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IN-VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *PITURANTHOS CHLORANTHUS* AND *ARTEMISIA VULGARIS* FROM TUNISIA

Soumeya Ben Nasr [1], Smail Aazza [2], Wissem Mnif [3, 4] and Maria Graça Miguel [*1]

Faculdade de Ciências e Tecnologia [1], Departamento de Química e Farmácia, Universidade do Algarve, Edifício 8, Centro para os Recursos Biológicos e Alimentos Mediterrânicos (MeditBio), Campus de Gambelas, 8005-139 Faro, Portugal.
Laboratory of Phytochemistry [2], National Agency of Medicinal and Aromatic Plants (NAMAP). BP. 159, Principal, 34000, Taounate, Morocco.
Department of Chemistry [3], Faculty of Sciences and Arts in Balgarn, University of Bisha, Bisha 61922, P.O. BOX 199, Kingdom of Saudi Arabia.
Université de Manouba [4], Institut Supérieur de Biotechnologie Sidi Thabet, LR Biotechnologie et Valorisation des Bio-Géo Ressources (BVBGR-LR11ES31), Biotechpole Sidi Thabet, Ariana 2020, Tunisia.

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Correspondence to Author:
Maria Graça Miguel

Associate Professor with Habilitation, Faculdade de Ciências e Tecnologia, Departamento de Química e Farmácia, Universidade do Algarve, Edifício 8, Centro para os Recursos Biológicos e Alimentos Mediterrânicos (MeditBio), Campus de Gambelas, 8005-139 Faro, Portugal.

E-mail: mgmiguel@ualg.pt

ABSTRACT: *Pituranthos chloranthus* and *Artemisia vulgaris* L. are two of the most important aromatic and medicinal species from the Apiaceae and Asteraceae families, respectively. They are traditionally used in certain pathologies in which inflammatory processes are involved. The present study investigates the potential of aqueous extracts of Tunisian *P. chloranthus* and *A. vulgaris* as a natural alternative source of antioxidant and anti-inflammatory activities using *in-vitro* techniques. The antioxidant activities of aqueous extracts were evaluated through several assays: capacity for scavenging free radicals (ABTS, DPPH, hydroxyl, superoxide, nitric oxide); and total antioxidant capacity by ferric reducing power activity and inhibition of lipid peroxidation by thiobarbituric acid reactive species (TBARS) method. The anti-inflammatory activity was evaluated through the lipoxygenase (LOX) inhibitory activity. *P. chloranthos* aqueous extract presented higher concentration of total phenols than *A. vulgaris* extract, nevertheless higher capacity for scavenging ABTS, superoxide, hydroxyl, and NO free radicals. In the presence of liver homogenate, both extracts had poorer antioxidant activity than in the remaining lipid substrates. *P. chloranthos* extract had a higher ability for inhibiting lipoxygenase twice higher than *A. vulgaris,* while it had lower capacity for reducing Fe^{3+} than *P. chloranthos* extract. Our results suggest that there are differences of antioxidant activity between both samples, but also the strength for inhibiting the oxidation is highly dependent on the method used.

INTRODUCTION: *Pituranthos chloranthus* (Bent. Et Hook.) (Apiaceae) is an aromatic plant from North Africa.

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This species grows naturally in central and southern Tunisia. The Arabian name of this species in this country is 'Guezzah' [1, 2] and 'Aljen' [3, 4]. However, there is also an indication that 'Guezzah' is the Arabian name of *P. scoparius* [5] or *P. tortuosus* [4, 6].

Over time, *P. chloranthus* has been used either in agriculture or as a remedy. In the agriculture, the stems can be used as a straw to dry figs and grapes or as insecticide or for disinfecting the underground

cisterns of the rainwater storage after suspending a tuft of the plant to the surface. In traditional medicine, the plant has been used for diverse purposes: treatment of pain, rheumatism, spasms, fever, digestive difficulties, hepatitis, urinary infections, and scorpion stings [2]. However, there are authors, who attribute some of these applications to *P. tortuosus* [6] or *P. scoparius* [5]. According to the review [5], *P. chloranthus* has only been traditionally used on diabetes and fever, whereas another author [7] indicates that this species is only used for combating headache after the application of cataplasms on head.

The chemical composition, the antimicrobial, antioxidant, antimutagenic and anti-genotoxic properties of the essential oils of *P. chloranthus* from Tunisia have been the aim of the study of several authors [1, 2, 4, 8, 9, 10, 11, 12]. Only very few reports [13] about the chemical composition of extracts of this species could be found: the flavonoids [isorhamnetin- 3- *O*- glucoside, isorhamnetin- 3- *O*-rutinoside, apigenin-6, 8-di-*C*-glucoside (vicenin-2), and tamarixetin-3-*O*-glucoside]. These flavonoids were obtained from extracts of the aerial parts of flowering *P. chloranthus* collected in Algeria [13]. From Tunisia, the authors [3] identified coumarins, two in the aerial parts (isoimperatorin and osthol) and two other coumarins in the roots (bergapten and nodakenetin). The antioxidant and anti-microbial activities of extracts of *P. chloranthus* have also been reported [14, 15].

Artemisia vulgaris L. (Asteraceae), known as mugwort or common wormwood, is an aromatic plant naturally growing in Europe, Africa and India [16]. In addition, Hooper [17] refers 'afsantin-e-hindi' as the Arabian name given to *Artemisia vulgaris* L, whereas USAID; Chemonics International, Inc [18] attributes 'Chih' as being the Arabian name for *Artemisia vulgaris*. On the other hand, there are also authors who attributed the name 'Chih' to the species *A. herba-alba* [19]. In folk medicine, *A. vulgaris* is generally used as an antispasmodic, antiseptic, anthelmintic, carminative, sedative, and in the treatment of menstrual problems, irregular periods, menopause and premenstrual syndrome [20, 21]. In some other regions of the world (Nepal, Pakistan), the leaves of *A. vulgaris* also have other applications, such as stopping nose bleeding, to treat oral ulcers, ophthalmic diseases, malaria, and fevers, to prevent and to treat some types of allergic reactions [22, 23].

Other properties have been reported for mugwort: antimicrobial [24, 25], antioxidant [16, 24, 25], anti-inflammatory [21, 26], hepatoprotective [27], hypo-lipidemic [21] and antihypertensive properties [26] although some authors have advised against the use of *A. vulgaris* for this purpose [28]. The antioxidant and antimicrobial activities of the mugwort extracts have led to use them for fabricating silver nanoparticles with high potential biomedical applications [29]. The chemical composition and biological properties of essential oils of *A. vulgaris* have been deeply investigated [16, 27, 30, 31, 32, 33]. Biological properties of extracts of this species have also been evaluated and reviewed [21, 24, 25, 34, 35] nevertheless their chemical composition has been much less scrutinized [16, 36, 37, 38].

As aforementioned, *A. vulgaris* and *P. chloranthus* have been used in folk medicine in certain pathologies in which inflammatory processes are involved. When there is an uncontrolled and excessive production of reactive oxygen species (ROS) a process called oxidative stress occurs. These ROS can react with biomolecules, such as lipids, proteins, and nucleic acids. Under hypoxia, mitochondrial respiratory chain produces nitric oxide (NO), which is able to generate other reactive nitrogen species (RNS), which in turn, can produce other reactive species.

The reaction of all these reactive species with cellular lipids induces excessive lipid peroxidation products, such as lipid-derived aldehydes (*e.g.* malonaldehyde, 4-hydroxy-2-nonenal) that can be responsible for several oxidative stress-induced inflammatory diseases [39, 40]. The capacity of compounds to prevent lipid peroxidation and/or generation of reactive species may contribute to retard, decrease or prevent inflammation.

The present work aimed at evaluating the potential antioxidant and anti-inflammatory activities using *in-vitro* techniques of aqueous extracts of Tunisian *A. vulgaris* and *P. chloranthus*.

MATERIALS AND METHODS:

Plant Material and Preparation of Extracts: Plant material was collected in 2014 from natural

populations located at the Center of Tunisia. The plant specimens were identified by Prof. Mohamed Chaieb, a botanist in the Faculty of Sciences of Sfax-Tunisia, and vouchers specimens (AVU-2 for *A. vulgaris* and PCH-3 for *P. chloranthus*) deposited in the Higher Institute of Biotechnology of Sidi Thabet-Tunisia. Extraction was performed by decoction of 5 g of dried aerial parts of Tunisian *A. vulgaris* and *P. chloranthus* for 1 h in 100 mL of distilled water. The aqueous phases were centrifuged for 10 min, at 2,000 g, at 20 ºC and the supernatant was removed and kept at -20 ºC until the determination of total phenols and *in vitro* antioxidant activities.

Determination of Total Phenols (Folin-Ciocalteau): The total phenol content of the extracts was determined according to [41]. Gallic acid was used as standard. The aqueous extracts (0.5 mL) and 2 mL of sodium carbonate (75 g/L) were added to 2.5 mL of 10% (v/v) Folin-Ciocalteau reagent. After 60 min at room temperature, the absorbance was measured at 765 nm. Tests were carried out in triplicate and the results presented as mg gallic acid equivalent (GAE)/g.

Determination of ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) Radical Cation Scavenging Capacity: The ABTS radical cation decolorization assay was carried out using the previously reported methods [41] with slight modifications. The aqueous extracts (10 µL) at various concentrations was mixed with 990 µL diluted $ABTS^{\cdot+}$ solution. The absorbance at 734 nm was measured after reaction at room temperature for 6 min. The ability to scavenge the $ABTS^{\cdot+}$ was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate and the inhibition percentage was plotted against extract concentration (w/v) and the results presented as IC_{50} values (concentration of extract able to prevent 50% of lipid peroxidation).

Determination of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (DPPH): The aqueous extracts (50 µL) at different concentrations were placed in a cuvette, and 2 mL of 60 mM methanolic solution of DPPH was added [41]. Absorbance measurements were made at 517 nm after 60 min of reaction at room temperature. The ability to scavenge the DPPH free radicals was calculated using the formula: Scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. The values of IC_{50} were determined as reported above. Tests were carried out in triplicate and the results presented as IC_{50} values.

Hydroxyl Radical Scavenging Activity: The assay of hydroxyl scavenging activity was performed as [42] with small modifications. Briefly, the reaction mixture was prepared using 10 mM $FeSO_4.7H_2O$, 10 mM EDTA, 10 mM 2-deoxyribose, 0.1 M phosphate buffer and sample at different concentrations to give a total volume of 1.8 mL. Finally, 200 µL of H_2O_2 was added to the mixture, which was incubated at 37 ºC for 4 h. After this period, 1 ml trichloroacetic acid (2.8%) and 1 mL thiobarbituric acid (1%) was added and boiled for 10 min to develop the pink colored malondialdehyde–thiobarbituric acid. After cooling, its absorbance was measured at 520 nm. The hydroxyl scavenging activity (%) was calculated using the following equation: Inhibition = $[(A_0 - A_1) / A_0] \times 100$ where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. The values of IC_{50} were determined as reported above. Tests were carried out in triplicate and the results presented as IC_{50} values.

Superoxide Anion Scavenging Activity (Non-Enzymatic Method): Measurements of superoxide anion scavenging activity of samples and positive control were based on the method previously described [41]. Superoxide anions were generated in a non-enzymatic phenazine methosulfate nicotinamide adenine dinucleotide (PMS/NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT). The method involved mixing the aqueous extracts (25 µL) at different concentrations with 25 µL nitroblue tetrazolium (0.42 mg/mL) and 25 µL NADH (1.32 mg/mL) and ethanol (125 µL) followed by 25 µL phenazine methosulfate (PMS) (0.25 mg/mL). The absorbance was measured at 560 nm, after 10 min of reaction.

The superoxide scavenging activity (%) was calculated using the following equation: Inhibition = $[(A_0 - A_1) / A_0] \times 100$ where A_0 is the absorbance of the control (without sample), and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate and the results presented as IC_{50} values.

Nitric Oxide (NO) Scavenging Capacity: The NO scavenging activity of aqueous extracts was determinate according to [41]. The aqueous extracts (75 µL) at diverse concentrations were added to 75 µL of 10 mM sodium nitroprusside into a 96-well plate and left standing at ambient temperature for 35 min. The reaction was initiated by adding Griess reagent [40 µL of sulfanilamide solution and 40 µL of N-1-napthylethylenediamine dihydrochloride (NED) solution] to the mixture. The absorbance was measured at 532 nm without previous incubation. The NO scavenging activity (%) was calculated using the following equation: Inhibition = $[(A_0 - A_1) / A_0] \times 100$ where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate, and the results presented as IC_{50} values.

Inhibition of Lipid Peroxidation by Thiobarbituric Acid Reactive Species (TBARS) Method: This assay was performed using three distinct lipid substrates: egg yolk, liver, and liposomes. The assay was carried out as described by [41]. Egg yolk or liver (100 mg/mL) (250 µL) homogenates were mixed with acetic acid (750 µL) and the solution of TBA (8 mg/mL) (750 µL) dissolved in sodium dodecyl sulfate (SDS) (11 mg/mL), and 100 µL aqueous extracts at different concentrations. The mixtures were heated for one hour in water bath at 95 °C. After cooling, the final volume was adjusted to 2 mL by adding butanol followed by centrifugation for 10 min at 3,000 *g*. The absorbance of the upper layer was read at 532 nm. The percentage of inhibition was calculated as follows: $[(A_0 - A_1 / A_0) \times 100]$, in which A_0 is the absorbance of the control reaction (without extract), and A_1 is the absorbance of the extracts. Analyses were run in triplicate. The IC_{50} values were determined (concentration of extract able to prevent 50% of lipid peroxidation). The liposome solution was made as follows: 0.4 g lecithin in 80 mL chloroform. This solution was dried and submitted to nitrogen flux for 30 s and then to vacuum for at least two hours until complete dryness. After that, the reaction was initiated by mixing 50 µL of different extract concentrations with 100 µL of liposome suspension, $FeCl_3$ (100 µL, 4 mM) and 50 µL of ascorbic acid (0.018 mg/mL). After incubation at 37 °C for one hour, 2 mL of TBA solution (0.6%) was added. The mixture was heated for 10 min at 95 °C. Afterward, 2 mL butanol was added and the final solution was centrifuged for 5 min at 3,000 g. The percentage of inhibition and the IC_{50} values were calculated as described above.

Ferric Reducing Power Activity: The reducing power of each extract at different concentrations was measured as previously reported [41]. Fifty microliters of each sample were mixed to 0.5 mL of potassium phosphate buffer (200 mM, pH 6) and 0.5 mL of potassium ferricyanide (1%). The mixtures were incubated in a water bath (50 °C), for 20 min. After this period, 0.5 mL of trichloroacetic acid (10%, w/v) was added to the cooled mixture and submitted to centrifugation at 3,000 g, for 10 min. Finally, distilled water (0.5 mL) and 0.1 mL $FeCl_3$ (0.1%, w/v) were added to the supernatant and the absorbance was measured at 700 nm. The ferric reducing power capacities of the extracts were expressed graphically by plotting absorbance against concentration.

Lipoxygenase (LOX) Inhibitory Activity: The inhibition of the lipoxygenase enzyme was performed as reported [41]. The reaction was started with the mixture of 10 µL of aqueous extracts at different concentrations, 5 µL of enzyme solution (0.054 g/mL), 50 µL of linoleic acid (0.001 M) and borate buffer 937 µL (0.1 M, pH 9). The measurement of the absorbance was recorded at 234 nm. The percentage of inhibition was calculated as follows: $[(A_0 - A_1 / A_0) \times 100]$, in which A_0 is the absorbance of the control reaction (without extract), and A_1 is the absorbance of the extracts. Analyses were run in triplicate. The percentage of inhibition and the IC_{50} values were calculated as described above.

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 23.0 software (SPSS Inc., Chicago, IL, USA). Statistical comparisons were

made with one-way ANOVA followed by Tukey multiple comparisons. The level of significance was set at $P<0.05$. The paired Student t-test was used in some tests to determine differences at 95% significance.

RESULTS: Table 1 depicts the content of phenols and antioxidant activities, measured through diverse methods, of *P. chloranthus* and *A. vulgaris* aqueous extracts. The antioxidant activity is expressed in IC_{50} (concentration of the extract that is able to prevent 50% lipid peroxidation or to scavenge free radicals or to inhibit lipoxygenase activity). The lower the IC_{50} values are, the higher the antioxidant activities will be.

TABLE 1: PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF *A. VULGARIS* AND *P. CHLORANTHUS*

Sample	Phenol	Hydroxyl	DPPH	ABTS	Superoxide	Egg yolk (TBARS)	Liposomes (TBARS)	NO	Liver (TBARS)	Lipoxygenase
P. chloranthos	1.36 ±0.02*	0.38 ±0.02^{de***}	0.44 ±0.01^{d,ns}	0.58 ±0.03^{c***}	1.57 ±0.05^{b***}	0.18 ±0.07^{f,ns}	0.12 ±0.01^{f***}	4.83 ±0.04^{a***}	0.36 ±0.03^{e***}	0.02 ±0.00^{g**}
A. vulgaris	0.91 ±0.18*	0.17 ±0.00^{d***}	0.47 ±0.03^{c,ns}	0.17 ±0.01^{d***}	0.57 ±0.02^{b***}	0.10 ±0.01^{e,ns}	0.06 ±0.01^{f***}	2.30 ±0.01^{a***}	0.57 ±0.01^{b***}	0.04 ±0.00^{f**}

Values in the same row followed by the same letter are not significant different ($P<0.05$) by the Tukey's multiple range test; Means ± standard deviation; *: Statistically significant differences between phenol content of *A. vulgaris* and *P. chloranthus* extracts ($p\leq0.05$; t-Student test); **Statistically significant differences between antioxidant activities of *A. vulgaris* and *P. chloranthus* extracts ($p\leq0.01$; t-Student test); ***Statistically significant differences between antioxidant activities of *A. vulgaris* and *P. chloranthus* extracts ($p\leq0.001$; t-Student test); ^{ns}: not significant between the capacity for scavenging DPPH free radicals and preventing lipid peroxidation in the presence of egg yolk of *A. vulgaris* and *P. chloranthus* extracts.

P. chloranthos aqueous extract presented higher concentration of total phenols than *A. vulgaris* extract, nevertheless higher capacity for scavenging ABTS, superoxide, hydroxyl, and NO free radicals, since IC_{50} values (0.17; 0.57; 0.17; and 2.30 mg/mL, respectively) were lower than those of *P. chloranthos* (0.58; 1.57; 0.38; and 4.83 mg/mL, respectively **Table 1**. When liposomes were used as lipid substrate, the capacity for preventing their peroxidation was better in the presence of *A. vulgaris* extract (IC_{50} = 0.06 mg/mL), nevertheless in the presence of liver homogenate, *P. chloranthos* extract had a higher capacity for preventing its oxidation (IC_{50} = 0.36 mg/mL). In addition, in the presence of egg yolk, the activities of both extracts were not significantly different **Table 1**. It is noteworthy the different ability of both extracts to act as antioxidants depending on the lipidic substrate. In the presence of liver homogenate, both extracts had poorer antioxidant activity than in the remaining lipid substrates. *P. chloranthos* extract had higher ability for inhibiting lipoxygenase (IC_{50} = 0.02 mg/mL) than *A. vulgaris* (IC_{50} = 0.04 mg/mL) **Table 1**.

Since, the activity unities are in all cases the same, IC_{50} values; it is possible to compare the effectiveness of samples as antioxidants in the diverse assays. The antioxidant activity is highly dependent on the method used. For example, and by descending order, the antioxidant activity of *P. chloranthos* extract was: lipoxygenase inhibition > prevention of lipid peroxidation in the presence of liposome or egg yolk homogenate > prevention of lipid peroxidation in the presence of liver homogenate > hydroxyl scavenging activity > DPPH scavenging activity > ABTS scavenging activity > superoxide scavenging activity > NO scavenging activity. For *A. vulgaris* extract, the order was: lipoxygenase inhibition or prevention of lipid peroxidation in the presence of liposome > ABTS scavenging activity or hydroxyl scavenging activity > DPPH scavenging activity > superoxide scavenging activity or prevention of lipid peroxidation in the presence of liver homogenate NO scavenging activity.

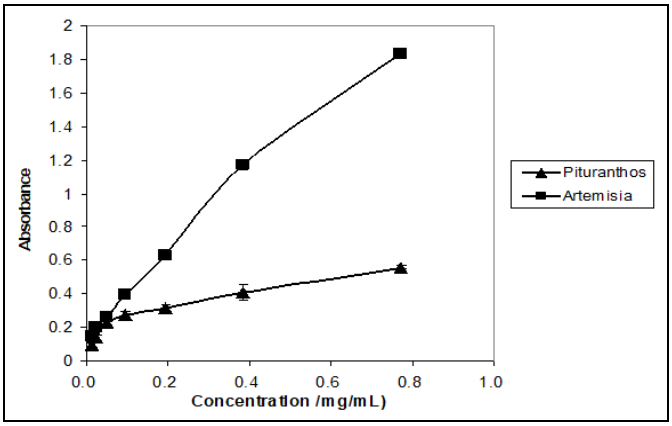

FIG. 1: REDUCING POWER OF *A. VULGARIS* AND *P. CHLORANTHUS* EXTRACTS

Fig. 1 presents the reducing power of *A. vulgaris* and *P. chloranthos* extracts. Higher absorbance means better capacity for reducing Fe^{3+}, and according to the results *A. vulgaris* extract had a better capacity for reducing these ions than *P. chloranthos* extract.

DISCUSSION: Solvent extraction is the method predominantly used for preparing extracts from plant material [43]. In the present work, the solvent used was water and the extraction method was decoction. With this procedure, the secondary metabolites extracted will be predominantly hydrophilic compounds. Generally, medicinal plants when used as folk medicines, and per os, were under aqueous form and not under other way using organic solvents, either an infusion or decoction. For this reason, water was the choice solvent, and decoction, the extraction method.

For evaluating lipid peroxidation, several substrates may be used such as homogenous lipids (fats and oils), or aqueous microheterogenous systems (micelles, liposomes) [44]. In the present work, pork liver homogenates, egg yolk and liposomes containing lecithin were used as lipid substrates. Lecithin liposomes were used as a model-system of biological membranes [45]. The modified Thiobarbituric Acid Reactive Species (TBARS) was the method used for measuring the antioxidant effectiveness of extracts.

Both aqueous extracts possessed the capacity for preventing lipid peroxidation, nevertheless such ability was dependent on the extract and lipidic substrate as well **Table 1**. The effectiveness of both extracts to prevent lipid peroxidation of liver was much weaker than those lipids of egg yolk or liposomes. Such difference may be correlated to the different lipidic profile of the substrate, as previously reported by some authors [46] when studying the antioxidant activity of essential oils, isolated from diverse aromatic plants, on egg yolk, chicken or rat livers. Generally, the antioxidant activity was associated with the amount of phenols in samples as well as with their structures, along with the method of antioxidant determination used [43, 47].

In the present work, two extracts obtained from two plants, using the same extraction procedure, had different amounts of phenols and distinct behaviors as antioxidants depending on the lipid substrates used. For example, *P. chloranthus* had a stronger ability to prevent lipid oxidation of liver, whilst *A. vulgaris* extract was a better antioxidant in the presence of liposomes ($p<0.001$; t-Student). In the case of egg yolk as lipidic substrate, there were no differences between extracts **Table 1**. These differences cannot only be attributed to the different phenol concentration of extracts (1.36 mg GAE/g and 0.91 mg GAE/g for *P. chloranthus* and *A. vulgaris* respectively). The amount of total phenol detected in *A. vulgaris* extract was within the range to that reported by [48] for methanolic extracts **Table 1** and **2**. *P. chloranthus* extract of the present work had higher amounts of total phenols (1.36 mg GAE/g) when compared to those previously reported **Table 1**.

The capacity for scavenging free radicals was also dependent on the extracts and type of radicals assayed. *A. vulgaris* extract was the best scavenger of almost all free radicals ($p<0.001$, t-Student) although its lower concentration of total phenols than *P. chloranthus* extract, with the exception of DPPH free radicals in which there were no differences between the extract **Table 1**.

After the research with the words "*Artemisia vulgaris* antioxidant" made in Web of Knowledge, it was possible to find the references listed in **Table 2**. According to the table, the capacity for scavenging DPPH free radicals (eight references) was the method mainly used by the authors, followed by FRAP (Ferric Reducing Antioxidant Power) (four references), ABTS method (two references) and finally TBARS, ORAC (Oxygen Radical Absorbance Capacity), NO and total antioxidant activity (one reference each).

Percentages of inhibition and IC_{50} values were ways to present the results for the DPPH method, and we can only compare our results with those also expressed as IC_{50} values. The results obtained in the present work (IC_{50} = 0.47 mg/mL) were closer to those found by [25]. Temraz and El-Tantawy [34] found IC_{50} = 11.4 µg/mL, much lower (and consequently much higher activity) than that obtained in the present work, though having the same extraction solvent used.

TABLE 2: REVIEW OF THE ANTIOXIDANT ACTIVITY OF *A. VULGARIS* AND *P. CHLORANTHUS* EXTRACTS

Artemisia vulgaris					
Extract	**Method**	**Total phenol content**	**Units**	**Activity**	**Units**
Hydroalcoholic	DPPH	28.62	mg GAE/g	IC_{50} = 976	µg/mL
Hydroalcoholic [25]	TBARS (egg yolk)	28.62	mg GAE/g	IC_{50} = 0.36	mg/mL
Methanol	DPPH	7920	µg GAE/g	IC_{50} = 16	µg/mL
Methanol [16]	ORAC	7920	µg GAE/g	5700	µmol Trolox/g
Methanol	DPPH	217.46	mg GAE/L	43.3%	0.25 mg/4 mL
Methanol [24]	ABTS	217.46	mg GAE/L	$TEAC^{*}$ = 4.40	mM/mg
Methanol [47]	DPPH	CE: 141.9	mg GAE/g	CE: IC_{50} = 22.2	µg/mL
		UE: 135.0	mg GAE/g	UE: IC_{50} = 26.5	
		SE: 123.4	mg GAE/g	SE: IC_{50} =28.1	
Methanol [52]	DPPH	321.23	mg GAE/g	92.33%	*
Aqueous	DPPH	19	mg GAE/g	IC_{50} = 11.4 (19)	µg/mL
Aqueous [34]	NO	19	mg GAE/g	IC_{50} = 125	mg/mL
Methanol	ABTS	956	µg GAE/g	TEAC = 2.86	µmol Trolox/g
Methanol [48]	FRAP	956	µg GAE/g	2.12	µmol Fe_2^+/g
HA-1 [53]	DPPH	18.17	µg GAE/mg	>.40 and < 50	Percentage inhibition
HA-2		9.24	µg GAE/mg		
HA-3		17.28	µg GAE/mg		
HA-4		9.37	µg GAE/mg		
HA-5		11.90	µg GAE/mg		
HA-6		11.36	µg GAE/mg		
HA-7		13.27	µg GAE/mg		
HA-8		26.29	µg GAE/mg		
HA-9		25.81	µg GAE/mg	>.80 and < 90	Percentage inhibition
				%inhibition >.8	Percentage inhibition
HA-1	FRAP			0	
HA-2					Ascorbic acid equivalent (mg/mL)
HA-3				> 130 and < 140	
HA-4					
HA-5					
HA-6					
HA-7					
HA-8					
HA-9					
					Ascorbic acid equivalent (mg/mL)
HA-1	Total antioxidant			> 170 and < 180	Ascorbic acid equivalent (mg/mL)
HA-2	activity			> 170 and < 180	Ascorbic acid equivalent (mg/mL)
HA-3				> 90 and < 100	Ascorbic acid equivalent (mg/mL)
HA-4				>80 and < 90	Ascorbic acid equivalent (mg/mL)
HA-5				>90 and < 100	Ascorbic acid equivalent (mg/mL)
HA-6				>80 and < 90	Ascorbic acid equivalent (mg/mL)
HA-7				>90 and < 100	
HA-8					
HA-9					
Methanol [54]	DPPH	86.62	mg GAE/g	80	Percentage inhibition (at 100 µg/mL)
	FRAP			2.104	Absorbance (λ = 700 nm), at 1000 µg/mL
Methanol [55]	FRAP	Roots: >40 and < 50	mg GAE/g	> 7 and < 8	M $FeSO_4$/mg
		Leaves: > 70 and < 80		> 9 and < 10	M $FeSO_4$/mg
		Inflorescences: > 100 and < 110		>10 and < 15	M $FeSO_4$/mg
Pituranthos chloranthus					
Methanol	DPPH	77.59	µg/mg	IC_{50} = 71.67	µg/mL
Aqueous [15]	DPPH	91.03	µg/mg	IC_{50} = 56.67	µg/mL
Hexane	DPPH	117	mg PyE/100 g	IC_{50} = 2.22	µg/mL
Ethyl acetate	DPPH	380	mg PyE/100 g	IC_{50} = 2.24	µg/mL
Methanol	DPPH	317	mg PyE/100 g	IC_{50} = 2.01	µg/mL
Water [14]	DPPH	372	mg PyE/100 g	IC_{50} = 4.59	µg/mL
Hexane	ABTS	117	mg PyE/100 g	TEAC = 0.20	mM Trolox equivalent of extract
Ethyl acetate	ABTS	380	mg PyE/100 g	TEAC = 0.36	
Methanol	ABTS	317	mg PyE/100 g	TEAC = 0.75	
Water [14]	ABTS	372	mg PyE/100 g	TEAC = 0.40	

CE: classical extraction; FRAP: Ferric Reducing Antioxidant Power; GAE: Gallic Acid Equivalent; ORAC: Oxygen Radical Absorbance capacity; PyE: Pyrogallol equivalent; SE: Sohlet extraction; TEAC: Trolox equivalent antioxidant capacity; UE: ultrasound extraction.
*The concentration responsible for this percentage is not given

The capacity for scavenging ABTS free radicals obtained in the present work is not possible to compare to those presented in **Table 2** since the ways used to express the activities are different. The capacity for scavenging NO radicals, expressed as IC_{50} values, observed by [34] for the aqueous extracts was significantly lower than our extracts (IC_{50} = 4.83 mg/mL), therefore presenting higher activity.

The same research made for *A. vulgaris* was repeated for *P. chloranthus* in Web of Knowledge, but in this case, no reference was found. Only research using Google allowed finding references; some of them are listed in **Table 2**. The capacity for scavenging DPPH and ABTS free radicals were the methods preferentially used by the authors. The activities found by the authors were 6 - 200-fold higher than those found in the present work.

Compounds that inhibit lipoxygenase may possibly prevent the formation of peroxides from unsaturated fatty acids containing a *Z,Z*-1,4-pentadiene system, and consequently the biosynthesis of leukotrienes that play an important role in inflammatory processes [49]. Such compounds have antioxidant and anti-inflammatory activity. The extracts of *A. vulgaris* and *P. chloranthus* had capacity for inhibiting lipoxygenase, although *P. chloranthus* possessed higher activity than *A. vulgaris* extract ($p<0.01$, t-Student).

P. chloranthus extract revealed to be better antioxidant acting through the inhibition of lipoxygenase and prevention of lipid peroxidation when the lipid substrate was liver than as scavenging free radicals **Table 1**. Among all tests assayed, the capacity for inhibiting lipoxygenase activity was also the best for *A. vulgaris* extract, followed by the inhibition of lipid peroxidation when using egg yolk and liposomes as lipidic substrates. In addition, this extract had also good capacity for scavenging hydroxyl and ABTS free radicals, not observed for *P. chloranthus* **Table 1**.

The antioxidant activities of *P. chloranthus* and *A. vulgaris* aqueous extracts, measured through diverse methods, found in the present work, were generally poorer when compared to the positive controls assayed in our laboratory in another time period [50, 51]. Briefly, the IC_{50} values found for BHT (butylated hydroxytoluene) used as positive control in ABTS, DPPH; TBARS (egg yolk as liposomes used as substrate) methods were 0.004 mg/mL; 0.089 mg/mL; 0.096 mg/mL; and 0.002 mg/mL, respectively. These values were lower than those obtained for plant extracts **Table 1**. Ascorbic acid used was used as positive control in the evaluation of the ability for scavenging superoxide anion radicals. The IC_{50} value was lower (better activity) (0.017 mg/mL) than the plant extracts **Table 1**. The IC_{50} value of mannitol, the positive control for the determination of the ability for scavenging hydroxyl radicals, was 0.0007 mg/mL, much lower (much better activity) than the samples **Table 1**. Nordihydroguaiaretic acid (NDGA) was the sole positive control studied in which the respective IC_{50} value was the same as that of P. *chloranthus* extract (IC_{50} = 0.02 mg/mL) **Table 1**.

CONCLUSION: The results observed in the present work not only show that there are differences of antioxidant activity between *A. vulgaris* and *P. chloranthus* extracts, but also the strength for inhibiting the oxidation is highly dependent on the method used, that is, it is possible to have samples that show better capacity for scavenging some free radicals and lower activity for scavenging other ones or preventing lipid peroxidation and *vice-versa*. The evaluation of the antioxidant activity of one sample can be a time-consuming process since several methods based in several mechanisms must be assayed. The oxidation process involves several steps and mechanisms, therefore diverse based-mechanisms assays must be done.

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