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Antioxidant activity of stems and leaves organic fractions of *Ecballium elaterium* L.

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ABSTRACT

The last decades are marked by the special interest in the development of medicinal plants at interest as a source of bioactive natural. As a result, many studies focus, increasingly, the therapeutic effects of natural antioxidants. In this context, we are interested in the study of antioxidant phenolics compounds, including flavonoids, capacity of a medicinal plant extracts (Ecballium elaterium L.). The plant has presented mean levels of polyphenols and flavonoids. Estimation of antioxidant activity of organic fractions was assessed by three methods: the reduction of iron, the DPPH free radical scavenging and hydrogen peroxide scavenging. The results showed that the polyphenols (including flavonoids) exhibit average antioxidant activity compared with reference antioxidants.

Key words: *Ecballium elaterium*; antioxidant activity; organic fractions; polyphenols; flavonoids.

INTRODUCTION

In recent years, the use of traditional medicine has spread in the world and has grown in popularity, not only the populations of developing countries have access, but also those countries where biomedicine occupies an important place in health systems. Natural substances from plants have multiple interests utilized in several industries (food, cosmetics ...). Occupying a prominent place in the group of polyphenols, flavonoids are ubiquitous secondary metabolites in plants. These compounds are known for their numerous biological activities, such as antiviral, anti-inflammatory and anticancer [1]. These numerous properties related to health, well described in epidemiological studies, mainly based on their antioxidant activities [2]. Recent work aimed to isolate new substances from plants and finding other ways of applications in different fields. For these reasons, we are interested in studying the antioxidant activities of polyphenols and flavonoids isolated from different organic fractions of : *Ecballium elaterium*

(L.). The few studies conducted on this plant, encouraged us to study the antioxidant properties of this plant to enrich knowledge on the biological activities of this plant.

E.elaterium (L.) (Cucurbitaceae) is a medicinal plant found abundantly in the wild South-West Europe and North Africa in stony ground, in the rubble and slope [3]. The plant is known as grass officinal herb and has a long tradition of uses in the Mediterranean basin. It is often used in dropsy (edema), especially pulmonary edema and also as a revulsive in brain diseases [4]. The fresh raw juice is frequently used in the treatment of sinusitis and jaundice by nasal aspirates [5]. Many biological activities of this species have been attributed to cucurbitacins and their glycosylated derivatives such as antiproliferative activity on various types of cancer cells [6-7]. But his most interesting potential activity can be antiviral [8].

MATERIALS AND METHODS

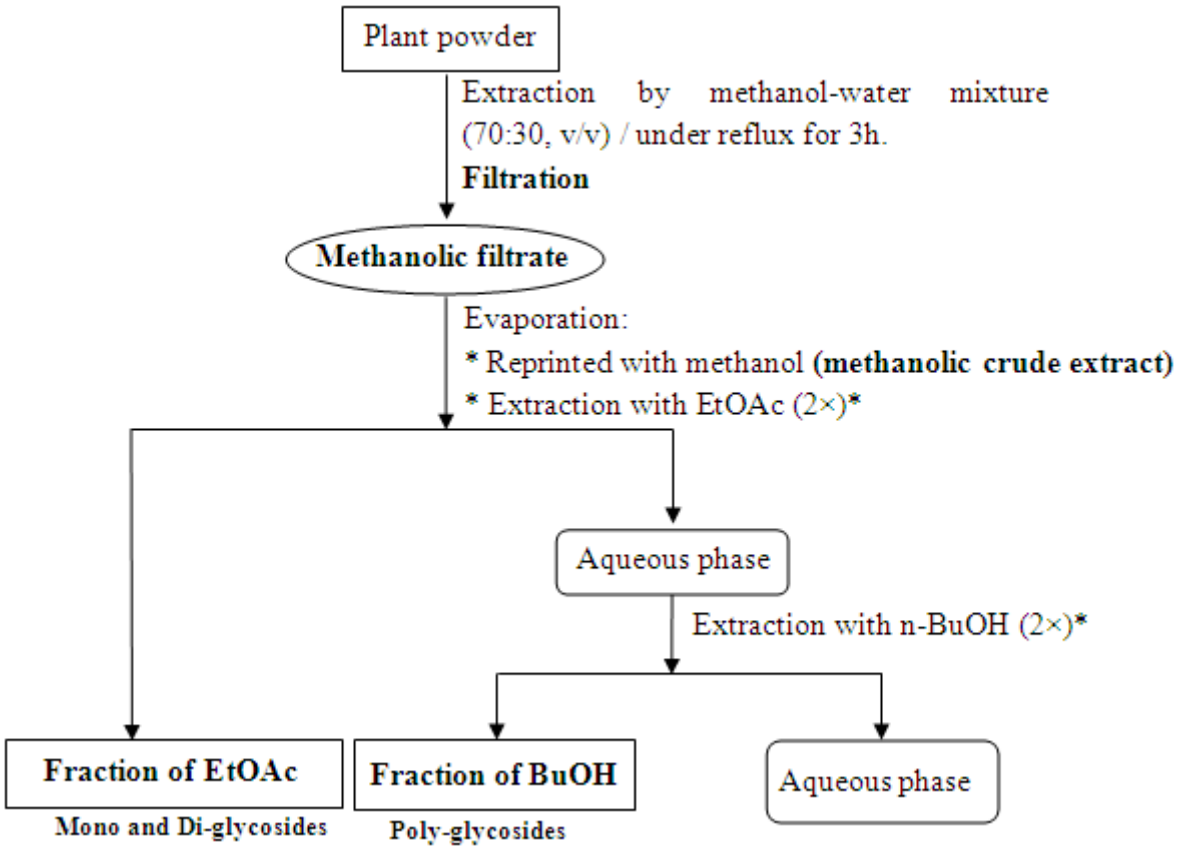

Fig. 1 Monitoring protocol for the extraction of polyphenols and obtaining fractions of ethyl acetate and n-butanol

2.1. Sample preparation

E.elaterium was collected in its natural habitat in the region of Bouhanek (west of Algeria) during the months of December (2007), and dried away from direct sunlight.

A dry powder of the plant (2g) of each part (leaves and stems) was extracted by the mixture methanol-water (70:30, v/v). The preparation is carried under reflux for 3h [9]. After cooling to room temperature, the methanolic extract is filtered and evaporated under reduced pressure using a rotary evaporator. The dry residue obtained after evaporation of the methanolic filtrate, were divided between 20 mL of ethyl acetate and the same volume of distilled water in a separating

funnel. After decantation of the two phases, the ethyl acetate phase is recovered and the aqueous phase is again divided with 20 mL of n-butanol. The operation is repeated twice for each step. The phases obtained are dried using a rotary evaporator. The dry residues were taken up by a few milliliters of methanol and kept at +4°C. Finally two fractions are obtained: fraction with ethyl acetate (EtOAc) and fraction with n-butanol (BuOH) (fig. 01).

2.2. Total phenolics and flavonoid content

Total phenolics content were estimated by the Folin-Ciocalteu method [10]. Results were expressed as mg gallic acid equivalent per gramme of dry extract (mg GAE/g). The total flavonoid content was determined by a colorimetric method as described in the literature [11]. Results were expressed as mg catechin equivalent per gramme of dry extract (mg CEQ/g).

2.3. Antioxidant activity

2.3.1. Ferric reducing antioxidant power assay

The reducing power of the different part of *E.elaterium* was determined according to the method of Yang *et al.* [12]. The EtOAc and BuOH fraction and ascorbic acid were used at different concentrations.

2.3.2. Determination of the scavenging effect on DPPH$^{\bullet}$ radicals

The determination of the capacity of the EtOAc and BuOH fractions to scavenging DPPH$^{\bullet}$ radicals was determined according to the method of El-Haci *et al.* [13]. The ascorbic acid was used as positive control.

2.3.3. Hydrogen peroxide-scavenging activity

The hydrogen peroxide scavenging activity of methanolic extract was determined according to the method of Ruch *et al.* [14].

RESULTS AND DISCUSSION

3.1. Total penolics and flavonoid content

The results are expressed as mg gallic acid equivalent per gram of dry plant material (mg GAE/g), using the linear regression equation of calibration curve of gallic acid. We noted varying levels of polyphenols: 48.22 ± 7.5 mg GAE/g and 10.71 ± 1.35 mg GAE/g for leaves and stems, respectively.

The determination of flavonoids was performed by the colorimetric method with aluminum chloride ($AlCl_3$). The results are expressed as mg catechin equivalent per gram of dry plant material (CEQ mg/g) using the linear regression equation of calibration curve of catechin. We noted varying levels of flavonoids: 45.43 ± 6.38 mg CEQ/g and 5.45 ± 1.29 mg CEQ/g for leaves and stems, respectively.

3.2. Antioxidant activity

3.2.1. *Ferric reducing antioxidant power assay*

The power reduction is one of the antioxidant mechanisms [15]. The presence of antioxidant compounds in a given medium cause the reduction of Fe^{3+} ferricyanide complex to Fe^{2+}. Indeed, the formation of Fe^{2+} can be followed spectrophotometrically by measuring the density of the blue iron complex of the reaction mixture at 700 nm. An increase in absorbance indicates increased reducing power of extracts tested [16].

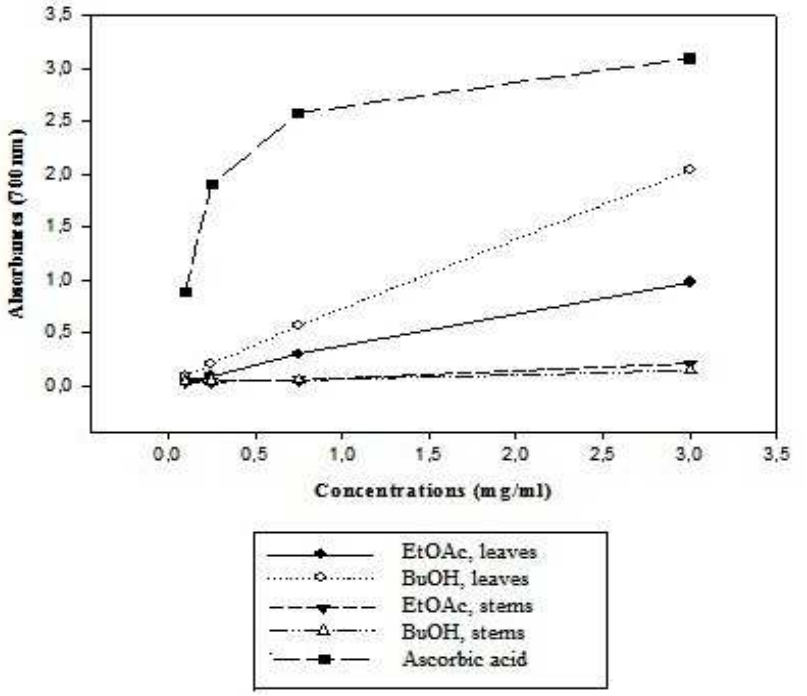

Fig. 2 Ferric reducing power of ethyl acetate and n-butanol fractions of *E.elaterium*

We note (fig. 02) that ethyl acetate fraction of leaves presented more activity to reduce the iron relative to other fractions, but significantly lower than the ascorbic acid, as reflected by the absorbances obtained at different concentrations. We also note that the stem showed very low activity for both fractions studied to reduce the iron.

The ability of reducing of a compound may serve as a significant indicator of its potential antioxidant activity [12]. Many publications have shown that there is a direct correlation between antioxidant activities and reducing power components of some plants [17]. The results obtained for a plant extract that has a high activity, suggesting that this one has a remarkable power to donate electrons to reactive free radicals, converting them into non-reactive species and, consequently, ending the chain reaction of free radicals.

3.2.2. DPPH radical scavenging

The radical DPPH$^{\bullet}$ is one of the most commonly used substrates for rapid and direct antioxidant activity because of its stability in radical form and simplicity of the analysis [18].

We note that EtOAc fraction of leaves showed high activity of DPPH radical scavenging. The BuOH fraction of the same part of *E.elaterium*, showed high activity of DPPH free radical scavenging, because it drew an exponential curve with the presence of a stationary phase that defines the almost total reduction of DPPH.

The results obtained show that the antioxidant activity of various fractions studied directly depends on the concentrations used which relate the presence of phenolic compounds contained in ethyl acetate, n-butanol fractions and antioxidant activity.

For each fraction studied (EtOAc and BuOH), we analyzed a dilution series to determine the concentration of each fraction required to reduce 50% of the free radical, also known as IC50 (table 1).

Table 1 The IC_{50} value of EtOAc and BuOH fractions of *E.elaterium* and ascorbic acid

		EtOAc fraction	BuOH fraction
		IC_{50} µg/mL	**[Final Concentration]**
E.elaterium	**Leaves**	39.60	14.54
	Stems	*	*
Acide ascorbique		**2.97**	

***:** *undetermined*

The interaction of flavonoids with many radicals has been used in several studies to identify the major elements of the antioxidant activity. Because of their low redox potential, flavonoids are thermodynamically capable of reduce free radical oxidants such as superoxide, peroxyl and hydroxyl hydrogen, as reported by Fabri *et al.* [19]. In our study, the BuOH fraction of leaves presented a remarkable antioxidant activity but it's average compared to that of ascorbic acid, suggesting that this plant has compounds endowed with antioxidant activity.

3.2.3. Hydrogen peroxide-scavenging activity

We studied the activity of hydrogen peroxide scavenging of methanol crude extracts of the two parts (leaves and stems) of *E.elaterium*, following the method described by Ruch *et al.* [14]. The results are reported in table 2.

Table 2 H_2O_2 scavenging activity of methanol extracts of *E.elaterium* (%)

Sample	Time (min)				
	0	10	20	30	40
Butylhydroxyanisole (BHA)	90,65	75,86	61,98	53,27	45,63
E.elaterium Leaves	39,50	33,36	26,99	05,08	04,25
E.elaterium Stems	15,43	15,13	09,24	07,59	04,04

At the concentration used (0.1 mg/mL), we note that the BHA has a powerful effect on the order of 90.65%. Regarding the extracts tested, the crude extract of the leaves has an effect of about 39.50%, followed by the crude extract of the stems with a percentage of 15.43%.

Polyphenols have been demonstrated to have a protective action in mammalian and bacterial cells against the cytotoxicity induced by hydrogen peroxide, including phenolic compounds like flavonoids such as quercetin, catechin and other polyphenols as esters of gallic acid and caffeic acid [20].

According to our results, we note that *E.elaterium* presented a mean activity relative to the reference antioxidant (BHA). This can be explained by the content of flavonoid in the plant (45.43 mg CEQ/g for the leaf part and 5.45 mg CEQ/g for the stem part), and polyphenols content (48.22 mg GAE/g for the leaf part and 10.71 mg GAE/g for the stem part), implying that the crude extract of this plant contains some polyphenols (hydroquinone, 4-hydroxy acetophenone, 4-hydroxy-3-methoxy-acetophenone, 2-nitroquinol the 4-hydroxyphenyl-Iignane and para-coumaric acid) and flavonoids (rutin) which have the ability to donate electrons to promote the conversion of H_2O_2 to H_2O [14-21].

CONCLUSION

In light of the results obtained, we can conclude that organic fractions of different parts studied of *E.elaterium*, had an average antioxidant activity compared with the reference antioxidants used, using three methods for studying the antioxidant activity. These results are to critically assess the activity of an extract or a fraction being due to the intrinsic activity of the active components and their relative abundance, a negative result may be due to the presence of a small amount of active compound in the extract as a large quantity of non-active compound. Therefore, a thorough phytochemical screening, extraction of various secondary metabolites, a study of their antioxidant and other biological activities, are recommended in order to explore the plant and, perhaps, to understand the mechanisms by which it is used in traditional medicine.

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