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PHYTOCHEMICAL SCREENING AND MINERAL PROFILING OF WILD AND CULTIVATED ROSEMARY (*ROSMARINUS OFFICINALIS* L.) FROM TAOUNATE REGION (NORTHERN MOROCCO)

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Abstract

In this paper, aerial parts of rosemary growing wild (WR) and cultivated (CR) were compared in terms of phytochemicals and mineral elements. Plant material was collected at flowering stage for both WR and CR. After drying at ambient temperature, samples were finely grounded and subjected to phytochemical screening and mineral profiling (expressed as mg/g of dry matter, mg/g DM). Phytochemical screening was also performed for aqueous extracts of rosemary leaves. The outcomes of this work demonstrated the presence of various phytochemicals (alkaloids, flavonoids, tannins, and saponins) with important variations between WR and CR on one hand and raw leaves and their aqueous extracts on the other hand. WR had higher content of phytochemicals except alkaloids in the case of leaves. Aqueous extracts showed their superiority over raw leaves in terms of phytochemicals except for tannins. Similar trends were observed for mineral profiling, WR displayed higher content of Ca (4.631), K (4.224), Fe (0.805), Mn (0.125), Zn (0.035), Pb (0.018), and Cu (0.012 mg/g DM). In contrast, samples belonging to CR were found to have the greatest records of Na (0.150) and Mg (1.920 mg/g DM). These results were confirmed via principal component analysis. WR seems to perform better in terms of main investigated elements as well as main studied phytochemicals. It could be concluded that rosemary may represent a good source of elemental minerals to enhance human diet and enrich foods.

Keywords: *Wild rosemary, cultivated rosemary, phytochemicals, minerals.*

Introduction

Morocco is among the most important botanic areas in northern Africa thanks to various factors including its geographical position, diverse geology, topography, climate, and ecoregion [1]. The Moroccan flora is estimated to encompass 978 endemic taxa, which form more than half of north African endemic species [2]. This endemic richness seems to be a result of the presence of mixed and well-differentiated environments as highlighted in Ranko *et al.* (2013) [3]. Rosemary known botanically as *Rosmarinus officinalis* L. grows wild primarily in the western Mediterranean region with more than 20 types, varieties, or cultivars [4-6]. According to the same authors, it has been used since ancient times for various medicinal, culinary, and ornamental purposes. In the field of food science, rosemary is well known to its essential oil used as food preservative, thanks to its antimicrobial and antioxidant properties, rosemary has many other food applications such as culinary, medicinal and pharmacology uses [7-10]. Many pharmacological activities of rosemary have been highlighted and well documented in several previous studies and considered as antimicrobial, anti-inflammatory, attenuating, antitumor, inhibitory, antiproliferative and antioxidant agent [11-14].

Rosemary nutritional value, and its bioactive compounds were reviewed by Ribeiro-Santos et al. (2015) [15]. A wide range of minerals, fatty acids, and vitamins are found in different plant parts. Likewise, several phytochemicals are highlighted depending on plant parts, processing techniques, geographical origin, among others [16-17].

Owing to their economic and medicinal interests along with consumer demand, several medicinal and aromatic plants are domesticated and cultivated for their production at a large scale. Rosemary became an important industrial crop [18]. Cultivation practices and environmental conditions can impact, to a large extent, morphological traits and biomass but also chemical composition [19-20].

Chemical composition and biological activities of rosemary has attracted the attention of many research works. However, little is known about the domestication of rosemary and its impacts on nutritional composition and secondary metabolites profiling. In this context, the present work aimed at comparing rosemary growing wild and cultivated under local conditions of Taounate region (northern Morocco) in terms of phytochemical screening and mineral composition.

Material and Methods

Plant material

Firstly, the plant species has been botanically identified and authenticated. At full blooming, the aerial parts (flowering tops) of *R. officinalis* were harvested in June from Taounate province (34°31'48" N, 4°42'36" W). Wild rosemary samples were collected from Aïn Mediouna, while the sampling of cultivated *R. officinalis* was carried out from Botanical Garden of National Agency for Medicinal and Aromatic Plants (ANPAM). Taounate region belongs to Central-northern Morocco, which is characterized by a Mediterranean climate humid in winter and semi-arid in summer.

Phytochemical screening

The samples were dried in a dark room to avoid the photo-oxidation, then crushed using an electric grinder to a fine powder, which was subjected to extraction via decoction according to Chanda et al. (2013) [21] with slight modifications. Briefly, 100g of leaves powder was added in 500 mL of distilled water for 5 min and then filtrated for the different reactions of phytochemical screening (alkaloids, saponins, flavonoids, and tannins) following Pandey and Tripathi (2014) [22]. The above-mentioned phytochemicals were also screened in powder leaves.

Elemental composition

Elemental composition was carried out on powder leaves in samples from both wild and cultivated rosemary. A total of nine elements (Ca, K, Na, Mg, Fe, Mn, Zn, Pb, and Cu) were determined according to Agusa *et al.* (2005) [23] with slight modifications by ICP-AES (Brand Horiba Jobin Yvon, type Activa). Homogenized samples of 0.1 g were digested through microwave with 1.5 mL of concentrated HNO_3. The instrument was calibrated using 0.1, 1.00, 10.00, and 25.00 mg/L concentrations from an ICP multi-elements Standard solution (Merck, 24 elements).

Statistical Analyses

All determinations and measurements were achieved, at least, in triplicates. Quantitative differences were assessed by general linear procedure followed by Duncan's test. Data statistical analyses were performed using the SPSS package version 23 (IBM, Armonk, NY, USA). Values were expressed as means ± standard deviations (SD). Differences were considered significant at 5% as a probability level. Principal component analysis (PCA) [24]. was carried out on mean values to discriminate among solvents and extraction techniques by means of STATGRAPHICS package version XVII (Statpoint Technologies, Inc., Virginia, USA).

Results and Discussion

Phytochemicals screening of both wild rosemary and cultivated rosemary are summarized in Table 1. As it is evidenced in this table, important variations were highlighted between WR and CR in terms of alkaloids, flavonoids, tannins, and saponins found in leaves and their aqueous extracts. Moreover, WR displayed higher levels of the investigated secondary metabolites in both leaves and their aqueous extracts except for alkaloids, which were similar in WR and CR leaves. Rosemary phytochemicals have been extensively investigated and reviewed by Ribeiro-Santos et al. (2015) [25]. Phytochemicals may vary widely depending upon various factors such as plant part (stem, leaves, flowers, etc), extraction conditions (time, solvents, extraction method, among others), processing techniques, and environmental conditions under which plants grow [26].

Additionally, aqueous extracts presented great levels as compared to raw leaves for most of studied phytochemicals, except tannins, which were similar in raw leaves and extracts in the case of WR. Such difference could be ascribed to the hot water used during decoction, allowing the release of more phytochemicals. Relatively high phytochemicals levels observed in rosemary growing wild may be explained by environmental conditions such as high temperature which favors biosynthesis of such phytochemicals [27].

Mineral composition of leaves of WR and CR are presented in Table 2. Similar trends were observed for mineral profiling, WR displayed higher content of Ca (4.631), K (4.224), Fe (0.805), Mn (0.125), Zn (0.035), Pb (0.018), and Cu (0.012 mg/g DM). In contrast, samples belonging to CR were found to have the greatest records of Na (0.150) and Mg (1.920 mg/g DM). Minerals are reported to influence human metabolism, affect general health and be linked to physiological functions within the human body and are, therefore, commonly examined in studies dealing with herbal issues [28]. Elemental composition found in our study was in agreement with published literature for rosemary and other plants [29-30] demonstrated that wild and cultivated plants belonging to the same species have significantly different content of individual major minerals and trace elements. This could be assigned primarily to soil fertility. Rosemary leaves may be seen as a good source of major minerals as well as some trace elements of crucial importance to human body physiology and to be used to enrich some foods.

PCA is one of the most popular multivariate statistical approaches. It is used to reduce the dimensionality of data sets and to project them into a reduced space. To this end, PCA approach is used widely used in many fields such as food science, agronomy, among others [31-32]. In our data, it was carried on mean values of minerals (Figure 1). The 9 plotted squares on this figure are related to minerals mean values. These seem to be distributed along the first (PC1) and the second component (PC2). Along the PC1, major minerals (Ca, K and Mg) whose concentration is over 1 mg/g were distributed on the positive side. Ca and K interacted with wild rosemary, while Mg was close to cultivated rosemary confirming, therefore, mean values comparison (**Table 2**). In contrast, the remaining minerals (whose concentration is below 1 mg/g) were distributed on the negative side of PC1. PC2 seems to separate wild rosemary, toward the positive direction of PC2, with higher levels of Ca, K, Fe, Mn, Zn, Pb and Cu. However, cultivated rosemary was plotted, on the negative direction of this component with great values of Mg and Na.

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Conclusion

Our outcomes demonstrate that rosemary aerial parts (leaves) is an important source of various minerals with large variations between wild and cultivated samples, wild rosemary had better minerals content. Similar trends were observed in the case of main phytochemicals. This could be assigned to pedoclimatic variations as well as domestication effects. Principal component analysis showed a good separation of wild rosemary and cultivated. Further investigations are needed to optimize the production of biomass, phytochemicals of interest under various cultivation conditions (water regime, soil fertility, etc).

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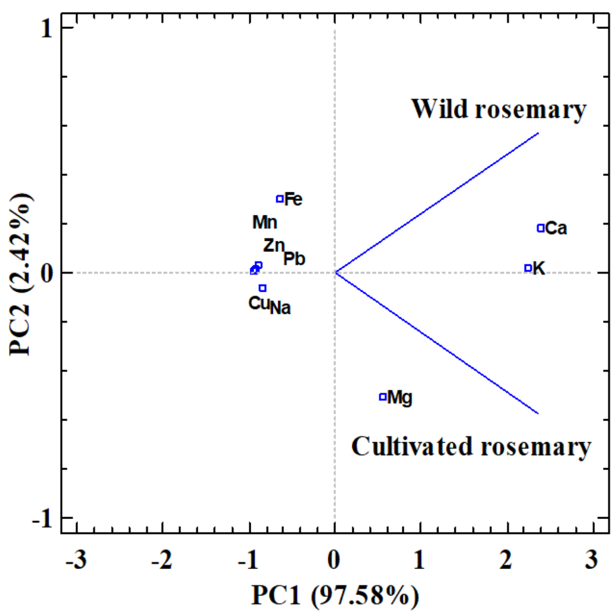
Table 1. Phytochemicals screening in wild rosemary (WR) and cultivated rosemary (CR). Low concentration (+), medium concentration (++), and high concentration (+++).

		Alkaloids	Flavonoids	Tannins	Saponins
WR	*Raw leaves*	+	++	+++	++
	Aqueous extracts	+++	+++	+++	+++
CR	*Raw leaves*	+	+	++	+
	Aqueous extracts	++	++	++	++

Table 2. Mean values (mg/gDM) of mineral elements of wild rosemary (WR) and cultivated rosemary (CR). Results are presented as mean of three replicates followed by Standard Deviation (SD). For each mineral element, values followed by different letters are significantly different at 5% as a probability level.

Mineral	WR	CR
Ca	4.631 ± 0.001 a	3.005 ± 0.003 b
K	4.224 ± 0.002 a	3.024 ± 0.002 b
Na	0.050 ± 0.001 b	0.150 ± 0.002 a
Mg	1.320 ± 0.003 b	1.920 ± 0.003 a
Fe	0.805 ± 0.002 a	0.005 ± 0.001 b
Mn	0.125 ± 0.001 a	0.025 ± 0.002 b
Zn	0.035 ± 0.001 a	0.005 ± 0.001 b
Pb	0.018 ± 0.001 a	0.001 ± 0.001 b
Cu	0.012 ± 0.001 a	0.001 ± 0.001 b

Figure 1. Principal component projections on PC1 and PC2. Blue squares plotted are mean values of mineral elements. Blue segments are related to both rosemary (wild and cultivated).



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