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Seasonal variations of ballota hirsuta benth flavonoids of tessala mount of the prefecture of Sidi Bel-Abbes (Western Algeria)

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ABSTRACT

Secondary metabolites are organic molecules synthesized by plants when interacting with their environment. To demonstrate this effect, we have followed the seasonal variation of the amount of flavonoids in Ballota hirsuta Benth which grows in Tessala Mountains.The results showed that the leavesaretherichest organs in flavonoids in the four seasons. The accumulation of flavonoids in the leaves was recorded much more in summer and in spring; however the low concentrations have been recorded in winter and autumn. The stalks and roots contain the lowest concentrations in flavonoids. This uneven variation of the flavonoids concentration among the three types of organs and between the different sampling seasons is closely linked to the environmental conditions and the phonological stages of the plant.

KEYWORDS

Ballota hirsuta Benth;
Flavonoids;
Seasonal variation.

INTRODUCTION

Phenolic compounds are secondary metabolites of higher plants. They have an active potential and a wide variety of chemical structure. They are recognized by their anti-inflammatory, antibacterial and antiviral activities[1]. Theycontribute effectively to the plants tolerance to various stresses: ultraviolet radiation, herbivores[2]. The accumulation rate of polyphenols varies depending on various environmental factors as well as on theplant growth stage.

The current study focused on theshaggy Horehound (*Ballota hirsuta* Benth.) characterized by a calyx with a folioseblade widely reticulate multifidus with5 main teeth and a pink corolla with bifid upper lip. The stems are woody and hairy of 24 to 60 cm in height and the lower leaves are sessile with very obtuse teeth whilethe upper leavesare cordate or truncated at the base[3].

The work of Ferreres et al. (1986)[4] reported that fourteen flavonoids have been identified, by a chromatographic analysis, in the aerial partsof the shaggy Horehound (Ballota hirsuta Benth), including eight aglycones (savigenin, Kumatokenin...) and six glucosides (luteolin-7-glucoside, quercetin-3-glucodide...).

The objective of this study is to follow the effect of the seasons on the quantitative variation of

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flavonoids in the leaves, stalks, and roots of the shaggy Horehound (Ballota hirsuta Benth.) of Tessala Mount of the prefecture of Sidi Bel-Abbes in Western Algeria.

MATERIALS AND METHODS

Plant material

The plant material is made up of the aerial and underground parts of shaggy Horehound (*Ballota hirsuta* Benth.), namely: the leaves, stalks and roots. Thesamplings of the studied species were collected from a thicket at an altitude of 789 m. The canopy is a natural formationconsisting of dwarf fan Palm (*Chamaeropshumilis*L.), fringed rue (*Rutachalepensis*L.), mountains rue (*RutaMontana*L.), grayish-white Germander (*Teucriumpolium*L.), silver Sage (*Salvia argentea*L.), ciliated thyme (*Thymus ciliatus* (Desf.) Benth), sea squill(*Urgineamaritma*L.),thorny Calycotome(*Calycotome intermedia*Presl.),green oak(*Quercus ilex* L.) and olive (*Olea europea* L.)[5].

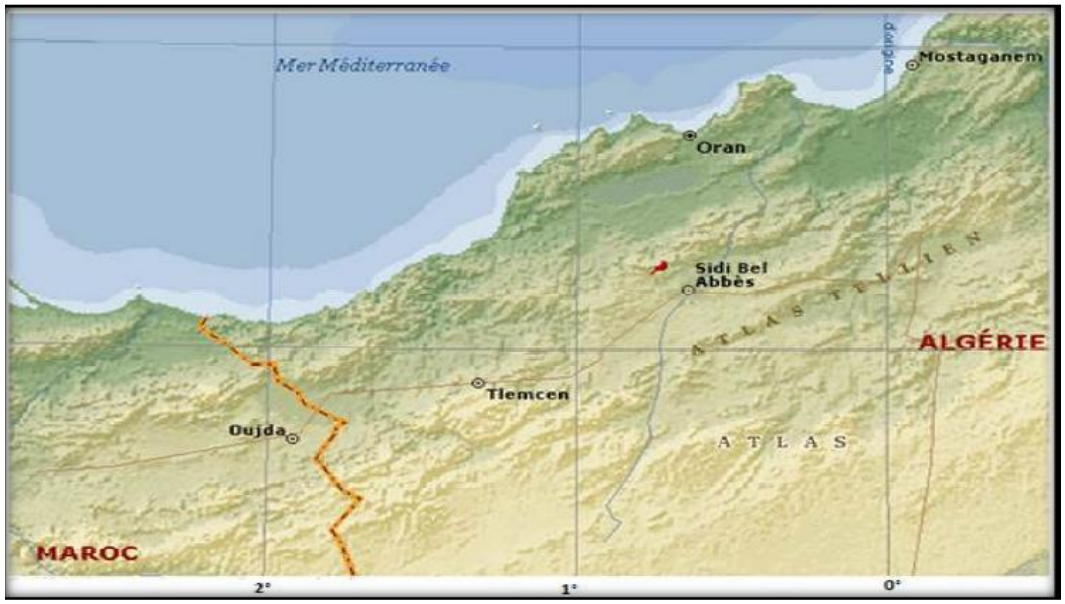
The climatic conditions prevailing in this region are characterized by annual thermal amplitude of 33°C where the minimum temperatures border 2.5°C and the maxima are around 35.5°C; the rainfall is between 335 and 409 mm. The relative humidity displays a maximum of 77% in winter and a minimum of 52% in summer. These climatic characteristics indicate that the region where the shaggy Horehound evolves is located in semiarid bioclimaticzone characterized by fresh winter[6]. The samples were collected during the four seasons of the year 2012 in a station at the following coordinates: X: 35 ° 16 ’14.6', Y:’0 ° 46’24.6 ‘ (Figure 1). The station benefits from optimal conditions of exposure to the Sun.

After being washed under running water, the plant material was dried at room temperature and away from light to maximally preserve the integrity of molecules. Thus, according to Bruneton (1987)[7], this method of drying does not degrade the phenolic compounds. The samples were then ground and sieved to obtain a homogeneousgranular structure.

Extraction of polyphenols

The performeddosage responds to two objectives, namely, the treatment of a large number of samples from very little vegetable matter and the transposition to any type of tissues (leaves, roots and stalks). Morphology of shaggy horehound is illustrated in Figure 2.

A quantity of 0.2 g of each plant organ was cold maceratedat 4 ° C in a mortar containing 10 ml of methanol at 80%. The mixture was centrifuged at 4000 rpm for 10 minutes. The supernatant was recovered after submitting the mixture tovortex agita-



Legend: Location site of shaggy horehound; scale 1/20.000

Figure 1 : Location site of shaggy horehound (*Ballota hirsuta* Benth) in the mountainsof Tessala(Western Algeria)

Figure 2: Morphology of shaggy horehound (*Ballota hursita*Benth) of Tessala Mount (Western Algeria)

tion. This operation was repeated twice to retrieve the maximum phenolic compounds. The supernatant from each of the three organs, constituting the hydroalcoholic extract, has been kept at -20 °C[8].

Quantification of flavonoids

The method of the aluminium trichloride (AlCl3) is generally used for the determination of the total content of flavonoid hydroalcoholic extracts[9]. A quantity of 500µl of hydroalcoholic extract was mixed with 1500 µl of distilled water and 150 µl of sodium nitrate to 5 %. The mixture was left at rest for 5 min in the dark at room temperature. It has been added a quantity of 150 µl aluminumtrichlorideto 10 %. A quantity of 500µlof soda of 1 M has been added to the new mixture which has required a rest of 11 min in the dark. The final mixture was submitted to vortex agitation and the absorbance was read using a spectrophotometer at 510 nm[10].

A calibration curve was carried out with the catechin at different concentrations (0-10 -20-30-40-50 mg/l) under the same operating conditions asthe samples used in the quantification of flavonoids. The results were expressed in milligram of catechin equivalent per gram of dry matter (mg EC/g MS).

Statistical analysis

The average concentration was determined on the basis of three measures. It is expressed as mean ± standard deviation. The analysis of variance witha classification criterion (ANOVA 1) was used to compare the amounts of flavonoids in the vegetative organs of the studied species. It has allowedcomparing the quantitative variation of flavonoids during the four seasons of sampling. The processing of the results was done using the software package of STATISTICA 6 and Excel 2007 spreadsheet.

RESULTS

The levels of flavonoids are significantly different in the three vegetative organs of the studied plant whatever the season of sampling. The difference is confirmed by the varianceanalysis test ($p < 0.05$). The highest quantitiesare recorded in the leaves, followed by the stalks and roots (Figure 3).

The leaves show higher concentrations in flavonoids during the seasons of summer (68.68 ± 1.57 mg CI /g of the MS) and spring (64.39 ± 1.33 mg CI /g of the MS). These levels reveal a decrease in autumn (51.04 ± 1.54 mg CI /g of the MS) and in

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winter (56.59 ± 1.15 mg CI /g of the MS). The difference observed was confirmed by the ANOVA test (F,obs = 93.456 > F,th = 4.066 ; P < 0.05). However, the quantitative difference in flavonoids between seasons is not significant in the case of stalks (F,obs = 0.136 < F,th = 5.143 ; P < 0.05) and roots (F,obs = 0.090 < F,th = 5.143 ; P < 0.05).

DISCUSSION

The contents of flavonoids in shaggy Horehound (Ballota hirsuta Benth) show variability depending on the seasons and the studiedorgan. Leaves synthesize and secrete significant quantities compared to stalks and roots regardless the season of sampling. Secretions are primarily due to specialized histological structures. Several categories of secretory tissues can coexist simultaneously within the same species and even within the same organ[11]. THEY are universally present in the foliar cuticle and epidermal cells of leaves where polyphenols are in high concentration[12]. Generally, the number and shape of trichomes (secretory canals) are very variablecharacters that are strongly influenced by environmental factors, such as temperature and light intensity[13].

The flavonoids provide the pigmentation of flowers, fruits and seeds to attract pollinators and protect the plant against summer radiation[14]. This may explain the important quantities of flavonoids recorded in spring and summer seasons; while the levels are more or less low in autumn and winter. The Shaggy Horehound (*Ballota hirsuta* Benth.) leaves contain a significant amount of phenylpropanoid (precursors of main flavonoids) in summer period. This quantity decreases during the winter period[15][16]. Other studies reported high levels of catechin in summer and anthocyanin in spring in samples of*Pennisetumpurpureum*[17].

The Sage (*Salvia officinalis* L.) leaves are richer in total flavonoids during the season of spring[18]. These results indicate that polyphenolic compounds are influenced byclimatic environmental factors[19]. The low levels are obtained in roots and stalks. Several studies demonstrated that the roots synthesize much more alkaloids than phenolic compounds[20].

The quantity of these metabolites in the methanolic extracts of the plant depends mainly on their origin[21]. Some factors such as the variety, the growing season, the harvest season, the climatic and environmental conditions, and the geographic location, greatly affect the metabolites content[22]. In fact, the phenolic content of a plant depends also on a number of intrinsic (genetic) and extrinsic factors

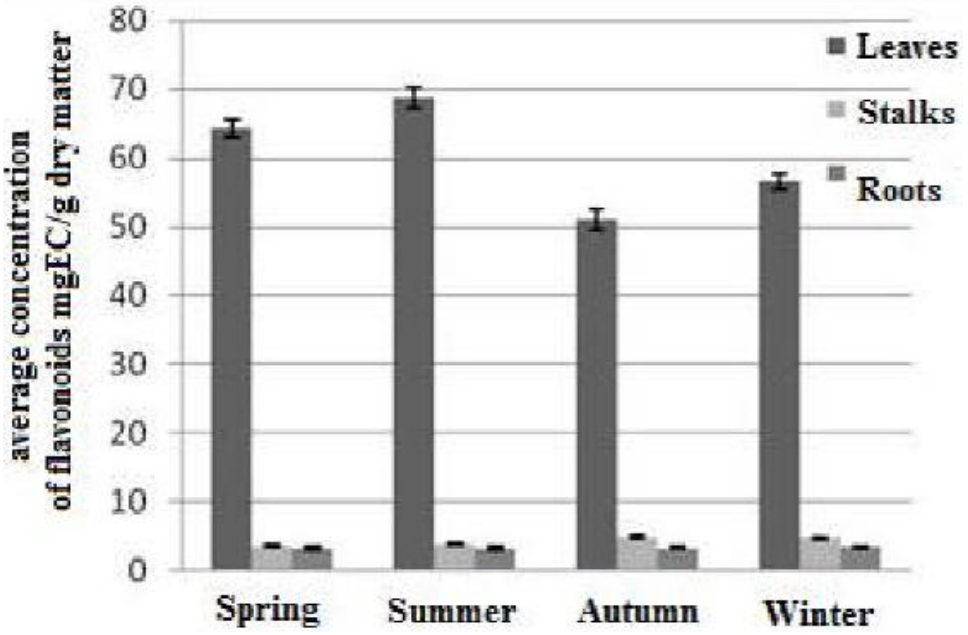

Figure 3 : Average concentrations of flavonoids in the vegetative organs of *Ballota hirsuta* Benth of Tessala Mount (Western Algeria)

(storage conditions)[23].

The difficulty in comparing the results obtained with those of the literature is due mainly to the conditions where the plant evolves as well as to the methods of extraction and quantification[24].

CONCLUSION

In the current study, the effect of climate on the quantitative distribution of the flavonoids in the three organs: leaves, stalks and roots during the four seasons has been highlighted. THE results show that the leaves accumulate relatively high amounts of flavonoids in the four seasons, compared with stalks and roots. The summer period is characterized by the dominance of the flavonoids in the leaves; this is closely linked to the effect of the climatic conditions (temperature etc.) on the trichomes development. The presence or absence of these sites of synthesis of flavonoids for each season must be seen alongside the quantity of metabolites secreted by each organ. The uneven distribution of flavonoids measured during the four seasons confirms that the secretion of secondary metabolites responds to abiotic stress (climate...).

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