NATURAL EXTRACTS FROM *PTEROSPARTUM TRIDENTATUM* AT DIFFERENT VEGETATIVE STAGES: EXTRACTION YIELD, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

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ABSTRACT: The aerial parts of *Pterospartum tridentatum*, a wild growing species in Portugal used in traditional medicine and gastronomy, were harvested at different stages (vegetative phase, flowering phase and beginning of dormancy) in two locations in Portugal (Malcata and Gardunha mountains), and the respective aqueous extracts have been studied. The influence of the seasonal variation in the extraction yield, total phenolic content and antioxidant activity was evaluated. The extraction was carried out in boiling water in consecutive steps. After each step, the aqueous extract was separated and fresh water was added maintaining the same plant material. The procedure was repeated seven times, within an overall time period of 180 minutes.
Higher extraction yields were achieved with plant stems collected at the vegetative phases, either from Malcata or Gardunha regions. The total phenolic content of the extracts from Malcata plants ranged from 273 mg to 400 mg gallic acid equivalent/g dry matter, which was quite similar to that determined for extracts from Gardunha (245 to 394 mg gallic acid equivalent/g dry matter). The antioxidant activity was determined by the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The greatest radical scavenging activity was observed in the flowers extracts, even though all extracts produced presented a good antioxidant activity. Furthermore, the antioxidant activity was not affected by the exposure of the plant material at 100ºC for long periods of time (180 min).
The results show that *Pterospartum tridentatum* has a great potential to be used as a new source of natural antioxidants for the food industry.
Key words: *Pterospartum tridentatum; Aqueous extracts; Extraction yield; Antioxidant activity; Phenolic content.*

INTRODUCTION

Researchers are looking for natural antioxidants as alternative to synthetic compounds, such as butylated hydroxytoluene (BHT), butylated hidroxyanisole (BHA) and *ter*-butylhidroxyquinone (TBHQ) that are widely used in the food and pharmaceutical industries. The interest in plant-derived food additives has grown, because the consumption of synthetic antioxidants has been related to the possible health risks.
Some plants synthesize secondary metabolites with biological activity, namely poliphenolic compounds and alkaloids. The amount and type of metabolites produced is dependent on the plant species and environmental factors that affect growth.
Polyphenols participate in plant defense mechanisms to counteract reactive oxygen species in order to avoid oxidative damage. They are often presumed to be safe for human consumption, due to their plant origin, and are used as health promoting ingredients (Moyo *et al*., 2010).
Pterospartum tridentatum L. Willk. [=*Chamaespartium tridentatum* (L.) P. Gibbs.; *Genista tridentata* L. is an European endemic Leguminosae (=Fabaceae) species belonging to the subfamily Papilionoideae and known as *carqueja* or *carqueija* in Portugal. This small shrub,

growing spontaneously up to 100 cm, is very common in the mountains of the north of Portugal and can usually be found in the understory of *Arbutus unedo, Pinus pinaster* and *Eucalyptus* forests. Some authors refer the use of *P. tridentatum* in popular medicine, claiming to possess digestive properties and activity against high blood pressure, cholesterol, diabetes and even obesity (Vitor *et al*., 2004). It is also used in gastronomy as a condiment in rice and rabbit stew to improve flavor. *Carqueja* is an underexploited natural source of compounds with biological activity, which should be fully characterized aiming to its valorization.
In the present study, the aerial parts of *P. tridentatum* were collected in two locations in Portugal (Malcata and Gardunha mountains), at three different vegetative stages. The effect of the harvest period and extraction time on the extract yield, as well as on the total phenolic content and antioxidant activity, was evaluated.

MATERIAL AND METHODS

Samples of the aerial parts of *Pterospartum tridentatum* were collected at different stages: vegetative phase (end of February), flowering period (in May) and beginning of dormancy (end of October). The shrubs were collected in two locations in Portugal: Gardunha and Malcata mountains.
Consecutive aqueous extraction steps were performed by refluxing a mixture of 25 g of plant samples and 100 mL of distilled water in a Clevenger apparatus. They were processed in consecutive time periods in boiling water: 15, 30, 60, 90, and 120, 150 and 180 minutes. After each time period, the aqueous phase was removed and the extraction continued after the addition of an equal amount of fresh water to the same plant material. The recovered aqueous phases were freeze-dried and a solid extract was obtained.
The total phenolic content (TP) of the extracts was evaluated by the method Ribéreau-Gayon (1970), measuring the absorbance at 280 nm. TP values were expressed as mg gallic acid equivalents per gram dry matter. All trials were carried out in duplicate.
The antioxidant activity of the solid extracts was determined by the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The freeze-dried extracts (0.2 mg) were dissolved in water (1 mL), and added to a methanolic DPPH solution (4 mL). After 40 min incubation period at room temperature, in the dark, the absorvance was measured at 517 nm. The radical scavenging activity (RSA) was calculated as follows: RSA (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$, where $Abs_{control}$ is the absorvance of DPPH radical in methanol and Abs_{sample} is the absorvance of DPPH radical after reaction with sample extract. Results were expressed as Trolox equivalents (mM Trolox/100 g extract dry mass).

RESULTS AND DISCUSSION

Extraction yield

The cumulative extraction yields presented some differences when comparing different harvesting seasons. Higher extraction yields were achieved from plant steams collected at the vegetative phases, either from Malcata or Gardunha regions (Figure 1). Regarding the extraction from plant parts collected in Malcata, a higher extraction yield was also obtained from the flowers (Figure 1A), when compared to that obtained from stems collected during flowering and in the beginning of dormancy. For all vegetative stages, there was an increase of the cumulative extraction yield over the consecutive batch extraction steps, for all the collected plant parts. This fact means that the plant material still contained unextracted compounds even after seven extraction steps.

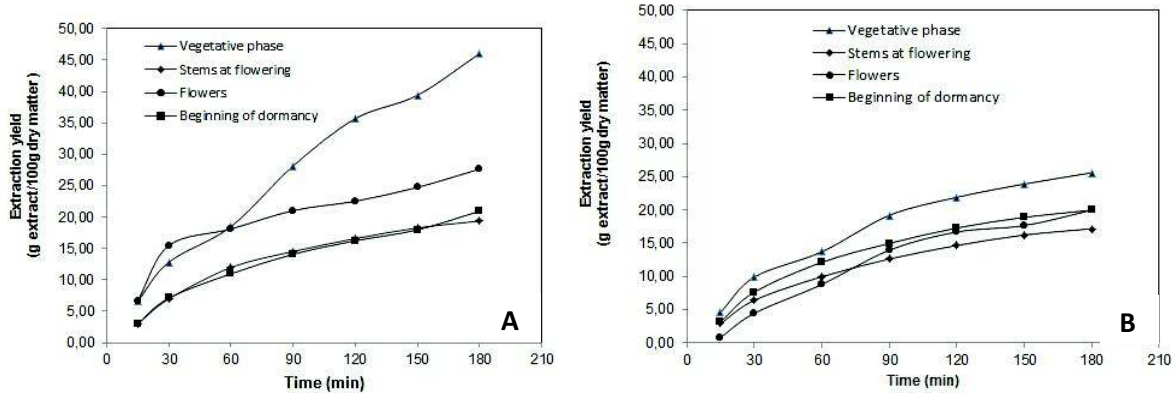

Figure 1. Cumulative curve of the extraction yield of *P. tridentatum* aereal parts collected in Malcata (A) and Gardunha (B) at various stages (stems at the vegetative phase; stems during flowering; flowers; stems in the beginning of dormancy).

The stems from flowering phase and beginning of dormancy showed lower yield values comparing to the plant parts harvested in the other phases. In the beginning of dormancy this fact may be explained by the plant investment in the previous period with fruiting and seed production. The lower extraction yield observed with stems from flowering phase may be due to the channeling of reserves for the floral organ.

In order to determine the most convenient extraction time (which is related with the number of batch extraction steps), the mass of extract recovered in each extraction step was converted as the percentage of the overall mass of extract obtained (Table 1). It was found that the mass of extract recovered after 60 minutes (three extraction steps), for Malcata and Gardunha plants, was between 40 and 65% of the overall mass recovered. After 90 minutes (four extraction steps) the percentage of extract recovered was between 61% to 79%, and after 120 minutes (seven extraction steps) it was on average more than 75%, which may be considered an acceptable value for the extraction time and energy costs involved. In most cases, the addition of an extra hour of extraction leads to a low increase on the cumulative extraction yield. For that reason, the extraction time of 120 minutes (five extraction steps) was considered to be a good compromise between the amount of extract recovered and the time and energy involved in the process.

Table 1. Mass of extract as percentage of the overall mass recovered as a function of extraction time.

	Malcata				**Gardunha**			
Time (min)	**Extract recovered (%)**				**Extract recovered (%)**			
	Vegetative phase	Flowering		Beginning dormancy	Vegetative phase	Flowering		Beginning dormancy
		Stem	Flower			Stem	Flower	
15	14.4	15.6	24.2	14.7	17.9	16.7	4.5	15.9
30	27.9	36.0	55.9	34.0	38.6	37.2	25.1	38.1
60	40.2	61.9	65.5	52.4	53.5	57.9	49.7	60.7
90	61.1	75.0	76.1	67.3	74.9	73.5	79.0	75.0
120	77.5	85.6	81.5	77.6	85.5	85.4	94.5	86.6
150	85.6	94.4	89.5	85.8	93.4	94.6	100.0	94.7
180	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

According to the results obtained by Luis *et al*. (2009) it is verified that the values of extraction yield of aqueous extracts in *Erica* spp. and *Cytisus scoparius* was 4.64 and 4.98% w/w (d.m.) respectively. In another study including *Carissa opaca* species (medicinal product

used in Pakistan) the extraction efficiency was 10.7 ± 0.99% w/w (d.m.) (Sahreen *et al*., 2010). These values are much lower than those observed in this work.

Total phenolic content

The total phenolic content of the extracts recovered from Malcata plants (Figure 2A) ranged from 273 mg to 400 mg gallic acid equivalent/g dry matter, which was quite similar to that determined for extracts from Gardunha (245 to 394 mg gallic acid equivalent/g dry matter) (Figure 2B). For both Malcata and Gardunha plant extracts, the lowest value was observed in flowers extracts after 180 minutes of extraction (eight consecutive extraction steps) and the highest value in vegetative phase after 150 minutes.

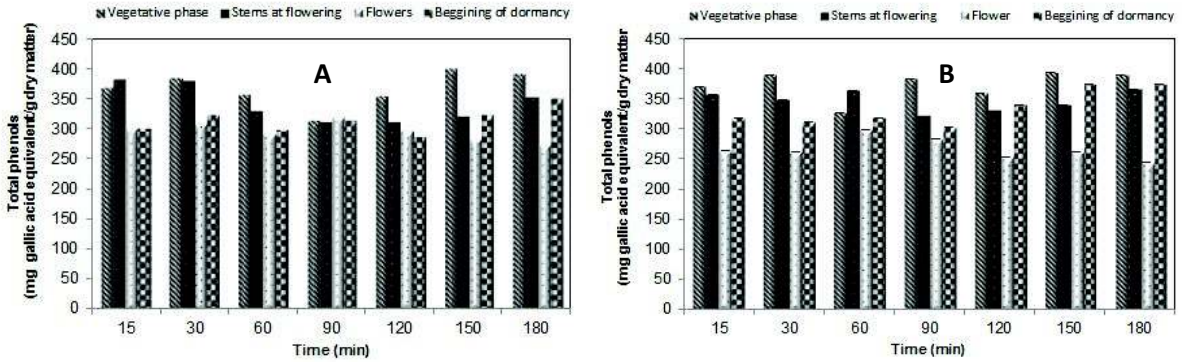

Figure 2 - Total phenols in the aqueous extract of *P. tridentatum* from Malcata (A) and Gardunha (B) (Vegetative phase; Stems at flowering; Flowers; Beginning of dormancy). Measurements were made in duplicate (SD ≤ 0.08).

We can consider that all extracts, from different vegetative phases and different extraction times, presented high levels of phenolic compounds when compared to other species described by Sousa *et al.* (2007). The phenols present in the species *Pterospartum tridentatum* show high levels at any stage of the vegetative cycle and were superior to other studied species such as *Harpephyllum caffrum*, *Sclerocarya birrea* (Ajila *et al*., 2010) and *Carissa opaca* (Saherr *et al*., 2010).

A similar study with five medicinal plants (*Terminalia brasiliensis*, *Terminalia fagifolia*, *Cenostigma macrophyllum*, *Qualea grandiflora*, *Copernicia prunifer)* carried out by Sousa *et al*. (2007) has shown much lower phenolic contents ranging from 11.55 mg gallic acid equivalent/g dry matter in the root extract of *C. prunifera* and 97.6 mg gallic acid equivalent/g dry matter in the leaf extract of *T. fagifolia*. Rockenboch *et al*. (2008) obtained values of total phenolic compounds in aqueous extract of *Physalis peruviana* fruit (47.8 mg gallic acid equivalent/g dry matter).

Antioxidant Activity

All extracts presented antioxidant activity, but higher AA values were obtained for the extracts recovered from plants harvested during the flowering period (Figure 3).

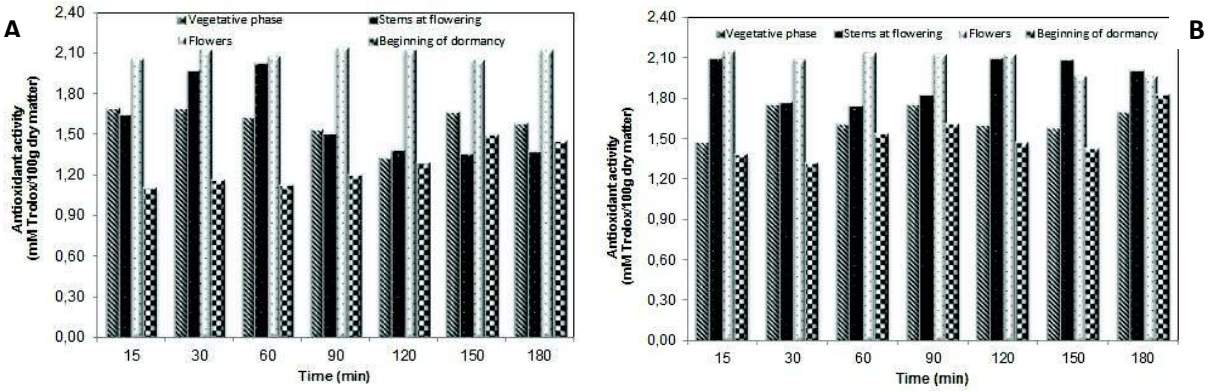

Figure 3 - Antioxidant activity of aqueous extracts of *P. tridentatum* from Malcata (A) and Gardunha (B) (Vegetative phase; Stems at flowering; Flowers; Beginning of dormancy). Measurements were made in triplicate (SD ≤ 0.02).

The values are consistent with the work reported by Luis *et al*. (2009), in which the AA of aqueous extract of *P. tridentatum* was 1.30 mMTrolox/100g dry matter.

CONCLUSIONS

The extraction process of *Pterospartum tridentatum* aerial parts presented a high extraction yield, being the highest yields achieved when processing flowers and plant stems harvested at the vegetative phase. The aqueous extracts presented high contents of total phenolic compounds, which did not change significantly with the harvesting season. In addition, consecutive extracts isolated from the same plant material over time have shown to possess similar total phenolic content and maintained their antioxidant activity. The results show that *Pterospartum tridentatum* has a great potential to be used as a new source of natural antioxidants for the food industry. Future studies will be focused on the identification of the major compounds present in the aqueous extracts and the correlation between the chemical species with the antioxidant activity. Vitor *et al*. (2004) identified some compounds such as isoflavones (namely prunetin, genistin and sissotrin) and the flavonol glycoside isoquercitrin.

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