *Proteus mirabilis* and a very good susceptibility to *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Panghal et al., 2011).

Antifungal activities of petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts of the Indian *A. tenuifolius* were tested against three fungal species showing potential antimicrobial activities (Menghani et al., 2012). Previous phytochemical studies led to the isolation of Asphorodin 1, a triterpenoidal diglycoside showing a potent inhibitory activity against the enzyme lipoxygenase (LOX) (Safder et al., 2009). In the frame of our ongoing project aimed at contributing to the valorization of the Tunisian flora by searching new natural products possessing beneficial biological activities, the present work has been focused on the chemical and biological investigation of butanol phase obtained from the organic extract of *A. tenuifolius* Cav. growing spontaneously in Tunisia. Thus, we report here the isolation and the characterization of *trans* N the isolation and the characterization of *trans* N -feruloyltyramine (1), luteolin (2), luteolin-7-*O*-*β*-D-glycopyranoside (3), apigenin (4) and chrysoeriol (5), isolated for the first time from butanol extract of *A. tenuifolius* Cav.

MATERIALS AND METHODS

Plant

Aerial parts of the narrow-leaved asphodel plant were collected during flowering period at the beginning of March, 2011 from the Kairouan region, center of Tunisia. The plant was identified by Dr. Ridha El Mokni, a member at the Laboratory of Botany and plant Ecology, Faculty of Sciences of Bizerta, Jarzouna, Bizerta, Tunisia, where a voucher specimen [AT (TC, Kair.) 2011-017] has been deposited.

General material

^{1}H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance (NMR) spectra were measured on a varian Inova spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0 or CD_3OD: δ_H 3.34, δ_C 52.0). Homonuclear ^{1}H connectivities were determined by the COSY experiment. Electrospray ionization mass spectrometry (ESI-MS) spectra were performed on a LCQ Finnigan MAT mass spectrometer. Medium pressure liquid chromatography was performed on a Büchi apparatus using a silica gel (230 to 400 mesh) column; HPLC were achieved on a Knauer apparatus equipped with a refractive index detector. LUNA (Phenomenex) columns were used.

Isolation and identification

Dried aerial parts of *A. tenuifolius* Cav. (1.5 kg) were extracted with methanol at room temperature three times to afford 150 g of crude extract after evaporation in vacuum of the solvent. The methanol extract was dissolved in water then successively extracted with CH_2Cl_2, EtOAc and butanol. Butanol phase (8.5 g) was subjected to column chromatography packed with silica gel 60 eluted with a solvent gradient of increasing polarity from hexane/EtOAc 1:1 to EtOAc and then to methanol. 79 fractions of 250 ml were collected and then joined into 27 groups (A_1 to A_{27}) on the basis of analytical thin-layer chromatography.

Isolation of N-feruloyltyramine (1)

The group A_{19} (171 mg) was subjected to HPLC separation on normal phase using a mixture of hexane/EtOAc (35:65) and affording eight subfractions (F_1 to F_8). Subfraction F_5 (7 mg) was further purified via HPLC using same solvent to afford 1 mg of compound 1 as a white solid.

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Isolation of luteolin (2)

Group of fractions A_{17} (107 mg) provided 20 mg of compound 2 as a yellow amorphous powder after precipitation in Ethyl acetate.

Isolation of acetylated derivative of luteolin glycoside (3)

70 mg from group A_{26} (158 mg) were acetylated using acetic anhydride in pyridine at room temperature. The acetylated fraction thus obtained was chromatographed on normal phase HPLC eluted with n-hexane/EtOAc (4:6) mixture (3 ml/min) to give eight subfractions G_1 to G_8. The most polar one (G_8) was further subjected to a second HPLC purification eluted with n-Hexane/EtOAc (2:8) mixture (0.6 ml/min) to afford 1.1 mg of compound 3 as a yellow amorphous powder.

Isolation of apigenin (4) and chrysoeriol (5)

Fractions A_{15} (26 mg) were purified by direct-phase HPLC column eluted using hexane/EtOAc mixture (6:4) with a flow rate of 3.5 ml/min. Thus 1.4 mg of compound 4 and 1.0 mg of compound 5 were isolated as yellow amorphous powders.

Spectral data

NMR analyses of N-feruloyltyramine (1): White solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.45 (1H, d, J = 15.6 Hz, H_{11}); 7.11 (1H, brs, H_{13}); 7.05 (2H, d, J = 8.4 Hz, $H_{3,5}$); 6.78 (1H, d, H_{16}); 6.71 (2H, d, J = 8.4 Hz; $H_{2,6}$); 6.39 (1H, d, J = 15.6 Hz, H_{10}); 3.88 (3H, s, H_{18}); 3.46 (2H, t, J = 7.6 Hz, H_8) and 2.74 (2H, t, J = 7.6 Hz, H_7).

NMR analyses of luteolin (2): Yellow amorphous powder, ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40 (1H, dd, J_1 = 8.8 Hz, J_2 = 2 Hz, $H_{6'}$); 7.39 (1H, d, J = 2 Hz, $H_{2'}$); 6.91 (1H, d, J = 8.8 Hz, $H_{5'}$); 6.55 (1H, s, H_3); 6.44 (1H, d, J = 2 Hz, H_8) and 6.21 (1H, d, J = 2 Hz, H_6).

NMR analyses of apigenin (4): Yellow amorphous powder, ^{1}H NMR (CD_3OD, 400 MHz) δ 7.86 (2H, d, J = 8.8 Hz, $H_{2',6'}$); 6.94 (2H, d, J = 8.8 Hz, $H_{3',5'}$); 6.60 (1H, s, H_3); 6.47 (1H, d, J = 2 Hz, H_6); 6.22 (1H, d, J = 2 Hz, H_8).

NMR analyses of Chrysoeriol (5): Yellowish amorphous powder. ESIMS m/z 299 $[M-H]^-$, ^{1}H NMR (400 MHz, CD_3OD), δ 7.52 (1H, d, J = 8 Hz, $H_{5'}$); δ 7.50 (1H, brs, $H_{2'}$); δ 6.95 (1H, d, J = 8.8 Hz, $H_{6'}$); δ 6.45 (1H, d, J = 2 Hz, H_8); δ 6.20 (1H, d, J = 2 Hz, H_6); δ 6.62 (1H, s, H_3); 3.98 (3H, s, $-OCH_3$).

Biological tests

Antimicrobial activity

Test microorganisms: Antimicrobial screening was performed using Gram-positive bacteria *Staphylococcus aureus* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212), Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923) and the fungi *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019) were provided from the laboratories of Parasitology-Mycology and of Bacteriology CHU. F. Bourguiba, Monastir, Tunisia.

Antifungal assay

Disc diffusion method was employed during the preliminary antifungal screening of crude extracts. Test strains suspension of 1 Mc Farland was prepared from fresh cultures. Plates were aseptically streaked with the tested micro-organisms and allowed to dry for a few minutes. Sterile filter paper Whatman discs (6 mm of diameter) were impregnated with 20 µl of crude extract solution, were then aseptically placed on the inoculated Sabouraud chloramphenicol plates. The plates were therefore incubated during 24 h at a temperature of 37°C. Tests were carried out in triplicates. The presence of a clear circular zone around the sample impregnated disc was used as an indicator of antifungal activity. The results were recorded by measuring inhibition diameter zones in mm. Disc impregnated with the solvent was used as negative control. For comparative purposes, standard drug fluconazole (40 µg/disc) was used as a positive control (Hammami et al., 2013).

Determination of minimum inhibitory concentration

Overnight broth cultures were adjusted to yield approximately 1 × 10^6 CFU/ml of bacteria. The Minimal inhibitory concentrations (MIC) were determined on the basis of the broth microdilution assay using liquid cultures in 96 well microplates from measuring bacterial growth. A sample from each extract (200 µl) was added to four wells of the first column of each plate and then serially diluted with dimethyl sulfoxide (DMSO) (10%) solution as doubling dilutions up to the well number eight of first column dilution factor (1:1). Each well was then inoculated with 50 ml of inocula. Four wells of one column from each plate were inoculated just with conidial suspension without any extract (positive control). Broth medium was used as a negative control. The microplates were incubated for 24 h at 37°C (clinical and Laboratory Standard Institute., 2008; Mousavi and Raftos, 2012).

RESULTS

Antifungal and antimicrobial effects of crude extracts

The three crude phases of the organic extract (methylene chloride, ethyl acetate and butanol) from aerial parts of *A. tenuifolius* Cavan. were evaluated for antimicrobial activity against some intestinal and skin bacterial pathogens (*S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*) and four *Candida* species: *C. albicans*, *C. prapsilosis*, *C. glabrata* and *C. krusei* using dilution and disc diffusion methods, respectively. The results illustrated in Table 1 indicated that butanol extract produced the strongest activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* (MIC = 0.729 and 0.156 mg/ml^{-1}, respectively). Also the other phases of the organic extract from aerial parts of *A. tenuifolius* Cavan. showed sig-nificant antimicrobial activity against *P. aeruginosa*, well known for its involvement in nosocomial infections and frequent resistance to antibiotics.

The disc diffusion antifungal assays showed that four *Candida* species were very sensitive to the polar butanol

Table 1. MIC of crude extracts from aerial parts of *Asphodelus tenuifolius* Cav. using the dilution assay.

Test organism	MIC (mg ml^{-1})		
	Methylene chloride	Ethyl acetate	Butanol
Gram+			
Staphylococcus aureus (ATCC 27853)	1.6	3.5	3.3
Enterococcus faecalis (ATCC 29212)	1.0	4.1	1.2
Gram-			
Escherichia coli (ATCC 25922)	1.8	3.7	0.72
Pseudomonas aeruginosa (ATCC 25923)	0.15	0.15	0.15

Table 2. Antifungal activity of organic extracts from aerial parts of *A. tenuifolius* Cav. using the disc diffusion method.

Test organism	Inhibition zone (mm)				
	Methylene chloride (20 mg/ml)	Ethylacetate (20 mg/ml)	Butanol (20 mg/ml)	Butanol (50 mg/ml)	Fluconazole
Candida albicans ATCC 90028	9±0	6±0	9.3±0.6	14±1.7	25
Candida prapsilosis ATCC 22019	6±0	6±0	8±0	16.6±2.9	30
Candida glabrata ATCC 90030	8±0	8±0	7±0	7±0	15
Candida krusei ATCC 6258	7.3±2.3	6±0	6±0	20±0	10

extract solution at a concentration of 50 mg/ml, thus inhibition zones were between 14 for *C. albicans* and 20 mm for *C. krusei*. Most important is the high bioactivity of butanol polar extract against *C. krusei* which seems more significant than that of the standard antibiotic fluconazole (Table 2). Overall, antimicrobial tests suggested that active compounds are mainly polar and dissolve in butanol. These results are consistent with those of some previous studies, indicating that the inhibitory activity is pathogen specific anddependent on the solvent, concentration of the crude drug and also on rate of diffusion and that alcohols are the most appropriate solvents for extraction of antimicrobial substances (Ahmed et al., 1998; Moorthy et al., 2013).

Encouraged by the results of antimicrobial tests, the butanol extract was subjected to extensive purification and five major polyphenols were isolated in the pure state and characterized through comparison of their spectroscopic data with those reported in the literature.

Characterization of pure compounds

Compound 1 gave ESI-MS ion peaks at *m/z* 314 and 336 attributable to pseudomolecular cations $[M+H]^+$ and $[M+Na]^+$, respectively in agreement with a molecular formula of $C_{18}H_{19}O_4N$. The ^1H NMR spectrum of compound 1 showed two triplets at δ_1 2.74 ppm (2H, t, J = 7.6 Hz) and δ_2 3.46 ppm (2H, t, J = 7.6 Hz*)* assignable to mutually coupled methylene groups at C-7 and C-8. In addition, signals for four aromatic protons of

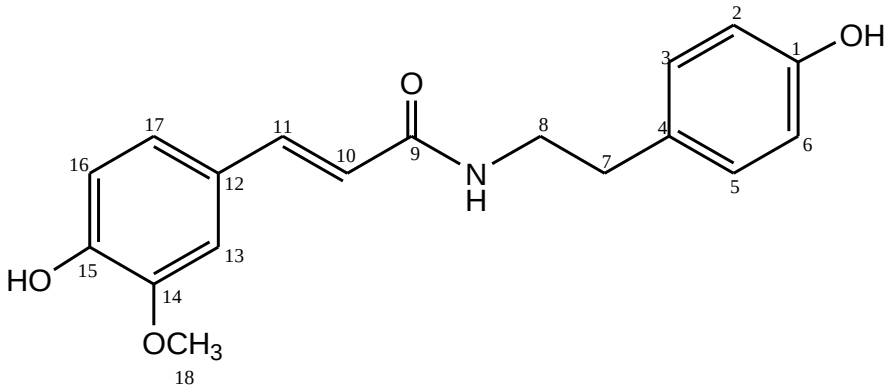


Figure 1. *Trans*-N- feruloyltyramine (1).

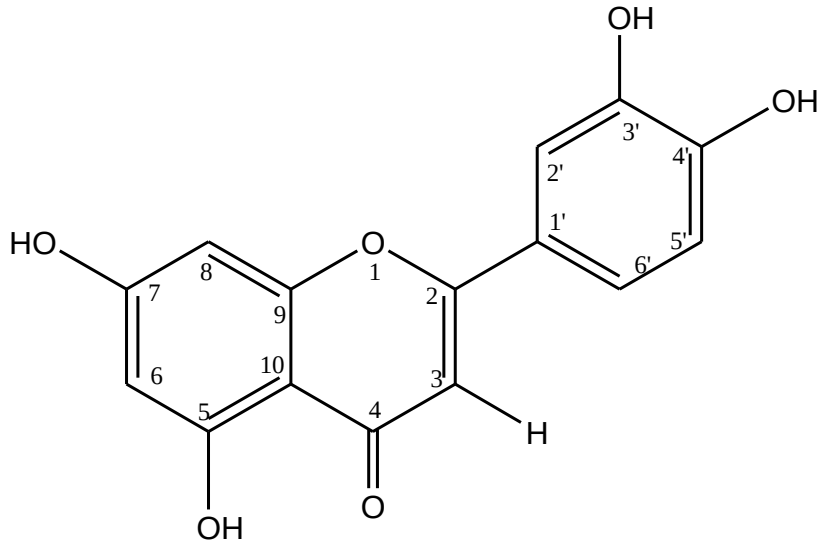


Figure 2. Luteolin (2).

a para-substituted ring at δ 6.71 ppm (2H, d, $H_{2,6}$) and δ 7.05 ppm (2H, d, $H_{3,5}$) as well as three others at δ 6.79 ppm (1H, d, J = 8.4 Hz, H_{16}); δ 7.02 ppm (1H, d, *J* = 8.4 Hz, H_{17}) and δ 7.11 ppm (1H, brs, H_{13}) assignable to protons of a trisubstituted aromatic ring. The ^{1}H NMR spectrum further showed typical signals of two olefinic protons of a *trans*-substituted double bond resonating at δ 7.45 (1H, d, J = 15.6 Hz, H_{11}) and 6.39 (1H, d, J=15.6*Hz*, H_{10}). In addition, a methoxy singlet at δ 3.88 was assigned to the group attached at C-14 on the basis of the ROESY cross-peak with the singlet at δ 7.11. Based on these analyses and on data of the literature (Fattorusso et al., 1999; Park, 2009; Al Taweel et al., 2012), compound 1 was identified as *trans*-N-feruloyltyramine (Figure 1) found for the first time in *A. tenuifolius* Cav.

In compound 2, ^{1}H NMR spectrum of exhibited protons of ABX system at δ 7.40 (dd, J_1 = 8.8 Hz, J_2 = 2 Hz); 7.39 (d, J = 2Hz) and 6.91 (d, J = 8.8*Hz*) of 1,3,4-trisubstituted phenyl unit, one singlet at δ 6.55 attributed to proton H_3 of flavonoids and two meta-coupled doublets at δ 6.42 and 6.21 (J = 2 Hz) characteristic of protons H_8 and H_6 from A ring of 5,7-dihydroxyflavonoid. These NMR data were in accordance with luteolin skeleton (Figure 2) isolated earlier from *A. fistulosus* L. and now found for the first time in *A. tenuifolius* Cav. (Owen et al., 2003). Luteolin has been previously described for its anti-inflammatory effects and its high inhibitory activity against synthesis of

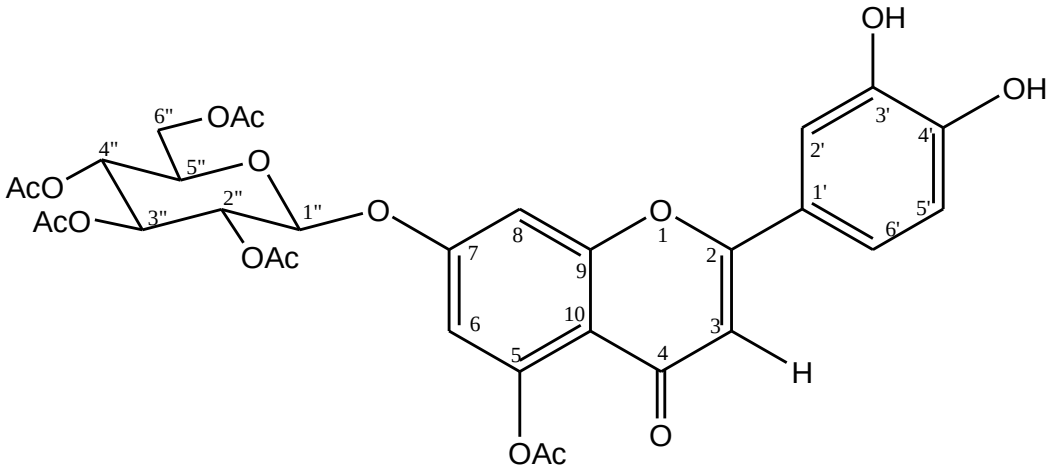


Figure 3. Luteolin 7-O- *β*-D-glycopyranoside tetraacetate (3a).

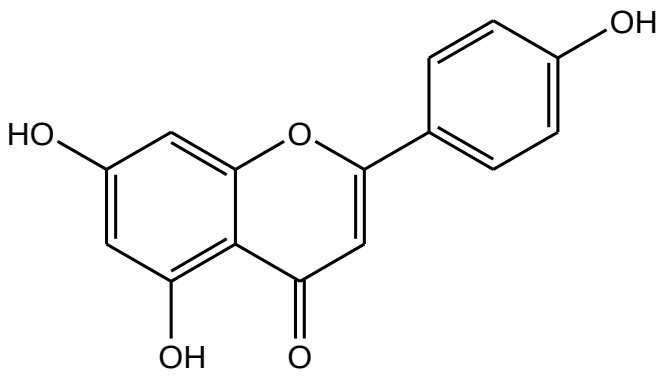


Figure 4. Apigenin (4).

both thromboxane and leukotriene (Odontuya et al., 2005).

In compound 3, luteolin glycoside has been identified via its acetylated derivative 3a (Figure 3). ^{1}H NMR spectrum displayed signals from luteolin skeleton acetylated on C_5 and a series of signals resonating between δ 4.00 and 5.40 ppm attributable to acetylated sugar moiety, whose signals and coupling constants indicated the presence of a glucopyranose unit. Thus, comparison of these data with those of the literature (Xizhi et al., 2011) allowed the identification of the structure of luteolin glucoside for the first time in *A. tenuifolius* Cavan. Luteolin and luteolin glucoside have been mentioned for their antidiabetic effects through inhibition of *α*-glucosidase and α-amylase (Elhawary et al., 2011).

In compound 4, ESI-MS gave ion peak at *m/z* 269 attributable to pseudomolecular anion $[M-H]^-$ in agreement with a molecular formula of $C_{15}H_{10}O_5$. The ^{1}H NMR spectrum of compound 4 exhibited two doublets of meta coupled aromatic protons at δ_H 6.47 (1H, , J = 2 Hz) and δ_H 6.22 (1H, d, J = 2 Hz) attributed to protons H-6 and H-8 of A ring of a flavones moiety. Signals of two vicinally ortho coupled aromatic protons at δ_H 7.86 (2H, d, J = 8.8 Hz) and δ_H 6.94 (2H, d, J = 8.8 Hz) were assigned to $H_{2'/6'}$ and $H_{3'/5'}$ of the ring B. Additionally, the singlet appearing at δ_H 6.60 was ascribed to vinyl proton H-3 belonging to C-ring. Comparison of these data with those of the literature (Ersoz et al; 2002) allowed us to assign the structure of the 5,7,4'-trihydroxyflavone apigenin to compound 4 (Figure 4). Antianxiety activity of apigenin have been previously studied by Suresh Kumar et al. (2006), indicating that this phenolic derivative exhibited significant anxiolytic activity in mice using elevated plus maze model of anxiety (Kumar and Sharma, 2006).

In compound 5, ESI-MS gave ion peak at *m/z* 299 attributable to pseudomolecular anion $[M-H]^-$ in agreement with a molecular formula of $C_{16}H_{12}O_6$. Analysis of its ^{1}H NMR spectrum revealed characteristic protons of one methoxy group at δ 3.98, the H-3 signal at δ 6.62 and aromatic protons at δ 6.45 (1H, d, J = 2 Hz); δ 6.20 (1H, d, J = 2 Hz); δ 6.95 (1H, d, J = 8.8 Hz); δ 7.52 (1H, d, J = 8 Hz) and δ 7.50 (1H, brs). These data pointed to the structure of a methylated derivative of luteolin 2. Comparison of these data with those of flavonoids skeletons (Kang et al., 2010) allowed to propose the structure of chrysoeriol (Figure 5) isolated for the first time from *A. tenuifolius* Cav.

DISCUSSION

Resistance to antimicrobial agents is becoming an urgent global problem and consistent research in the field of

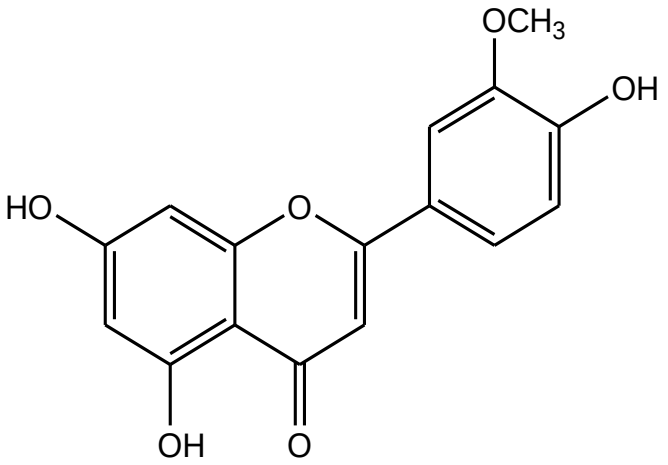


Figure 5. Chrysoeriol (5).

anti-infective agents is strongly needed. In the frame of our research program aimed at exploiting the potential of Tunisian endemic flora, we have demonstrated that extracts obtained from the spontaneous plant *A. tenuifolius* Cav., widely used as culinary ingredient, has a consistent activity against some bacterial and fungal strains. The analysis of the considerably potent antimicrobial butanol phase revealed that it was mainly composed by polyphenols and in this paper we have described in detail those described for the first time from this species.

Flavonoids have been reported to possess many biological and medicinal activities including enzyme inhibition, anti-inflammatory, cytotoxic-antitumor and others. However, flavonoid-rich plants have also been extensively used for their antimicrobial activities (Cushnie and Lamb, 2005) and the best example is given by propolis, whose antimicrobial (antibacterial and anti-fungal) properties have been unambiguously attributed to its flavonoid content (Grange and Davey, 1990). Several hypotheses have been made for the mechanism of antibacterial action of flavonoids but the inhibition of nucleic acid synthesis and the alteration of the membrane function seem to be the better demonstrated mechanisms (Cushnie and Lamb, 2005).

Although we have not separately tested the antimi-crobial activity of the flvonoids isolated from *A. tenuifolius* Cav., on the basis of the above considerations, their involvement in the determination of the activity of the extract seems very likely. On the other hand, it is unlikely that a single flavonoid would be the sole responsibility of the evidenced activity, while the entire flavonoid fraction, exerting a combined and possibly synergistic effect, can be identified as the active part of the plant.

Conclusion

On the basis of the data reported in the present study and those in the literature, five phenolic derivatives were isolated for the first time from antimicrobial bioactive butanol extract of the narrow-leaved asphodel (*A. tenuifolius* Cav., Asphodelaceae) growing spontaneously in Tunisia. The compounds were identified as follows: *trans*-N-feruloyltyramine (1), luteolin (2), luteolin-7-*O*-*β*-D-glycopyranoside (3), apigenin (4) and chrysoeriol (5). These compounds may be mainly responsible for the antimicrobial activity of the butanol extract. However, further studies into the activity of pure isolated compounds are needed to evaluate the potential health and food protecting benefits.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

A guide to Medicinal Plants in North Africa (2005). IUCN Centre for Mediterranean Cooperation Malaga Spain.

Ahmed I, Mehmoud Z, Mohammad F (1998). Screening of some Indian Medicinal Plants for their antimicrobial properties. J. Ethnopharmacol. 62:183-193.

Al Taweel AM, Perveen S, El-Shafae AM, Fawzy GA, Malik A, Afza N, Iqbal L, Latif M (2012). Bioactive phenolic amides from *Celtis Africana*. Molecules 17(3):2675-2682.

Clinical and Laboratory Standard Institute (2008). Reference Method for Broth dilution antifungal susceptibility testing of yeasts: Approved standard-third edition M 27-A3.

Cuénod A, Pottier-Alapetite G, Labbé A (1954). Flore analytique et synoptique de la Tunisie. Cryptogames vasculaires. Gymnospermes et Monocotylédones. p. 287.

Cushnie TPT, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agent 26:343-356.

Díaz Lifante Z (1991). *Asphodelus cirerae*, a forgotten species of *Asphodelus* sect. *Verinea* (*Liliaceae*). Morphological, palynological, karyological and ecogeographical characterization. Flora Mediterr. 1:87-109.

Elhawary SS, Eltantawy ME, Sleem AA, Abdallah HM, Mohamed NM (2011). Investigation of phenolic content and biological activities of *Scabiosa atropurpurea* L. World Appl. Sci. J. 15(3):311-317.

Ersoz T, Harput US, Saracoglu I, Calis I (2002). Phenolic compounds from *Scutellaria pontica*. Turk. J. Chem. 26:581-588.

Fattorusso E, Lanzotti V, Tagliatela-Scafati O (1999). Antifungal N-feruloylamides from roots of two *Allium* species. Plant Biosyst. 133(2):199-203.

Grange JM, Davey RW (1990). Antibacterial properties of propolis (beeglue). J. R. Soc. Med. 83:159–160.

Hammami S, Ben Salem A , Mastouri M, Falconieri D, Gorcii M, M'Henni MF, Marongiu B, Mighri Z (2013). Essential oil composition and antimicrobial activities of aerial parts from Tunisian *Anacyclus clavatus* (Desf). J. Med. Plants Res. 7(2):71-75.

Kang J, Li Z, Wu T, Jensen G, Schauss, AG, Wu X (2010).Anti-oxidant capacities of flavonoid compounds isolated from acai pulp (*Euterpe oleracea* Mart.). Food Chem. 122:610-617.

Kumar S, Sharma A (2006). Apigenin: the anxiolytic constituent of *Turnera aphrodisiaca*. Pharm. Biol. 44(2):84-90.

Le Floc'h E, Boulos L, Vela E (2010). Catalogue synonymique commenté de la flore de Tunisie. P 503.

Menghani E, Bhatnagar K, Saraswat P, Soni M (2012). Isolation and characterization of bioactives from arid zone plants. Int. J. Pharm. Res. Dev. 4(02):113-118.

Moorthy KK, Subramaniam P, Senguttuvan AJ (2013). In vitro antifungal activity of various extracts of leaf and steam parts of *Solena amplexicaulis* (Lam.) Gandhi. Int. J. Pharm. Pharm. Sci. 5(3):745-747.

Mousavi SM, Raftos D (2012). In vitro Antifungal activity of a new combination of Essential oils against some filamentous Fungi. Middle East. J. Sci. Res. 11(2):156-161.

Odontuya G, Hoult JRS, Houghton PJ (2005). Structure-activity relationship for anti-inflammatory effect of Luteolin and its derived glycosides. Phytother. Res. 19:782-786.

Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. Food Chem. Toxicol. 41:703-713.

Panghal M, Kaushal V, Yadav JP (2011). *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann. Clin. Microbiol. Antimicrob. 10:21.

Park JB (2009). Isolation and characterization of N-Feruloyltyramine as the P-selection Expression suppressor from Garlic (*Allium sativum*). J. Agr. Food Chem. 57(19):8868-8872.

Ruíz Rejón C, Blanca G, Cueto M, Lozano R, Ruíz Rejón M (1990). *Asphodelus tenuifolius* and *A. fistulosus* (*Liliaceae*) are morphologically, genetically and biologically different species. Pl. Syst. Evol. 169:1-12.

Safder M, Imran M, Mehmood R, Malik A, Afza N, Iqbal L, Latif M (2009). Asphorodin, a potent lipoxygenase inhibitory triterpene diglycoside from *Asphodelus tenuifolius*. J. As. Nat. Prod. Res. 11(11):945-950.

Xizhi F, Suixu X, Mei D (2000). Two novel flavonoids from *Ixeris sonchifolia*. J. Chin. Pharm. Sci. 9(3):134-136.