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**RESEARCH ARTICLE**

# Metabolic profile of the bioactive compounds of *Ormenis africana* Jord. and Fourr. (Asteraceae) an endemic species from Tunisia

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## *Abstract*

The profiles and bioactivities of phenolics extracted from leaves of *Ormenis africana* Jord. and Fourr. were investigated. The amount of total phenolics, measured by Folin-Ciocalteu method, was high (50±0.408 mg GAE/100g DM). The total flavonoid content is about 42.56 ± 0.980 QE/g of dry mass. The ability of *O. africana* leave to act as a natural antioxidant was assessed by means of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals scavenging and ferric reducing power (FRAP). *Ormenis* displays significant activity towards Ferric reducing power, even superior to that measured towards DPPH radicals (52 ± 3.1 vs 46 ± 0.5 µm TE, respectively). $ABTS^{+}$ radical cation was found to be in the same range as DPPH radicals (36 ± 2 µm TE). Phenolic profile and composition of leave extract were determined for the first time. The analysis was performed by HPLC-PDA-ESI/MS in negative mode. The main phytochemicals in *O. africana* were identified as mono-caffeoylquinic acid and dicaffeoylquinic I. Due to the well established antioxidant activity of phenolic compounds, *O. africana* leaves could be selected for its incorporation into functional beverages or products with bioactive properties related to oxidative stress.



**Abbreviations**: HPLC-PDA-ESI/MS: reversed phase HPLC with on-line photodiode array detection and electrospray ionization mass spectrometry method, UV: The ultraviolet, Mw: Molecular weight, DPPH: Free radical scavenging activity, FRAP: Ferric ion reducing antioxidant power, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

## Introduction

In recent years there is strong current interest in discovering new edible natural products with medicinal properties. Since 1960, phytochemicals previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. The use of herbal remedies is often justified by their long history of usage. But, this is does not necessarily guarantee that the product in question is efficacious with reasonable specificity.

Tunisian flora includes more than 2150 species (Pottier-Alapetite, 1979; 1981) growing on various bioclimatic zones ranging from lower humid to Saharan. Actually, 200 to 350 spontaneous species are considered as medicinal and aromatic plants (MAPs) and used in traditional phytotherapy mainly by rural communities having developed a large local knowledge (Le Floc'h, 1983; Boukef, 1986).

The present work juxtaposes ancient practices related to Tunisian MAPs and their various parts with relevant modern studies of their biological activities, emphasizing potential antioxidant activities. Antioxidant compounds are usually employed in the food industry to prevent undesirable changes due to oxidation reactions. Actually, there is a wide interest in finding phytochemicals that could replace synthetic antioxidants such as BHT and BHA which are commonly used in foods, packaging for foods, and medicines because of its possible secondary effects.

*Ormenis africana* (Jord. and Fourr.) Lit. et Maire (Asteraceae) is an endemic specie of the North African region that grows naturally in the rocky slopes on the mountains (Pottier Alapetite, 1980). It has been used since ancient times for medicinal, food and spice purposes, and known for its healing effects (Yawar, 2001). It is traditionally used for its hypoglycemic effect as well as for the treatment of stomacal pain. Inflorescences of this plant are mixed with honey and used for the treatment of the cardialgia ulcer and stomacal pain (Ben Mansour et al., 2011). To the best of our knowledge there is only a single report on the antioxidant capacity of *O. Africana* extract (Ben Mansour et al., 2011).

In the present paper, we report a detailed qualitative profile of flavonoids present in *O. africana* methanolic extract. On top of that, we report also on the total phenolic and flavonoid contents and antioxidant capacity of this little-known plant.

## 2. Materials and methods

*2.1. Plant materiel*

*Ormenis africana* samples were collected in region of Kef Mountain in April 2012 from plants growing under upper semi-arid bioclimatic zone. Ten plants were sampled at distances exceeding 50 m to avoid the sampling of closely related individuals. Botanical identification of this species was carried out by Prof. M. Boussaid, Biologist (National Institute of Applied Science and Technology, Tunisia). After the harvest, voucher specimens were deposited at the herbarium of INSAT.

*2.2. Extraction*

Plant was dried in air shade at room temperature, and the dry plant was powdered. 10 g of powders was extracted in a soxhlet system with 100 ml of two solvents: hexane (6h) and methanol (6h). The two organic extracts were concentrated by rotary evaporation under vacuum at 35°C. Finally, the solvent was evaporated under reduced pressure, and the powder thus obtained was resuspended in 10 ml methanol. For the chemical analyses and the antioxidant activities, methanolic extracts were re-suspended in the methanol and filtered through a 0.22 µm filter paper discs (Glass Microfibre filters, Whatman; 6 mm in diameter), and stored in deep freeze (-20°C) until further treatment.

*2.3. Total flavonoids determination*

The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand et al. (1994). A diluted solution (4 ml) of extract was mixed with a solution (4 ml) of aluminum trichloride ($AlCl_3$) in methanol (2%). The absorbance was read at 415 nm after 15 min against a blank sample consisting of a methanol (4 ml) and extract (4 ml) without $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100g of dry weight.

*2.4. Determination of total phenolic compounds by the Folin-Ciocalteu method*

Total phenols were determined by using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products and slightly modified by Dewanto et al. (2002). An aliquot of each diluted sample extract (0.5 ml) was mixed with 2 ml Folin-Ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate solution (7.5%) was added. After incubation (90 min) in dark, the absorbance at 760 nm was read versus the prepared blank. The total phenolic content of the plant parts was expressed as milligrams of gallic acid equivalents per 100 g of dry weight through the calibration curve with gallic acid. All samples were analyzed in three replications.

*2.5. Antioxidant activities of methanolic extract*

The antioxidant activity of methanolic extract was assessed using both free radical scavenging activity (RSA) and of a stable ABTS radical cation.

*2.5.1. DPPH radical scavenging assay*

The antioxidant ability was assessed using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The assay was carried out following a literature procedure (Barreca et al., 2010). *O. africana* leave was centrifuged at 2800 g for 10 min and the supernatant was collected for analysis. Supernatant (50 µl) was mixed with 63 µm of DPPH in methanol, to a final volume of 4 ml. The control contained all the components except juice supernatant. The changes in absorbance at 517 nm were monitored after 50 min of incubation. The inhibition percentage I(%) of radical-scavenging activity was calculated as:

I(%) = 100× ($A_0$- $A_S$)/ $A_0$, where $A_0$ is the absorbance of the control and $A_S$ is the absorbance of the sample after 50 min of incubation. All tests were run in triplicate and the results expressed as means ± standard deviation (SD). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in the 1.0–100 µm range was chosen as a standard antioxidant and leave activity expressed as µm of Trolox equivalents (TE), referring to 1 ml of extract.

*2.5.2. Free radical-scavenging ability by the use of a stable ABTS radical cation*

The method is based on the ability of antioxidant molecules to quench the long-lived 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$), compared with that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). A stable stock solution of $ABTS^{\bullet+}$ was produced by reacting a 7 mmol aqueous solution of ABTS with 2.45 mmol ammonium persulfate (final concentration), and allowing the mixture to stand in the dark at room temperature for 12–16 h before use (Pellegrini et al., 1999). At the beginning of the analysis day, an $ABTS^{\bullet+}$ working solution was obtained by diluting the stock solution with ethanol, to an absorbance of 0.70 ± 0.02 at 734 nm, verified with a Varian Cary 50 UV–vis spectrophotometer (Varian, Waldbronn, Germany). *O. africana* leave was centrifuged at 2800 g for 10 min and the supernatant was collected for analysis. Ten microlitres of supernatant was mixed with the working solution, to a final volume of 1 ml. The inhibition percentage I(%) of radical scavenging activity was calculated as:

I(%) = 100× ($A_0$- $A_S$)/ $A_0$, where $A_0$ is the absorbance of the control and $A_S$ is the absorbance of the sample after 4 min of incubation. Trolox (in the range from 1.0 to 50 µm) was used as a reference standard. TEAC were expressed as µm of Trolox equivalents (TE), referring to 1 ml of extract.

*2.5.3. Ferric reducing antioxidant power (FRAP) assay*

The FRAP assay was performed according to a literature procedure with minor modification (Benzie and Strain, 1996). The fresh working FRAP reagent was prepared daily by mixing 25 ml acetate buffer (300 mmol, pH 3.6), 2.5 ml TPTZ solution (10 mmol in 40 mmol HCl) and 2.5 ml of $FeCl_3$ $6H_2O$ solution (20 mmol). The reagent was warmed to 37°C, then 1500 µl were placed in a cuvette and the initial absorbance was read. A 50 µl volume of extract was added to the cuvette and the absorbance was measured after 4 min at 593 nm. All tests were run in triplicate and the results expressed as means ± standard deviation (SD). Trolox was chosen as a standard antioxidant, and extracts' activity were expressed as µm of Trolox equivalents (µm TE) referring to 1 ml of extract.

*2.6. LC-PDA-ESI/MSD conditions*

The phenolic compounds of *O. africana* were identified using a 3100 mass detector (Waters Co., Milford, MA) and an Alliance e2695 HPLC system (Waters Co.) equipped with a 2998 photodiode array detector (PDA), in addition to an XTerra MS C18 column (3.5µm, 150 x 4.6 mm, Waters, Milford, MA, USA).

The analysis was conducted at a flow rate of 0.5 ml/min at a detection wavelength of 200-600 nm and the oven temperature was 25°C. The mobile phases used were 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B) and the gradient varied from 14% to 26% B in 40 min, to 15% B at 60 min, to 0% B at 75 min and finally to 14% B at 75 min and held at 14% B from 75 to 80 min.

The DAD was set at 280, 320 and 360 nm to provide real time chromatograms and the UV/Vis spectra from 200 to 600 nm were recorded for plant component identification.

Mass spectra were acquired using electrospray ionization in the negative ionization (NI) modes. The MS parameters were each set to a cone voltage of 70 V, source temperature of 120 °C, desolvation temperature of 350 °C, and a desolvation N2 gas flow of 780 l/h. The range of molecular weights was m/z 100–1000 in full scan mode.

## Result and Discussion

*3.1. Chemical composition: phenolics and flavonoids*

This is the first study which recorded the phenolic and flavonoid content of methanolic extracts from *O. Africana*. The amount of total phenolics, measured by Folin-Ciocalteu method, was high (50±0.408 mg GAE/100g DM). It is lower than that observed for inflorescence ethanolic extract (312.07 ± 4.81 mg GAE/g dray matter (Ben Mansour et al., 2011). The amount of flavonoids was 42.56 ± 0.980 QE/g of dry mass. The results of Ben Mansour et al. (2011) show that ethanolic extracts contain 73.72 ± 1.98 QE/g of dry mass. The difference between our results and their results was probably due to the difference of the part of the plant used (we have used leaves and they have used inflorescences) and/or of the used solvent. There is no other study in the literature which investigated the phenolic and flavonoid content in this species. Previous works showed that Asteraceae species (*Achillea millefollium, Echinaceae purpurea*, …) exhibited high levels of polyphenols (Wojdyło et al., 2007). In the literature it was reported that phenolic contents on methanol extracts of 112 traditional Chinese medicinal plants ranged from 0.22 to 50.3 g of gallic acid equivalent/100 g DW (Cai et al., 2004).

*3.2. Antioxidant activity*

The ability of *O. afriana* extract to act as a natural antioxidant was assessed by means of DPPH and ABTS radicals scavenging and ferric reducing power (FRAP).

The results (Table 1) show that, in line with other flavonoid-rich Asteraceae extracts (Ben Mansour et al., 2011). *O. africana* displays significant quenching activity towards $DPPH^{\cdot}$ radicals, even superior to that measured towards $ABTS^{\cdot+}$ radical cations (46 ± 0.5 vs 36 ± 2 µm TE, respectively). Ferric reducing power was found to be in the same range as $DPPH^{\cdot}$ radicals bleaching (52 ± 3.1 µm TE).

The antioxidant activity shown by *Ormenis* leave is remarkable in light of the relatively high amount of flavonoids. However, given the well-known ability shown by flavonoids (and flavones, in particular) to act as radical scavengers (Barreca et al., 2009; Bellocco et al., 2009). The different values obtained from the three assays are a consequence of the evidence that the test species quenched/reduced by leave of *O. africana* (i.e., DPPH, $ABTS^{+}$ and $Fe^{3+}$) react according to different mechanisms and kinetics. In fact, even though these antioxidant ability assays proceed via electron transfer (ET) rather than hydrogen atom transfer (HAT) mechanisms, they have been found to be influenced by both the pH and the solvent mixtures in which they are carried out (Huang et al., 2005).

Several reports have shown close relationship between total phenolic contents and antioxidative activity of the fruits, plants and vegetables (Shahidi and Marian, 2003; Bouaziz et al., 2009). The chemical composition and chemical structures of active extract components are important factors governing the efficacy of natural antioxidants.

**Table 1**. Total Phenolics and flavonoïds and antioxidant capacity in methanolic extract of *Ormenis africana*.

| Sample | TPC[b] | TF[c] | Antioxidant Capacity | | |
|---|---|---|---|---|---|
| | | | ABTS[d] | DPPH[d] | FRAP[d] |
| Methanolic extract | 50 ±0.408 | 42.56 ± 0.980 | 36 ±2 | 46 ±0.5 | 52 ±3.1 |

[a]: Results are reported as mean ± standard deviation of three replicates (95% confidence); [b]: mg gallic acid equivalents/g dry weight; [c]: mg quercetin equivalents/g dry weight; [d]: µm Trolox equivalents (TE).

TPC: total phenolic compounds, TF: total flavonoids.

**Table 2**. Peak assignments of the methanol extract of *O. africana*.

| Peak No. | RT (min) | Mw | UV λmax (nm) | $[M+H]^+$ *(m/z)* | $[M-H]^-$ *(m/z)* | Identification | Occurrence |
|---|---|---|---|---|---|---|---|
| **1** | 4.35 | 192 | 205/262 | - | 191 | Quinic acid | ++ |
| **2** | 6.79 | 152 | 205 | - | 151 | Unknown | ++ |
| **3** | 11.09 | 354 | 244, 300sh, 326 | 355.23 | 353.15 | mono-caffeoylquinic acid | ++++ |
| **4** | 13.01 | 594 | 256, 266, 348 | - | 593.13 | Luteolin-7-O-rutinoside | + |
| **5** | 18.43 | 306 | 208, 270 | - | 305 | epigallocatechin | + |
| **6** | 20.25 | 780 | 241, 326 | - | 779.3 | Unknown | + |
| **7** | 24.48 | 594 | 264,346 | - | 593.13 | Kaempferol 3-O-rutinoside | +++ |
| **8** | 27.23 | 448 | 269,348 | - | 447.27 | Orientin | +++ |
| **9** | 31.4 | 578 | 268, 329 | - | 577.12 | Apigenin-7-*O*-rutinoside | + |
| **10** | 33.5 | 516 | 244, 300sh, 326 | 517.27 | 515.06 | dicaffeoyquinic acid I | ++++ |
| **11** | 37.54 | 516 | 244, 300sh, 326 | - | 515.13 | di-caffeoyquinic acid II | + |

*3.3. HPLC–DAD–ESI/MS analyses for phenolic compounds identification*

Data from the LC-DAD–ESI/MS were used to identify the phenolic acids and flavonoids. Three DAD chromatograms (360, 320, and 280 nm) are shown in Fig 1. The retention times (TR), UV $\lambda_{max}$ values, and the molecular ions of the flavonoids and phenolic acids are listed in Table 2 for each peak.

The identification of flavonoids was performed according to following rules: (1) Most aglycones in *Santolina chamaecyparissus* L. are luteolin, kaempferol, Hispidulin, quercetin, patuletin, jaceocidin and apigenin; (2) The spectra of flavonoids typically consist of two absorption maxima in the ranges 240-285 nm (band II) and 300-400 nm (band I). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoid and its oxygenation pattern; 3) The flavonoids (M) easily formed $[2M-H]^-$, $[2M+H]^+$ or $[2M+Na^+]^+$ in

ESI-MS. The structures of compounds were finally identified by comparison with literature when reference standard is unavailable (Stobiecki, 2000; March et al., 2006; Tolonen and Uusitalo, 2004; De Rijke et al., 2004). Phenolic acids were identified according to a hierarchical scheme for characterizing chlorogenic acids that has been developed by Clifford et al. (2003; 2005; 2008) and Plazonić et al. (2009).

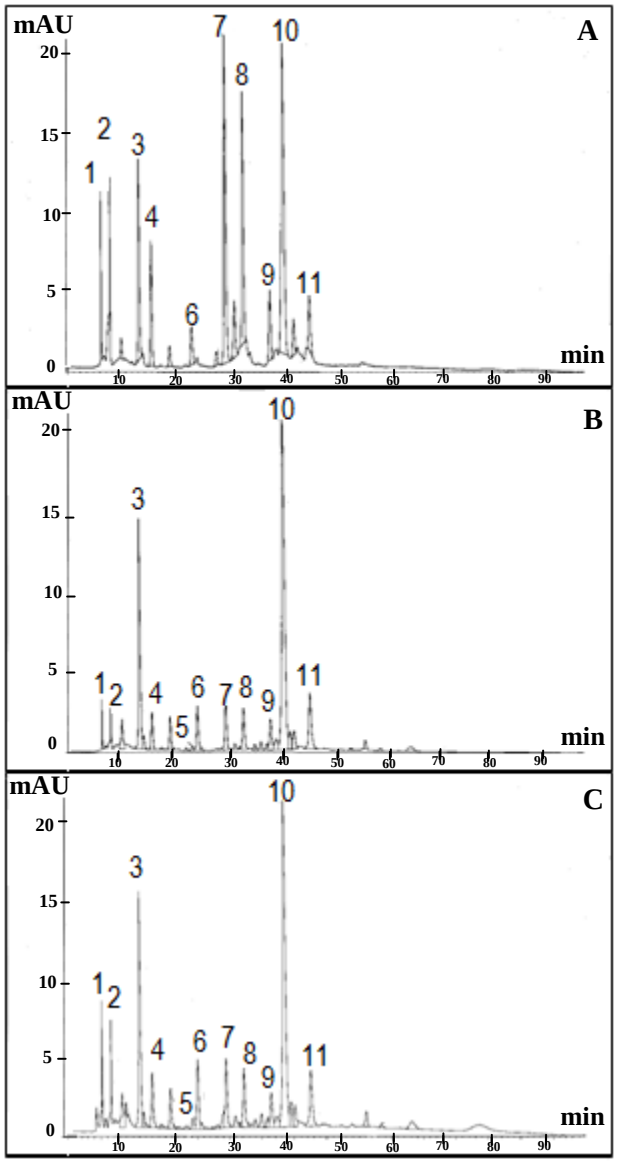


**Figure 1**. The LC chromatograms of Tunisian *O. africana* extract with UV detection at 360 (A), 320 (B), and 280 (C).

The hydroxycinnamic derivatives detected in this work belong to mono- and di-caffeoylquinic acid compounds. These data agree with the results in the literature cited (Jin et al., 2007; Pinelli et al., 2007).

The major peaks (**3** and **10**) eluted at 11.09 and 33.5 min, were identified as mono-caffeoylquinic acid and dicaffeoylquinic acid I (Table 2). The ESI-MS spectra of both compounds are shown in Fig 2.

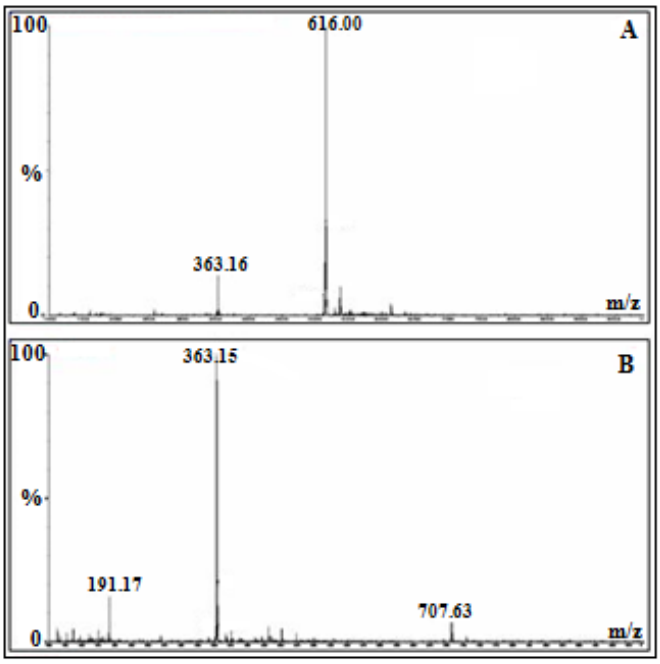


**Figure 2.** ESI-MS spectra for: **(A)** compound 3; **(B)**: compound 10 in the negative mode.

Compound **3** (TR = 11.09 min) give a $[M-H]^-$ ion at m/z 353 and showed a fragment ion at m/z 191 which represents a quinic acid, resulting from the neutral loss of caffeic acid $[M-H-162]^-$. It showed also a $[M+H]^+$ ion at m/z 355 and a fragment ions at m/z 163 and m/z 731 $[2M+Na]^+$. Therefore, this compound has been assigned as mono-caffeoylquinic acid.

Compound **10** (RT = 33.5 min) give $[M-H]^-$ ion at m/z 515 and $[M+H]^+$ ion at m/z 517 with a fragment ion at m/z 353 $[M-H-162]^-$ and at m/z 355 $[M+H-162]^+$ and identical molecular formula ($C_{25}H_{23}O_{12}$), was identified as isomer of dicaffeoylquinic acid. In addition, compound **11** (RT = 37.5 min) give $[M-H]^-$ ion at m/z 515 and fragment ion at m/z 353$[M-H-162]^-$ and identical molecular formula ($C_{25}H_{23}O_{12}$), was identified as isomer of dicaffeoylquinic acid, named dicaffeoylquinic I. The second isomer (compound **11)**, eluted at RT = 37.54 min, is dicaffeoylquinic II.

Flavonoids were detected on leave extracts of *O. africana*. The main aglycones detected were flavones apigenin and luteolin and flavonol kaempferol. The important flavonoids are Kaempferol 3-O-rutinoside (compound **7**) and Orientin (compound **8**) (Table 2). However, two components with retention times of 6.79 (m/z 151) and 20.25 (m/z 779) were not identified.

*Ormenis africana* is considered as synonym of *Santolina chamaecyparissus* L.. The later was shown to accumulate 7-0-glycosides of apigenin, luteolin, chrysoeriol (Becchi et al., 1976), patuletin, and axillarin (Becchi and Combier, 1976). Becchi and Carrier (1980) reported hispidulin, pectolinaringenin, nepetin, and jaceosidin. Luteolin 7-methyl ether and 5,4'-dihydroxy-6,7-dimethoxyflavone were added to the list by Wollenweber et al., 1989). Bioactivity-guided fractionation of the methanol extract from the leaves of *Santolina insularis* had led to the isolation of six flavonoids: hispidulin, nepetin, cirsimaritin, rhamnocitrin, luteolin and luteolin 7-O-β-d-glucopyranoside (Cottiglia et al., 2004).

The important constituents, considered as chemical markers of *Santolina* species (chrysoeriol, nepetin, jaceosidin, patulitin,…) were not detected on our work suggesting a high chemical differentiation between *O. africana* and its synonymous species *Santolina chamaecyparissus*. *O. africana* has a specific position as an endemic North African species. Endemism may play a major role in flavonoid diversity. Flavonoids have been used extensively in phytogeographical investigations. In an investigation of flavonoid diversity and endemism in *Parthenium* L. species, Mears (1980) reported that narrow endemic were characterized by depauperate flavonoid profiles composed largely of methylated aglycones; while, in contrast, wide ranging species ware characterized by high flavonoid diversity with a variety of glycosides and few methylated aglycones. It was hypothesized that this reduction of fiavonoid profile in narrow endemics is a result of the founder effect and structural changes related to natural selection in populations.

## Conclusion

The present study represents a contribution to the chemical characterization of phenolic extracts from wild endemics with reported antioxidant activity and traditionally used for several medicinal applications. The phenolic components and bioactivities of extracts from *O. africana* leaves were reported first time. The major detected phenolics are hydroxycinnamic derivatives. Leaves of *O. africana* were found to be an effective antioxidant in several in vitro assays. In order to find new chemical particularities of this endemic species, further chemical analyses and biological activities must be investigated.

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## References

Arvouet-Grand, A., Vennat, B., Pourrat, A. and Legret, P. (1994) : Standardisation d'un extrait de propolis et identification des principaux constituants. Standardization of a *Propolis* extract and identification of the main constituents. J. Pharm. Belg., 49(6): 462-468.

Barreca, D., Bellocco, E., Caristi, C., Leuzzi, U. and Gattuso, G. (2010): Flavonoid composition and antioxidant activity of juices from chinotto (*Citrus myrtifolia* Raf.) fruits at different ripening stages. J. Agr. Food Chem., 58(5): 3031-3036.

Barreca, D., Laganà, G., Tellone, E., Ficarra, S., Leuzzi, U. and Galtieri, A. (2009): Influences of flavonoids on erythrocyte membrane and metabolic implication through anionic exchange modulation. J. Membrane Biol., 230(3): 163-171.

Becchi, C., Rouet, A. and Stora, R. (1976): Renormalization of gauge theories. Ann. Phys., 98, 287-321.

Becchi, M. and Carrier, M. (1980): 6-methoxy flavones of *Santolina chamaecyparissus.* Planta Med., 38: 267-268.

Becchi, M. and Combier, H. (1976): Sur deux glucosides flavoniques des feuilles de *Santolina chamaecyparissus*. Planta Med., 30(8): 388-394.

Bellocco, E., Barreca, D., Laganà, G., Leuzzi, U., Tellone, E. and Kotyk, A. (2009): Influence of L-rhamnosyl-D-glucosyl derivatives on properties and biological interaction of flavonoids. Mol. Cell. Biochem., 321(1-2): 165-171.

Ben Mansour, R., Gargouri, B., Bouaziz, M., Elloumi, N., Belhadj, J.I., Ghrabi, Z. and Lassoued, S. (2011): Antioxidant activity of ethanolic extract of inflorescence of *Ormenis Africana* in vitro and in cell cultures. Lip. Health Dis., 10:78.

Benzie, I.F.F. and Strain J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay. Anal. Biochem., 239(1): 70-76.

Bouaziz, M., Dhouib, H., Smaoui, S., Loukil, S., Boukhris, M. and Sayadi, S. (2009): Polyphenols, antioxidant and antimicrobial activities of some wild medicinal plants collected in southern Tunisia. Afr. J. Biotechnol., 8(24): 7017-7027.

Boukef, M.K. (1986): Les plantes dans la médecine traditionnelle tunisienne "Médecine traditionnelle et pharmacopée". Agence de coopération culturelle et technique, pp. 350.

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004): Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Clifford, M.N., Johnson, K.L., Knight, S. and Kunhert, N. (2003): Hierarchical scheme for LC-$MS^n$ identification of chlorogenic acid. J. Agr. Food Chem., 51(10): 2900-2911.

Clifford, M.N., Kirkpatrick, J., Kunhert, N., Roozendal, H. and Salgado, P.R. (2008): LC-$MS^n$ analysis of the cis isomers of chlorogenic acid. Food Chem., 106: 379-385.

Clifford, M.N., Knight, S. and Kunhert, N. (2005): Discriminating between the six isomers of dicaffeolquinic acid by LC-$MS^n$. J. Agr. Food Chem., 53(10): 3821-3832.

Cottiglia, F., Dhanapal, B., Sticher, O. and Heilmann, J. (2004): New chromanone acids with antibacterial activity from *Calophyllum brasiliense*. J. Nat. Prod., 67: 537-541.

De Rijke, E., De Kanter, F., Ariese, F., Gooijer, C. and Brinkman, U.A.T. (2004): Liquid chromatography coupled to nuclear magnetic resonance spectroscopy for the identification of isoflavoneYglucosideYmalonates in *T. pratense* L. leaves. J. Sep. Sci., 27: 1061Y1070.

Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. (2002): Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem., 50: 3010-3014.

Huang, D., Ou, B. and Prior, R.L. (2005): The chemistry behind antioxidant capacity assays. J. Agric. Food Chem., 53(6): 1841-1856.

Jin, C.C., Fang, D., Bo, Z., Li, Y. and Zhong, L.L. (2007): Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: Mechanism and structure–activity relationship. Food Chem., 104: 132-9.

Le Floc'h, E. (1983): Contribution à une étude ethnobotanique de la flore tunisienne. Imp. Off. Rep., Tunisia.

March, R.E., Lewars, E.G., Stadey, C.J., Miao, X.S., Zhao, X. and Metcalfe, C.D. (2006): A comparison of flavonoid glycosides by electrospray tandem mass spectrometry. Internat. J. Mass Spectrom., 248: 61-85.

Mears, J.A. (1980): Flavonoid diversity and geographic endemism in *Parthenium*. Bioch. Syst. Ecol., 8: 361-370.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C.A. and Lester, P. (1999): Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying the 2,20-azobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. Meth. Enzymol., 299: 379-389.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E. and Romani, A. (2007): Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. Food Chem., 105: 1695-1701.

Plazonić, A., Bucar, F., Maleš, Ž., Mornar, A., Nigović, B. and Kujundžić N. (2009): Identification and Quantification of Flavonoids and Phenolic Acids in Burr Parsley (*Caucalis platycarpos* L.), Using High-Performance Liquid Chromatography with Diode Array Detection and Electrospray Ionization Mass Spectrometry. Molecules 14: 2466-2490.

Pottier-Alapetite, G. (1979-1981): Flore de la Tunisie. Angiosperrnes Dicotylédones: apétales - dialypétales - gamopétales. Première et deuxième partie. - Ouvrage publié par le Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture. Tunisie, 1190 p.

Shahidi, F. and Marian, N. (2003): Phenolics in food and nutraceuticals. CRS Press L.L.C., Boca Raton, F.L., 1: 144-150.

Singleton, V.L. and Rossi, J.A. (1965): Colorimetry of total phenolic with phosphomolibdic phosphotungstic acid reagents. American J. Enol. Vitic., 16: 144-158.

Stobiecki, M. (2000): Application of mass spectrometry for identification and structural studies of flavonoid glycosides. Phytochem., 54(3): 237-256.

Tolonen, A. and Uusitalo, J. (2004): Fast screening method for the analysis of total flavonoid content in plants and foodstuffs by high-performance liquid chromatography/electrospray ionization time-of-flight mass spectrometry with polarity switching. Rapid Comm. Mass Spectrom., 18: 3113-3122.

Wojdyło, A., Oszmiański, J. and Czemerys, R. (2007): Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940-949.

Wollenweber, E., Maan, K. and Valant-Vetscher, K. (1989): External flavonoid aglycones in Artemisia and some further *Anthemideae* (Asteraceae). Fitoterapia 60: 460-463.

Yawar, A. (2001): Spirituality in medicine: what is to be done? J. Royal Soc. Med., 94: 529-533.