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PHENOLIC COMPOUNDS PROFILING AND ANTIOXIDANT CAPACITY OF FIVE ECOTYPES OF TUNISIAN JERUSALEM ARTICHOKE

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ABSTRACT

In the present investigation, acetonic extracts from tubers of Tunisian Helianthus Tuberosus L. were assayed for their chemical composition and antioxidant activity. The phenolic composition of the acetonic extracts was determined by reverse phase high performance liquid chromatography (RP–HPLC). The phenolic composition of all extracts was characterized by its richness in trans-cinnamic acid, flavonose, carnosic acid and warfarin. Total phenolic content (TPC) measured by the Folin–Ciocalteu assay in Jerusalem artichoke extracts of five ecotypes ranged from 502.36 µ mol/100 g FW (ecotype 2) to 2640.15 µ mol / 100 g FW (ecotype4). A positive correlation between total phenols contents and antioxidant activity was recorded for all ecotypes with the extract of ecotype 4 showed highest antioxidant activity in DPPH radical scavenging assay (3. 62 µ mol/g MF). Our findings demonstrate that the acetonic extracts of Helianthus Tuberosus L. possess significant antioxidant activity and may be suggested as a new potential source of natural antioxidant.

Keywords: Helianthus Tuberosus, Jerusalem artichoke, Tunisia, phenolic composition, antioxidant activity.

1. INTRODUCTION

Currently the concept of functional food is a new quality orientation. It is the result of our society evolution toward a power supply which also responds well to the nutritional requirements or organoleptic characteristics, but also possess a beneficial potential for health. A food is said "functional" if it has effects on one or several functions targets of the body, beyond its strength's nutrients, improving the state of well-being or the health status of individuals, or reducing the risk of a disease (Roberfroid, 2005). Based on this definition and by its specific composition in various nutrients, the Jerusalem artichoke belongs today to this class of foods.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a species of the Asteraceae family, genus Helianthus, known for the remarkable genetic variability of its clones and genotypes (Puttha et *al*., 2013). *Helianthus tuberosus* L.(Asteraceae), a perennial plant, is a sunflower species originating from the Ohio and Mississippi River valleys in the United states that has been introduced and become naturalized as an economic crop worldwide in temperate areas.

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Helianthus tuberosus has a 5-10 tall stems, large leaves, bright yellow sunflower, and fleshy tubers resembling those of potatoes.

In Mediterranean regions it spontaneously grows virtually everywhere and does not require any kind of fertilizer or organic matter and should not be subjected to pesticides. Its rapid and vigorous growth allows good natural control against weeds, which hardly exceed the plant (Rosati, 2010).

The tubers of H. tuberosus have been utilized not only as a food but also as a raw material in the bioethanol industry for its high content of inulin, a fructan that can be easily hydrolyzed. **(**Annalisa et *al*.,2010). It is indeed long been recognized that the Jerusalem artichoke presents a profile very interesting to compounds beneficial to human health. The tubers of Jerusalem artichoke can be used for human nutrition, view its low-calorie content and the presence of inulin which also has positive effects on health (Roberfroid, 2005).

Helianthus tuberosus is mainly cultivated for use as green or brackish fodder as crops in marginal areas, especially in relation to hardiness and low production costs (Shanzhao et al., 2013) and for the production of sugars and soluble fiber. The plant is futhermore an excellent resource for bioenergy production, such as methane, bioethanol, anaerobic digestion and biogas from pyrolysis (Kim and Kim, 2014). *Helianthus tuberosus* can be appreciated not only as a biomass crop resource but also for its nutritional and medical qualities as an accessible source of protein and essential amino acids (Cieślik et al., 2011), minerals (Terzić et al., 2012) and a number of functional ingredients such as inulin, oligofructose and fructose. Besides, it has both nutritional and functional attributes, particularly beneficial for individuals with type 2 diabetes and obesity (Yang et al., 2015).As the majority of the plants, Jerusalem artichokes contain antioxidant compounds that include vitamins, carotenoids and flavonoids (Vadakkemuriyil *et al*., 2013). In recent years many studies have shown that free radicals are the main cause of degenerative diseases, many phenols have a biological protection against these radicals (Shao *et al*., 2008). The composition of these bioactive compounds can be affected by internal and external factors. Such modification of this composition, under the influence of one factor or another, could lead to the modification of the characteristic biological properties of plant (Kalleli et al., 2019). Thus, the objective of this investigation was to determine the phytochemical and antioxidant potential of *Helianthus tuberosus* tubers from Tunisian regions in order to find the ideal origin producing more bioactive compounds.

2. MATERIEL AND METHODS

Plant Materiel

The plant material is a collection of different stations of the Kef (Tunisia) region that belong to the same bioclimatic floor.

Extract preparation

The extraction was carried out using the method developed by Fattouch et al. (2008) with minor modifications. Each 1g of tuber was mixed with 10ml of cold aqueous acetone 70%. The homogenate was sonicated for 10min and then centrifuged at 8000g for 15min at room temperature. Supernatants were concentrated using a rotary evaporator (40°C) under vacuum to a

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final volume of 3 ml. To prevent oxidation of the polyphenols, extraction was achieved rapidly, and the final extract solutions were stored at -20°C until analyzed.

Determination of total phenolic content

The total phenol content (TPC) was measured through spectrophotometric determination with the Folin-Ciocalteu method as reported by Tuberoso *et al*. (2007). The absorbance was read at 725 nm on a10 mm quartz cuvette using a Varian Cary spectrophotometer against a blank. The TPC results, expressed as milligrams of gallic acid equivalent (GAE) per 100g of fresh weight (FW) were obtained using a calibration curve of a freshly prepared gallic acid standard solution (10-200mg /l). All of the measurements were taken in triplicate, and means and standard deviation value were calculated.

Antiradical activity (DPPH Test)

This essay is based on the ability of the antioxidant to scavenge the radical cation DPPH. The method described by Tuberoso et al,(2007) was used starting from 50µLof water- diluted extract. The simple was mixed with 2ml of DPPH 0.04 Mm in methanol. Antiradical activity was measured as the relative decrease in absorbance at 517nm as the reaction between DPPH and antioxidant. Reading was carried out with a Cary spectrophotometer. A calibration curve in the rang 0.02-0.8 Mm was used for Trolox, and data were expressed as Trolox equivalent antioxidant capacity (TEAC, µmol/g fw).

Identification of phenolic compounds by HPLC

The HPLC used throughout our experiment is Hypersil ODS-C18 (5µm;4,6mm*250mm). The mobile phase consists of solvent B: acetonitrile and solvent C: which contains water at 0,2% formic acid. The injection volume used is 50 µl and the UV spectrum was 280 nm.

Statistical Analysis

All testes and analyses were run in triplicate and averaged. Quantitative presented data are means ± standard deviation (SD). One-way analysis of variance (ANOVA) with Dunnett's post-test was performed using Graph Pad Prism version 4.00 for windows. Difference of $P<0.05$were considered significant.

3. RESULTS

Total phenolic content

The total phenol contents of Jerusalem artichoke extracts from five provenances are shown in Figure 1. There were significant differences ($p < 0.05$) in total phenolic contents between these different provenances varying from 502.36 to 2640.15 µ soft / 100g FW. The highest content of total phenolic was found in the ecotype 4 (2640.15 µ soft / 100g FW).

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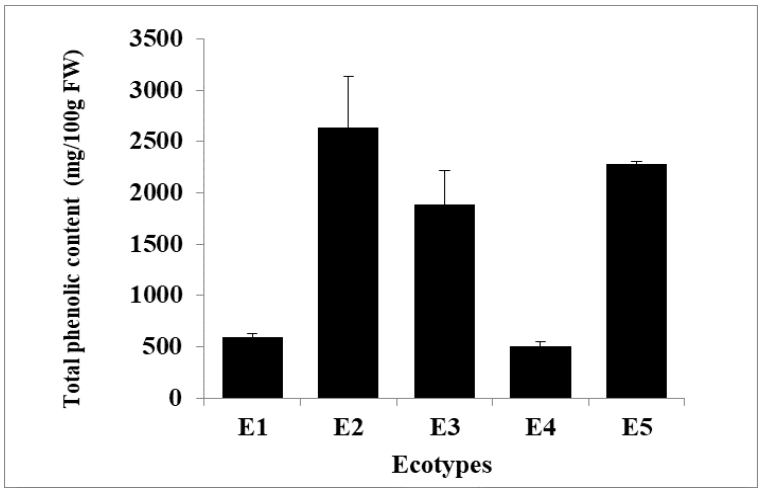

Figure 1. Total phenolic content of Jerusalem artichoke ecotype.

Values are mean of three replicates.

Antioxidant activity (Capacity of inhibition of free radical DPPH)

Antioxidant activity of the extracts of five Jerusalem artichokes ecotypes are shown in figure 2. Results showed that Jerusalem artichokes extracts from the different provenances had different Capacity of inhibition of free radical DPPH. Ecotype 4, is characterized by the highest antioxidant activity TEAC=3,62 µmol/g FW. Ecotypes 1, 2, 3 and 5 recorded respectively (2,32); (1,50); (1,55) and (2,79 µmol /g FW). Indeed, a positive correlation between the total phenols content and the antioxidant activity was recorded for all the ecotypes.

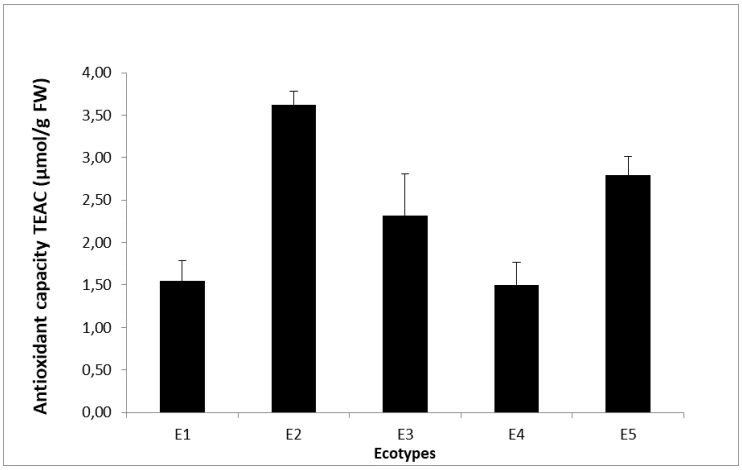

Figure 2. Antioxidant capacity of different phenolic extracts of five Jerusalem artichoke ecotypes (DPPH free radical inhibition capacity). Values are mean of three replicates.

Separation and identification of the phenolic compounds by HPLC

The results of phenolic identification compounds by HPLC showed a heterogeneity between the various ecotypes in regard to this composition. Indeed, thirteen made up was identified by

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comparison of UV and time of retention with compounds of reference. There are some peaks which remain to be identified by more precise means. Identified compounds are: Flavonol, chalcone, carnosoic acid, flavonose, genestein, uteolin, warfarin, flavone, gallic acid, 5,7dihydroxyflavone, naringenin 7-O-glucoside, trans cinnamic.

Ecotype 4 has the most diverse phenol composition with the majority of phenolic compounds, which is correlated with its polyphenol content and antioxidant activity relative to other ecotypes. Carnosoic acid is the common phenolic compound of all ecotypes with a higher amount based on peak air (221) for ecotype 5.

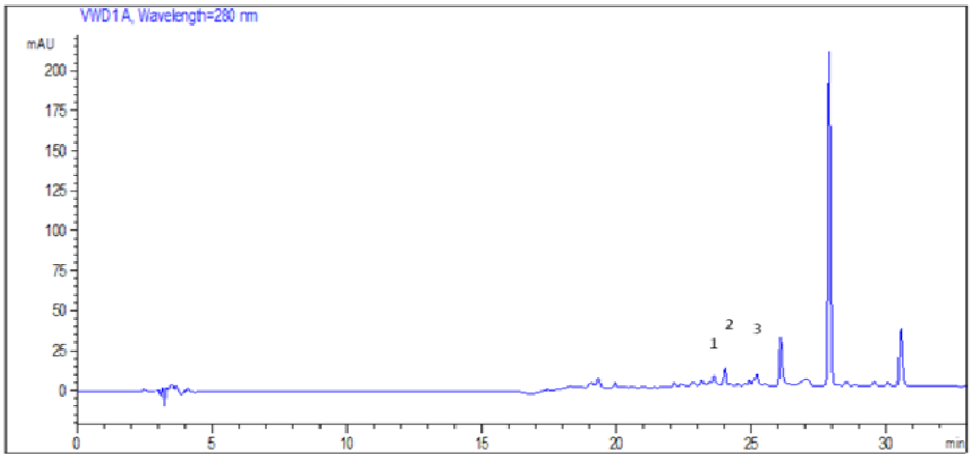

Figure 3. Chromatogram of Jerusalem artichoke tuber phenolic extract from ecotype 1: flavonol (1), Chalcone (2) and carnosic acid (3)

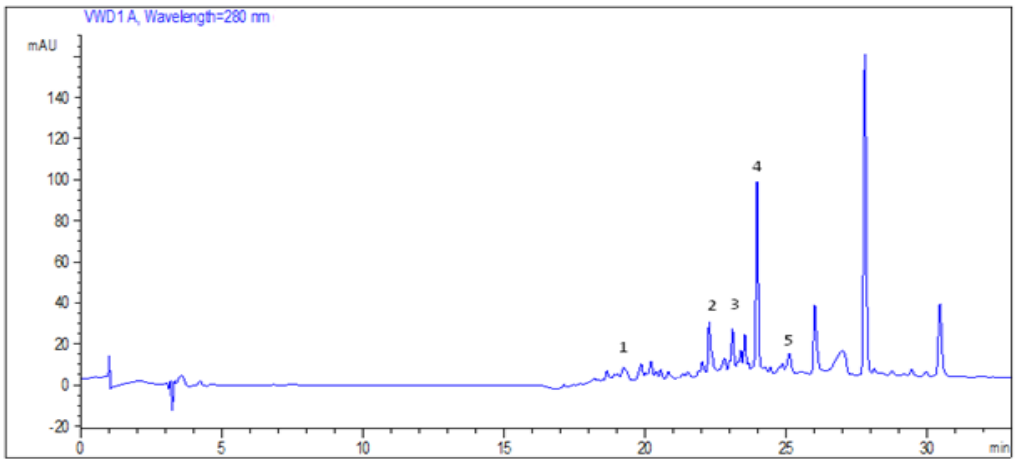

Figure 4. Chromatogram of Jerusalem artichoke tuber phenolic extract from ecotype 2: flavonol (1), Chalcone (2) and carnosic acid (3)

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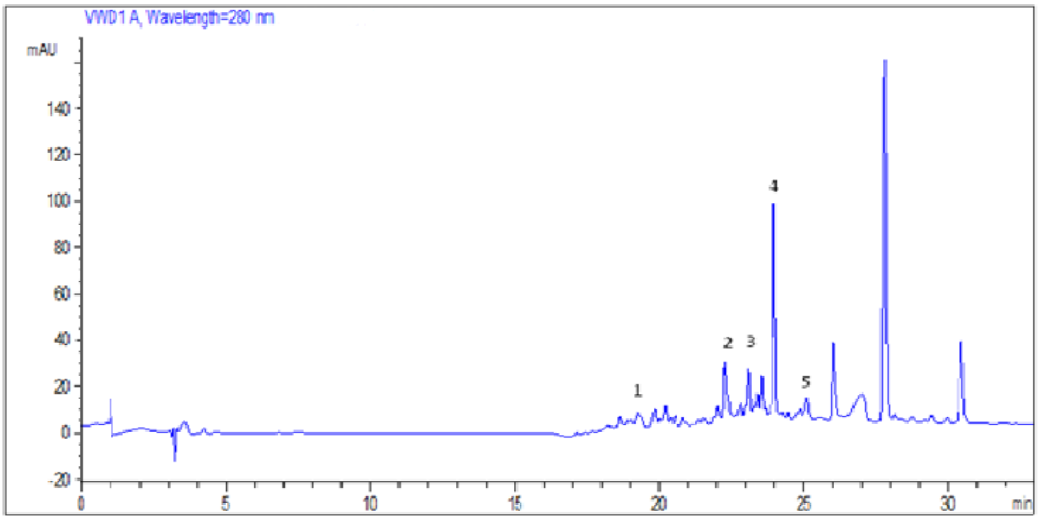

Figure 5. Chromatogram of Jerusalem artichoke tuber phenolic extract from ecotype 3: Uteolin (1), Warfarin (2), 5,7dihydroxyflavone (3), flavonose (4) and carnosic acid (5)

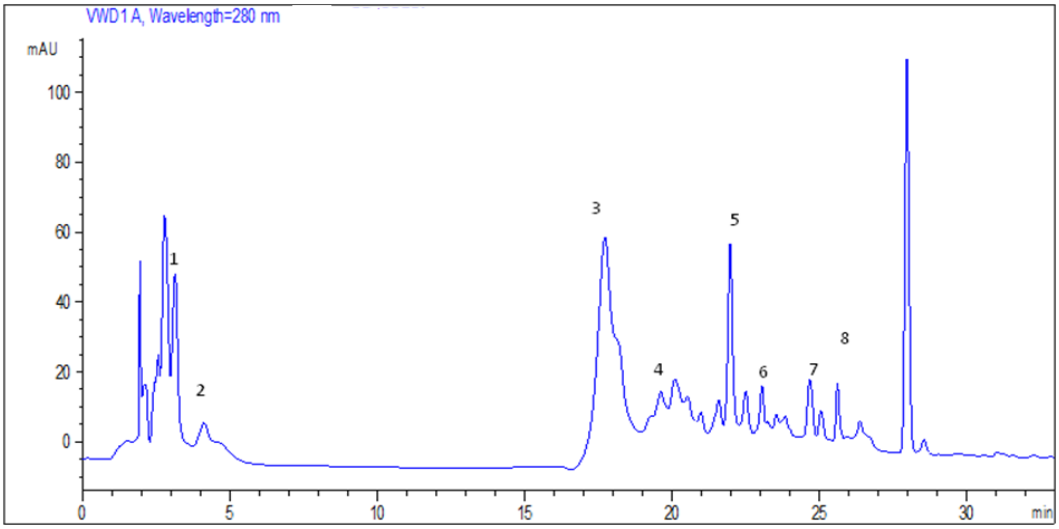

Figure 6. Chromatogram of Jerusalem artichoke tuber phenolic extract from ecotype 4: gallic acid (1), procatechic acid (2), naringenin-7-O-glucoside (3), trans cinnamic acid (4), Warfarin (5) , 5,7dihydroxyflavone (6), chalcone (7) and carnosic acid (8).

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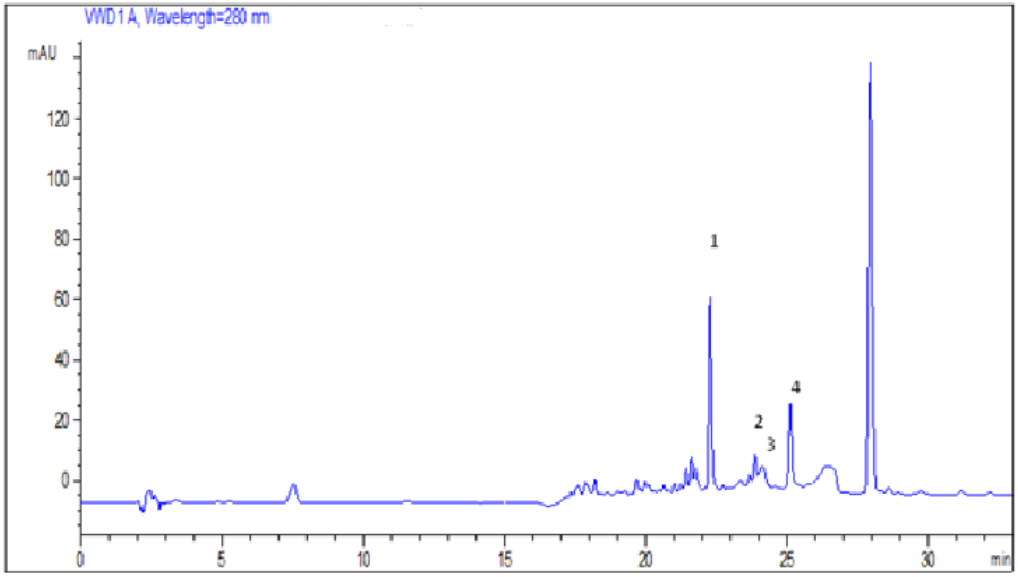

Figure 7. Chromatogram of Jerusalem artichoke tuber phenolic extract from ecotype 5: warfarin (1), flavonose (2), chalcone (3) and carnosic acid.

Table1. The main phenolic compounds in the tubers extracts of Jerusalem artichoke and their availability in the different ecotypes.

Ecotypes	**E3**	**E4**	**E1**	**E2**	**E5**
Phenolic compounds					
Flavonol	-	-	+	-	-
Chalcone	-	+	+	+	+
Carnosoic acid	+	+	+	+	+
Flavonose	+	-	-	+	+
Genestein	-	-	-	+	-
Uteolin	+	-	-	-	-
Warfarin	+	+	-	-	+
Flavone	+	-	-	+	-
Gallic acid	-	+	-	-	-

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Naringenin-7-0-glucoside	-	+	-	-	-

(+) Present ;(-) Absent

4. DISCUSSION

Since about fifteen years, the search for natural antioxydants or extracts with antioxidant capacity caused much interest. Thus a whole series of plants had reviewed, and in particular the spices. Many compounds responsible for the antioxydant capacity were identified. They are especially phenols and polyphenols

Concerning the antioxydant capacity of Jerusalem artichokes, our results were based on the inhibition capacity of free radical DPPH. Indeed, the estimate of inhibition capacity of free radical DPPH by extract phenolic from Jerusalem artichokes is a suitable model of the antioxydant capacity estimation (Sanchez-Moreno, 2002). The antioxydant activities of the extracts of plant are closely dependent has their polyphenol contents (Ratheeet *et al*., 2007). It explains the important correlation between the quantity out of polyphenols and the antioxydant capacity of the various extracts, in our study (R^2=0.96037). These results agree with those of Yuan *et al.* (2012) and Fujia *et al.* (2013). The antioxidant potency and the content of the most important total phenols have been recorded in ecotype 4, but these remain lower than those recorded in other research. Indeed, Naczk *et al*. (2003) showed that Jerusalem artichoke leaves are richer in polyphenols and have a higher antioxidant value than tubers. Yuan *et al*. (2012) reported very high antioxidant potency in leaves of Jerusalem artichokes (304.74 mg/ml) with high polyphenols content (266.69 mg/g). The identification of phenolic compounds by HPLC of the tubers extracts has shown us that the composition in phenols is different from that of the leaves reported by (Lee *et al*., 2010; Peng *et al*., 2000; Tolonen et al., 2002; Yuan et al., 2012).Yuan et al. (2012) identified six phenolic compounds in Helianthus tuberosus leaf extracts: 3- O-caffeoylquinic acid (3-CQA), caffeic acid (2), 3,5-dicaffeoylquinic acid (3,5-DiCQA, 3), 1,5- dicaffeoylquinic acid (1,5-DiCQA, 4), 4,5-dicaffeoylquinic acid (4,5- DiCQA, 5) and 3,5-

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dicaffeoylquinic acid ether. As for our results for tuber extracts, ten main compounds were identified with heterogeneity between the ecotypes and which are totally different to the ecotypes (Table1).

5. CONCLUSIONS

This study revealed a significant variation in the biochemical composition of Tunisian Jerusalem artichoke ecotypes. Ecotype 4 has the most diverse phenol composition in which most of the phenolic compounds are found, which correlates with its polyphenol content and its antioxidant capacity compared to other ecotypes. The presence of carnosic acid in the extracts of Jerusalem artichoke tubers can put the species as an object of industrial development to enhance its composition especially that despite the diversity of plants that have an excellent ability to inhibit oxidative reactions, only rosemary has been the subject of industrial development. In addition, the presence of warfarin or coumaphene in both E3 and E4 ecotypes suggests the use of extracts of both ecotypes as bio-insecticides and also in pharmaceutical uses, although coumaphene-based drugs have an anticoagulant effect.

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