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Nutritional value and therapeutic effect of *Mentha pulegium* L. and *Artemisia abrotanum* L.

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ABSTRACT

This work is an informative study investigated the nutrient contents and the antioxidant activity of *Mentha pulegium* L. and *Artemisia abrotanum* L. two plants largely used in North African traditional medicine as well as in pharmaceutical and agro-alimentary industries. These plants have been used as herbal tea, or powder in herbal remedies, to treat painful menstruation, and gastrointestinal disorders. Recently they were extensively used during the pandemic of Covid-19. Results revealed that both plants were not, only, a good source of essential minerals, like Calcium, Iron, and Magnesium. But they were also rich sources of crude fibre and protein. Vitamin C amount was found 180.94 ±3.01 mg/g100g, with an IC_{50} value 54.45±25.53 µg/mL 10^{-1} in *M. pulegium* and 171.64±3.0 mg/100g with IC_{50} value 60.61± 19.71 µg/mL 10^{-1} in A. *abrotanum*. The antioxidant study showed a high activity that paves the way for the possibility of new health-related uses.

KEYWORDS: Antioxidant activity, Dietary supplement, Herbal medicine, North Africa

INTRODUCTION

Mentha pulegium L. and *Artemisia abrotanum* L. were the most used plants during the Covid-19 epidemic worldwide, especially in the Maghreb (El Alami *et al*., 2020; Kshirsagar *et al*., 2021). *M. pulegium* L., commonly called *pennyroyal*, is a perennial, herbaceous plant of the Lamiaceae mint family growing spontaneously in humid areas of plains, and mountains (Rodrigues *et al*., 2013; Politeo *et al*., 2018). *A. abrotanum* L., commonly known as *Southernwood*, belongs to the Asteraceae family (Chopra *et al*., 1956). In Tunisia, they are found in mountain sand slopes (Pottier-Alapetite, 1981).

M. *pulegium* and A. *abrotanum* are considered as a foodstuff by the European Herbal Infusions Association (ETC & EHIA, 2016). They are known as herbaceous plants, often used as cooking ingredients for their flavouring quality and also in traditional medicine (Roby *et al*., 2013). These plants possess potential health benefits due to their nutrient contents (Woo & Robinson, 2016). Very few have scientifically explored the nutritional value and usefulness of *M. pulegium* and *A. abrotanum* (Fernandes *et al*., 2010; Pinela *et al*., 2017; Pereira *et al*., 2020).

The present study aimed to explore, the nutritional value of *pennyroyal* and *southernwood to* ascertain the macro and micro nutrients and study their antioxidant activity to test their potential health benefits and verify their safety for human consumption.

MATERIALS AND METHODS

Plant Samples

Pennyroyal and *Southernwood* were collected in July 2019 and 2020 from two different areas in the region of Sousse in Tunisia. Plants were identified by Prof. Slim Rouz at the Department of Ecology and Vegetal Biology in the higher school of Agriculture, Mogran, A voucher specimen (M.P-01.03) was deposited in the laboratory of Biophysics Metabolic of the faculty of Medicine Ibn El Jazzar Sousse, Tunisia. Authentication was confirmed using the description of the flore (Pottier-Alapetite, 1981).

Preparation of the Plant for Testing

The aerial parts (leaves and stems) of each plant were dried at room temperature in the shade. Then, they were powdered in a rotating knife grinder. Herb powders were sieved and stored in sterile containers until use.

Nutritional Value Analyses

Extraction samples were performed by Atomic Absorption spectrometry Shimadzu this method is basically used for high level screening it related each atomic emission to each element was measured.

Determination of Citric Acid Equivalent

The citric acid equivalent was determined by titration according to (AFNOR BS EN 12147, 1997).

Determination of Fats

The rate of fats was determined according to the (AFNOR V03-030, 1991) the nature of the solvents chosen (mixture of hexane and isopropanol). The operating protocol does not include any heating and or hydrolysis step.

Determination of Total Sugars

Sugars were extracted in dilute ethanol. After eliminating the ethanol, the sugars were determined before and after inversion using the Luff-Schoorl method (Dekker, 1950).

Determination of Crude Fibre

Crude fibre was determined according to the standard reference of AFNOR V03-040 (1993). It is a conventional method for determining raw cellulose in agricultural and food products.

Determination of Iron, Calcium, and Magnesium

Minerals content were obtained after mineralization and calcination at 450°C in reference to the AFNOR EN 1134 (1994) for Calcium and magnesium and AFNOR EN 14082 (2003) for Iron.

Protein Assay

Total nitrogen was determined by volume following mineralization, according to the Kjeldahl methods (Bradstreet, 1954) the percent nitrogen obtained by the analysis is converted to percent protein by multiplying it by a factor of 6.25.

Determination of Vitamins

Vitamins A, B1, B2 were determined, respectively, according to the standard reference of AFNOR NF EN 12823-2 (2001), AFNOR NF EN 14122 (2003) and AFNOR NF EN 14152 (2003). Vitamin C was extracted by using a solution of met phosphoric acid and determined by HPLC using UV detection at 265nm in reference to the AFNOR V03-135 (2011).

Test of Nitrites, Nitrates, and Cyanides

Nitrates and Nitrates, were determined, according to the standard reference AFNOR ISO 7890-3 (1988) and AFNOR ISO 6777 (1984) respectively, and Cyanides was determined according to the photometry method WTW 250344 (1984) (Fisher Scientific, 2015).

Extraction Procedures for the Antioxidant Activity

Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was conducted according to Hatano *et al*. (1988) with slight modifications. The dried plant extract was diluted in pure methanol at different concentrations, ranging from 1 to 50µg mL^{-1}. One mL of this extract was added to 0.5mL of a 0.2$mmol^{-1}$ DPPH methanol solution. The mixture was shaken vigorously and was left standing at room temperature for 30 min in the dark. Absorbance was measured against the blank at 517nm using HPLC agilent1200 spectrophotometer. For each dilution of the extract, DPPH scavenging activity was calculated as scavenging activity (%) = [(A517 of control–A517 of sample)/ A517 of control] ×100.

The dynamic of this activity allows for determining the extract concentration required to provoke a 50% of inhibition. All samples were analysed in three replications.

Statistic Analysis

The experimental results were performed in triplicate used Excel software (Microsoft Office 2013). The data were recorded as mean ± standard deviation (n=3) on a dray weight, and $p < 0.05$ was regarded as significant.

RESULTS

Determination of Nutritional Value (Dietary Crude Fibre, Total Sugar, Protein, and Total Fats) of Both Plants.

Southernwood was riche in crude fibre and protein, with respectively, 19.41 ±0.02 % and 2.64 ±0.02 %, compared to *Pennyroyal* 17.76 ±0.01% of crude fibre and <0.1 of protein (Figure 1). Total sugar was similar in both plants with 15.93 ±0.01 % in *Pennyroyal* and 14.35% in *Southernwood.* Furthermore, total fats ranged between 3.30 ±0.03 % in *Pennyroyal* and 9.93 ±0.01 % in *Southernwood* (Figure 1).

Determination of Mineral Composition (mg/kg) in Both Plants

Pennyroyal and *Southernwood* were rich sources of iron with 147.8 ± 3.1 mg/kg in *Pennyroyal* and 168.01 ± 3.01 mg/kg in *Southernwood*, calcium with 5123.86 ± 3 mg/kg in *Pennyroyal* and

4324.10 ± 3 mg/kg, and magnesium with 364.03 ±2.99 mg/kg in *Pennyroyal* and 595.60 ± 3 mg/kg in *Southernwood* (Figure 2).

Determination of Core Nutrients in Leaves Powder of *Pennyroyal* and *Southernwood*

The citric acid equivalent content ranged between 1.12 ±0.01 g/100 g in *Pennyroyal* and 0.55 ±0.01 g/100 in *Southernwood*. Vitamin B1 content was the highest in *Southernwood* and it was very low in *Pennyroyal*. Similar amounts of vitamin B2 (riboflavin) were recorded in both plants with a difference did not exceed 0.07 mg in 100 g. However, with regard to Beta-Carotene (vit A) content, did not exceed 1 mg in both plants (Table 1).

Both plants showed a value of nitrate and nitrite under 0.1 mg/kg. As for the cyanide level, it was 2.26 ± 0.07mg/kg in *Pennyroyal* and 1.93 ±0.06 mg/kg in *Southernwood*. Approximate values in vitamin C were recorded in both plants. The highest amount of mean ascorbic acid was found in *Pennyroyal* with 180.94 ±3.01 mg/g100g and it was found to be 171.64±3.0 mg/100g in *Southernwood* (Table 1).

Antioxidant Activity in Both Plants

The antioxidant activity expressed as IC_{50} value in methanol leaves extracts of Pennyroyal and Southernwood. *Pennyroyal* showed high antioxidant activity with 54.45±25.53 µg/mL10^{-1} compared to *Southernwood* which showed activity with IC_{50} an value of 60.61± 19.71 µg/mL 10^{-1} (Table 2).

DISCUSSION

To the best of our knowledge nutritional composition, mineral levels, antioxidant activity of the aerial parts of *Pennyroyal* and *Southernwood* provenance from Tunisia were not reported previously.

The basic nutritional composition and mineral levels was shown that powder of aerial parts of *Southernwood* was richer in crude fibre and protein compared to *Pennyroyal*. These results were confirmed by Pinela *et al.* (2017) in regard to *Pennyroyal* that showed closer amount of protein. The total sugar presented in this study was closer to the results found by Fernandes *et al.* (2010) in Portuguese *Pennyroyal*, with total sugar represents an approximate percentage of 10% in total kg. Both plants were a rich source of minerals. Hence, an exception was observed for the higher content of magnesium and iron in *Southernwood*. Whereas, high amount of calcium (Ca) was found in *Pennyroyal*. Our results were inconsistent with those of Karagiannidis *et al.* (2010). Where minerals nutrient in Greece *Pennyroyal* is higher for iron with 237.18 mg/kg, but it recoded very low levels of Magnesium (Mg) with 0.78 mg/kg as well as of calcium with 1.83 mg/kg compared to Tunisian *Pennyroyal* (Karagiannidis *et al.*, 2010).

The obtained results show that the aerial parts of *Pennyroyal* and *Southernwood* are safe for consumption, with nitrite and nitrate amounts being under permissible levels in both plants.

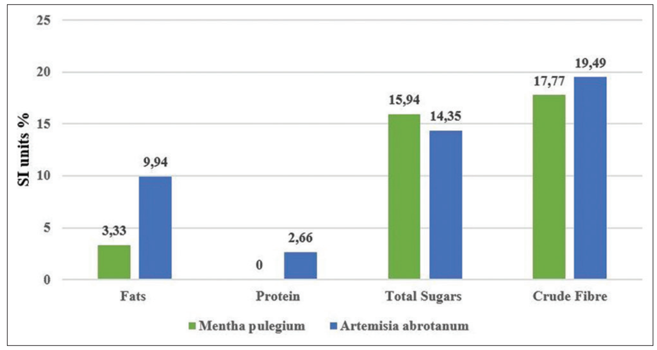

Figure 1: Proximate composition of Core nutrients (dietary crude fiber, total sugar, protein, and total fats) content in dray powder of the aerial parts of *Mentha pulegium* L. and *Artemisia abrotanum* L.

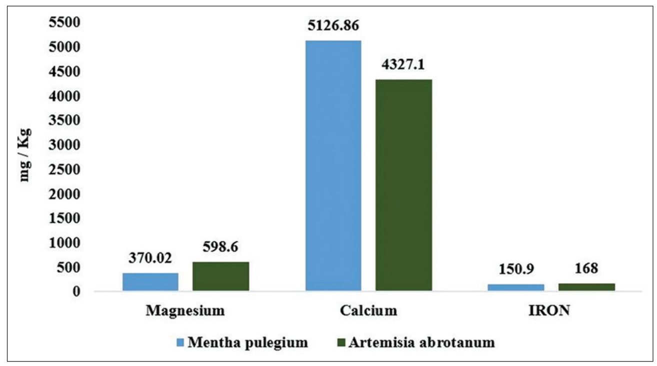

Figure 2: Mineral composition presented in milligram/kilograms (mg/kg) of dray powder of the aerial parts of *Mentha pulegium* L. and *Artemisia abrotanum* L.

Table 1: The Core nutrients content in dray leaves powder of *Pennyroyal* and *southernwood*

Parameters sought	SI Units	*Pennyroyal*	*Southernwood*
Citric acid equivalent	g/Citric acid	1.12 (±0.01)	0.56 (±0.01)
Vitamins			
Vitamin A (beta- carotene)	mg/100g	0.58 (±0.01)	0.94 (±0.01)
Vitamin B1	ug/100g	0.10 (±0.01)	6.91 (±0.3)
Vitamin B2	mg/100g	0.52 (±0.01)	0.45 (±0.02)
Vitamin C	mg/100g	180.94 (±3.01)	171.64 (± 3.0)
Anti-nutriments			
Nitrates	mg/kg	<0.1*	<0.1*
Nitrites	mg/kg	<0.1*	<0.1*
Cyanides	mg/kg	2.26 (±0.07)	1.93 (±0.06)

** P values<0.05, Pennyroyal: Mentha pulegium* L., *Southernwood: Artemisia abrotanum* L.

Table 2: Antioxidant activity expressed as ic_{50} value in methanol leaves extracts of *Pennyroyal* and *Southernwood*

	*IC_{50} µG/ML
Pennyroyal	54.45±25.53
Southernwood	60.61±19.71

*Data expressed as mean (of three replicates) ± sd, *ic_{50} ∝g/mL: half-maximal inhibitory concentration expressed microgram/ millilitre, Pennyroyal: Mentha pulegium* L., *Southernwood: Artemisia abrotanum* L.

Vegetables with nitrate concentration in the range of 1000-4000 mg/kg are classified as high nitrate containing vegetables (WHO, 2003; Anjana *et al.*, 2007). Study made by the Committee on the toxicity of chemicals in food suggests that humans are sensitive to cyanide toxicity, in doses higher than 3.5 mg/kg. Therefore, our results showed that cyanide concentration in the powder of the aerial parts of both plants was lower than the toxic level. The value content of vitamin B2 (riboflavin) was similar in both plants. A study made by Powers (2003) prove that B2 is involved in diverse reduction-oxidation reactions as electron carriers in the respiratory electron transfer chain, and the former is necessary for the oxidation of fatty acids (Powers, 2003). However, both plants had a similar amounts of vitamin C. According to study made by Aazza *et al.* (2013) vitamin C found in Portuguese *Pennyroyal* was three time less than results found in Tunisian *Pennyroyal* (Aazza *et al.*, 2013). The Ascorbic acid (vitamin C) is a water-soluble nutrient and acts as a perfect antioxidant Doba *et al.* (1985). According to Khadim *et al.* (2021) the human body needs vitamin C as an essential nutriment to improves the absorption of iron from plant-based foods and helps the immune system work properly to protect the body from diseases, also it helps body to make collagen, a protein required to help wounds heal (Khadim *et al.*, 2021).

The richness of both plants in ascorbic acid stimulated our curiosity to test the antioxidant activity in both plants. The results of antioxidant activity of methanol extract of the aerial parts of Tunisian *Pennyroyal* and *Southernwood* showed significant potential to inhibit free radical activity. The same findings were reported by Scherer and Godoy (2009) with regard to *Pennyroyal*, and by Elansary *et al.* (2020) with respect to *Southernwood.*

Results of this study were four time lower than the IC_{50} value in the methanol extract of Algerian *Pennyroyal* (187.37 ug/mL) according to the study performed by Bouhaddouda *et al.* (2016). And three times higher than the IC_{50} value in Turkish *Pennyroyal* (16.92 ug/mL) according to Gülçin *et al.* (2020). Moreover, the results of this study were two time higher than IC_{50} value of methanol leaves of *Southernwood* provenance from northern Saudi Arabia (27.1±2.3 μg/mL) according to Elansary *et al.* (2020). The Ascorbic acid (vitamin C) is a water-soluble nutrient and acts as a perfect antioxidant (Doba *et al.*, 1985). According to the US National institutes of health the human body needs vitamin C as an essential nutrients to improve the absorption of iron from plant-based foods and helps the immune system work properly to protect the body from diseases, also it helps body to make collagen, a protein required to help wounds heal (National Institutes of Health, 2019). Studies made by Frei *et al.* (1988, 1989, 1991) confirmed our suggestion by showing that vitamin C "ascorbic acid, AA" is a powerful antioxidant preventing lipid peroxidation in plasma exposed to various types of Oxidative stress.

CONCLUSION

To the best of the authors' knowledge, this is the first study investigating the nutritional values of *Southernwood* and *Pennyroyal* for human health. To summarize, *Pennyroyal* and *Southernwood* are not only a good sources of essential minerals, such as Calcium (Ca), Iron (Fe), and Magnesium (Mg), but they are also a rich source of crude fibre, sugar, vitamin (C), and riboflavin (vit B2). *Pennyroyal* and *Southernwood* are safe and effective, and they have an important role for human use. The antioxidant study showed a high activity that paves the way for the possibility of new health-related uses.

AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and design. Mejda Selmi, Latifa Lassoued, Chahra Chbili, Ben Fredj Maha and Ridha Charfeddin performed material preparation, data collection and analysis. Mejda Selmi wrote the first draft of the manuscript and all authors commented on previous versions of manuscript. All authors read and approved the final.

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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