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ORIGINAL ARTICLE

Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob tree barks growing in Morocco

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Abstract We evaluated the *in vitro* antioxidant property and phytochemical constituents of the crude ethyl acetate and methanol extract of the three genders of carob tree barks (spontaneous male, spontaneous female, and grafted female). The scavenging activity on DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined, as well as the phenolic contents (Folin–Ciocalteu method) of both the extracts. The highest antioxidant activity and the higher amounts of total phenols were shown in methanol crude bark extract for the three genders. Variety significantly affected the phenol content and the antioxidant activity, with the spontaneous male variety globally showed a higher polyphenol concentration and antioxidant activity than the grafted female and spontaneous female.

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1. Introduction

Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruits and vegetables and the risks for chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation, and cancers (Chen et al., 2005; Middleton et al., 2000; Prior, 2003; Saleem et al., 2002; Zhang et al., 2005). These physiological functions of fruits and vegetables may be partly attributed to their abundance of phenolics. Phenolic

compounds are a complex group of substances that have attracted considerable attention due to their roles in providing flavor and color characteristics of food and in human health (Visioli and Galli, 1998; Spanos and Wrolstad, 1992; Ingram et al., 1997). Many of the benefits associated with consumption of phenolic-rich foods are associated with their antioxidant activities (Vinson et al., 1993; Sawa et al., 1999). Antioxidant activities of plant polyphenols have been claimed to have beneficial health functions for retarding, aging, and preventing cancer and cardiovascular diseases (Scalbert et al., 2005). For these reasons, the natural antioxidants have recently become a major area of research.

Carob (*Ceratonia siliqua* L.), is a slow-growth evergreen tree, a plant rich in phenols and polyphenols. Due to its interesting morphophysiological characteristics, such as resistance to drought and salinity, adaptation to poor soils (Battle and Tous, 1997), it has been introduced to the Mediterranean basin. Carob tree requires little maintenance (Fletcher et al., 1997) and it is considered to be an important component of vegetation for environmental, economic, and social reasons (Battle and Tous, 1997). The fruits are used for diverse purposes as fodder. They are more important in food industry and are a source of many products such as gum, sugar, and alcohol (Carlson, 1986; Tous, 1992). World carob pod production is estimated about 310,000 tones per year and the principal producing countries are Spain, Italy, Portugal, Morocco, Greece, and Cyprus (Battle and Tous, 1997). The morphological and physiological traits are traditionally used for the identification and the management of carob cultivars (Tous and Batlle, 1990; Gharnit et al., 2004). The carob is a dioecious species with some hermaphroditic forms; thus male, female, and hermaphrodite flowers are generally borne on different trees. Many investigators have studied the carob trees, pods (beans), and their seeds (Brito de Carvalho, 1987; Charalambous and Papaconstantinou, 1966;Thompson, 1971; Marakis et al., 1993, 1980). Recently, investigators isolated and identified the major polyphenols in carob fibers (Owen et al., 2003; Papagiannopoulos et al., 2004) and other studied the variation and the composition of phenolic compounds of carob pods grown in different regions of Morocco (Rakib et al., 2010).

To our knowledge, there are no such phytochemical reports concerning Moroccan cultivars, so the present investigation was designed to determine the phytochemical screening of the main secondary metabolites classes, the total phenolic content, and antioxidant activity of barks of spontaneous male, Spontaneous female, and grafted female carob trees growing in Morocco.

2. Experimental

2.1. Plant materials

This work was conducted on Dkar (spontaneous female (F)), unproductive Dkar (spontaneous male (M)), and Lanta (grafted female (L)) of carob tree barks. Plant materials were collected in 2007 from the Province of Chafchaouen (NW of Morocco) and identified by Professor A. Ennabili. Authenticated voucher specimens were deposited in the Herbarium of The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fès, Morocco.

2.2. Preparation of the extracts

Dried coarse powder of the bark was placed into the extractor of a Soxhlet. The extraction was carried out by using solvents of increasing polarity starting from hexane, dichloromethane, ethyl acetate, and methanol. At the end of the extraction the respective solvents were concentrated by evaporation.

2.3. Phytochemical screening

The ethyl acetate and methanol extracts were screened for phytochemical constituents (alkaloids, flavonoids, saponins and tannins) using simple qualitative methods of Paris and Nothis (Paris and Nothis, 1996). The total phenolic contents was determined spectrophotometrically using the Folin–Ciocalteu method. This reagent is based on the Slinkard and Singleton method (Slinkard and Singleton, 1977) and the early work of Singleton and Rossi (Singleton and Rossi, 1965) is a colorimetric oxidation/reduction method for phenolic compounds. Briefly, extracts were reacted with Folin–Ciocalteu reagent and then neutralized with sodium carbonate solution (25%). After 2 h, the absorbance of the resulting solution was measured at 764 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard as gallic acid graph: Absorbance = 0.0007 gallic acid (μg) − 0.0642 (R^2: 0.9964). All tests were carried out in triplicate and the results are given as gallic acid equivalents (GAE).

2.4. Determination of free radical scavenging activity by DPPH method

The antioxidant potential of the ethyl acetate extract and the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Briefly, 100 μl of various concentrations of the extract in methanol was added to 10 ml of a methanol solution of DPPH (1.01×10^{-2} M). The mixture was vigorously shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm by using a double-beam UV–vis Camspec M550 spectrophotometer. A mixture of 100 μl of methanol and 10 ml of DPPH solution was used as the control. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\%\text{Inhibition} = [(A_B - A_S)/A_B] \times 100 \text{ (Blois, 1958)}$$

where A_B is the absorbance of the control reaction (containing all reagents except the test compound), and A_S is the absorbance of the test compound. Butylatedhydroxytoluene (BHT) was used as positive control. The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (4.0, 2.0, 1.0, 0.5 and 0.25 mg/L).

3. Results and discussion

3.1. Phytochemical screening

Phytochemical screening of the crude ethyl acetate and methanolic extract of the three categories of carob tree barks

revealed the presence of flavonoids and tannins. Alkaloids and saponins were not detected; the result of phytochemical test has been summarized in Table 1. The result of total phenol contents is shown in Table 2. The total phenolic contents from the ethyl acetate and methanolic extracts of the three varieties of *Ceratonia siliqua* L. barks varied from 0.46 to 0.76 (g/l GAE). In this research, the methanolic extract has the highest phenolic content than ethyl acetate extract. These phenolic compounds and most other reported bioactive compounds are generally more soluble in polar solvents.

Table 1 Phytochemicals detected in extracts of three genders of carob barks.

Varieties	Phytochemicals	Ethyl acetate extracts	Methanolic extracts
Spontaneous female	Alkaloids	–	–
	Flavonoids	+	+
	Tannins	+	+
	Saponins	–	–
Spontaneous male	Alkaloids	–	–
	Flavonoids	+	+
	Tannins	+	+
	Saponins	–	–
Grafted female	Alkaloids	–	–
	Flavonoids	+	+
	Tannins	+	+
	Saponins	–	–

Key: + = present; – = absent.

Table 2 Total polyphenolic contents of three genders of carob barks.

Categories	Sample	Total phenolic content (g/l GAE)
Spontaneous female	EtOAc	0.46
	MeOH	0.54
Spontaneous male	EtOAc	0.52
	MeOH	0.76
Grafted female	EtOAc	0.58
	MeOH	0.62

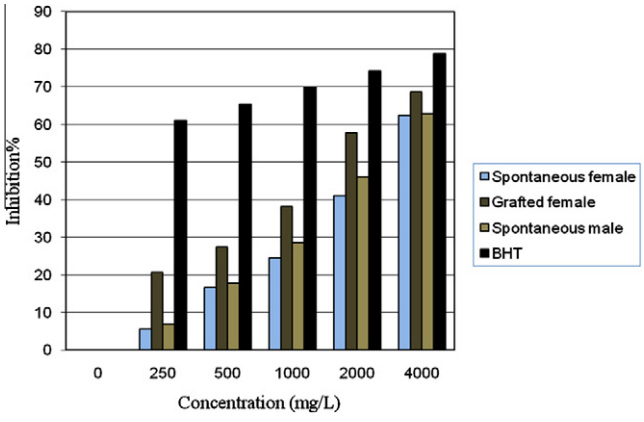

Figure 1 Antioxidant activity of ethyl acetate extract.

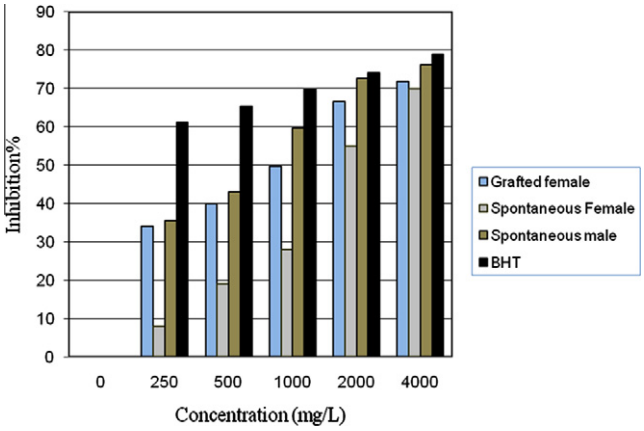

Figure 2 Antioxidant activity of methanol extract.

Table 3 IC_{50} (g/l) values of fractions of three genders of carob barks.

Categories	Sample	IC_{50} (g/l)
Spontaneous female	EtOAc	2.8
	MeOH	1.8
Spontaneous male	EtOAc	2.5
	MeOH	0.7
Grafted female	EtOAc	1.6
	MeOH	1
–	BHT	0.2

3.2. Determination of free radical scavenging activity by DPPH method

The antioxidant activity of each extract is tested with DPPH scavenging assay. Six samples from the three categories of *Ceratonia siliqua* L. barks were investigated for their antioxidant activity. The result of the DPPH scavenging assay is shown in Figs. 1 and 2. It is found that antioxidant compounds in ethyl acetate and methanol extracts have the free radical scavenging ability.

Thus, among the three *Ceratonia siliqua* L. barks categories tested, the methanolic extracts showed a very good scavenging effect compared with ethyl acetate extracts. As shown in Table 3, the results of DPPH analyses for the methanol extract demonstrated that the most active radical scavengers were found in the spontaneous male (IC_{50} = 0.7 g/l) followed, respectively by grafted female (IC_{50} = 1 g/l), and spontaneous female (IC_{50} = 1.8 g/l). In the ethyl acetate extracts, the sequence for DPPH radical scavenging ability was grafted female (IC_{50} = 1.6 g/l) followed by spontaneous male (IC_{50} = 2.6 g/l), and spontaneous female (IC_{50} = 2.8 g/l). The presence of flavonoids and tannins in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterai, 1997).

4. Conclusion

This study confirms that the barks of the carob tree contain high amounts of polyphenol compounds. It also showed that

the amounts of those compounds were significantly affected by the variety of the tree. The phenolic compounds are commonly found in the plant kingdom, and they have been reported to have multiple biological effects including antioxidant activity.

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