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ORIGINAL ARTICLE

Contribution to the study of the chemical variability of the essential oils of the seeds and the stems in some populations of *Pituranthos tortuosus* (Coss.) Maire from Tunisia

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Abstract

Purpose The aim of this study was to investigate the variation of the chemical composition of the seeds and stems essential oils of *Pituranthos tortuosus* (Coss.) Maire collected from three regions of Tunisia (Bousaid (Sfax), Rgeb (Sidi Bouzid) and Majoura (Gafsa)).

Method The seeds and the stems were hydrodistillated during 4 h by using a Clevenger-type apparatus. The obtained essential oils were analyzed by GC and GC-EI-MS.

Results The chromatographic analysis indicated the detection of sixty-seven components accounting from 97.5% to 99.7% of the whole of each essential oil with a predominance of monoterpene hydrocarbons (from 38.2% to 66.1%). So, a strong predominance of the monoterpene fraction was observed in the seeds (75.8%; 60.6%; 90.8%) and the stems (89.7%; 91.7%; 89.7%) essential oil of *P. tortuosus* collected from Majoura, Bousaid and Rgeb, respectively. However, sabinene (19.4%; 11.8%; 24.7%) and terpinen-4-ol (16.9%; 22.9%; 19.2%) were the most abundant compounds of the essential oil of seeds and stems of Rgeb as well as the essential oil of the stems of Majoura, respectively, whereas the major components of the seeds of *P. tortuosus* collected from Majoura were limonene (25.1%), sabinene (18.6%) and apiol (13.4%). The stems essential oils of Bousaid were dominated by terpinen-4-ol (32.3%), sabinene (23.6%) and *p*-cymene (10.9%) while dillapiole (34.7%), limonene (14.3%), sabinene (12.6%) and terpinen-4-ol (10.4%) were the main components of the essential oil of the seeds collected from Bousaid.

Conclusion Based on the main compounds, the essential oils of seeds and stems of *P. tortuosus* from the three regions of Tunisia displayed different chemical compositions.

Keywords *Pituranthos tortuosus* · Essential oil · Location · Seeds · Stems

1 Introduction

For a long time, medicinal and aromatic plants were used for their beneficial properties to human health. They were the major sources of drugs since they contain bioactive compounds with several pharmacological effects. Actually, some biological activities have been reported for several extracts or essential oils of these plants, such as antioxidant activity [1]. In this context, various studies have shown that essential oils of several plants (thyme, clove, cinnamon, basil, eucalyptus, and chamomile) are ideally natural sources of antioxidants as it is described by Shaaban et al. [2]. Medicinal and aromatic plants can also be used as food or condiment for the preparation of traditional food recipes [3]. In fact, many essential oils or compounds from these plants have shown interesting nutraceutical properties. Regarding

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these properties, some essential oils (rosemary, oregano, and thyme) may be used as potential agents for applications to different food systems (meat, dairy, and dressing) as well as pharmaceutical industries [4].

Tunisia, a North African country, was characterized by a greatest diversity of medicinal and aromatic plants as it has more than 500 species of plants and a total of 2,163 varieties [5]. Among them, the genus *Pituranthos* (Apiaceae) includes about 20 species spread throughout North Africa [6, 7]. In Tunisia, three species (*P. chloranthus, P. scoparius* and *P. tortuosus*) were mentioned by Pottier Alapetite [8]. One of them is *Pituranthos tortuosus* (Coss.) Maire known in Arabic as ''Guezzah''. It grows spontaneously in central and southern Tunisia [9]. This small aromatic plant is used in traditional medicine as an anti-asthmatic and against scorpion stings [10].

Previous works were carried out not only on several phytochemical investigations of *Pituranthos tortuosus* such as the isolation and the characterization of ester and furocoumarins from the roots of Egyptian *Pituranthos tortuosus* [11] but also on the biological activities of many extracts of Tunisian *Pituranthos tortuosus* which were assessed distinctively through the antifungal, larvicidal and insecticidal properties of the essential oil of this species [12] as well as its anticancer effects [13, 14].

Although many studies were done to investigate the chemical composition of aerial parts of *P. tortuosus* essential oil growing wild in Egypt [15–17] and Tunisia [13, 18, 19], to the best of our knowledge, the literature data show that only one study has been carried out on the chemical variability of essential oil of the Tunisian species depending on the different plant parts (stems, flowers and seeds) [20]. In fact, the chemical composition of essential oils from aerial parts of *P. tortuosus* grown in some regions of Egypt has shown a large chemical variability even in their main compounds (β-myrcene, sabinene, trans-iso-elemicin and terpinen-4-ol [15]; dillapiole [16]; camphene [17]). In Tunisia, the chemical variation of *P. tortuosus* essential oils have essentially been considered the effect of the geographical locations. However, sabinene, limonene, myrtenol and terpinen-4-ol were the most abundant constituents in essential oils of aerial parts of *P. tortuosus* collected from central Tunisia [18]. Along the Tunisia coast, the chemical composition of essential oil of *P. tortuosus* aerial parts was characterized by the dominance of sabinene, α-pinene, limonene and terpinen-4-ol [13]. In the southern part of Tunisia (Medenine), the main compounds of the essential oil of the aerial parts of *P. tortuosus* were sabinene and myrcene [19].

More recently, Aloui et al. [20] reported that the flowers and seeds essential oils of *P. tortuosus* collected from Boughrara region (South-East of Tunisia) were characterized by the predominance of dillapiole, limonene, sabinene and 3-n-butylphtalide, while sabinene, dillapiole, terpinen-4-ol and *p*-cymene were found to be the major compounds in the stems essential oils at the flowering stage. Furthermore, the same authors [20] showed that the stems essential oils were dominated by sabinene, limonene, p-cymene, and terpinen-4-ol at the fruiting stage.

In continuous of our work on the study of the chemical variability of essential oil of different organs of *P. tortuosus* growing wild in the south-east of Tunisia as described by Aloui et al. [20], the present study aimed to determine the chemical composition of the stems and seeds of the essential oils of *P. tortuosus* collected from three regions of Tunisia (Gafsa, Sfax and Sidi Bouzid) in order to highlight a wider chemical variability of the essential oils of this plant.

2 Material and methods

2.1 Plant material

The stems and seeds of *Pituranthos tortuosus* were collected during the fruiting stage (Juin 2019) from three regions of Tunisia (Fig. 1): Majoura (34°36′31′′N, 9°13′29′′E, at 500 m altitude, South-west of Tunisia, Gafsa) and Bousaid (34°12′53′′N, 9°59′46′′E, at 168 m altitude, South-east of

Fig. 1 Geographical mapping of the three collection regions of seeds and stems of *Pituranthos tortuosus* (Majoura, Bousaid and Rgeb)

Tunisia, Sfax). They were characterized by a lower arid climate (150 mm of rain/year) while Rgeb (34°59′10′′N, 9°49′00′′E, at 333 m altitude, Central of Tunisia, Sidi Bouzid) was known by an upper arid climate (250 mm of rain/year). The plant was identified according to the flora of Tunisia [9].

2.2 Essential oil extraction

The plant's stems and seeds were dried in the shade at the laboratory, until the constancy of the mass (for twenty days). Then, they were submitted to hydrodistillation for 4 h by using a Clevenger-type apparatus (100 g of the dry matter of each organ of the three provenances). All samples were carried out in triplicates. The essential oils were stored at 5 °C in the dark until analysis.

2.3 Essential oils analysis

2.3.1 Gas chromatography with flame ionization detection (GC/FID)

All hydrodistillated essential oils were diluted up to 5% in n-hexane HPLC grade and then injected in a GC apparatus. Gas chromatography–flame ionization detector (GC-FID) analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and connected to a flame ionization detector (FID). During the process of analysis, the oven temperature was programmed from 60 to 240 ℃ at 3 ℃/min. The injector' and the detector's temperatures were 250 and 280 °C, respectively. The samples (0.2 µl) were put in the injector at a ratio of 1:30. The carrier gas (helium, 99.995% of purity) moved along the column at an output of 1 ml/min. The retention indices (RI) were calculated for separate compounds relative to C_8–C_{25} n-alkanes mixture [21].

2.3.2 Gas chromatography-electron impact-mass spectrometry analysis (GC-EI-MS)

The isolated essential oils were also analyzed by gas chromatography coupled with electron impact-mass spectrometry (GC-EI-MS) using a Varian CP-3800 gas chromatograph. The fused DB-5 capillary column (the same of that used in the GC-FID analysis) was connected to a Varian Saturn 2000 ion trap mass detector. The oven temperature was programmed from 60 to 240 ℃ at 3˚C/min. The injector and the transfer line temperatures were 250 and 280 °C, respectively. The essential oil volume was 0.2 µl (5% n-hexane HPLC grade solution) at a split ratio (1:30). The carrier gas (helium, 99.995% of purity) circulate in the column at an output of 1 ml/min. The identification of the constituents was based on the comparison of the retention times with those of the authentic samples, on comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 14 and ADAMS) and laboratory-developed library mass spectra built up from pure substances and components of known oils and MS literature data [22–25].

2.4 Statistical analysis

The experiments were realized in triplicate. One-way analysis of variance was conducted by using the SPSS software for Windows™ (version 20, IBM SPSS Statistics). Duncan's multiple range test ($p < 0.05$) was used to compare the average responses among the samples.

3 Results and discussion

3.1 Essentials oils yields

The distilled essential oils of seeds and stems of *P. tortuosus* collected from three regions of Tunisia (Bousaid (Sfax), Rgeb (Sidi Bouzid) and Majoura (Gafsa)) were found to be yellowish in which their yields were ranging from 0.09 to 0.9% as shown in Table 1. However, the results of the table indicated that the essential oil yields of seeds of Majoura (0.9%) was significantly higher ($p < 0.05$) than the other one checked in Bousaid (0.57%) and much higher in Rgeb (0.1%). Nevertheless, the oil yields of the stems of Bousaid (0.6%) was higher than obtained for the stems of Rgeb (0.1%) and slightly higher than the value registered for the stems of Majoura (0.48%). Furthermore, Table 1 showed that the yield of seeds of Majoura location (0.9%) was higher than those of the correspondent stems (0.48%) while both organs present almost the same yield of the essential oil for each provenance of Bousaid and Rgeb. By comparing the current results with those described in the literature, Aloui et al.[20] reported that the yields of essential oils of the stems (0.7%) and seeds (1.4%) of *P. tortuosus* collected from Boughrara (South East of Tunisia) was higher than the values reported for the same organs collected from all studied provenances (Bousaid, Rgeb and Majoura). These variability in essential oil yields proved that several factors such as the influence of the different plant parts as well as the geographical location had a significant effect on the changes of the essential oil yields of other plants [26, 27].

3.2 Essential oils chemical composition

The chemical composition of stems and seeds essential oils of *P.tortuosus* harvested from the three provenances: Bousaid, Rgeb and Majoura was investigated by using GC/

Table 1 Chemical composition of the seeds and stems essential oils of *P. tortuosus* collected from Majoura, Bousaid and Rgeb

N°	Conpounds†	RI‡	Gafsa: Majoura		Sfax: Bousaid		Sidi Bouzid: Rgeb	
			Seeds	Stems	Seeds	Stems	Seeds	Stems
1	Nonane	900	1.2	1.0	0.7	–	1.3	0.2
2	α-Thujene	931	1.4	2.5	1.5	2.0	2.5	1.2
3	α-Pinene	941	5.7	4.9	3.4	3.6	6.9	4.8
4	Camphene	954	0.7	–	0.4	–	–	–
5	Sabinene	976	**18.6**	**24.7**	**12.6**	**23.6**	**19.4**	**11.8**
6	β-Pinene	982	–	–	0.4	0.7	2.4	1.7
7	β-Myrcene	992	1.3	1.2	0.8	0.7	1.0	0.6
8	α-Phellandrene	1005	2.4	1.8	0.1	–	0.4	0.4
9	δ-3-Carene	1011	0.4	0.4	0.2	–	0.1	0.1
10	α-Terpinene	1018	1.4	3.8	0.5	–	2.4	2.7
11	*p*-Cymene	1027	–	**7.6**	**4.0**	**10.9**	**8.0**	**6.0**
12	Limonene	1032	**25.1**	**2.6**	**14.3**	**2.6**	**2.9**	**1.9**
13	α-Ocimene	1042	1.0	0.6	0.2	0.2	0.2	0.2
14	β-Ocimene	1052	1.1	1.1	0.2	–	0.1	0.1
15	γ-Terpinene	1062	4.6	6.9	2.1	0.4	5.2	4.7
16	*trans*-Sabinene hydrate	1070	0.7	1.0	0.8	1.6	2.3	1.4
17	α-Terpinolene	1087	2.4	3.3	1.1	–	2.5	2.0
18	*cis*-Sabinene hydrate	1100	0.5	1.0	1.1	1.6	2.4	1.5
19	*cis*-*p*-Menth-2-en-1-ol	1124	0.6	2.0	1.0	2.4	1.9	2.5
20	α-Campholenal	1125	–	–	0.1	–	0.7	1.4
21	*trans*-*p*-Menth-2,8-dien-1-ol	1128	–	–	–	–	2.1	–
22	1-Terpineol	1138	0.6	1.1	–	–	–	0.2
23	*cis*-Verbenol	1142	–	–	–	–	0.2	0.4
24	Camphor	1143	–	–	–	–	–	0.7
25	*trans*-*p*-Menth-2-en-1-ol	1144	–	–	0.5	1.6	–	3.1
26	*trans*-Verbenol	1145	–	–	–	–	1.1	1.3
27	Citronellal	1155	–	–	0.2	1.2	–	–
28	Sabinaketone	1157	–	–	–	0.4	0.7	0.7
29	Pentyl benzene	1158	–	–	–	–	0.6	–
30	β-Pinene oxide	1159	–	–	–	–	–	0.8
31	Pinocarvone	1163	–	–	–	–	0.2	0.3
32	Terpinen-4-ol	1178	**5.4**	**19.2**	**10.4**	**32.3**	**16.9**	**22.9**
33	*p*-Cymen-8-ol	1183	0.5	3.3	0.4	0.9	1.6	0.6
34	α-Thujenal	1184	–	–	–	–	–	0.2
35	α-Terpineol	1191	–	–	0.7	2.4	1.5	1.9
36	*cis*-Piperitol	1195	–	0.7	0.3	0.9	1.5	2.2
37	Decanal	1204	–	–	0.2	0.2	0.3	0.4
38	*trans*-Piperitol	1207	–	–	0.2	0.9	1.0	1.5
39	*trans*-Carveol	1218	–	–	–	–	0.1	0.2
40	*cis*-Carveol	1229	–	–	–	–	0.3	0.3
41	Cumin aldehyde	1240	–	–	–	–	0.6	0.7
42	Neral	1241	–	–	–	–	–	0.2
43	Carvacrol methyl ether	1245	–	0.8	0.3	0.8	0.7	0.8
44	(*E*)-2-Decenal	1260	–	–	0.2	–	0.5	0.6
45	n-Decanol	1272	–	–	–	1.8	–	–
46	*p*-Menth-1-en-7-al	1276	–	–	–	–	0.4	0.6
47	α-Terpinen-7-al	1284	–	–	–	–	0.4	0.8

Table 1 (continued)

N°	Conpounds†	RI‡	Gafsa: Majoura		Sfax: Bousaid		Sidi Bouzid: Rgeb	
			Seeds	Stems	Seeds	Stems	Seeds	Stems
48	Bornyl acetate	1285	1.4	–	3.1	0.7	–	–
49	*p*-Cymen-7-ol	1289	–	–	–	–	1.0	1.1
50	*cis*-2,3-Pinanediol	1316	–	–	–	0.5	–	–
51	2,3,5,6-Tetramethyl phenol	1319	–	–	–	–	0.6	4.4
52	*p*-Mentha-1,4-dien-7-ol	1333	–	–	–	–	–	0.5
53	Methyl eugenol	1403	–	–	–	0.7	0.5	1.3
54	β-Bisabolene	1509	–	–	–	–	–	0.1
55	Myristicin	1522	–	–	1.4	–	–	–
56	Croweacin	1526	0.4	–	–	–	–	–
57	Citronellyl butyrate	1530	–	–	–	–	0.1	0.2
58	Elemicin	1558	2.7	2.9	–	–	–	–
59	Spathulenol	1577	0.4	0.5	0.4	1.3	1.3	2.0
60	1-Tridecanol	1582	–	–	0.2	–	0.3	0.4
61	Globulol	1583	–	–	–	0.4	–	–
62	Carotol	1594	0.2	–	0.3	–	–	–
63	Dillapiole	1634	–	**1.9**	**34.7**	–	–	–
64	α-Eudesmol	1652	–	–	0.8	0.5	0.9	0.9
65	Apiol	1674	**13.4**	–	–	–	–	–
66	3-N-Butyl phthalide	1658	4.6	1.5	–	–	–	–
67	Butylidiene phthalide	1685	0.5	0.8	–	–	–	–
Total identified (%)			99.2	99.1	99.7	97.8	98	97.5
Yields (ml/100 g dried mass)			0.90^{a}	$0.48^{b'}$	0.57^{b}	$0.60^{a'}$	0.10^{c}	$0.09^{c'}$
Grouped compound (%)								
Monoterpenes			75.8	89.7	60.6	91.7	90.8	89.7
Monoterpene hydrocarbons			66.1	61.4	41.8	44.7	54.0	38.2
Oxygenated monoterpenes			9.7	28.3	18.8	47.0	36.8	51.5
Sesquiterpenes			0.6	0.5	1.5	2.2	2.2	3.0
Sesquiterpene hydrocarbons			–	–	–	–	–	0.1
Oxygenated sesquiterpenes			0.6	0.5	1.5	2.2	2.2	2.9
Phenyl propanoids			16.5	4.8	36.1	0.7	0.5	1.3
Others			6.3	4.1	1.5	3.2	4.5	3.5

†Compounds are listed in order of their elution from a DB-5 column and their percentages were obtained by flame ionization detector peak-area normalization

‡RI represented retention indices calculated against C_8–C_{25} n-alkanes mixture on the DB-5 column

a,b,c,a',b',c' values with the same superscript letters are non-significant at $p < 0.05$

Values in bold font represented the major compounds

FID and GC-EI-MS techniques. The percentages and the retention indices of the identified compounds of these oils are listed in Table 1 in the order of their elution on the DB-5 capillary column. Sixty-seven compounds, representing 97.5–99.7% of the total essential oil were identified and separated regarding their chemical structures into 6 classes (Table 1). As shown in Table 1, a strong predominance of the monoterpene fraction was observed in the seeds (75.8%; 60.6%; 90.8%) and the stems (89.7%; 91.7%; 89.7%) essential oil of *P. tortuosuss* collected from Majoura, Bousaid, and Rgeb, respectively. This richness in monoterpene compounds was recently reported by Aloui et al. [20] in stems (95.6%) and seeds (46.3%) of the essential oil of *P.tortuosus* collected from Boughrara (South East of Tunisia). Similarly, another study reported that the essential oils of aerial parts of *P. tortuosus* collected from central Tunisia (Monastir) at November and April months were rich in monoterpene (88.2%; 74.3%), respectively

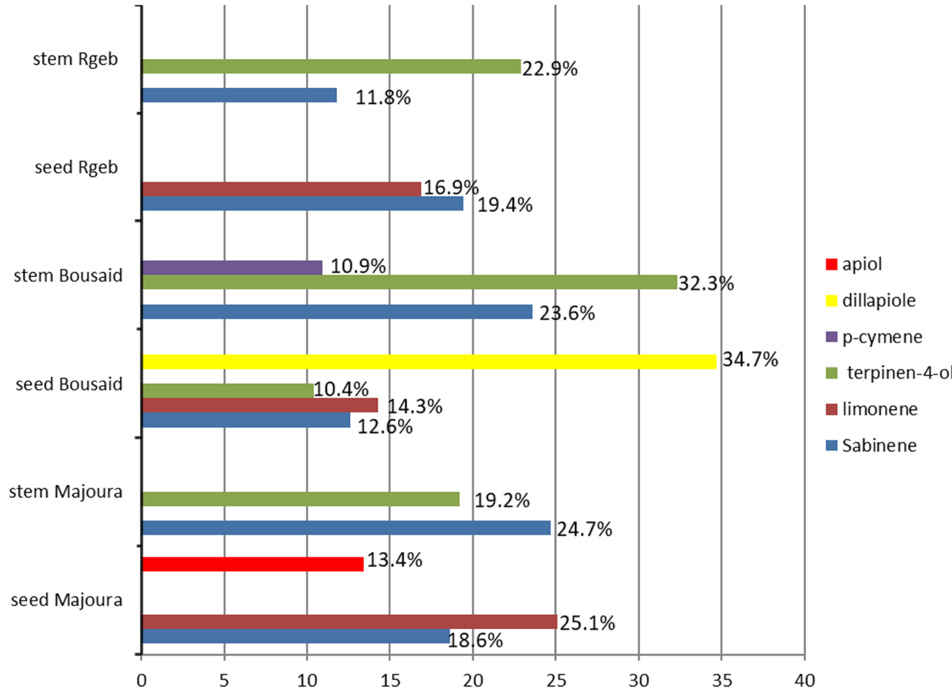

Fig. 2 Main compounds of seeds and stems essential oils of *Pituranthos tortuosus* collected from Majoura, Bousaid and Rgeb

[18]. Among the monoterpene compounds, hydrocarbon monoterpenes in seeds of Majoura constitute the higher percentage (66.1%) when compared to the other studied oils. However, the oxygenated monoterpenes of seeds of this location present the lowest percentage (9.7%) while the stems of Rgeb have the highest rate of oxygenated monoterpenes (51.5%), and the lowest rate of hydrocarbon monoterpenes (38.2%).

Among the identified compounds in the class of hydrocarbon monoterpenes, sabinene (18.6%; 12.6%; 19.4%) was found to be a major compound in the seeds essential oils of Majoura, Bousaid and Rgeb, respectively; whereas limonene (25.13%; 14.3%) was identified as the main compound only in the seeds essential oils of Majoura and Bousaid. Moreover, the predominance of sabinene (24.7%; 23.6%; 11.8%) was reported in the stem's oils of Majoura and Bousaid, Regeb, respectively. An overview of the literature demonstrated that the essential oil of stems of *P. tortuosus* collected from Boughrara at fruiting stage was also dominated by hydrocarbon monoterpenes in which sabinene (32.9%) and limonene (17.9%) were the most abundant compounds [20]. However, it is interesting to note that p-cymene (10.9%) was identified as a main compound solely in the stems of Bousaid. Compared to the literature, Aloui et al. [20] reported that this compound was found to be a major compound in the stems essential oil at the flowering (7.1%) and the fruiting stage (16.9%).

Regarding the oxygenated monoterpenes class, terpinen-4-ol was identified as the main compound in the essential oil of stems of Majoura, Bousaid and Rgeb (19.2%; 32.3%; 22.9%) as well as in the seeds of Bousaid and Rgeb (10.4%; 16.9%), respectively. These findings were in accordance with those of Aloui et al. [20]. who mentioned the presence of this compound in the stems at the flowering (10.9%) and the fruiting stage (10.7%).

In addition, Table 1 showed that the seeds of Majoura was dominated by dillapiole (34.7%) while the seeds of Bousaid contains apiol with a relatively high rate (13.4%). For a further comparison to the literature, the essential oil of aerial parts *P. tortuosus* collected from the north of Egypt was dominated by dillapiole (94.7%) [16]. Nevertheless, the chemical composition of essential oil of this plant harvested from southern Sinai of Egypt was rich in camphene (31.0%) [17].

In order to clarify the quantitative and the qualitative differences among all the studied essential oils of *P. tortuosus*, even in their major constituents, the main compounds of *P. tortuosus* seeds and stems essential oil of Majoura, Bousaid and Rgeb were represented in Fig. 2. From this data, we could be noted that there was a large variability of the chemical composition of *P. tortuosus* essential oils with respect to the different plant organs and location. In fact, as far as the main compounds are concerned, the seeds essential oils of Majoura and Bousaid were composed of limonene/sabinene/apiol and dillapiole/limonene/sabinene/terpinen-4-ol, respectively while the stems essential oil of the same provenances (Majoura and Bousaid) were represented by sabinene/terpinen-4-ol and terpinen-4-ol/sabinene/p-cymene, respectively. The decrease in the percentage of sabinene and terpinen-4-ol on the seeds essential oils of Majoura and Bousaid could be explained by the appearance of other major compound such as limonene and apiol in the seeds of Majoura as well as dillapiole and limonene in the stems of Bousaid, respectively. These changes in percentages of the main compounds between different plant organs were closely related to the metabolic and physiological changes of the plant, which are concomitant with the occurring morphological modification along its development [27]. Nevertheless, both seeds and stems of Rgeb location were represented by sabinene/terpinen-4-ol with different percentages for each organ. Therefore, the essential oil of seeds and stems of *P. tortuosus* collected from Majoura, Bousaid displayed different chemical compositions while both seeds and stems of Rgeb location exhibited similar essential oil ones.

4 Conclusion

The current study's finding highlights, for the first time, distinctive data on the chemical variability of the essential oils of the seeds and stems of *P. tortuosus* widely growing in three regions of Tunisia (Bousaid (Sfax, South-east of Tunisia), Rgeb (Sidi Bouzid, Central of Tunisia) and Majoura (Gafsa, South-west of Tunisia)). The yields of all studied essential oils were ranging from 0.09% to 0.9%. With reference to the different plant organs and location, the studied plant was characterized by a large chemical variability of volatile compounds with a strong predominance of the monoterpene fraction. Based on the major compounds, the seeds essential oils of the provenances of Majoura and Bousaid were represented by limonene/sabinene/apiol and dillapiole/limonene/sabinene/terpinen-4-ol, respectively. In contrast, the stems essential oil of the same locations (Majoura and Bousaid) were formed with sabinene/terpinen-4-ol and terpinen-4-ol/sabinene/p-cymene, respectively. Nevertheless, both seeds and stems of Rgeb location were represented by sabinene/terpinen-4-ol with different percentages for each organ. These differences among the studied essential oils composition suggest a wide range chemical variability of *P. tortuosus* growing wild in other regions of Tunisia.

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Code availability Software application.

Compliance with ethical standards

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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