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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT PROPERTIES OF LEAVES EXTRACTS FROM *POSIDONIA OCEANICA* (L.) DELILE AND THEIR ALLELOPATHIC POTENTIAL ON TERRESTRIAL PLANT SPECIES

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ABSTRACT. Dichloromethane, chloroform, ethyl acetate and ethanol extracts obtained from *Posidonia oceanica* (L.) leaves, were examined in order to determine their total phenolic, flavonoid, flavonol, anthocyanin and condensed tannins contents as well as their antioxidant and allelopathic activities. The qualitative phytochemical analysis of crude extracts confirmed the presence of coumarins in dichloromethane, chloroform and ethyl acetate extracts. The antioxidant activity estimated using the DPPH assay was significantly more pronounced for the ethyl acetate extract (IC_{50} = 1.19±0.018 mg/mL) than that of the other extracts. The allelopathic effect against the seeds of *Carum carvi* (L.) and *Foeniculum vulgare* (Mill.) indicated that, depending on concentration, the extracts from the leaves of *P. oceanic* (L.) inhibited or stimulated at different concentrations the germination, shoot and root elongation of seedlings growth. The results of this study suggest that *P. oceanica* (L.) extracts could be useful as a natural source of health-promoting effects and herbicides.

KEY WORDS: *Posidonia oceanic* (L.), Phytochemical profile, Phenolic compounds, Antioxidant activity, *Carum carvi* (L.), *Foeniculum vulgare* (Mill)

INTRODUCTION

Marine species have been considered as approximately half of the total biodiversity and a source of potentially active novel natural products [1]. The seagrass *Posidonia oceanica* (L.) Delile represents one of the most widespread species endemic to the Mediterranean Sea. *P. oceanica* plant has leaf bundles consisting of probably seven leaves 8–11 mm broad and 20–80 cm long. Rhizomes have a thickness varying between 5 and 10 mm, they are carrying out the roots which attach the plant to the substratum and allow the absorption of nutrients from the sediment [2]. The leaves of *P. oceanica* (L.) have been tested by humans for various traditional applications. For instance, they were used as a material for manufacturing mattresses. Apart from these benefits, the dead leaves of *P. oceanica* (L.) were also used a long time ago, as compost by farmers on the Mediterranean coasts. Over the past fifty years, experiments have been carried out regularly in Italy, Tunisia, and Greece with a view to produce *Posidonia*-based compost [3].

In this context, fresh leaves have good nutritional value. In Italy, for instance, the use of leaves powder as an additive to poultry feed has improved egg production and their weights [4]. In Tunisia, around 1920, attempts to feed livestock on leaves, mixed with fodder, had a controversial success. This species, biosynthesizes polyphenol compounds, having an important role in protecting plants against competitors, predators and pathogens [5]. In folk medicine, the seagrass has been used by Egyptians to treat skin diseases [6]. Previous phytochemical studies showed that the profile of secondary metabolites of leaves is mainly characterized by phenolic derivatives. Chicoric acid is reported as a major constituent of the leaves [7]. Other phenolic constituents, such as vanillic aldehyde, *p*-coumaric and cinnamic acids, are also abundant in *P.*

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oceanica (L.) species [8]. The main flavonoids are proanthocyanidins [9], quercetin, isorhamnetin [10] and phytosteroids such as stigmasterol [11].

Studies performed on *P. oceanica* (L.) leaves have recently demonstrated their vasoprotector effects, antidiabetic and antioxidant potential [12]. Hammami et *al*. indicated that *P. oceanica* (L.) leaves possess antibacterial activities against various pathogenic bacteria [2]. In addition, *P. oceanic* (L.) has several antifungal activities against certain dermatophytes and yeasts [13]; antileishmanial [14]; and cytotoxic activities [15].

Phenolics compounds are antioxidants with redox properties, allowing them to act as hydrogen donors and singlet oxygen quenchers. In recent years, there is a wide interest in extracting phytochemicals from natural sources that could replace toxic synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used in food industry [16]. Antioxidants such as flavonoids, phenolics, flavonols, proanthocyanidins, and anthocyanins are found in various plant products [17]. For this reason, there is a growing interest in separating these plant antioxidants and using them as natural antioxidants.

Several plants also exhibit allelopathic potential [18, 19] because they biosynthesize a great variety of substances generically named allelochemicals, such as alkaloids, flavonoids, steroids, tannins, phenolic derivatives and coumarins [20-23]. When released into the environment, these chemicals can positively or negatively affect the lives of other coexisting plants [24]. The allelochemicals affect plant growth and development and can be employed successfully against pathogens to reduce weed and enhance yield in crops [25]. The present study was conducted to assess the polyphenols, flavonoids, flavonols, proanthocyanidins, anthocyanins and coumarins contents in different extracts of Tunisian *P. oceanic* (L.) leaves; and to investigate their antioxidant and allelopathic properties.

EXPERIMENTAL

Chemicals and reagents

Analytical grade ethanol, ethyl acetate, chloroform, dichloromethane and methanol were acquired from Merck Life Science (Merck KGaA, Darmstadt, Germany). The reagents used in the contents assay were Folin-Ciocalteu, Na_2CO_3, gallic acid, aluminum chloride, NaOH, quercetin, $NaNO_2$, vanillin, catechin and HCl, obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). DPPH was purchased from Merck Life Science (Merck KGaA, Darmstadt, Germany).

Plant material

Leaves of *P. oceanic* (L.) were harvested from Monastir (Tunisia) in March 2017. A voucher specimen (FSM-PC-Mon 2017) was deposited at the herbarium of the Faculty of Sciences of Monastir, Tunisia. The leaves were dried in shade during ten days and then ground using an electric Moulinex type crusher. The resulting powder was stored in hermetically sealed glass vials until use.

Preparation of extracts

Samples of 100 g of *P. oceanic* powdered dead leaves were successively extracted under reflux using different solvents of increasing polarity: dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), ethyl acetate ($C_4H_8O_2$) and ethanol (C_2H_5OH), during a time of 90 min for each solvent, considering the corresponding boiling temperatures. The resulting solutions were filtered through a Whatman filter paper No. 2, then evaporated under vacuum using a rotavapor (Heidolph VV2000), to obtain four crude extracts symbolized E_1, E_2, E_3 and E_4.

Phytochemical analysis of organic extracts

Estimation of total polyphenols contents (TPC). The polyphenols contents were determined according to the Folin-Ciocalteu method [26]: a portion of 100 µL of each extract (1 mg mL^{-1}) was mixed with 500 µL of the FC reagent. Five minutes later, 400 µL of Na_2CO_3 at 7.5% were added. The mixture thus obtained was incubated for 1 h, and the absorbance was measured at 765 nm using a UV/Visible spectrophotometer. The total phenolics contents were deduced using a standard calibration curve at different concentrations of gallic acid; $y = 0.168x + 0.122$; $R^2 = 0.994$. The polyphenols contents of the extracts tested are expressed as mg of gallic acid equivalent (GAE) per g DW.

Determination of total flavonoids contents (TFC). The total flavonoids contents of the extracts were determined on the basis of the aluminum chloride colorimetric method [27]. 500 µL extract (1 mg.mL^{-1}) was mixed with 1 mL $NaNO_2$ (5%). After standing for 6 min, 1 mL of 10% $AlCl_3$ and 10 mL of NaOH (1 M) were added to the mixture. Absorbance was measured at 510 nm, using a UV/Vis spectrophotometer. Total flavonoids contents was calculated as quercetin equivalent (mg QE/g DW) using the equation obtained from the curve; $y = 0.296x + 0.024$; $R^2 = 0.968$, where x is the absorbance and y is the quercetin equivalent. The values were determined after triplicate analyses.

Total flavonols contents (TF). Total flavonols content was determined using the method described by Mbaebie *et al.* [28]. A volume of 2 mL of the plant extract (at a concentration of 1 mg mL^{-1}), was mixed with 2 mL of 10% aluminum chloride ethanolic solution and 3 mL of 50 g L^{-1} of sodium acetate solution. The mixture was incubated at 20 °C for 2.5 h, after which the absorption was measured at 440 nm. Total flavonols contents were calculated as quercetin (mg QE/g DW) using the following equation of the curve $y = 4.454x + 0.167$; $R^2 = 0.971$, where x is the absorbance and y is the quercetin equivalent.

Determination of condensed tannins contents (proanthocyanidin) (CTC). Total proanthocyanidins contents were determined using the vanillin assay [29]. A mixture of 3 mL of vanillin-ethanol solution (4%) with 1.5 mL of hydrochloric acid was added to 0.5 mL of each tested extract (at a concentration of 1 mg.mL^{-1}). The resulting solutions were incubated at 30 °C during 15 min. The absorbance of each solution was measured at 500 nm. Total proanthocyanidins contents were expressed as mg of catechin equivalents per g DW (mg CE/g DW) using the following equation of the curve $y = 0.967x + 0.046$; $R^2 = 0.969$, where x is the absorbance and y is the catechin equivalent.

The anthocyanins contents assay. The anthocyanins contents of the extracts were analyzed according to the method described by Chung *et al.* [30]. The extracts were mixed with acidified methanol (1% HCl/methanol) for 2 h at room temperature in the dark and then centrifuged at 1000×g for 15 min. The anthocyanins contents in the supernatant were measured simultaneously at 530 and 657 nm. The absorbance values at 530 and 657 nm were indicated as A_{530} and A_{657}, respectively. The extinction coefficient of 31.6 M^{-1} cm^{-1} was used to convert absorbance values into concentrations of anthocyanins. The concentrations were calculated using the following equation:

Anthocyanin content (µmole/g) = $[(A_{530} - 0.33 \times A_{657})/31.6] \times$ [volume (mL)/weight (g)].

Test for coumarins. Test for coumarins (NaOH test): 1 mLof extract was mixed with 1.5 mL of 10% NaOH. The chemical reaction produced a yellow color, indicating the presence of coumarins [31].

Antioxidant activity evaluation

The anti-radical activities of the extracts E_1, E_2, E_3 and E_4 against $DPPH^{\bullet}$, were evaluated using a colorimetric method described by Molyneux and Songklanakarin [32]. Each sample at different concentrations (2, 1, 0.5, 0.25 and 0.125 $mgmL^{-1}$ in ethanol) was mixed with 0.5 mL of $DPPH^{\bullet}$ (0.1 mM in ethanol). The solutions were incubated for 30 min at room temperature. The absorbance of each sample at different concentrations was noted at 517 nm. The inhibition percentage of the $DPPH^{\bullet}$ radical by the tested sample was calculated according to the equation of Yen and Duh [33]:

$$\% I = 100 \times [(Ac - As) / Ac]$$

where %I is the $DPPH^{\bullet}$ inhibition percentage; Ac and As are the absorbance values of the control and the test sample, respectively. $DPPH^{\bullet}$ scavenging activity is presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of $DPPH^{\bullet}$ present in the test solution. Quercetin was used as standard. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

Allelopathic potential

The inhibitory potential of *P. oceanic* extracts on the seed germination, the hypocotyls and root lengths of *Foeniculum vulgare* (Mill.) (Fennel) and *Carum carvi* (L.) (Carvi) seeds were investigated. Extracts were dissolved in methanol to compare their phytotoxic effects. 5 mL of each extract dissolved at different concentrations (1, 0.5, 0.25, 0.125 and 0.06 mg mL^{-1}) were added in sterile Petri dishes (9 cm diameter) lined with double-sterile filter paper (Whatman No. 2). The dish-filter paper solution was kept to evaporate in the air for 24 h. Ten healthy seeds of *F. vulgare* (Mill.) and *C. carvi* (L.) were surfaces sterilized for 20 min in 1% NaClO before use. Then, 5 mL of distilled water was added to each Petri dish, those were sealed with parafilm to prevent water loss and stored in the dark at 25 °C for 7 days. Distilled water was used as a positive control. Three replicates for each extract solution at different concentrations were performed.

After 7 days, the germination percentages were determined: % G = (NGS/NTS) ×100; where % G: germination percentage NGS: number of germinated seeds; NTS: total number of seeds. Then, the seedlings of *F. vulgare* (Mill.) and *C. carvi* (L.) seeds were collected; the shoots (SL) and roots lengths (RL) were measured. The inhibitory or stimulatory effects were calculated using the following equation from Kordali [34] Inhibition (+)/stimulation (-) % = [(Control–sample)/control]×100; where sample (extract effect) is the parameter (%G, RL, SL) measured in the presence of *P. oceanic* extracts and control (control effect) the parameter measured in the presence of distilled water.

Statistical analysis

Each assay was done three times from the same extract in order to determine its reproducibility. Data were calculated using the software using the SPSS version 22.0 for Windows. Quantitative differences were assessed by ANOVA procedure followed by Duncan's multiple range tests. Values were expressed as means ± standard deviations (SD). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Yields and phytochemical analysis of organic extracts

The yields of *P. oceanica* different extracts (dichloromethane, chloroform, ethylacetate and ethanol) are reported in Table 1. It is apparent from this work that the highest yield was obtained

by ethanol extraction (2.16%), followed by dichloromethane (1.43%), chloroform (0.87%) and ethyl acetate (0.44%), respectively. This variation in the yields of different extracts might be ascribed to the different availability of extractable components depending on their polarities and solubility in each solvent. To divulge the importance of natural products and phytochemicals, specifically flavonoids in managing various diseases, including oxidative stress, inflammation, and cancer, this work sheds light on *P. oceanic* leaves as a potential source of flavonoids. Flavonoids are a group of naturally occurring secondary metabolites with variable phenolic structures [35], possessing potential beneficial effects on human health. They are well known for their various biological activities, which are attributed to their high antioxidant activity [36]. The aim of this study was to determine the total contents of polyphenolic compounds in crude extracts from *P. oceanic* leaves. The total phenolics, flavonoids, flavonols, anthocyanins, and proanthocyanidins bioavailability in *P. oceanic* leaves varied significantly depending on the solvents of extraction (Table 1). The ethyl acetate extract had the highest contents of polyphenols and flavonoids: (85.71±0.59 mg EAG/g DW) and (74.43±4.25 mg QE/g DW), respectively. However, the percentages were so lower in chloroform: (16.26±1.49 mg EAG/g DW) and (16.32±1.11 mg QE/g DW), respectively (Table 1). The total flavonols contents varied between 9.19±0.31 and 14.91±0.28 mg QE/g DW for all extracts, with the richest amount detected in dichloromethane (Table 1). Results presented in Table 1 show the presence of a remarkable amount of tannins in chloroform (73.94±0.02 mg CE/g DW) and dichloromethane extracts (65.4 ± 1.09 mg CE/g DW) Furthermore, anthocyanins contents in the dichloromathane extract was found to be the highest (0.88 µmole anthocyans/g crude extract) as compared with other extracts. For coumarin derivatives, dichloromethane, chloroform and ethyl acetate extracts showed positive responses. Though flavonoids are usually among the most intensely studied natural products, there are only two reports on flavonoids from *Posidonia* [37], who showed that the flavonoid derivatives: proanthocyanins, flavonols (kaempferol, quercetin, isorhamnetin, and myricetin) were identified in leaves collected from the North coast of Corsica/France.

Table 1. Yields in percent of dry matter, total phenolics (TPC), flavonoids (TFC), flavonols (FC), condensed tannins (CTC) and anthocyanins content in various extracts from *P. oceanica* (L.).

Extracts	Yield (%)	Total phenolics content TPC (GAE)[e]	Total flavonoids content TFC (QE)[e]	Total flavonols content FC (QE)[e]	Total condensed tannins CTC (CE)[e]	Anthocyanins[f]	Coumarins
(E_1)	1.43	63.9 ± 5.18[b]	59.68 ± 4.25[b]	14.91 ± 0.28[a]	65.4 ± 1.09[a]	0.88± 0.15[a]	+
(E_2)	0.87	16.26 ± 1.49[d]	16.32 ± 1.11[c]	13.85 ± 0.39[a]	73.94 ± 0.02[a]	0.6 ± 0.05[b]	+
(E_3)	0.44	85.71 ± 0.59[a]	74.43 ± 4.25[a]	9.19 ± 0.31[b]	0.00 ± 0.00[b]	0.1 ± 0.041[c]	+
(E_4)	2.16	20.02 ± 1.57[c]	16.89 ± 2.58[c]	9.34 ± 0.21[b]	0.00 ± 0.00[b]	0.41± 0.048[b]	-

[e]GAE Gallic Acid Equivalents, QE Quercetin Equivalents, CE Catechin Equivalents. [f]µmole Anthocyans/g crude extract, (+) presence, (-) absence. Means in each column followed by different letters are significantly different ($p < 0.05$), as established by Duncan's test.

Antioxidant activity

To evaluate the power of the leaves in protecting against oxidative stress, we tested the antioxidant properties of four crude extracts from the leaves of *P. oceanica* (L.) growing in Tunisia. Table 2 shows significant differences in the radical-scavenging activities of the extracts, expressed as inhibition concentration (IC_{50}), depending on the nature of the solvent. The ethyl acetate extract exhibited the best activity (IC_{50} = 1.19±0.055 mg mL^{-1}), whereas, chloroform was identified as the least active extract (3.04±0.067 mg mL^{-1}). The radical scavenging DPPH activities of all extracts from *P. oceanic* (L.) leaves, were lower than that of the synthetic antioxidant quercetin (IC_{50} = 0.047±0.00 mg mL^{-1}). Comparing the IC_{50} values, we found that the extracts with ethyl acetate (E_3), dichloromethane (E_1) are more antioxidant and it

is illustrated by: IC_{50} = 1.19 and 1.22 mg.mL^{-1}, respectively. The highest activity observed for ethyl acetate extract may be due to its richness in polyphenolic (85.71±0.59 mg EAG/g DW) and flavonoid compounds (74.43±4.25 mg QE/g DW) which possess structural features that enable them to have antioxidant activities. On the other hand, the dichloromethane (E_1) extract is rich in flavonols (14.91±0.28 mg QE/g MS), tannins (65.4±1.09 mg EC/g MS) and anthocyanins (0.88 µmol/g) these compounds are found to possess antioxidant effects [38-40].

Table 2. IC_{50} values (mg/mL) of *P. oceanic* (L.) leaves extracts.

Extracts	IC_{50} (mg mL^{-1}) ± SD[h]
Dichloromethane extract (E_1)	1.22 ± 0.061[c]
Chloroformic extract (E_2)	3.04 ± 0.067[a]
Ethyl acetate extract (E_3)	1.19 ± 0.055[c]
Ethanolic extract (E_4)	1.78 ± 0.018[b]
Quercetin	0.047 ± 0.00

Means in each column followed by different letters are significantly different ($p < 0.05$), as established by Duncan's test. [h]IC_{50}: concentration (in mg mL^{-1}) of extracts required to inhibit the formation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by 50%. Each value in the table is the mean ± standard deviation (SD) (n = 3).

Our results are comparable to previous reports which attested an important in vitro antioxidant activity of various extracts of *P. oceanic* (L.). In Tunisia the work reported by Kesraoui *et al.* [41] showed that the radical-scavenging activity of bound phenolic compounds (BPC) (IC_{50} = 10.71±0.001 µg mL^{-1}) and free phenolic derivatives (FPC) (IC_{50} = 17.63±0.09 µg mL^{-1}) extracted from fresh leaves can be considered high compared to our results (Table 2). While previous work in Italy indicated that the ethanol extract from dried leaves has antioxidant activity (IC_{50} = 6.01±0.55 mg mL^{-1}). Barletta *et al.* [42] which is less than that of ethanolic extract (IC_{50} = 1.84±0.018 mg mL^{-1}) in the present study. These differences in the antioxidant activity can be due to the difference in the qualitative and quantitative composition of extracts which depend on the geographical area of growth and the period of harvest.

Allelopathic potential

The phytotoxic effects of *P. oceanic* dried leaves crude extracts (dichloromethane, chloroform, ethyl acetate and ethanol) at five different concentrations are summarized in Tables 3 and 4. Indeed, the allelopathic effects were significantly different ($p < 0.05$) (Tables 3 and 4). The results of the present study showed a varying degree of inhibitory and stimulatory effects of different extracts on seed germination, root length and shoot length. The inhibition percentage varied based on the nature of the solvent and the concentration of the tested extract. The maximum toxicity of the four crude extracts was registered on the seventh day. The seed germination of *C. carvi* (L.) was completely inhibited by dichloromethane extracts (100%), at concentrations equal to (C_0 = 1 mg.mL^{-1}) (Table 3). The significant allelopathic effect was also observed in ethyl acetate extract (64.28% and 57.14%) at the concentrations (C_0 = 1 mg.mL^{-1} and C_1 = 0.5 mg.mL^{-1}), respectively. The inhibition of the shoot and root lengths varied from 14.12% (ethyl acetate extract at 0.25 mg mL^{-1}) to 100% (at 1 mg mL^{-1} of dichloromethane extract), and from 8.8% (ethyl acetate extract at 0.06 mg/mL) to100% (at 1 mg.mL^{-1} of dichloromethane extract), respectively. This inhibitory activity can be mainly due to toxic compounds present in the crude extracts. Indeed, the reduction in seed germination may be attributed to the reduced rate of cell division and cell elongation due to the presence of the allelochemicals. The main classes of allelochemicals (coumarins and polyphenols) are present in chloroform and ethyl acetate extracts of *P. oceanic* (Table 1). A number of various chemicals constituting the organic crude extracts are implicated in allelopathic relationships due to their phytotoxic nature. Other studies highlight extract's ability to exert a negative effect on

germination due to their richness in polyphenols and especially in tannins [43]. Polyphenols can inhibit cell division, which subsequently interferes with the normal growth and development of the whole plant [44, 45]. The natural herbicide coumarin is useful in sustainable agriculture because of its phytotoxic potential [46]. The stimulatory effect on seeds germination was observed in dichloromethane (-21.44% at 0.06 mg.mL^{-1}). With regard to seedling growth; shoots and roots growth was stimulated or inhibited by the tested extracts. The highest stimulation of hypocotyl growth was observed by dichloromethane extract (-113% at 0.25 mg.mL^{-1}), (-102% at 0.125 mg.mL^{-1}) and (-82.8% at 0.06 mg.mL^{-1}). The maximum roots stimulation was observed for dichloromethane extract (-80.3% at 0.125 mg.mL^{-1}). Previous researchers signaled that several inhibitory allelochemicals are known for their stimulatory activity on growth at low levels [47]. Moreover, polyphenolic compounds such as flavonoids (amentoflavone) have been cited as stimulators of the growth of other plants [48].

Table 3. Inhibition percentage % (I) of seed germination (G), shoot length (SL) and root length (RL) of *C. carvi* (L.) by organic extracts from *P. oceanic* (L.) dried leaves. Means in each column followed by different letters are significantly different ($p < 0.05$), as established by Duncan's test.

C.carvi(L.)				
Concentration (mg mL^{-1})	Extracts	% (I) Germination (G)	% (I) Shoot length (SL)	% (I) Root length (RL)
1	E_1	100^{a}	100^{a}	100^{a}
	E_2	42.86^{c}	31.30^{b}	63.38^{b}
	E_3	64.28^{b}	91.22^{a}	93.66^{a}
	E_4	42.86^{c}	-89.69^{c}	14.08^{c}
0.5	E_1	35.72^{a}	-14.50^{c}	51.41^{b}
	E_2	50.00^{a}	46.95^{a}	32.39^{c}
	E_3	57.14^{a}	30.53^{b}	65.49^{a}
	E_4	35.71^{a}	-79.39^{d}	-35.91^{d}
0.25	E_1	21.44^{a}	-112.98^{d}	22.18^{b}
	E_2	14.29^{a}	-69.08^{c}	23.94^{b}
	E_3	42.85^{a}	14.12^{a}	51.05^{a}
	E_4	21.43^{a}	-60.69^{b}	-26.4^{c}
0.125	E_1	21.44^{a}	-101.53^{d}	-80.28^{d}
	E_2	14.29^{a}	-32.44^{c}	-49.29^{c}
	E_3	21.43^{a}	30.15^{a}	30.28^{a}
	E_4	21.44^{a}	-5.72^{b}	-16.9^{b}
0.06	E_1	-21.44^{b}	-82.82^{c}	-49.64^{d}
	E_2	7.14^{a}	-14.50^{b}	-19.36^{c}
	E_3	14.29^{a}	30.15^{a}	8.8^{a}
	E_4	14.29^{a}	-5.17^{b}	-6.69^{b}

Moreover, the allelopathic effects of *P. oceanic* leaves on *F. vulgare* (Mill) germination and seedling growths depend on the solvent and concentration. The inhibition effects were observed in dichloromethane and chloroform extracts at different concentrations; the highest inhibition was (75.00% at 1 mg.mL^{-1}). Contrariwise the highest stimulatory effect on seeds germination was observed inethanolic extracts (-33.33% at 0.25 mg.mL^{-1}) and chloroform (-25.00% at 0.125 mg.mL^{-1}). We noticed that the *P.oceanica* leaves extracts have an important herbicidal activity on seedling growth of *F. vulgare*; the highest inhibition rate of growth shoot (95.84% of chloroform extract at 1 mg.mL^{-1}) and root (100% of ethylacetate at 1 mg mL^{-1}). The differences in allelopathic activity shown in Tables 3 and 4 can be due to the difference in the quantitative and qualitative composition of extracts. The results of the phytochemical characteristics and phytotoxic effect of the *P. oceanica* (L.) leaves extracts to support the conclusion that this species contains phytotoxic compounds that can negatively or positively affect germination and seedling growth on *C. carvi* (L.) and *F. vulgare* (Mill.) seeds.

Table 4. Inhibition percentage % (I) of seed germination (G), shoot length (SL) and root length (RL) of *C. carvi* (L.) by organic extracts from *P. oceanic* (L.) dried leaves. Means in each column followed by different letters are significantly different ($p < 0.05$), as established by Duncan's test.

F. vulgare (Mill.)				
Concentration (mg mL^{-1})	Extracts	% (I) Germination (G)	% (I) Shoot length (SL)	% (I) Root length (RL)
1	E_1	75.00^{a}	88.70^{b}	90.33^{c}
	E_2	75.00^{a}	95.84^{a}	95.07^{b}
	E_3	25.00^{b}	82.73^{c}	100.00^{a}
	E_4	41.66^{ab}	87.53^{b}	97.29^{ab}
0,5	E_1	75.00^{a}	77.01^{bc}	77.67^{c}
	E_2	66.67^{a}	93.51^{a}	95.7^{b}
	E_3	33.33^{b}	78.96^{b}	100.00^{a}
	E_4	41.66^{b}	76.36^{c}	95.07^{b}
0,25	E_1	41.66^{a}	70.78^{b}	64.67^{b}
	E_2	25.00^{a}	40.13^{c}	87.29^{a}
	E_3	$33,33^{a}$	69.35^{b}	91.23^{a}
	E_4	$-33,33^{b}$	85.97^{a}	90.49^{a}
0,125	E_1	$33,33^{a}$	64.41^{b}	54.66^{b}
	E_2	-25.00^{b}	14.28^{c}	87.29^{a}
	E_3	25.00^{a}	66.49^{b}	95.07^{a}
	E_4	-16.67^{b}	71.56^{a}	90.49^{a}
0,06	E_1	25.00^{a}	52.34^{a}	14.00^{c}
	E_2	-8.33^{a}	7.14^{c}	76.05^{a}
	E_3	8.33^{a}	15.58^{b}	66.37^{ab}
	E_4	8.33^{a}	58.18^{a}	57.75^{b}

CONCLUSION

This study investigates the global chemical composition and antioxidant and allelopathic activities of various crude extracts from the leaves of *Posidonia oceanica*. Our results clearly showed that the ethylacetate extract, with the highest antioxidant capacity (IC_{50} = 1.19±0.33 mg mL^{-1}) and important levels of polyphenols and flavonoids contents is presumed to significantly contribute to anticioxidant activity. Thus, *P. oceanic* ethylacetate extract may serve as a valuable source of natural antioxidants. Further studies should be directed to identify its medicinally active components in order to prepare natural pharmaceutical products of high value. Furthermore, the high herbicidal activity of *P. oceanic* extracts on seedling growth of *F. vulgare* (Mill.) and *C. carvi* (L.), reinforces the possible incorporation as a bio-herbicide for weed management and control. Obviously, long-term field studies and further phytotoxic assays are needed to support these suggestions.

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