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Comparative study of wild chamomile plants from the north-west of Morocco: Bioactive components and total antioxidant activity

Amina EL MIHYAOUI[1,2*], María Emilia Candela CASTILLO[1], Antonio CANO[1], Josefa HERNANDEZ-RUIZ[1], Ahmed LAMARTI[2] and Marino Bañon ARNAO[1]

[1]Department of Plant Biology (Plant Physiology), Faculty of Biology, University of Murcia, 30100-Murcia, Spain.
[2]Laboratory of Plant Biotechnology, Department of Biology, Faculty of Sciences, Abdelmalek Essaadi University, Tetouan, Morocco.

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Morocco presents numerous plants due its localization between the Mediterranean Sea and Atlantic Ocean and the variability of its mountainous terrain, with a rich range of medicinal plants. *Cladanthus mixtus* (L.) Oberpr. & Vogt and *Matricaria chamomilla* L., two Asteraceae (Compositae) from Beni Hassane region in north-western Morocco are both used in traditional medicine for the treatment of different ailments. The phenolic and flavonoid contents of roots, stems, leaves, and flowers of both species were determined by using the Folin Ciocalteu and the aluminum chloride methods, respectively. Antioxidant activity of their aqueous and organic compounds was performed applying the robust and widely used method ABTS radical scavenging assay. *C. mixtus* had a higher phenolic content (115.25 mg GAE g $^{-1}$ DW) than *M. chamomilla* (82.99 mg GAE g^{-1} DW). However, flavonoid content of both plants was very close (33.53 and 32.45 mg QE g^{-1} DW in *M. chamomilla* and *C. mixtus*, respectively). The total phenolic and flavonoid content was high in flowers of both plants. Generally, for all the studied organs of the chosen plants, hydrophilic antioxidant activity was significantly higher than the lipophilic antioxidant activity. The correlation results show that these bioactive components in both plants are the main factor influencing the antioxidant activity.

Key words: *Cladanthus mixtus*, *Matricaria chamomilla*, Asteraceae, phenolic content, flavonoid content, antioxidant activity.

INTRODUCTION

Medicinal plants are frequently used as remedies in developing countries. For example, in Africa, more than 80% of the inhabitants depend on traditional medicines for their medication (Emmanuel and Didier, 2012; Komoreng et al., 2017; Sevindik et al., 2017). Also, the African continent is characterized for having a wide

*Corresponding author. E-mail : amina.el@um.es. Tel: +34 603 22 07 55.

variety of plant species, used mainly for medicine, among 300 000 plant species identified, more than 200 000 species live in tropical Africa and are used for their healthy properties. Morocco is characterized for having a good geographical position (a country with Mediterranean and Atlantic coasts) which has allowed it to have an interesting plant diversity (Scherrer et al., 2005; Ghanmi et al., 2011). Effectively, amongst more than 7 800 species listed in North Africa, Morrroco presents the greatest biodiversity (Dobignard and Chatelain, 2010-2013), Herbal medicine is an important cultural tradition in Morocco (Benkhnigue et al., 2010) and plays a central role in the daily life of many rural and urban Moroccans (Jouad et al., 2001; Eddouks et al., 2002; El-Hilaly et al., 2003; Merzouki et al., 2003; Tahraoui et al., 2007; Jamila and Mostafa, 2014; Eddouks et al., 2017).

Bioactive compounds of plants are natural products that show some Health-related and interesting activities such as anti-hyperglycemia, anti-inflammatory, anti-microbial, antioxidant, anti-cancer and, antiviral activities, and more generic actions such as pain reliever, nerve relaxant, tranquilizer, spasmodic, etc. (Winklhofer-Roob et al., 2003; Wang et al., 2014; Baser and Buchbauer, 2015; Akhlaghi and Foshati, 2017; Altemimi et al., 2017; Desmarchelier and Borel, 2017; Pehlivan et al., 2018; Ashktorab et al., 2019; Bohn, 2019; Mohammed et al., 2019; Agati et al., 2020; Mu et al., 2021). Considering their great efficacy and low toxicity, natural products have been extensively studied and introduced as a chemopreventive therapy for many diseases including cancer (Al-Hrout et al., 2018; Amin et al., 2021)

Cladanthus mixtus (L.) Oberpr. and Vogt. (synonymous with *Ormenis mixta* subsp. *Mixta*, *Anthemis mixta* L., Moroccan chamomile, simple leaved chamomile), is a traditional herbal medicine, widespread in Morocco, Algeria, Northern and Eastern part of the Mediterranean basin. The infusion of Moroccan chamomile leaves and flowers is used in Moroccan traditional medicine for treatment of different ailments, also as an anxiolytic and to rebalance the central nervous system. The essential oil showed significant antimicrobial, anti-inflammatory, antinociceptive, antioxidant and antibacterial effects (Satrani et al., 2007; Zrira et al., 2007; Merghoub et al., 2009; Hajjaj et al., 2016).

Matricaria chamomilla L. (synonymous with *Matricaria recutita*, German chamomile, chamomile and little chamomile) is commonly used as a medicinal plant due to its anti-inflammatory, analgesic, anti-microbial, anti-allergic, anti-hyperglycemia, anticancer, and anti-spasmodic effects in pregnant women, and for its sedative properties. Generally, essential oil of chamomile plant is used in pharmaceutical, cosmetic, and food industries (Wu et al.,2012; Dadashpour et al., 2018; Abai et al., 2019; Suroowan et al.,2019; Asadi et al.,2020, Hoferl et al., 2020; Ahanni Arazi and Danesh, 2021; Heidarianpour et al., 2021).

Many scientific studies have shown that free radicals play a major role in the development of different diseases and dysfunctions, such as cancer, heart disease, aging, cataracts and immune system deterioration (García-Conesa and Larrosa, 2020; Sevindik, 2021). Free radicals can be eliminated by antioxidants, which inhibit the rate of lipid and protein oxidation and protect cells from oxidative damage (Asimi et al., 2013; Mohammed et al., 2020b). Antioxidant drugs are used for the prevention and treatment of oxidative stress related disease such as diabetes, Alzheimer's disease, atherosclerosis, heart stroke and cancer (Devasagayam et al., 2004; Khalipha et al., 2012; Mohammed et al., 2020a). Antioxidant activity in plant extracts is a widely used parameter to characterize the possible beneficial properties of plants (Shahidi and Ambigaipalan, 2015). The most widely used methods for measuring antioxidant activity are those that involve the generation of free radical species, and the presence of antioxidant compounds, which determine the disappearance of these free radicals (Arnao et al., 1999; Pehlivan et al., 2021). For the analysis of *in vitro* and *in vivo* antioxidant activity numerous methods have been developed, but only a few fast and reliable methods exist for a large number of plant samples. An extensive study on antioxidant activity measurement methods can be consulted in Apak et al. (2018).

We applied the end-point method using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) as chromogen for the estimation of the total antioxidant activity (TAA) developed by our group (Arnao et al., 2001a). This method allows the calculation of the contribution of lipophilic (in organic media) and hydrophilic (in buffered media) compounds to total antioxidant activity (TAA), referred as hydrophilic and lipophilic antioxidant activities. This is a robust method, widely used and applied to diverse biological samples (Cano and Arnao, 2018). Although this method is originated for plant foodstuffs, it has also been applied to characterize other diverse biological materials such as plant extracts (Arnao, 2000; Arnao et al., 2001a, b; El Omari et al., 2003) and animal samples (Espinosa et al., 2012; Rubio et al., 2016).

In this work, we present a comparative study of bioactive components of German and Morrocan chamomile plants, studying the lipophilic and hydrophilic antioxidant activities in the different plant organs (root, stem, leaf, and flower). Also, total phenolic and flavonoid contents were determined in these two chamomile species. To the best of our knowledge, this is the first comparative study on these two plants from north-west Morocco (Tangier-Tetouan-Al Hoceïma region).

MATERIALS AND METHODS

Plant material and preparation of extracts

C. mixtus and *M. chamomilla* plants were collected at full maturity from Beni Hassane (Tangier-Tetouan-Al Hoceïma region, north-west of Morocco) in May 2018 and transported to the laboratory. Table 1 shows the plant's geographical localization and GPS

Table 1. Geographical location and GPS coordinates of studied plant species.

Plant species	GPS coordinates	Locality
Matricaria chamomilla	N 35° 21' 18.865" W 5° 22' 11.677"	Beni Hassane (Tangier-Tetouan-Al Hoceïma region)
Cladanthus mixtus	N 35° 21' 20.865" W 5° 22' 12.677"	

coordinates. Plant identification was done by Prof. Lamarti in Tetouan (Morocco). After separating the organs (roots, stems,leaves, and flowers) in fresh plants, the material was dried in an oven until stabilization of dry weight at 50°C in order to preserve the integrity of its natural composition as much as possible. After, it was ground in a Microtron-MB550 (KINEMATICA AG, Germany), at 8,000 rpm. The powder obtained was composed of particles with a diameter of around 0.2 mm, and stored in the dark at room temperature.n

A mixture of 0.1 g of homogeneous powder, 5 ml of 50 mM Na phosphate buffer (pH 7.4) and 8 ml of ethyl acetate was crushed in a Euroturrax T20 (IKA, Germany) for 2 min and transferred to a decantation funnel. The solid residue (totally colorless) was discarded. The aqueous phase was collected and analyzed as soon as possible for hydrophilic antioxidant activity, and aqueous phenolic and aqueous flavonoid contents. The organic phase (ethyl acetate) was collected and stored at −20°C until analysis to measure lipophilic antioxidant activity, and organic phenolic and organic flavonoid contents. In all cases, three replicate extraction were performed.

Total phenolic content

Folin–Ciocalteu's reagent was applied to determine the total content of phenolic compounds in the samples as described by Singleton and Rossi (1965) with some modifications. Fifty microliters of sample (hydrophilic or lipophilic phase) was placed in a glass test tube, then 950 µl of distilled water, 50 µl of 1 M sodium carbonate and 50 µl of Folin Ciocalteu reagent were added. The mixture was left to stand in a water bath for 15 min at 30°C, and the absorbance was measured at 715 nm. Gallic acid was used as standard. The results were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW). Photometric measurements were recorded on a Perkin-Elmer Lambda-2S UV-VIS spectrophotometer (Loughborough, UK). Experiments were conducted in triplicates.

Total flavonoids content

The aluminum chloride colorimetric method, modified by Woisky and Salatino (1998), was used to determine total flavonoid content. Quercetin, used as standard, was dissolved in 80% ethanol and then diluted to 25, 50, 100 and 150 µg·mL^{-1}. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. A similar step was applied to the plant samples for the flavonoid content analysis. The results were expressed as mg of quercetin equivalents per gram of dry weight (mg QE·g^{-1} DW). Experiments were conducted in triplicates.

Hydrophilic and lipophilic antioxidant activities using ABTS radical scavenging assay

Hydrophilic and lipophilic antioxidant activities were measured using our ABTS/H_2O_2/HRP decoloration methods (Arnao et al., 1999). These methods are based on the capacity of a sample to scavenge the ABTS radical cation ($ABTS^{\cdot+}$) compared to a standard antioxidant (Trolox) in a dose-response curve. The $ABTS^{\cdot+}$ blue color changes to pale yellow. This method allows to measure separate antioxidant activity due to hydrophilic and lipophilic compounds, being able to operate mathematically with both values, obtaining the sum as the total antioxidant activity (TAA=HAA+LAA). The hydrophilic antioxidant activity (HAA) is measured in buffered medium and the lipophilic antioxidant activity (LAA) is measured in organic medium.

For the determination of the lipophilic antioxidant activity (LAA), the reaction mixture contained 1 mM ABTS, 75 µM hydrogen peroxide (H_2O_2), and 6 µM horseradish peroxidase (HRP) type VI in acidified ethanol (pure ethanol with phosphoric acid, 0.7%, w/v), in a total volume of 1 ml, at 25°C. In this case, 40 µl of the organic phase obtained during the plant extraction was added to the reaction medium and the decrease in absorbance at 730 nm was measured after 6 min. The total time needed to carry out each assay was approximately 6 min, including ABTS radical generation by peroxidase, the addition of antioxidant, and acquisition of the final absorbance value. The absorbance decrease was measured from the difference between the A_{730} values before and 6 min after sample addition. Antioxidant activity was calculated as moles of $ABTS^{\cdot+}$ quenched by 1 mole of Trolox. LAA was expressed as Trolox equivalents per g of dry weight (mg TE·g^{-1} DW). Experiments were conducted in triplicates.

For hydrophilic antioxidant activity (HAA), the reaction mixture contained 2 mM ABTS, 60 µM H_2O_2, and 0.2 µM HRP in 50 mM Na-phosphate buffer (pH 7.5) in a total volume of 1 ml. The reaction was monitored at 730 nm until a stable absorbance was obtained. The assay temperature was 25°C. Then, 10 µl of the aqueous sample was added to the reaction medium and the decrease in absorbance, which is proportional to the $ABTS^{\cdot+}$ quenched, was determined after 6 min, as Trolox equivalents per g of dry weight (mg Trolox·g^{-1} DW). Experiments were conducted in triplicates. The concentrations of ABTS, H_2O_2, and Trolox were determined by measuring their absorbance using ε_{340}= 36 mM^{-1} cm^{-1} for ABTS, ε_{240}= 43.6 M^{-1} cm^{-1} for H_2O_2 and ε_{403}= 100 mM^{-1} cm^{-1} for HRP.

Statistical analysis

For statistical analysis of the results, the SPSS program (Chicago, USA) was used applying the One-way ANOVA to evaluate the statistical differences among the group and the Tukey multiple range test to establish significant differences between the evaluated parameters.

Table 2. Aqueous and organic phenolic contents in different organs of *Matricaria chamomilla* and *Cladanthus mixtus.*

Organ	*Matricaria chamomilla*		*Cladanthus mixtus*	
	Aqueous phase	**Organic phase**	**Aqueous phase**	**Organic phase**
Root	4.41* ± 0.12^{d}	4.16 ± 0.43^{b}	11.31 ± 0.32^{c}	6.48 ± 0.28^{c}
Stem	13.45 ± 0.52^{b}	8.13 ± 0.48^{a}	18.09 ± 1.84^{b}	6.86 ± 0.23^{bc}
Leaf	10.56 ± 0.64^{c}	9.21 ± 1.05^{a}	25.15 ± 0.10^{a}	8.31 ± 0.67^{b}
Flower	21.78 ± 0.49^{a}	11.29 ± 0.82^{a}	22.60 ± 0.42^{a}	16.45 ± 0.04^{a}

*Values are the mean ± SD of three replicates expressed as mg gallic acid equivalents (GAE)·g^{-1} DW. Different letters indicates significant differences between the organs for the same column at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of phenolic content of *M. chamomilla* and *C. mixtus*

Phenolic compounds, such as flavonols, flavones, and phenolic acids, are synthesized by plants in the so called secondary metabolism. *M. chamomilla* is known to have high phenolic content (Gee and Johnson, 2001; Guimarães et al., 2013) that may contribute to its antioxidant activity (Lim and Quah, 2007; Wojdyło et al., 2007).

Table 2 shows the phenolic content of different organs of *M. chamomilla* and *C. mixtus.* Aqueous phenolic content was higher in all organs of both plants compared to organic phenolic content. The calculations were made using a calibration curve that showed a good linearity for gallic acid with a correlation coefficient R^2 of 0.9988. Results showed, for the same phase, significant differences ($p < 0.05$) between the organs.

M. chamomilla showed values of aqueous phenolic content between 4.41 and 21.78 mg GAE·g^{-1} DW, with the flowers showing the highest content, followed by the stems, leaves, and roots. Organic phenolic content was between 4.16 and 11.29 mg GAE·g^{-1} DW, with the flowers showing the highest level followed by the leaves, stems, and roots. Consequently, total phenolic content, as the sum of the aqueous and organic phenolic contents, was highest in flowers (40%), followed by the stems (26%), leaves (24%) and roots (10%) (Figure 1).

The total phenolic content of flower extract of *M. chamomilla* found in this work (as the sum of the aqueous and organic phenolic contents = 33.07 mg GAE·g^{-1} DW) is slightly higher than that found in the flower extract from a similar study with italian chamomilla (2,689.2 mg GAE·100 g^{-1} DW) (Formisano et al., 2015), and much higher than in a study with Egyptian chamomille (3.7 mg GAE·g^{-1} DW) (Roby et al., 2013), and than in a commercial chamomile product from United Arab Emirates (21.4 mg GAE·g^{-1} DW) (Al-Dabbagh et al., 2019). Thus, chamomile flower from this study still has the highest value of total phenolic content, possibly due to that the applied bi-phasic extraction method is more exhaustive.

The extracts of *C. mixtus* had the highest total phenolic content compared to *M. chamomilla* (Table 2). The aqueous phenolic content ranged between 11.31 and 25.15 mg GAE·g^{-1} DW, with the leaves showing the highest content, followed by the flowers, stems, and roots. The organic phenolic content ranged between 6.48 and 16.45 mg GAE·g^{-1} DW, following the order: flower s> leaves > stems > roots. Therefore, the highest total phenolic content was observed in flowers (34%), followed by leaves (29%), stems (22%), and roots (15%) (Figure 1). In a previous report, extracts from aerial parts of *C. mixtus* (collected from Bouznika, Morocco) showed total phenolic content of 38.2 mg GAE·g^{-1} DW in the aqueous extracts, and of 19.5 mg GAE·g^{-1} DW in methanolic extracts (Elouaddari et al., 2019), showing lower content than the one found in this study (65.84 and 31.62 mg GAE·g^{-1} DW, respectively).

Determination of flavonoid content of *M. chamomilla* and *C. mixtus*

Table 3 shows the flavonoid content of different organs of *M. chamomilla* and *C. mixtus.* To quantify flavonoid content in plant extracts, we used quercetin as standard, obtaining a linear calibration curve and a correlation coefficient R^2 of 0.9994. Results showed that aqueous flavonoid content was significantly higher in all organs for both plants compared to organic flavonoid content. *M. chamomilla* showed values of aqueous flavonoid content between 2.11 and 7.97 mg QE·g^{-1} DW, and of organic flavonoid content between 0.50 and 5.65 mg QE·g^{-1} DW.

In *C. mixtus*, aqueous flavonoid content was between 3.70 and 10.20 mg QE·g^{-1} DW, and the organic flavonoid content between 0.99 and 3.74 mg QE·g^{-1} DW. Both plants showed the highest flavonoid content in theflowers, followed by leaves, stems, and roots. *M. chamomilla* and *C. mixtus* showed close proportions of total flavonoid content in the flowers (41 and 43%, respectively) and in stems (15 and 17%, respectively) (Figure 2).

In Italian chamomile flowers, a total flavonoid content of 710.7 mg QE·100 g^{-1} DW was observed (Formisano et al., 2015), which is lower than in our flowers extract of *M.*

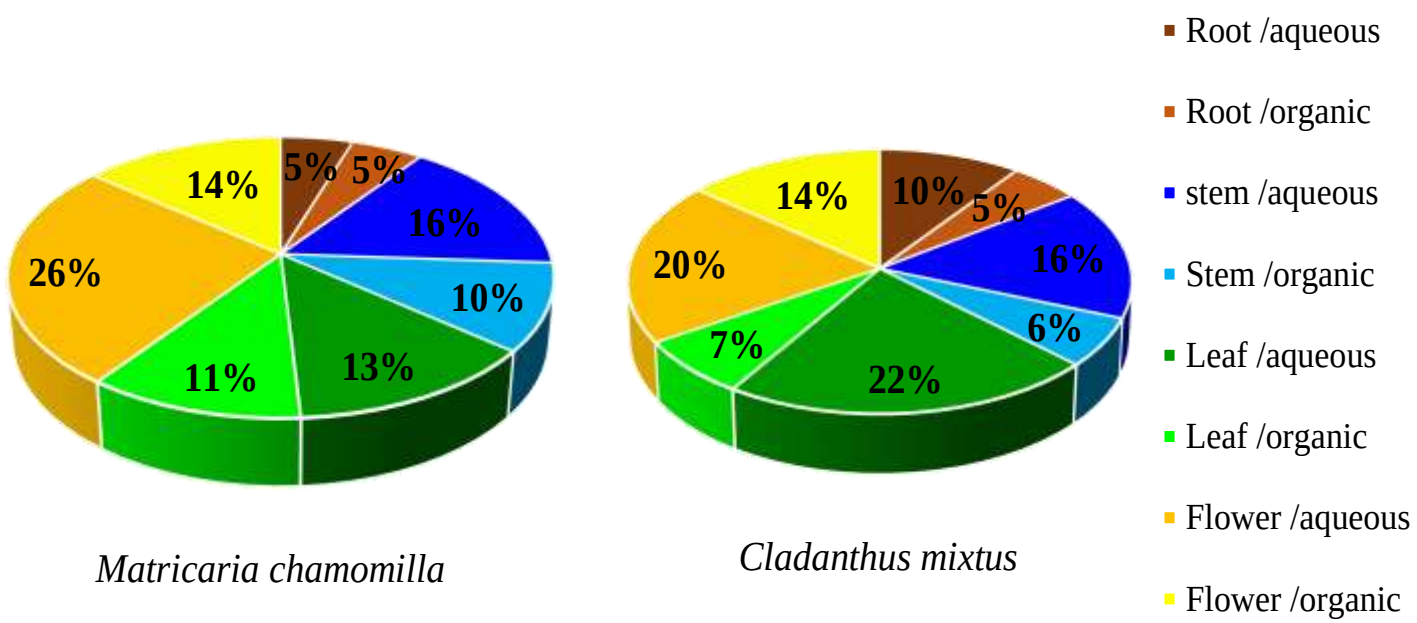

Figure 1. Percentage of phenolic compounds in different organs of *Matricaria chamomilla* and *Cladanthus mixtus*.

Table 3. Aqueous and organic flavonoid content in different organs of *Matricaria chamomilla* and *Cladanthus mixtus.*

Organ	*Matricaria chamomilla*		*Cladanthus mixtus*	
	Aqueous phase	Organic phase	Aqueous phase	Organic phase
Root	*2.11 ± 0.23^{c}	0.50 ± 0.04^{b}	3.70 ± 0.11^{c}	0.99 ± 0.02^{c}
Stem	3.98 ± 0.23^{b}	1.12 ± 0.41^{b}	4.34 ± 0.35^{c}	1.36 ± 0.08^{c}
Leaf	6.78 ± 0.24^{a}	5.42 ± 0.16^{a}	5.34 ± 0.09^{b}	2.78 ± 0.17^{b}
Flower	7.97 ± 0.36^{a}	5.65 ± 0.05^{a}	10.20 ± 0.07^{a}	3.74 ± 0.10^{a}

*Values are the mean ± SD of three replicates expressed as mg quercetin equivalents (QE)·g^{-1} DW. Different letters indicates significant differences at $p < 0.05$ differences between the organs for the same column at $p < 0.05$.

chamomilla (13.62 mg QE·g^{-1} DW). In a previous study of aerial parts of Moroccan *C. mixtus,* the flavonoid content was 3.2 and 2.7 mg QE·g^{-1} DW in methanolic and aqueous extracts, respectively (Elouaddari et al., 2019). In our case, *C. mixtus* contained a flavonoid content of 19.88 and 7.88 mg QE·g^{-1} DW, in aqueous and organic extracts, respectively. Globally, a total flavonoid content in aerial parts of 27.76 mg QE·g^{-1} DW was determined in our estimations, a much greater amount than in the mentioned study of Elouaddari et al. (2019).

Determination of the antioxidant activity of *M. chamomilla* and *C. mixtus*

Preformed ABTS radical (ABTS^{*+}) is considered to be an excellent chromogen for use in measurement of the antioxidative properties of pure products and biological samples (Arnao et al., 1999, 2001b). Figure 3 shows hydrophilic antioxidant activity (HAA) and lipophilic antioxidant activity (LAA) of different organs of *M. chamomilla* and *C. mixtus.* Results of *M. chamomilla* showed that HAA ranged from 9.28 to 18.02 mg TE·g^{-1} DW, while LAA ranged from 1.47 to 3.51 mg TE·g^{-1} DW. Therefore, TAA (HAA+LAA) was formed mainly of HAA (87%) and the rest (12%) of LAA (Figure 4).

Differences of antioxidant activity were noted between the studied organs of *M. chamomilla*. Concerning HAA, the order was as followed: roots (18.02 mg TE·g^{-1} DW) ≥ flowers (17.57 mg TE·g^{-1} DW) > stems (12.27 mg TE·g^{-1} DW) > leaves (9.28 mg TE·g^{-1} DW). For LAA the order was as followed: flowers (3.51 mg TE·g^{-1} DW) > stems (1.49 mg TE·g^{-1} DW) ≅ roots (1.48 mg TE·g^{-1} DW) ≅ leaves (1.47 mg TE·g^{-1} DW). Significant difference was

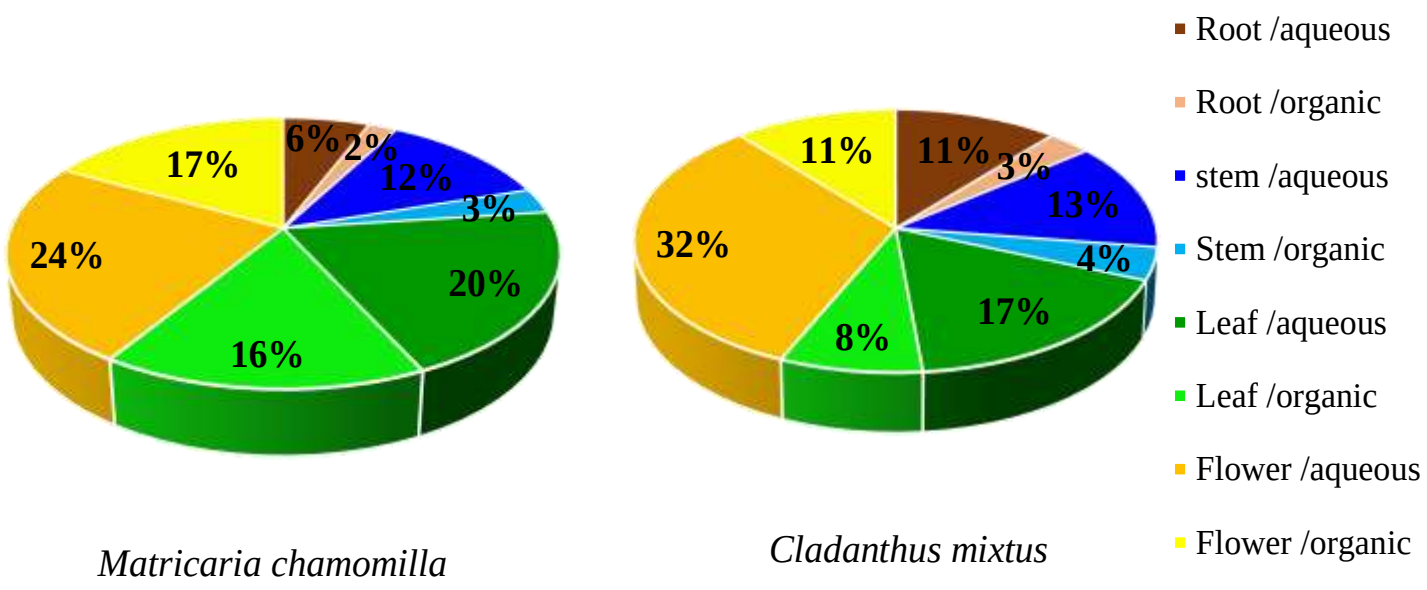

Figure 2. Percentage of flavonoid compounds in different organs of *Matricaria chamomilla* and *Cladanthus mixtus*.

reported between LAA of flowers and other organs ($p < 0.05$). Consequently, the flowers of *M. chamomilla* showed the highest TAA while leaves had the lowest TAA.*C. mixtus* also showed greater HAA than LAA, ranging from 12.89 to 23.40 mg TE·g^{-1} DW for HAA, and from 1.34 to 3.99 mg TE·g^{-1} DW for LAA. Thus, TAA was formed of 89% HAA and 11% LAA (Figure 4). Significant difference was noted between HAA of the studied organs ($p < 0.05$), with stems showing the highest HAA (23.40 mg TE·g^{-1} DW), followed by flowers (20.32 mg TE·g^{-1} DW), roots (14.11 mg TE·g^{-1} DW) and leaves (12.89 mg TE·g^{-1} DW). As in *M. chamomilla,* the flowers of *C. mixtus* had the highest LAA. Significant difference was noted between LAA of flowers and other organs ($p < 0.05$), with the order: flowers > roots > leaves > stems.

Relationship of the phenolic and flavonoid contents with the antioxidant activity of *M. chamomilla* and *C. mixtus*

Some natural products may act to regulate cell growth, differentiation, apoptosis, and oxidative stress (Hamza et al., 2018; Kamal et al., 2018, Mohammed et al., 2020b; Amin et al., 2021; Sevindik, 2021). Phenolic molecules are known for their antioxidant activity. For example, Gallic acid is reported to have antioxidant, antimicrobial, antitumor and anti-inflammatory properties activities (Lima et al., 2016). Since phenolics and flavonoids may contribute to the antioxidant activity of extracts, we have studied the correlation between these compound contents and the antioxidant activity (Tables 4 and 5).

The correlation coefficient is a statistical measure of linear association between two variables, ranging between -1 and 1. A correlation value of 1 or -1 indicates that two variables are perfectly related in a positive or negative linear sense, and a value of 0 indicates that there is no linear relationship between the two variables (Asuero et al., 2006). Regarding phenolic content and antioxidant activities (Table 4), in stems, leaves, and flowers of *M. chamomilla,* an excellent significant positive correlation was obtained between HAA and aqueous phenolics ($r^2 = 0.98$, $p < 0.05$), and an acceptable significant positive correlation was obtained between TAA and total phenolic content ($r^2=0.87$, $p < 0.05$). In roots, stems, and flowers, a significant positive correlation was observed between LAA and organic phenolics ($r^2=0.70$, $p < 0.05$). In the case of *C. mixtus*, a good significant positive correlation was observed between aqueous phenolics and HAA ($r^2= 0.85$, $p < 0.05$) in stems, leaves, and flowers (Table 4). Between organic phenolics and LAA, an extremely significant positive correlation was found in all organs ($r^2= 0.92$, $p < 0.05$). Lower significant positive correlation ($r^2=0.53$, $p < 0.05$) was observed in roots, stems, and flowers of *C. mixtus.* Therefore, it can be concluded that stems and flowers always showed a good correlation between their phenolic compounds and antioxidant activities.

Regarding flavonoid content and antioxidant activities (Table 5), a significant positive correlation was also reported in roots, stems, and leaves of *M. chamomilla* between aqueous flavonoid content and HAA ($r^2=0.91$, $p < 0.05$), and between total flavonoids content and TAA ($r^2=0,81$, $p < 0.05$). Also, a significant positive correlation was observed between organic flavonoid content and LAA ($r^2= 0.98$, $p < 0.05$) in roots, stems, and flowers. In *C. mixtus*, a great significant positive correlation was found between HAA and aqueous flavonoid content (r^2 =

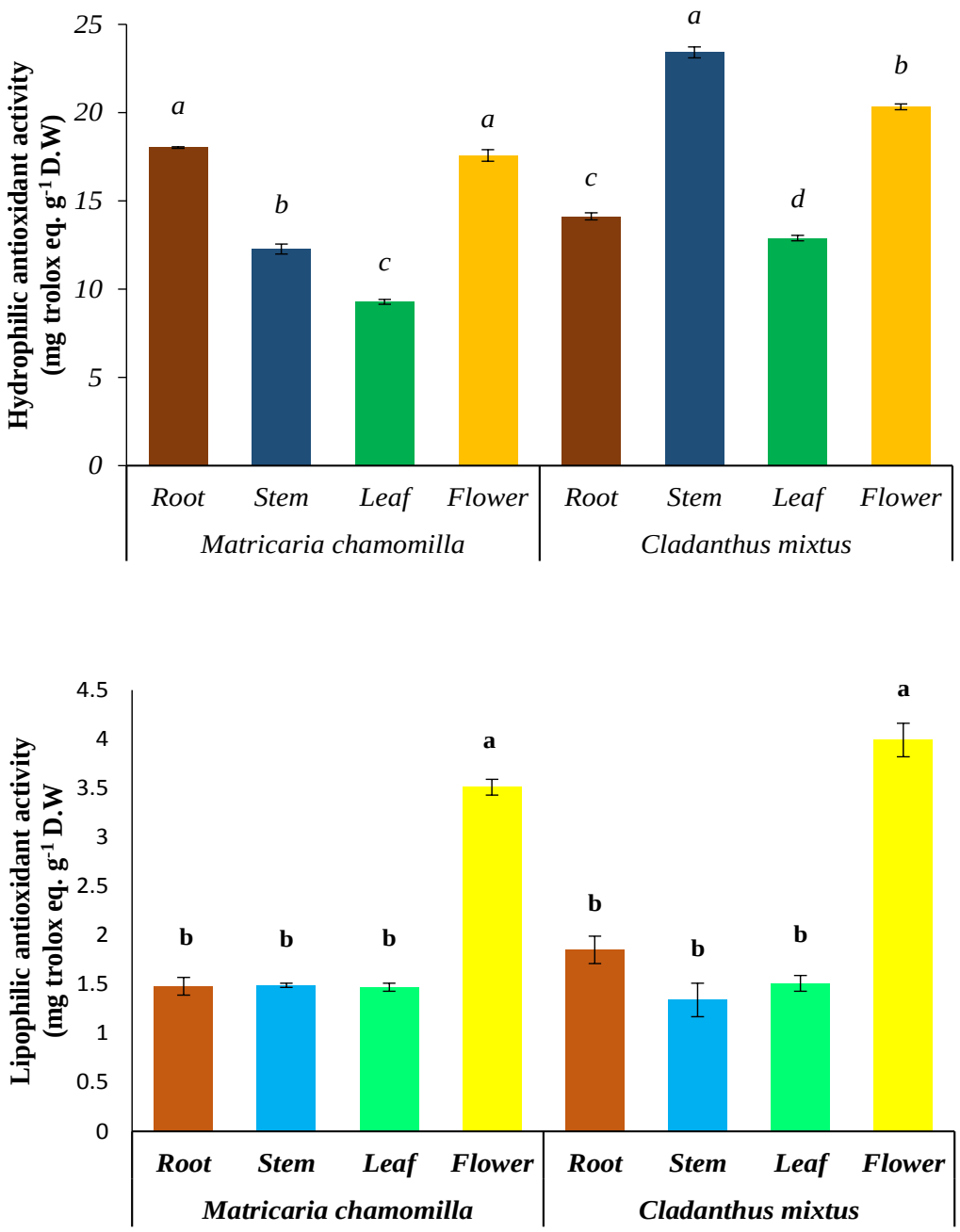

Figure 3. Lipophilic and hydrophilic antioxidant activities of different organs extracts of *Matricaria chamomilla* and *Cladanthus mixtus*. Different letters indicate significant differences between the organs for one plant at $p < 0.05$. Values are means ± S.D. for 3 replicates.

0.91; $p < 0.05$) in roots, stems, and flowers. Also, a good significant positive correlation was observed between organic flavonoid content and LAA ($r^2 = 0.84$, $p < 0.05$), and, in a lesser extent, between total flavonoid content and TAA ($r^2 = 0.75$, $p < 0.05$) of roots, leaves, and flowers.

This analysis confirms that phenolic and flavonoid contents are the main contributors to the total antioxidant activity in our study. Nonetheless, it is important to take into consideration that different phenolic compounds can contribute in different ways to TAA, resulting in different antioxidant activities. Accordingly, it is very important to obtain real TAA measurements by methods or tests that quantify antioxidant activities of aqueous and organic antioxidant compounds in their respective media (Cano and Arnao, 2018).

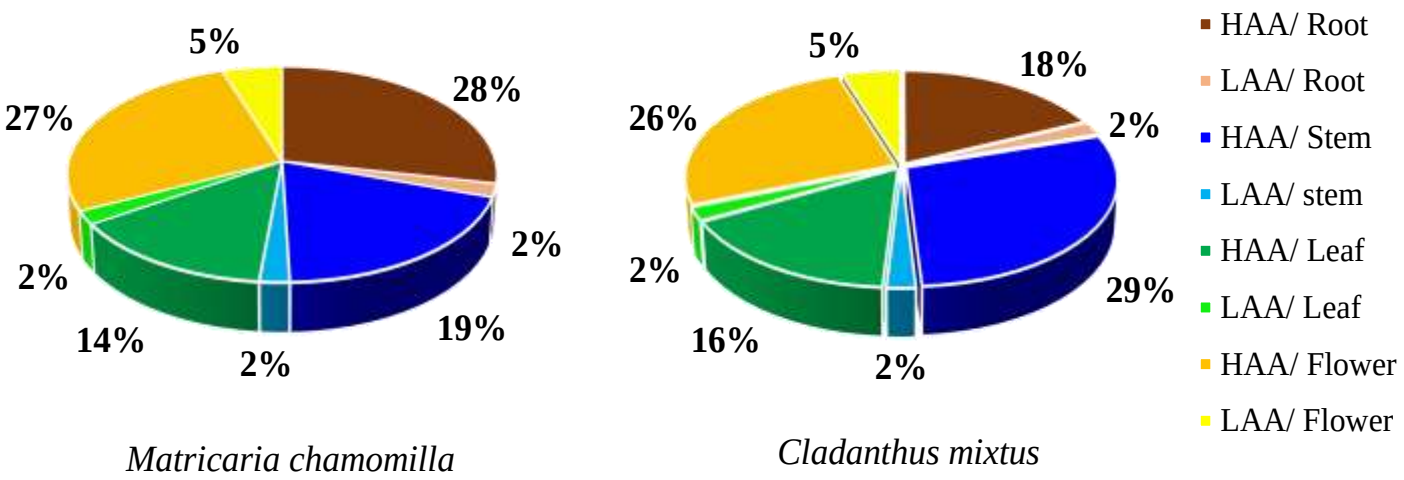

Figure 4. Percentage of antioxidant activity in different organs of *Matricaria chamomilla* and *Cladanthus mixtus*.

Table 4. Relationship between phenolic contents and antioxidant activities (HAA, LAA, and TAA) of different organs of *Matricaria chamomilla* and *Cladanthus mixtus.*

Matricaria chamomilla	**Relationship between parameter**		
	Organ	**Equation**	**R^2***
Aqueous phenolics vs HAA	Stem, leaf, flower	y = 0.7165x + 2.1017	0.98
Organic phenolics vs LAA	Root, stem, flower	y = 0.0543x + 1.1914	0.70
Total phenolics vs TAA	Stem, leaf, flower	y = 0.6345x - 1.4994	0.87
Cladanthus mixtus			
Aqueous phenolics vs HAA	Stem, leaf, flower	y = -1.3937x +49.462	0.85
Organic phenolics vs LAA	Root, stem, leaf, flower	y = 0.2528x - 0.2369	0.92
Total phenolics vs TAA	Root, stem, flower	y = 0.3338x + 12.572	0.53

* indicates significant correlation at p< 0.05.

Table 5. Relationship between flavonoid contents and antioxidant activities (HAA, LAA and TAA) of different organs of *Matricaria chamomila* and *Cladanthus mixtus.*

Matricaria chamomilla	**Relationship between parametres**		
	Organ	**Equation**	**R^2***
Aqueous flavonoids vs HAA	Root, stem, leaf	y = -1.8111x +20.972	0.91
Organic flavonoids vs LAA	Root, stem, flower	y = 0.4135x + 1.1562	0.98
Total flavonoids vs TAA	Root, stem, leaf	Y = -0.8049x +20.021	0.81
Cladanthus mixtus			
Aqueous flavonoids vs HAA	Root, stem, flower	y = 0.9002x + 0.5625	0.91
Organic flavonoids vs LAA	Root, leaf, flower	y = 1.0854x + 8.8089	0.84
Total flavonoids vs TAA	Root, leaf, flower	y = 0.9875x + 9.4099	0.75

*Indicates significant correlation at $p < 0.05$.

Conclusion

This study investigated the bioactive components and the total antioxidant activity levels measured in hydrophilic and lipophilic solutions of different organs of *C. mixtus* and *M. chamomilla*. The results indicate that hydrophilic

antioxidant activity, using ABTS radical scavenging assay, was significantly higher than the lipophilic antioxidant activity, in both plants. In addition, total phenolic and flavonoid contents of both plants are also highly relevant. Therefore, it can be concluded that *C. mixtus* and *M. chamomilla* are rich in phenolic and flavonoid contents and have a strong antioxidant activity, mainly in flowers. The correlation analysis results show that the phenolic and flavonoid contents in both plants are the main factors influencing the antioxidant activity. This study suggests that both plants present interesting health-related bioactivities, but more specific bioassays should be made to determine the therapeutic and food applications of interest.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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