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Phytochemical Characterization and Radical Scavenging Activity of Aqueous Extracts of Medicinal Plants from Portugal

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AFV and MM conceived and designed the experiments, author AS performed the statistical analysis, authors AFV and MOS performed the experiments, and authors AFV and AC wrote the first draft of the manuscript. A.F.V. and A.C. managed the analyses of the study. Author MBPPO managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aim of this study was to evaluate the total phenolic and flavonoid contents of four medicinal plants from Portugal usually consumed as tea infusion, *Melissa officinalis* (Lamiaceae) – lemon balm, *Matricaria chamomilla* (Asteraceae) – chamomile, *Olea europaea* (Oleaceae) – olive leaves, *and Aloysia triphylla* (Verbenaceae) – lemon verbena.
Place and Duration of Study: Air-dried leaves of four of the most consumed medicinal plants from Portugal were analyzed in the Department of Pharmacognosy (UFP) and Laboratory of Bromatology (FFUP) and, Department of Pharmacy, and Chemical Laboratory, Health Technology Research Center (CITS) between June 2010 and September 2011.

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Methodology: Qualitative phytochemical analysis was done for several phytoconstituents (alkaloids, steroids, terpenoids, flavonoids, and tannins). Total phenolic, flavonoid, and tannin contents were quantified by UV-Vis spectrophotometry. It was also analyzed the possible correlation between antioxidant activity (*in vitro*) and the synergistic effect between different phytochemicals, using the free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$).
Results: Steroids, terpenoids, flavonoids and tannin, were present in *Melissa officinalis and Matricaria chamomilla*. In *Olea europaea* leaves, all the chemical constituents were present except alkaloids, and terpenoids. *Aloysia triphylla* showed the presence of all the constituents. Total phenol contents ranged from 12.91mg.100g^{-1} to 87.25 mg.100g^{-1} and flavonoid contents ranged from 25.17mg.100g^{-1} to 57.28mg.100g^{-1}. The screening of the leaf of the four selected medicinal plants indicates that the presence of high phenolic content may be due to the presence of tannins and flavonoids which are known to possess antioxidant activities. A slight correlation has been observed between total phenolics and antioxidant activity.
Conclusion: Our findings provided evidence that aqueous extracts of these tested plants from Portugal contain medicinally important bioactive compounds. Results showed that plants from Portugal usually used as tea infusions are a good source of phytochemical compounds presenting antioxidant activity, so their consumption must be incremented in younger generations which usually consume other less beneficial drinks.

Keywords: Matricaria chamomilla; Melissa officinalis; Olea europaea; Aloysia triphylla; phytochemicals; antioxidant activity; medicinal plants from Portugal.

1. INTRODUCTION

Medicinal plants have always been associated with cultural behavior and traditional knowledge. Many studies have demonstrated that medicinal plants contain various bioactive compounds with antioxidant activity, which are responsible for their beneficial health effects. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010).

Herbal medicine is the major stay of about 75-80% of the world population, mainly in the developing countries, for primary health care due to a better cultural acceptability, better compatibility with human body and few side effects (Kommu et al., 2011).

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenis of several diseases. The oxidation of lipid, protein, DNA, carbohydrate, and other biological molecules by toxic ROS may cause DNA mutation and/or damage target cells and tissues, and this often results in cell senescence and death. Cancer chemoprevention by using natural antioxidants approaches has been suggested to offer a good potential in providing important benefits for public health, and is now considered by many physicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the carcinogenesis process (Grassi et al., 2009). Bioactive compounds that display antioxidant effects, have specific applications in preventing oxidative stress-related injury, which

characterizes the pathogenesis of cardiovascular disease (Ferri and Grassi, 2010; Grassi et al., 2009; Valko et al., 2007). In addition to ascorbic acid (vitamin C), vitamin E, carotenoids and polyphenols have shown strong antioxidant capacity (Gao et al., 2010; Sun et al., 2010; Alim et al., 2009; Giovanelli and Buratti, 2009), medicinal plants also contain some organic compounds which provide definite physiological action on human body and these bioactive substances include tannins, alkaloids, terpenoids, steroids and flavonoids (Edoga et al., 2005). Nevertheless, phytochemicals are extensively found at different levels in plants, directly influenced by geographic and climatic conditions (Vinha et al., 2012). Knowledge of the chemical constituents of autochthonous plants from different countries is desirable for such information may be valuable for the synthesis of complex chemical substances.

Lemon balm leaves, *Melissa officinalis* (*M. officinalis*), are often used as herbal teas. It contains some phenolic and flavonoid compounds such as rosmarinic acid (Herodez et al., 2003) and is widely used as herbal tea to treat or to relieve nervous disturbance of sleep and functional gastrointestinal disorders (Marongiu et al., 2004).

Chamomilla recutita (*C. recutita*) is an annual herbaceous plant indigenous to Europe and Western Asia. Dried flower heads of the plant are widely used in traditional and herbal medicine because of its anti-inflammatory, spasmolytic, antipeptic, sedative, antibacterial and antifungal properties (Pereira et al., 2009; Avallone et al., 2000).

Aloysia triphylla (*A. triphylla*) has long been used in traditional medicine. *A. triphylla* has been reported to have a gentle sedative action and helps to counter depression (Pascual et al., 2001). An infusion of aerial parts of *A. triphylla* is used as antipyretic, antispasmodic and diuretic agent (Ragone et al., 2007).

Olea europaea (*O. europaea*) leaves have been widely used in traditional remedies in European and Mediterranean countries and they have been used in the human diet as an extract, an herbal tea, due to their antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties (El and Karakaya, 2009).

According with some authors the quantity and the composition of bioactive compounds, present in plants are influenced by genotype, extraction procedure and environmental conditions (Vinha et al., 2012; Dai and Mumper, 2010; Mukhopadhyay et al., 2006).

For all the reasons mentioned above, the aim of the present study was to determine the contents of phenolics, and flavonoids in aqueous extracts of four medicinal plants from Portugal, usually consumed as teas: *Melissa officinalis* (lemon balm), *Matricaria chamomilla* (chamomile), *Olea europaea* (olive leaves), and *Aloysia triphylla* (lemon verbena) using spectrophotometric methods, as well as to examine the DPPH scavenging activity of each plant extract. A possible correlation between antioxidant activity and total phenolic contents and antioxidant activity with flavonoid contents were also analyzed.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh herbal plant materials were purchased from a reputable medicinal plant producer in the region located in North of Portugal (position: 41º 52’ 26’’ N; 8º 50’ 26’’ W, altitude: 16 m), characterized as a Mediterranean climate. Four medical plants from Portugal were studied:

Melissa officinalis, *Matriarcia chamomilla*, *Olea europaea*, and *Aloysia triphylla*, the most commonly used in Portuguese traditional medicine. The collected plants material (leaves and/or dried flowers) were air-dried in darkness at room temperature (20ºC+/-2ºC). The dried plant material was cut up and stored in tightly sealed dark containers until needed.

2.2 Qualitative Analysis of Phytochemicals

Chemical tests performed in the screening and identification of phytochemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures as described by Sofowara (1993); Trease and Evans (1989); Harborne (1998).

2.2.1 Preparation of reagents

Maeyer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Dragendorff's reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

2.2.2 Test for alkaloids

About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity and/or precipitate formation.

2.2.3 Test for steroids

About 0.5 g of the methanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

2.2.4 Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result.

2.2.5 Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration identifies the presence of flavonoids (Shinado's test).

2.2.6 Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal

method, which includes a conical flask and filter paper. The 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish green or a blue black coloration, which showed the presence of tannins.

2.3 Quantification of Phytochemical Compounds and DPPH Scavenging Activity Assay

Chemical tests for the screening and identification of bioactive chemical constituents in the four medicinal plants from Portugal under study were carried out in aqueous extracts.

2.3.1 Plant extracts

The air-dried plant material (5 g) was coarsely crushed in small pieces of 2-5 mm using the cylindrical crusher and extracted with water and ethanol, respectively. Each infusion was prepared using 5 g of dried herbal tea in 200 ml of distilled water and (equivalent to 1 tea cup) at approximately 100ºC+/-1ºC for 10 min. Each aqueous extract was filtered through a paper filter (Whatman, No. 1) and stored under refrigeration in glass flasks tapered with screw plastic lid. Both the infusion time and the solvent initial temperature used in this study have been described by several authors as efficient conditions to extract phytochemical compounds, such phenolic and flavonoid compounds from herbs (Katalinic et al., 2006; Su et al., 2006).

2.3.2 Total phenolic compounds analysis

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent (Velioglu et al., 1998) with slight modifications. 200µl of each extract (aqueous and ethanolic, respectively) was mixed with 1.5 ml of Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water), and allowed to stand at 22ºC±1ºC, for five minutes. A 1.5 ml sodium bicarbonate solution (8%) was added to the mixture. After 90 min at 22ºC±1ºC, absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Shimadzu, model UV-1800). Total phenolic amounts were quantified by calibration curve obtained from measuring the absorbance of a know concentrations of galic acid standard (15-300 µg/ml; R^2 =0.9985). The concentrations were expressed as mg of galic acid equivalents (GAE) per 100g of dry weight. The total phenolic assay was measured four times for each extract of tea samples analyzed and results were shown in Table 2.

2.3.3 Total flavonoids assay

Total flavonoids content was measured by the aluminum chlorite assay, previously described by Zhishen et al. (1999). An aliquot (1 ml) of aqueous and ethanolic extracts and standard solution of catechin [50, 100, 150, 200, 250 and 300 mg/ml] was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml of $NaNO_3$ (5%). After 5 min at quiescent, 0.3 ml 10% $AlCl_3$ was added, and at 6^{th} min, 2 ml of NaOH (1 M) was added and total volume was made up to 10 ml. Resultant solution was mixed well and absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/ 100g dry weight (DW). Total flavonoid assay was measured four times for each plant extract and results are shown in Table 2.

2.3.4 Total of tannins content

The content of tannins was determined according to a procedure previously described (Schneider, 1976). A volume of 2 ml of medicinal plant extract was mixed with 8 ml of water and 10 ml of acetate buffer. The mixture prepared in such a way represented the solution 1 (S1). 10 ml of this solution (S1) was posterior shaken with 50 mg of casein during 60 min and after filtered. This filtrate solution represented the solution 2 (S2). A quantity of 1 ml of each solution (S1, S2), previously prepared, was mixed separately with 0.5 ml of Folin-Denis reagent and then both solutions were diluted to 10 ml with sodium carbonate decahydrate solution (33%). The absorbance of such prepared solutions was measured against a blank sample at 720 nm. The tannins content was evaluated upon three independent analyses. Absorbance values obtained for S1 correspond to total polyphenol content. Differences between absorbances of solutions S1 and S2 correspond to content of casein-absorbed tannins in medicinal plant samples. The content of tannins was expressed as mg catequin equivalents (EC)/ 100g dry weight (DW).

2.3.5 Evaluation of DPPH$^{\bullet}$ scavenging activity

The use of simplified models to evaluate antioxidant activity is very important for studies aiming to determine this biological property of foods, and some researchers have suggested the use of more than one method (Kulisic et al., 2004; Koleva et al., 2002). Among the various models of antioxidant activity, both in terms of scavenging activity of radicals and oxidation inhibition in lipidic system, two are frequently used because they are quick, sensitive, and do not demand equipment or reagents that are difficult to find: DPPH$^{\bullet}$ scavenging activity method and β-carotene/linoleic acid method (Koleva et al., 2002). Besides the advantages above mentioned, and the fact that the antioxidant activity determination may be influenced by antioxidant affinity, DPPH$^{\bullet}$ scavenging activity method has been proven to be good because its results are not affected by substrate polarity.

The samples were analyzed with DPPH$^{\bullet}$ solution at 517 nm, according to the method reported by Brand-Williams et al. (1995). Results of the DPPH$^{\bullet}$ scavenging activity (RSA) which is defined by following expression:

$$RSA\ (\%) = 100x(A_0 - A_t)/A_0 \quad (1)$$

Where, A_0 is the initial absorbance and A_t is the value of absorbance of the antioxidant measured at t=30 min. All determinations were performed in triplicate.

2.4 Statistical Analysis

Data are reported as mean ±standard deviation of three measurements. Statistical analyses were performed using the statistical package SPSS v 15.0 (SPSS for Windows; SPSS Inc., Chicago, IL). One-way ANOVA was used to compare two or more groups, and post-hoc Dunnett's test was performed for simultaneous paired comparisons. Differences at $P < 0.05$ (95% confidence level) were considered to be statistically significant. Simple linear regression analysis was used to evaluate the relationship between compounds amount and DPPH$^{\bullet}$ scavenging activity.

3. RESULTS AND DISCUSSION

Medicinal and aromatic plants incorporate a broad range of interrelated and complexly interwoven subjects, including agriculture, commerce, ecology, pharmacology, and social issues.

In the present study several phytochemical constituents and free radical scavenging activity of medicinal plants, were evaluated. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites and may vary with plant species. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. However, they possess biological properties such as antiapoptotic, antiaging, anticarcinogenic, antiinflammatory, antiatherosclerotic, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation (Han et al., 2007; Singh et al, 2007).

Table 1 shows the phytochemical analysis of different chemical constituents of selected plant species under study on qualitative basis. Results revealed the presence of medically active compounds in the four plants studied.

Steroids, terpenoids, flavonoids and tannin, were present in *Melissa officinalis and Matricaria chamomilla* but alkaloids were found absent. In *Olea europaea* leaves, all the chemical constituents were present except alkaloids, and terpenoids. *Aloysia triphylla* showed the presence of all the constituents. Alkaloids have been associated with cytotoxic properties and steroids have been reported with antibacterial properties (Raquel, 2007).

Catalan and Lampasona, (2002) studied alkaloids, terpenoids, steroids, flavonoids and tannins in *Aloysia triphylla* and found that these chemical constituents were found in significant amount, namely in terpenoids, and flavonoids. Gonzala et al. (2004) in a phytochemical screening showed alkaloids, tannins and flavonoids presence in the aqueous extracts of *Melissa officinalis*. Olive tree (*Olea Europaea*) products are essential elements of Mediterranean diet. Qualitative results showed total absence of alkaloids and terpenoids, although, olive leaves are recognized for their high flavonoid contents and also for the presence of a potentially bioactive compound oleuropein, a secoiridoid, which can constitute up to 6–9% of dry matter in the leaves (El and Karakaya, 2009, Vinha et al., 2005).

Plants which are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, polyphenols) are generally superior in their biological activities suggesting that this strength is dependent on the diversity and quantity of such constituents (Geyid et al., 2005). The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous studies.

Table 1. Qualitative analysis of five major photochemical present in leave extracts of tested plants from Portugal

Phytochemicals*	M. officinalis	M. chamomilla	O. europaea	A. triphylla
Alkaloids	-	-	-	+
Steroids	+	+	+	+
Terpenoids	+	+	-	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+

(+) Presence of phytochemical compound; (-) Absence of phytochemical compound.

The phenolic, flavonoid and tannins contents like antioxidant properties of many medicinal plants of the Lamiaceae family, such as lemon balm, thyme and lavender (Fecka and Turek, 2008; Bozin et al., 2007; Marongiu et al., 2004), Asteraceae family, and Verbenaceae family (Pereira et al., 2009; Katalinic et al., 2006) have been evaluated, but the polyphenolics contents of olive leaves (Oleaceae) are still in some extent unexplored, especially in Portugal, where olive tree and related products have a high economic and impact.

The Table 2 presents the total phenol contents (TPC), flavonoid contents (TFC) and tannin contents (TTC) determined in medicinal plant using aqueous extracts.

Total phenol contents (TPC) in aqueous extracts prepared with the above mentioned herbs were determined by Folin-Ciocalteau method. TPC ranged from 12.91 mg EAG.100g^{-1} to 87.25 mg EAG.100g^{-1} for *O. europaea* and *A. triphylla,* respectively. Amoung samples, lemon verbena leaves contained relatively higher total phenolics content showing statistically significant results (P <0.05) between them. Our results are not in agreement with Pereira et al. (2009) who described *Melissa officinalis* with higher phenolic content. These results may improve the relationship between the plant metabolic synthesis and the climacteric conditions and also to justify the evidence in phytochemical content variations presented in the same medicinal plant produced in different countries, with specific soil and climacteric conditions.

The diversity of polyphenolics constituents and their different distribution in plants may explain the different ranges obtained for the total phenolic and flavonoid contents analyzed. Flavonoid content was found in all selected medicinal plant species. However, the flavonoid content was different among the selected plants. The maximum flavonoid content (57.28 mg CE/ 100g) was observed in *Melissa officinalis* while minimum flavonoid content (25.17 mg CE/ 100g) was recorded in *Olea europaea* leaves. According to flavonoid content, the selected medicinal plant species were ranked as: *Melissa officinalis* (lemon balm) > *Aloysia triphylla* (lemon verbena) > *Matricaria chamomilla* (chamomilla) > *Olea europaea* (olive leaves).

The tannin content was found in all selected medicinal plant species. Maximum quantity of tannin was observed in *Olea eurpaea* (37.01 mg CE/ 100g), followed by *Matricaria chamomilla* (16.11 mg CE/ 100g), *Melissa officinalis* (11.34 mg CE/ 100g), and *Aloysia triphylla* (10.26 mg CE/ 100g), respectively. Studies have shown that tannins possess antidiarrhoeal (Amabeoku, 2009; Hayet et al., 2008) and antimicrobial activities (Corrales et al., 2009) and, the difference in tannin levels of our plants was statistically significant (P < 0.05) except for *Melissa officinalis* and *Aloysia triphylla* leaves (P > 0.05).

Low concentration of tannins is known to “tan” the outermost layer of the gastric mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation The gastro protective effect of tannins was experimentally confirmed when the administration of tannins was found to significantly lower stomach free radical concentrations in rats (Ramirez, Roa, 2003). Thus, the fact that the group of antimicrobial plants possesses a higher proportion of plants with high tannin levels compared to the other therapeutic indications, the present study showed that *Olea europaea* may present more antimicrobial activity (P < 0.01).

Table 2. Phytochemical contents: Total phenol contents (TPC) (mg gallic acid equivalents (EAG)/100g); flavonoid content (TFC) (mg catechin equivalents (CE)/ 100g); and tannin contents (TTC) (mg catequin equivalents (EC)/ 100g)

Plants	TPC	TFC	TTC
M. officinalis	69.66±1.08^{b}	57.28±4.02^{a}	11.34±3.05^{c}
M. chamomilla	43.35±4.71^{c}	41.32±0.87^{b}	16.11±2.99^{b}
O. europaea	12.91±7.91^{d}	25.17±3.53^{c}	37.01±0.48^{a}
A. triphylla	87.25±3.21^{a}	43.38±0.38^{b}	10.26±2.99^{c}

**Values expressed are means±S.D. of triplicate measurements (n=3). ^{a,b,c,d}Compare results between rows by comparison different medicinal plant extract for each phytochemical amount, with different lowercase letters were significantly different (P Y 0.05).*

The chemical complexity of extracts, often a mixture of dozens of compounds with differences in functional groups, polarity and chemical behavior, could lead to scattered results, depending on the assay employed. Therefore an approached with multiple assays in screening work is highly advisable. DPPH$^{\bullet}$ scavenging activity method has been widely used to evaluate the antioxidant activity of natural products from natural plants.

DPPH$^{\bullet}$ stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract (Koleva et al., 2002).

Fig. 1 shows the amount of each plant extract analyzed needed for 50% inhibition (IC_{50}). IC_{50} of BHT and catequin (standard compounds) were 0.058 and 0.014 mgml^{-1}, respectively. The highest radical scavenging activity was observed by *Aloysia triphylla* (IC_{50} = 0.021 mgml^{-1}) significantly higher than BHT (synthetic antioxidant) ($P < 0.05$). The radical scavenging activity (Table 3) measured in the four medicinal plant extracts decreased in the following order: *Aloysia triphylla* > *Melissa officinalis* > *Olea europaea* > *Matricaria chamomilla*. Most of the plants extracts at different concentrations exhibited more than 70% scavenging activity. The radical scavenging effect of *Aloysia triphylla* at 0.15 mg/ml was similar to the synthetic antioxidant used in this study (BHT) at 0.58 mg/ml. *M. chamomilla* was the medicinal plant with lowest antioxidant activity (44.5%). Therefore, the antioxidant effect of *A. triphylla* was near 4 times greater than BHT. *Melissa officinalis* from Portugal presented excellent radical scavenging, however lower antioxidant activity than other previously described by Hussain et al. (2011) and Marongiu et al. (2004).

The screening of the leaf of the four selected medicinal plants indicates that the presence of high phenolic content may be due to the presence of tannins and flavonoids which are known to possess antioxidant activities. A slight correlation has been observed between total phenolics and antioxidant activity. Also a relationship between total phenolic content and antioxidant activity has been reported by Javanmardi et al. (2003) did not found any such kind of correlation between antioxidant activity and phenolic content in plant extracts. In contrast, relationship was not observed between flavonoids content and antioxidant activity. Our results are in agreement with Miliauskas et al. (2004).

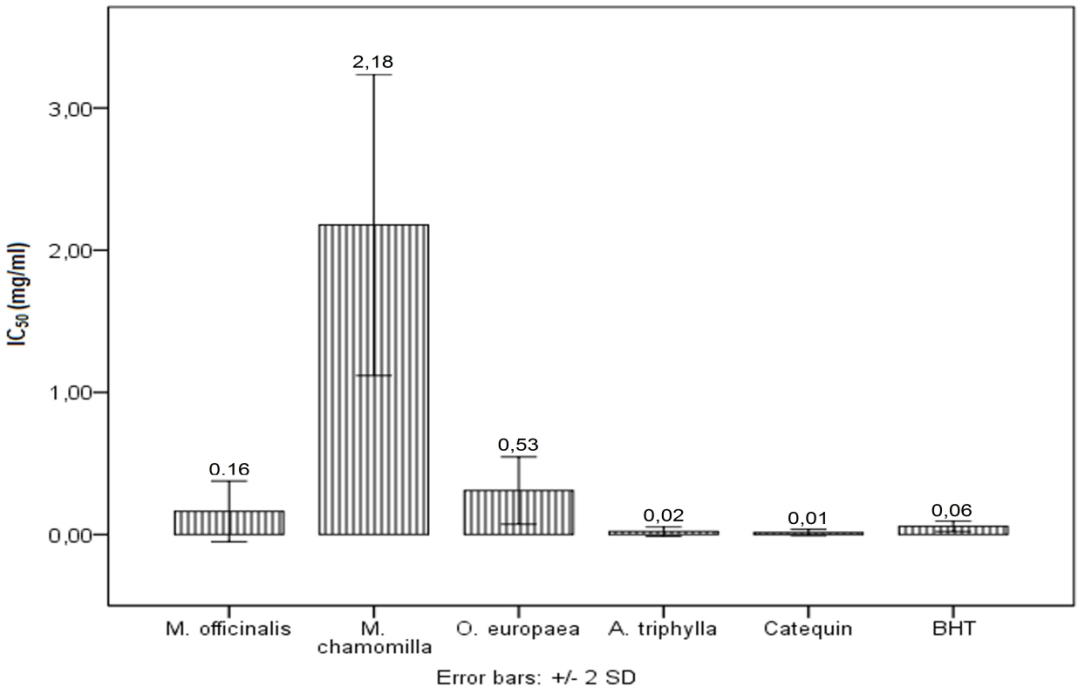

Fig. 1. IC50 values of dried plant extracts were measured by DPPH• scavenging activity. BHT and catequin were used as standard compounds.

Table 3. Comparison of DPPH$^{\delta}$ scavenging activity (%) of tested plants extracts and BHT and catequin as standard compounds (mg/ml)

Plants	(mg/ml)	DPPH Assay (%)*	IC_{50} ($mgml^{-1}$)*
M. officinalis	0.75	89.7±1.04	0.16±0.11
M. chamomilla	4.02	44.5±1.02	2.18±0.53
O. europaea	0.82	79.7±1.50	0.31±0.02
A. triphylla	0.15	94.1±0.82	0.021±0.02
Catequin	0.014	94.6±0.69	0.014±0.01
BHT	0.58	93.4±0.81	0.058±0.02

Values expressed are means±S.D. of triplicate measurements (n =3).
Mean Value ± Standard Deviation of three replicates (n=3)

4. CONCLUSION

Today, antioxidative properties of extracts from plants have become a great interest due to their possible uses as natural additives to replace synthetic ones. This study was designed to investigate the phytochemical characterization and evaluate the *in vitro* antioxidant activity of four medicinal plants from Portugal usually consumed as tea infusion. Phenolic compounds, flavonoids and proanthocyanidins (tannins) were detected in the aqueous extract of each plant analyzed. Results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive.

The tested medicinal plants presented high antioxidant activity, oxidation inhibition and free radical scavenging, thus indicating possible benefits on human health when present in diet. Moreover, this is the first study with these medicinal plants from Portugal, demonstrating their antioxidant potential.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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