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Antioxidants from Medicinal Plants Used in the Treatment of Obesity

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Authors' contributions

This work was carried out in collaboration between all authors. The study design was by authors AAS, ADC and TRM, authors FFL and PMBC performed the experiments, authors JMF and RMF managed the literature searches. Authors AAS, JMF and ADC were involved in the writing process of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The objective of this work was to quantify phenolic compounds, flavonoids, vitamin C, total carotenoids, β-carotene and lycopene and to measure the antioxidant activity in the medicinal plants *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., and *Tournefortia paniculata* Cham. (marmelinho) and of the phytotherapic made with the combination of these plants.
Place and Duration of Study: Chemistry Department of Federal University of Lavras – UFLA, Brazil between June 2011 and September 2012.
Methodology: Phenolic compounds, flavonoid, vitamin C, total carotenoids and β-carotene and lycopene contents were quantified by UV-Vis spectrophotometer and antioxidant activity by ABTS and β-carotene/linoleic acid methods.
Results: High contents of phenolic compounds were found in marmelinho (36.19g $100g^{-1}$ dry matter – DM), followed by carqueja (4.03g $100g^{-1}$DM) and calunga (1.62g $100g^{-1}$DM); of flavonoids in marmelinho (480.30mg $100g^{-1}$DM) and carqueja (173.68mg $100g^{-1}$DM); of vitamin C in marmelinho (652.80mg $100g^{-1}$DM) and *G. cambogia* (127.63mg $100g^{-1}$DM); and of carotenoids in marmelinho (23.16 mg 100 g^{-1}). The antioxidant activity, in µmol

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trolox g^{-1}, by the ABTS method, was considered moderate in the aqueous (728.80) and ethanolic (731.06) marmelinho extracts, and weak for the other plants. However, by the β-carotene/linoleic acid method, the aqueous and ethanolic marmelinho extracts show great antioxidant potential at all tested concentrations (above 80% inhibition), and those of carqueja, calunga and the ethanolic of the phytotherapic, at the concentrations of 40,000 and 20,000mg L^{-1}, also showed good antioxidant potencies (over 60% inhibition).
Conclusion: Those five species of plants showed antioxidant activity with potential for use in pharmaceutical and food preparations, with possible health benefits.

Keywords: Phenolic compounds; flavonoids; vitamin C; carotenoids; antioxidant potential.

1. INTRODUCTION

In recent years, the effects of antioxidants have been investigated in relation to illnesses. The research has tried to explain the benefits of those substances for the prevention and treatment of various types of diseases [1,2].

Antioxidants are substances that combat free radicals, which are extremely reactive species that cause the oxidation of various biomolecules (lipids, proteins and nucleic acids) present in our organism causing diverse pathologies, such as cancer, neurodegenerative disorders, cardiovascular diseases, diabetes and other chronic diseases, and the free radicals may be the cause or the aggravating factor of their general picture [3]. Thus, research seeks alternatives to reduce the harmful effects of free radicals and improve the body's antioxidant capacity, as a form of treatment and prevention of diseases and their complications.

In view of the epidemiologic growth of these diseases, different foods and plants are studied by their substances, which are capable of neutralizing the effects of free radicals, such as phenolic compounds and vitamin C (hydrophilic antioxidants), vitamin E and carotenoids (lipophilic antioxidants), and certain minerals, such as zinc and selenium [4].

In that context, plants that are popularly known as having some therapeutic purpose, and that have not been the object of studies proving their effects, have become the central objective of research that seeks the development of new pharmaceuticals to aid in the treatment of diseases.

Aloe vera (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., *Tournefortia paniculata* Cham. (marmelinho) are plants used, isolated or associated together, as aids in the treatment of obesity [5,6]. When associated, they are used in an attempt to obtain better results, as is the phytotherapic Moder diet, prepared from the combination of these plants to help in the treatment of obesity, with few studies related to their antioxidant properties which, if proven, could help in the treatment of various other diseases, some directly related to obesity, such as diabetes, cardiovascular diseases, hypertension, among others, which can be caused or worsen by free radicals [7].

Based on the above, the objective of this work was to quantify the phenolic compounds, flavonoids, vitamin C, total carotenoids, β-carotene and lycopene and to measure the antioxidant activity of the medicinal plants aloe, calunga, carqueja, *G. cambogia* and marmelinho and of the phytotherapic made from the combination of these plants, with the

purpose of evaluating the possible use of these plants to combat free radicals and consequently in the treatment of various illnesses caused by them.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Baccharis trimera (Less.) DC (carqueja) and *Tournefortia paniculata* Cham. (marmelinho) leaves, and the trunk barks of *Simaba ferruginea* St. Hil. (calunga) were acquired in the municipal market of Belo Horizonte, Minas Gerais, Brazil, in January 2011, in three replicates, and transported to the Biochemistry Laboratory in the Chemistry Department of Federal University of Lavras (UFLA). The marmelinho and carqueja leaves were washed under running water and distilled water and soon afterwards placed together with the bark obtained from the calunga trunk in forced-air ovens for drying for 48 hours, at temperatures ranging from 30ºC to 35ºC. After drying, the leaves and the bark were ground in a Willy type mill and the flours stored in hermetically sealed flasks until the analyses. The commercial powder of *Aloe vera* (L.) Burm. (aloe) (mucilage) and that of *Garcinia cambogia* Desr. (fruit) were acquired from FLORIEN, a pharmaceutical supply distributor. The aloe powder was obtained from the lyophilization of the plant mucilage, while that of *G. cambogia* from drying by spray dryer.

The flour of the plants were mixed for the elaboration of a simulated phytotherapeutic, from the combination of aloe, calunga, carqueja, *G. cambogia* and marmelinho in proportions of 1:1,5:0,5:1,5:0,5, respectively; the same combination is used in the elaboration of the phytotherapeutic known by the trade name 'Moder Diet'.

2.2 Moisture Determination

The moisture determination was carried in the medicinal plant flours according to the Association of Official Analytical Chemists - AOAC [8] method, that consists of the water loss by dehydration, at temperatures ranging from 100ºC to 105ºC.

2.3 Phenolic Compounds

The extraction of the phenolic compounds was carried out with 1 g of sample in 50mL of 50% methanol, under reflux three consecutive times, at 80ºC and the extracts collected, evaporated up to 25mL and submitted to phenolic compound measurement, using the Folin-Denis reagent, and tannic acid as a standard [8].

2.4 Total Flavonoids

The total flavonoid contents were measured using the same extracts of the phenolic compound analyses, using the aluminum chloride colorimetric method, with catechin used as a standard [9].

2.5 Vitamin C

The extraction of the ascorbic acid was carried out with 0.5g of sample in 50mL of oxalic acid and 0.1g of diatomaceous earth, under agitation, for 15 minutes. After filtration

(Whatman Nº 40), the vitamin C in the extract was dosed, using ascorbic acid as a standard [10].

2.6 Total Carotenoids

For the determination of total carotenoids, the extraction was made according to Higby [11], using 0.5 g of sample in a 40 mL extraction solution of isopropyl alcohol:hexane 3:1. The content was transferred to a 125 mL separation funnel wrapped in aluminum, where the volume was completed with distilled water. It was left at rest for 30 minutes, followed by washing of the material and the discard of the aqueous phase. This operation was repeated three more times. The content was filtered with cotton sprayed with anhydrous sodium sulphate 99% to a 25 mL volumetric flask wrapped with aluminum, where 5 mL of acetone were added and the volume completed with hexane. The readings were made at 450 nm and the results expressed in mg 100 g^{-1}, calculated by the formula:

Total carotenoids = (A_{450} x 100)/(250 x L x W), where:

A_{450} = absorbance, L = cuvette width in cm, and W = ratio of the mass of the original sample and the final dilution volume in mL.

2.7 β-carotene and Lycopene

For the determination of the β-carotene and lycopene, the same extract was used as in the total carotenoid analysis, in which those extracts were taken for absorbance readings in a spectrophotometer at four wavelengths: (453, 505, 645 and 663 nm) [12]. For the calculations of the β-carotene and lycopene concentrations, the following equations were used:

β-carotene (mg 100 g^{-1}) = 0.216 A_{663} – 1.22 A_{645} – 0.304 A_{505} + 0.452 A_{453}.
Lycopene (mg 100 g^{-1}) = - 0.045 A_{663} + 0.204 A_{645} + 0.372 A_{505} – 0.0806 A_{453}.

2.8 Antioxidant Activity

2.8.1 Extract preparation

The extraction of the antioxidants was conducted using two extragents: water (1:25, w/v) and ethanol (1:25, w/v). For each extragents the samples were maintained under agitation for 1 hour and soon afterwards, filtered in filter paper. All of the extractions were carried out in three replicates, protected from light and subsequently submitted to antioxidant activity (AA) detection process by the methods described below.

2.8.2 β-carotene/linoleic acid method

Starting from the raw extracts of the samples (40,000 mg L^{-1}) dilutions in 20,000 and 10,000 mg L^{-1} were prepared. The methodology used was developed by Rufino et al. [13], with modifications.

For the preparation of the β-carotene/linoleic acid solution system, 50 µL of β-carotene diluted in chloroform (20 g L^{-1}) were used, to which 40 µL of linoleic acid were added, as well as 530 µL of tween 20 (emulsifier) and, for solubilization, 1 mL of chloroform. In a flask

covered with aluminum for protection against light, the chloroform was evaporated in a rotary-evaporator and 100 mL of oxygen saturated water (distilled water treated with oxygen for 30 minutes) were added, and the combination was agitated until that the solution system presented a yellow-orange coloration. In test tubes, 2,5 mL of that solution system were added to 0.2 mL of each dilution of the sample used for the test. Control tubes were made containing 2.5 mL of the solution system with 0.2mL of BHT (butyl-hydroxytoluene - synthetic antioxidant), quercetin and rutin, which are flavonoids with proven antioxidant action all at the concentration of 200mg L^{-1}. In laboratory tests, it was found that the concentration of 200 mg L^{-1} of BHT is the one that provides the greatest protection for the system, when compared to others; therefore, its use is suggested. After homogenization, their readings were taken in a spectrophotometer at 470 nm, using water for calibration of the spectrophotometer; this was considered to be the reading at time zero (initial). The tubes were placed in a water bath, at 40°C and readings were taken after 2 hours.

2.8.3 ABTS method

The methodology used was developed by Re et al. [14], with modifications by Rufino et al. [15]. Four different dilutions of the obtained extracts were conducted for the assays and subsequent construction of the analytical curves.

Analytical curves were made with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and with ascorbic acid, besides tests for comparison with the standards BHT, and with rutin and quercetin, prepared in the concentration of 200mg L^{-1}.

2.9 Statistical Analysis

The data are the average of three replicates ± standard deviation analyzed by one-way analysis of variance (ANOVA) and when this analysis showed a significant difference, the Skott-Knott test ($P<0.05$) was used for the comparison of means. All statistical tests were carried out using R (version 2.15.2) statistical software [16].

3. RESULTS AND DISCUSSION

3.1 Antioxidant Substances

The levels of antioxidants in the flours of the medicinal plants and in the phytotherapic are shown in Table 1. Phenolic compounds were found in all the plants, and marmelinho showed the highest contents (36.19g $100g^{-1}$ dry matter - DM) and *G. cambogia* the lowest (0.09g 100 DM g^{-1}). The phenolic compound content in the phytotherapic was considered relatively high (4.56g 100 DM g^{-1}) being lower only than those of marmelinho.

The contents of phenolic compounds in the carqueja leaves (4.03g $100g^{-1}$ DM) were higher than those observed in other studies with this plant, whose levels ranged from 0.045 to 2.67g $100g^{-1}$ DM (5,17,18). These differences may be due to the different ways of preparing the plant (maceration and infusion) and to the use of other extraction agents, such as ethanol, ethyl acetate, butanol, among others.

Table 1. Levels of antioxidants, in dry matter, of medicinal plants and the phytotherapic

Plants	Phenolic compounds (g100 g^{-1})	Flavonoids (mg100 g^{-1})	Vitamin C (mg100 g^{-1})	Total Carotenoids (mg 100 g^{-1})	β-carotene (mg100 g^{-1})	Lycopene (mg100 g^{-1})
Aloe vera	0.15±0.01 d	ND[1]	13.37±0.60 f	0.54±0.11 e	ND	0.52±0.01 d
Calunga	1.62±0.03 c	55.75±2.76 d	44.40±0.96 d	1.50±0.02 d	ND	0.34±0.04 d
Carqueja	4.03±0.21 b	173.68±3.60 b	19.27±0.38 e	13.67±0.74 b	3.59±0.50 b	1.97±0.15 b
Garcinia cambogia	0.09±0.00 d	ND	127.63±0.70 b	0.21±0.01 e	ND	0.20±0.08 d
Marmelinho	36.19±0.91 a	480.30±4.73 a	652.80±8.66 a	23.16±0.52 a	10.09±0.94 a	3.28±0.36 a
Phytotherapic[2]	4.56±0.20 b	82.10±3.70 c	121.50±1.10 c	4.30±0.70 c	1.36±0.01 c	0.79±0.01 c

Data are the average of three replicates ± standard deviation. Same letters in columns do not differ among themselves by the Scott-knott test (P<0.05). Moisture levels of medicinal plant flour in g 100 g^{-1}: Aloe vera = 8.53; calunga = 8.42; carqueja = 8.56; Garcinia cambogia = 3.94; marmelinho = 9.90. [1]Nd: Not detected. [2]Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in proportion 1:1.5:0.5:1.5:0.5, respectively, these data being obtained by estimation.

For aloe, the levels exceeded those recorded by Moniruzzaman et al. [19], which was 0.0008g $100g^{-1}$ DM. In the same study, the authors also found that the aloe leaves have higher phenolic contents than the gel, indicating the use of aloe leaves as antioxidants. For *G. cambogia*, the levels were lower than those recorded by Subhashiniet al. [20], which was 7.5g of pyrocatechol $100g^{-1}$ DM, and also than those recorded by Jantan et al. [21] in 22 methanol extracts from different parts (leaves, branches, bark and fruits) of 9 *Garcinia* species, with levels ranging from 0.44 to 6.28g of gallic acid $100g^{-1}$ DM and, in both studies, the Folin-Ciocateu method was used. These different results are probably due to the pattern used in the dosage, to the different extractors, different species, plant parts used and even to the origin of the samples.

For calunga and marmelinho, no records about the phenolic content of these plants were found in the literature, so the comparison will be made with other medicinal plants. The contents of phenolic compounds in marmelinho (36.19g 100 DM g^{-1}) were very high, and superior to those related by Ghimire et al. [22] who, in 24 medicinal plants of Nepal, verified phenolic compounds, in g 100 DM g^{-1}, between 2.38 (*Drymaria cordata*) and 32.12 (*Amamum subulatum*); by Wojdylo et al. [23], who registered contents between 0.07 (*Carum carvi*) and 15.15 (*Echinacia purpurea*) in 32 Polish herbs; and to those of Gan et al. [24] who, in 40 medicinal plants, found contents between 0.04 (*Curcuma* aromatic) and 7.57 (*Sanquisorba officinalis*). The phenolic compounds in marmelinho were surpassed only by *Acacia catechu* Willd with 41.47g 100 DM g^{-1}, in a study conducted with 133 medicinal plants of 64 different families from India [25], highlighting the high levels of phenolics in marmelinho, and may represent several possible fields for the application of these phenolics, adding value to this plant.

The phenolic compounds act as antioxidants, due to their redox properties that allow them to act as reducings agents, hydrogen donors and metal chelators. Besides their role as antioxidants, these compounds present a wide spectrum of medicinal properties, such as antiallergic, anti-inflammatory, anti-bacterial and anti-thrombotic, plus present cardioprotective and vasodilator effects [26], showing a broad field of application for the phenolics in these plants.

Marmelinho showed the highest flavonoid content (480.30mg 100 DMg^{-1}), followed by carqueja (173.68mg 100 DMg^{-1}) and calunga (55.75mg 100 DMg^{-1}). Flavonoids were not detected neither in aloe nor *G. cambogia.* However, for the phytotherapic we estimated a flavonoid content of 82.10mg 100 DMg^{-1}.

The flavonoids in carqueja were higher than those in Borella and Fontoura [27], who recorded levels between 12.43 and 47.50mg of rutin $100g^{-1}$ DM in 8 commercial samples of carqueja leaves. This difference is probably due to the pattern used, besides factors related to cultivation, manure, collection site, plant age, among others. Studies with aloe [19] and with *G. cambogia* [20] showed the presence of flavonoids in the extracts of these plants, differently from the present work, in which flavonoids were not detected. These differences may be due to several factors, such as the environmental, site collection, preparation and handling of the extracts; besides, tampering and/or forgeries could have occurred during this process.

The flavonoid levels of marmelinho were higher than those related by Sumazian et al. [28] in leaves of 6 vegetables from Malaysia whose levels, in mg 100 DMg^{-1}, ranged from 42 (*Whitania somnifera*) to 405 (*Curcuma tames*), to those of various parts of medicinal plants, in 100 g DM: *Alcea kurdica* flowers (22 mg), *Valerian officinalis* root (110 mg), *Stachys*

lavandulifolium flowers (402mg), and lower in relation to the *Lavandula officinalis* (618mg) and *Melissa officinalis* leaves (1,000mg) [29]. For the leaves of 11 plants analyzed by Djeridane et al. [4], the flavonoids of marmelinho surpassed those of 6 plants with levels between 162 (*Ruta montana*) and 454mg 100 DMg^{-1} (*Globularia alypum*) and they are inferior to those related for 24 medicinal plants from Nepal that presented contents, in mg 100 DMg^{-1}, between 1.353 (*Withania somnifera*) and 10.033 (*Artemesia vulgaris*) [22].

The consumption of foods and plants rich in flavonoids is associated with the risk reduction of various chronic diseases, and their protecting effect is due, partly, to their antioxidant properties and capacity to reduce oxidative stress [30]. Epidemiological data confirm a significant relationship between the high ingestion of flavonoids and the decrease of carcinogenic risk, cardiovascular diseases, myocardial infarction and total LDL concentrations [31,32].

Vitamin C was also found in all the analyzed plants, presenting contents that varied from 13.37 to 652.80 mg 100 DMg^{-1}. The estimated vitamin C level of the phytotherapic (121.50 mg 100 DMg^{-1}) was only lower to those of *G. cambogia* and marmelinho. With the discovery of the antioxidant action of that vitamin, the ingestion of substances with high vitamin C content has been recommended. As such, those plants are shown as good vitamin C sources with potential for use as antioxidants.

The total carotenoid contents varied from 0.20 to 23.16mg 100 DMg^{-1}. The highest amount of) they form a strong defense against the free radicals as they act in different cell total carotenoids, in mg 100 DM g^{-1}, was registered in marmelinho (23.16), followed by carqueja (13.67) and the phytotherapic (4.30). *G. cambogia* presented the lowest carotenoid content. The carotenoids act with lipophilic antioxidants and together with the vitamin C and the phenolic compounds (hydrophilic antioxidants) they form a strong defense against the free radicals as they act in different cell compartments.

β-carotene was not detected in the plants aloe, calunga and *G. cambogia*, while the lycopene was found in all the plants; marmelinho and carqueja showed the highest levels of those substances. The estimated β-carotene content (0.79) and lycopene (1.36) in the phytotherapic were only smaller than that of marmelinho and carqueja.

Studies conducted by Zhao et al. [33] indicate that the supplementation of carotenoids reduces DNA injury and that the combination of carotenoids (β-carotene and lycopene) ingested and reached via diet or in high doses of an individual carotenoid (12 mg) could protect against DNA injury. The β-carotene is highly liposoluble and widely transported with LDL cholesterol (75%) and HDL cholesterol (25%). It possesses an antioxidant function as a free radical scavenger, to reduce the extent of cell nucleus injury and to inhibit lipidic peroxidation induced by the free radical enzyme sources, such as xanthine oxidase [34].

3.2 Antioxidant Activity

The results of the (AA) by the ABTS method, calculated by the analytical curve of trolox and by the standard vitamin C curve, in flours of the medicinal plants, the phytotherapic and the three standards are presented in Fig. 1. Out of the analyzed plants, marmelinho was the one that showed the highest antioxidant potential, probably due to the higher levels of phenolics, flavonoids, vitamin C and carotenoids in the leaves. For this plant practically no difference in AA between the aqueous and ethanolic extracts, in trolox equivalent, as well as vitamin C. For calunga, carqueja and the phytotherapic, the aqueous extracts showed a higher AA than

the ethanolic extracts, both for the trolox equivalent, and for the vitamin C, which can be explained by the fact that the ABTS test shows the best results when in the presence of hydrophilic antioxidants. Aloe and *G. cambogia* did not show AA by the ABTS method, probably due to the low content of phenolic compounds and the absence of flavonoids in the extracts of these plants, which are substances that have strong action in capturing the ABTS radical.

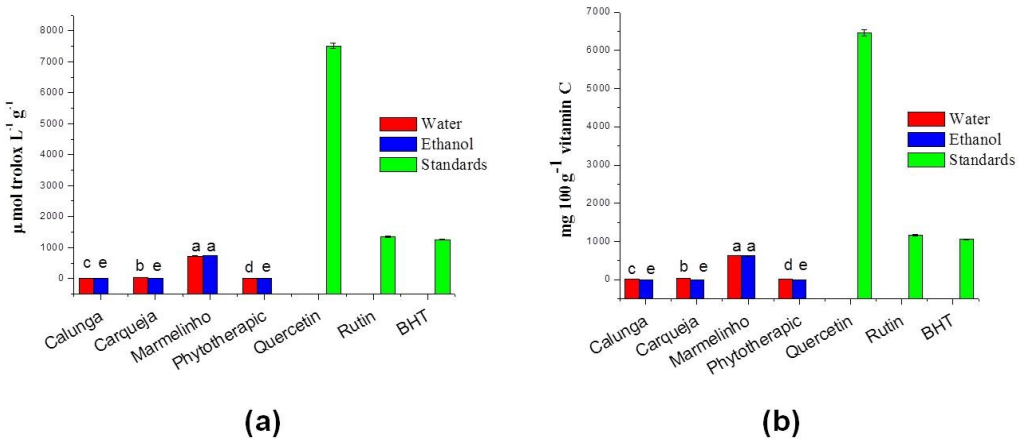

Fig. 1. Antioxidant activity by ABTS method of aqueous and ethanolic extracts of medicinal plants, the phytotherapic and three standards, expressed in (a) µmol trolox L^{-1} g^{-1} and (b) mg g^{-1} de vitamin C.

Data are the average of three replicates ± standard deviation. Same letters in columns do not differ among themselves by the Scott-Knott test (P<0.05). Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in a proportion of 1:1.5:0.5:1.5:0.5 respectively.

The low antioxidant potential shown by the phytotherapic in the ABTS test may be due to the lower proportion of marmelinho in the constitution of the phytotherapic (10%), since this plant showed a good antioxidant potential, and also to a negative synergistic action in the combination of these plants.

It was observed that, when compared to the BHT and rutin standards, the antioxidant potential, in trolox and vitamin C equivalents, of the aqueous and ethanolic extracts of marmelinho reached 58% and 54% on average, of the potential of those standards. In relation to quercetin, the potential of marmelinho was much smaller, with only 9.7% of its potential (in trolox equivalent, as well as vitamin C). The antioxidant potential of the extracts of the other plants and of the phytotherapic was considered low. However, as in the extracts of marmelinho, the antioxidant substances are not in the isolated form as are the standards, this average potential of approximately 56% can be considered a good antioxidant potential.

The good antioxidant potential shown by the marmelinho leaves is evidenced when compared to other studies, in which, independently of the extract, surpassed that Wojdylo et al. [23] who in 32 Polish herbs, verified potentials between 0.0045 (*Archangelica officinalis*) and 3.46 (Syzygium aromaticum) µmol trolox g^{-1}. It also surpassed that detected by Bouayed et al. [29] in several parts of medicinal plants, in mg g^{-1} vitamin C: 2.8 (*Alcea kurdica* flowers), 7.36 (*Valerian officinalis* root), 15.4 (*Stachys lavandulifolium* flowers), 19.2 (*Lavandula officinalis* leaves) and 19.3 (*Melissa officinalis* leaves). It is surpassed only by 11

of 132 Indian medicinal plants analyzed by Surveswaran et al. [25] and by two of 40 medicinal plants studied by Gan et al. [24]. Such results were probably due to the presence of different antioxidants, ways and extractors used in the preparation of these plants, which implies a greater efficiency in the extraction of these compounds, and thus, a greater antioxidant potential.

The lipidic oxidation inhibition results, by the β-carotene/linoleic acid method for the MPF, the phytotherapic and the three standards after 2 hours of reaction, are shown in Table 2. All the plants demonstrated lipidic oxidation inhibition potential, except the ethanolic extract of *G. cambogia*.

Marmelinho was the plant with the highest antioxidant potential at the analyzed concentrations, and, at concentrations of 40,000 and 20,000 mg L^{-1}, the aqueous and ethanolic extracts showed practically the same antioxidant potential, whereas at the concentration of 10,000 mg L^{-1}, the ethanol extract showed the greatest potential.

The calunga and carqueja extracts (aqueous and ethanolic) at concentrations of 40,000 and 20,000 mg L^{-1} also showed good antioxidant potentials (over 60% inhibition). The phytotherapic showed a great antioxidant potential, especially its ethanolic extract, with an inhibition potential at the concentrations of 40,000 and 20,000 mg L^{-1}, which was only inferior to the antioxidant potential of marmelinho, evidencing the occurrence of a good antioxidant potential by the combination of the plants.

The β-carotene/linoleic acid method shows a better response to antioxidants with apolar character. As marmelinho showed the highest levels of carotenoids (lipophilic antioxidant) and also of other antioxidants, it presented the highest antioxidant potential among the plants analyzed. For calunga, carqueja and the phytotherapic, the greatest inhibition potential of lipid oxidation found in the ethanolic extracts of these plants occurred probably due to the greater removal of antioxidants with apolar character, provided by the alcohol in relation to water. The low antioxidant activity in the aloe and *G. cambogia* extracts probably occurred due to the low levels of carotenoids in the extracts of these plants.

In order to demonstrate the response of polar and apolar antioxidant groups compared to the ABTS and β-carotene/linoleic acid methods, observing Fig. 1 (ABTS Test), it is possible to observe that quercetin and rutin, hydrophilic antioxidants, have a greater antioxidant potential than BHT (lipophilic antioxidant), i. e. in this test, polar antioxidant groups showed better responses. Now, by the β-carotene/linoleic acid method (Table 2), BHT shows a greater the antioxidant potential, exceeding that of quercetin and rutin, i. e. nonpolar antioxidants present better results.

When compared to the standards, the marmelinho extracts were higher at all of the tested concentrations, as well as the ethanolic extract of the phytotherapic in concentrations of 40,000e 20,000mg L^{-1}, while the carqueja (aqueous and ethanolic) and the calunga (ethanolic) extracts at the concentration 40,000mg L^{-1} presented the same inhibition potential as the quercetin. In relation to rutin, all the plants showed antioxidant potential above that of this standard.

Table 2. **Antioxidant activity of aqueous and ethanolic extracts of the medicinal plants, the phytotherapic and three standards, in % inhibition by the β-carotene/linoleic acid method**

Plants	Water			Ethanol		
	40,000mg L^{-1}	**20,000mg L^{-1}**	**10,000mg L^{-1}**	**40,000mg L^{-1}**	**20,000 mg L^{-1}**	**10,000 mg L^{-1}**
Aloe vera	50.14±2.33 eA	39.44±0.37 eC	41.73 ± 0.09 dB	16.55±1.67 eD	12.27± 2,09 eE	14.27±1.10 eE
Calunga	61.97±1.67 cB	58.05±0.85 bB	57.66±0.54 bB	70.75±0.17 dA	64.37±0,59 dA	46.67±3.41 cC
Carqueja	70.91±1.17 bA	60.21±1.32 bB	55.09±7.33 bB	73.53±1.37 cA	68.21±2,26 cA	49.67±0.29 cC
Garcinia cambogia	57.21±1.28 dA	54.13±3.08 cA	20.78 ± 0.28 dB	ND[1]	ND	ND
Marmelinho	99.98±1.10 aA	96.71±1.53 aA	86.08±7.44 aB	100.00±1.40 aA	100.00±0,34 aA	95.71±1.25 aA
Phytotherapic[2]	49.05±0.25 eD	48.41±1.50 dD	43.54±0.61 cE	87.23±0.34 bA	81.22±0,41 bB	56.58±4.15 bC
BHT (200 mg L^{-1})	75.93±1.15					
Quercetin (200 mg L^{-1})	70.48±0.71					
Rutin (200 mg L^{-1})	8.71±1.29					

Data are the average of three replicates ± standard deviation. Lowercase letters in the column compare between plants and uppercase letters on the line compare between concentrations. Same letters do not differ among themselves by the Scott-Knott test (P<0.05).[1]ND: Not detected.
[2]Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in a proportion of 1:1.5:0.5:1.5:0.5 respectively.

The different results of antioxidant activity observed in the extracts of the plants were probably due to the different antioxidant compounds present in the extracts of these plants, and to the difference principle employed by each antioxidant method used in this work. Given this situation, some plants showed a good antioxidant potential to a method and a weak potential to the other, emphasizing the importance of using more than one technique to reflect the antioxidant capacity of a sample.

Studies performed with these plants confirm this antioxidant potential. Rajasekaran et al. [35] observed that an oral administration of the alcoholic extract of *Aloe vera* gel (300mg kg^{-1}) to diabetic rats reversed the high levels of lipid peroxidation and hydroperoxides in the tissues of these rats to nearly normal levels. The treatment also resulted in a significant increase in the levels of reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidney of diabetic rats; these results show the antioxidant property of the *Aloe vera* extract.

In vitro studies showed that the *Aloe vera* gel has a potential for capturing free radicals [19,36], which was not observed in the present study, and a potential for combating the inhibition of lipid oxidation [36], corroborating the results of this study. Such differences may be due to several factors, such as environmental, collection site, plant age, preparation and handling of the extracts. Yun et al. [37] analyzing *Aloe vera* at various ages, found that at age four this plant shows a greater antioxidant potential than at ages below, which shows that the development phase plays a vital role in the antioxidant composition of the *Aloe vera* gel.

For carqueja, Pádua et al. [39] reported a good antioxidant potential *in vit*ro and *in vivo* for the hydroethanolic extract of this plant in a study performed with neutrophils of Fisher rats. Mendes et al. [40] reported that the hydroalcoholic extract of carqueja presentes a moderate antioxidant activity. In the same study the authors observed a reduced antiulcer activity in rats (ulcer induction by stress). Dias et al. [41] and Morais et al. [2], analyzing the antioxidant potential of carqueja by the DPPH method, which has the same principle of capturing free radicals as the ABTS method, found a good antioxidant potential for the carqueja extracts. These results differ from those found for carqueja in the present study, in which it was possible to observe a low antioxidant potential for the extracts (aqueous and ethanolic) by the ABTS method. This difference is probably due to factors such as the collection site, plant age, ways and extractors used in the preparation of the plant.

For *G. cambogia*, Subhashini et al. [20] registered a great antioxidant potential for the aqueous extract obtained from the bark of its fruits by the methods of scavenging free radicals (DPPH), reduced iron (FRAP), total reactive antioxidant potential (TRAP) and lipid peroxidation. Results that differ from those found for the extracts (aqueous and ethanolic) of *G. cambogia* in the present study, when using the ABTS method, and show similar results when it comes to lipid oxidation. The different results among these studies may be due to the way the extracts were prepared, which is an extremely important factor in order to have a good antioxidant activity. In this work, crude extracts were used, while in the work of Subhashini et al. [20], concentrated extracts were used, which can justify these differences. Other factors, such as the geographic origin, plant age, and even tampering and/or forgeries can be considered. In the literature consulted, no studies on the antioxidant potential of calunga and marmelinho were found.

4. CONCLUSION

The studied plants showed an antioxidant potential, and the antioxidant activity by the ABTS method was considered moderate in marmelinho extracts, and weak in the other plants. For the β-carotene/linoleic acid method, the aqueous and ethanolic extracts of marmelinho, carqueja and calunga, and only the ethanolic extract of the phytotherapic presented a good antioxidant potential. Thus, those plants show potential to be used as antioxidant sources in pharmacological and food preparations, with possible health benefits.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Halliwell B. The antioxidant Paradox. Lancet. 2000;355(9210):1179-1180.
2. Morais SM. Antioxidant action of teas and seasonings more consumed in Brazil. Rev Bras Farmacogn. 2009;19(1):315-320.
3. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence. Lancet. 1994;344(8924):721-724.
4. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006;97(4):654-660.
5. Souza SP, Pereira LLS, Souza AA, Santos CD. Inhibition of pancreatic lipase by extracts of *Baccharis trimera* (Less.) DC. Asteraceae: evaluation of antinutrients and effect on glycosidases. Rev Bras Farmacogn. 2011;21(3):450-455.
6. Simão AA, Corrêa AD, Chagas PMB. Inhibition of digestive enzymes by medicinal plant aqueous extracts used to aid the treatment of obesity. J Med Plant Res. 2012;6(47):5826-5830.
7. Villareal DT, Apovian CM, Kushner RF, Klein F. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Am J Clin Nutr. 2005;82(5):923-934.
8. AOAC. Official methods of analysis of the association of the analytical chemists (17 ^{ed}) Association of Oficcial Anlytical Chemists. Washington, DC. USA; 2005.

9. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555-559.
10. Strohecker R, Henning HM. Analisis de vitaminas: metodos comprobados. Madrid: Paz Montalvo; 1967.
11. Higby WK. A simplified method for determination of some the carotenoid distribution in natural and carotene-fortified orange juice. J Food Sci. 1962;27(1):42-49.
12. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomatoes fruit. J Japan Soc Food Sci Technol. 1992;39(10):925-928.
13. Rufino MSM, Alves RS, Brito ES, Filho JM, Moreira AVB. Determination of total antioxidant activity in fruit by the method β-caroteno/ácido linoleic. Fortaleza: Embrapa Agroindústria tropical; 2006.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9/10):1231-1237.
15. Rufino MSM, Alves RS, Brito ES, Morais SM. Determination of total antioxidant activity in fruits by capturing the free radical $ABTS^{+}$. Fortaleza: Embrapa Agroindústria Tropical; 2007.
16. R Core Team. R: A language and environment for statistical computing. Viena: R Foundation for Statistical Computing; 2012. ISBN 3-900051-07-0. Available: http://www.R-project.org/.
17. Freitas MSM, Martins MA, Carvalho AJC, Carneiro RFV. Crescimento e produção de fenóis totais em carqueja [*Baccharis trimera* (Less.) DC.] em resposta a inoculação em fungos micorrizícos arbusculares, na presença e na ausência de adubação mineral. Rev Bra Plant Med. 2004;6(3):30-34.
18. Oliveira CB, Comunello LN, Lunardelli A, Amaral RH, Pires MGS, Silva GL, Manfredini V, Vargas CR, Gnoatto SCB, Oliveira JR, Gosmann G. Phenolic enriched extract of *Baccharis trimera* presents anti-inflammatory and antioxidant activities. Molecules. 2012;17(1):1113-1123.
19. Moniruzzaman M, Begum R, Sohel A, Amrita B, Ibrahim K, Siew H. *In Vitro* Antioxidant effects of *Aloe barbadensis* miller extracts and the potential role of these extracts as antidiabetic and antilipidemic agents on streptozotocin-induced type 2 diabetic model rats. Molecules. 2012;17(11):12851-12867.
20. Subhashini N, Nagarajan G, Kavimani S. *In vitro* antioxidant and anticholinesterase activities of *garcinia combogia.* Int J Pharm Pharm Sci. 2011;13(3):129-132.
21. Jantan I, Farra AJ, Fadlina CS, Khalid R. Inhibitory effects of the extracts of *Garcinia* species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents. J Med Plant Res. 2011;5(13):2699-2709.
22. Ghimire BK, Seong ES, Kim EH, Ghimeray AK, Yu CY, Ghimire BK, Chung MA. Comparative evaluation of the antioxidant activity of some medicinal plants popularly used in Nepal. J Med Plant Res. 2011;5(10):1884-1891.
23. Wojdylo A, Osmianski J, Czermerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105(3):940–949.
24. Gan R, Xu XR, Song FL, Kuang L, Li HB. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. J Med Plant Res. 2010;4(22):2438-2444.
25. Surveswaran S, Cai Y, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 2007;102(3):938–953.

26. Balasundram N, Sundram K, Sammar S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191-203.
27. Borella JC, Fontoura A. Avaliação do perfil cromatográfico e do teor de flavonoides em amostras de *Baccharis trimera* (Less.) DC. Asteraceae (carqueja) comercializadas em Ribeirão Preto, SP, Brasil. Rev Bras Farmacogn. 2002;12(2):63-67.
28. Sumazian Y, Syahida A, Hakiman M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J Med Plant Res. 2010;4(10):881-890.
29. Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, Soulimani S. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. Food Chem. 2007;104(1):364-368.
30. Halliwell B, Rafter J, Jenner A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? Am J Clin Nutr. 2005;81(1):2685-2765.
31. Kris–Etherton PM, West SG. Soy protein with or without isoflavones: in search of a cardioprotective mechanism of action. Am J Clin Nutr. 2005;81(1):5-6.
32. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Technol Metall. 2005;40(3):255-260.
33. Zhao X, Aldini G, Johnson EJ, Rasmussen H, Kraemer K, Woolf H, Musaeus N. Modification of lymphocyte DNA damage by carotenoid supplementation in postmenopausal women. Am J Clin Nutr. 2006;83(1):163-169.
34. Chao JCJ, Huang CH, Wu SJ, Yang SC. Effects of β-carotene, vitamin C and E on antioxidant status in hyperlipidemic smokers. J Nutr Biochem. 2002;13(7):427-434.
35. Rajasekaran S, Sivagnaman K, Subramanian S. Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharm Pharmacol. 2005;57(2):241-246.
36. Saritha V, Anilakumar KR, Farhath K. Antioxidant and antibacterial activity of *Aloe vera* gel extracts. Int J Pharm Biol Arch. 2010;1(4):376-384
37. Yun HU, Juan XU, Qiuhui HU. Evaluation of Antioxidant Potential of *Aloe vera* (*Aloe barbadensisMiller*) Extracts. J Agr Food Chem. 2003;51**(**26):7788-7791.
38. Padua BC, Silva LD, Rossoni Junior JV, Humberto JL, Chaves MM, Silva ME, Pedrosa ML, Costa DC. Antioxidant properties of *Baccharis trimera* in the neutrophils of Fisher rats. J Ethnopharmacol. 2010;129(3):381-386.
39. Mendes FR, Tabach R, Carlini EA. Evaluation of *Baccharis trimera* and *Davilla rugosa* in tests for adaptogen activit. Phytother Res. 2007;21(6):517-522.
40. Dias LFT, Melo ES, Hernandes LS, Bacchi EM. Atividade antiúlcera e antioxidante *Baccharis Trimera* (Less) DC (Asteraceae). Rev Bras Farmacogn. 2009;19(1):309-314.

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