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presented a higher total antioxidant capacity reaching 168.28 ± 0.01 mg α-tocopherol/g DW for the methanolic extract and 187.48 ± 0.09 mg α-tocopherol/g DW for the ethyl acetate extract. A strong positive correlation between TAC and condensed tannins (r = 0.886) was observed.

Table 4. Total antioxidant capacity (TAC) (mg GAE/g DW) and reducing power (EC50 in µg/mL), DPPH (IC_{50} in µg/mL) of Artemisia absinthium extracts.

	Bizerte	**Zaghouan**	**Kasserine**	**Gabes**	**Tozeur**	***P***
TAC (mg GAE/gDW)	35.11±0.02 1^{c}	21.38±0.01 2^{e}	33.82 ± 0.011^{d}	42.01 ±0.028^{b}	85.24± 0.069^{a}	0.000***
DPPH (IC_{50}=µg/ml)	52.828±4.77^{d}	31.459±1.41^{e}	56.014±3.68^{b}	107.659±3.47^{a}	54.630±3.23^{c}	0.000***
PR (EC50=µg/mL)	788.66±5.63^{d}	922.52±3.85^{b}	1366.45±29.59^{a}	832.67±20.38^{c}	654.05±14.51^{e}	0.000***
BHT	10.77 ± 2.98					
Ascorbic acid	37.30					

IC_{50} and EC_{50} values represent the mean of three replicates (n=3); letters (a-e) are significantly different at P < 0.05. significant at P< 0.001 %; *P:* probability.

DPPH-radical scavenging activity

DPPH radical scavenging activity is the fast technique to evaluate the antioxidant activity. The dose-dependent curve of the five wormwood extracts at different concentrations (25, 50, 1000 and 250 µg/ml) are given in Fig. 5. As can be seen in Table 4, the antiradical activity of the methanolic extracts from *A. absinthium* was highly influenced by the region factor. DPPH effect of the five wormwood extracts increased in the order of Gabes Kasserine Tozeur Bizerte Zaghouan, respectively. The methanolic extract of Zaghouan had an IC_{50} of 31.46 ± 1.42 µg/mL which was higher than that of BHT (IC_{50} = 10.77 ± 2.98 µg/mL). Msaada *et al.* [24] reported that DPPH activity of Tunisian *A. absinthium* extracts was highly affected by the region factor. In fact, the IC_{50} values varied from 9.38 ± 0.82 to 44.26 ± 1.92 µg/mL according to the sampling site. Moreover, the ethanolic extract of Romanian wormwood had a higher DPPH activity compared to our study with IC_{50} = 0.57 ± 0.05 mg/mL [51]. Mahmoudi et *al.* [45], reported an anti-radical activity lower than our results with an IC_{50} of 612 ± 30.6 µg/mL of the methanolic extract from Iranian wormwood aerial parts at the flowering stage. However, Riahi *et al.* [25] reported a DPPH scavenging power for Tunisian wormwood extract more important than that of our work (IC_{50} = 11.65 ± 0.95 µg/mL).

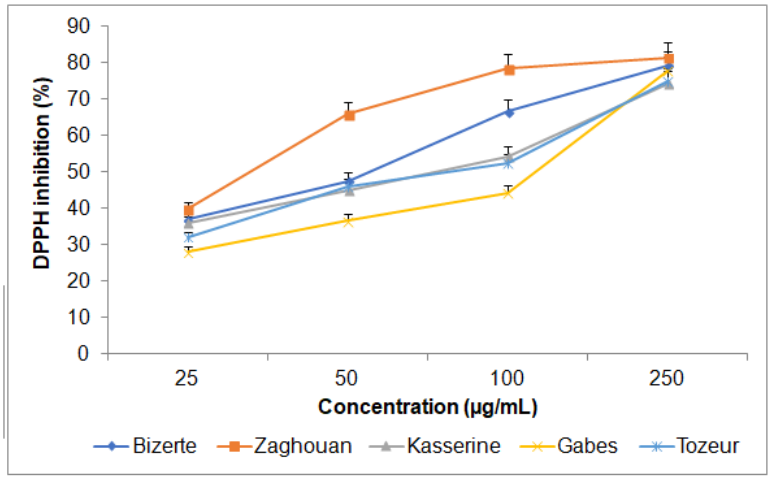

Fig. 5. DPPH radical scavenging percentage of *Artemisia absinthium* extracts from different regions.

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Ferric-reducing antioxidant power

In ferric reducing power assay, the dose-dependent curve of wormwood extracts at different concentrations (250, 500, 1000 and 1500 µg/mL) are given in Fig. 6. The ferric reducing power of wormwood extracts was significantly influenced ($P < 0.05$) by the region factor. The EC_{50} values varied from 654.05 ± 14.51 µg/mL to 1366.45 ± 29.59 µg/mL (Table 4). The aerial parts of *A. absinthium* collected from Tozeur showed the best reducing power with EC_{50} = 654.05 ± 14.51 µg/mL. The ferric reducing power of *A. absinthium* aerial parts extracts was found to increase in the order: Kasserine > Zaghouan > Gabes > Bizerte >Tozeur. A strong negative correlation between ferric reducing power assay (FRAP) and total contents of phenolic compounds (r = - 0.8618) was found. These results suggested that flavonoids played a significant role in the antioxidant activity. Msaada *et al.* [24] reported that the methanolic extracts of wormwood, growing in Boukornine, showed an important ferric reducing power with EC_{50} = 2.16 ± 0.05 µg/mL.

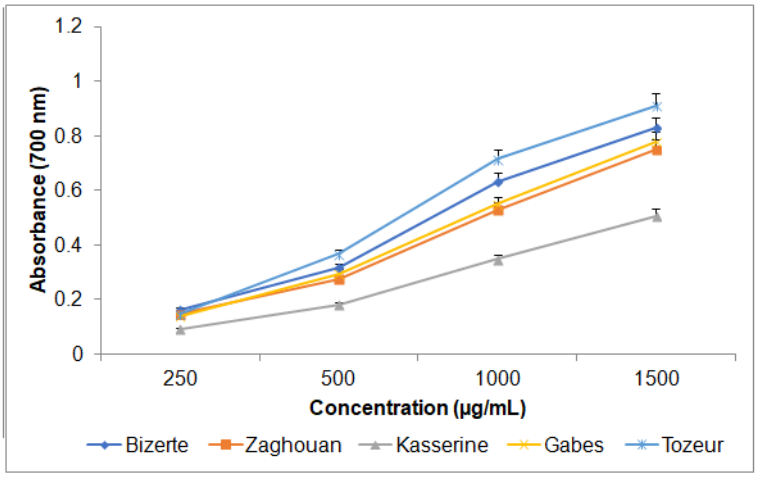

Fig. 6. Ferric reducing power activity of *Artemisia absinthium* extracts from different regions.

Antibacterial activity of the essential oil

The evaluation of antibacterial activity of the five EOs of *A. absinthium* against the different bacteria strains was carried by the agar disc-diffusion method based on the determination of the inhibition zone (IZ). As it is seen in Table 5, IZ was highly affected by the region factor ($P < 0.001$).

For Gram-negative bacteria strains *E. coli* and *S. marcescens*, they were sensitive to the five EOs. However, *P. aeruginosa* was relatively resistant. Indeed, the highest activity was observed against *E. coli* strain with IZ = 31 ± 1.96 mm for *A. absinthium* EO of Kasserine region. This strain also presented a sensitivity towards *A. absinthium* EOs of Bizerte (IZ = 28.70 ± 2.61 mm), Gabes (IZ = 27.30 ± 2.36 mm) and Tozeur (IZ = 24.7 ± 0.65 mm) regions. The positive control streptomycin had an IZ = 18.33 ± 1.3 mm. However, *A. absinthium* EO of Zaghouan region was less active (IZ = 17.3 ± 2.85 mm). In agreement with our results, the EO of wormwood aerial parts collected from North Khorasan in Iran had a strong antibacterial activity against *E. coli* (IZ = 30 ± 0.11 mm). *S. marcescens* strain showed a higher sensitivity for *A. absinthium* EO of Zaghouan (IZ = 19.70 ±0.65 mm), Gabes (IZ = 18.00 ±1.13) and Bizerte (IZ = 17.00 ± 0.00 mm) regions. Nevertheless, *A. absinthium* EOs of Kasserine (IZ = 16.30 ± 0.65 mm) and Tozeur (IZ = 14.00 ±1.13 mm) were less active. The positive control streptomycin had an IZ = 14.00 ± 0.01 mm. The antibacterial test showed a normal development of *P. aeruginosa* strain. However, a low antibacterial activity was detected for the EOs of Bizerte (IZ = 8.00 ± 1.13 mm) and Kasserine regions (IZ = 8.30 ± 0.65 mm). The positive control streptomycin had an IZ = 17.66 ± 0.65 mm.

Concerning Gram-positive bacteria strains, Gabes EO exhibited the highest antibacterial activity against *B. licheniformis* (IZ = 26.30 ± 1.73 mm), followed by Zaghouan (IZ = 25.00 ± 1.13 mm) and Bizerte

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(IZ = 20.70 ± 2.36 mm) regions. The EOs of these regions were more powerful than the positive control streptomycin (IZ = 16.00 ± 0.02 mm). As shown in Table 5, *A. absinthium* EO of Bizerte region was the most active against *S. aureus* with IZ = 23.30 ± 3.27 mm. *A. absinthium* EOs of Gabes, Kasserine and Zaghouan regions showed a moderate antibacterial activity with IZ = 21.70 ± 3.27 mm, 20.30 ± 1.73 mm and 17.30 ± 2.36 mm, respectively. IZ of the positive control streptomycin was 15.00 ± 0.05 mm. For *E. hirae*, the highest antibacterial activity was observed with *A. absinthium* EO of Zaghouan region with IZ = 23.30 ± 3.27 mm which was greater than that of the positive control (IZ = 13.00 ± 1.13 mm). Msaada *et al.* [24] mentioned that IZ of Tunisian *A. absinthium* EO against *S. aureus* varied from 18.00 ± 1.13 to 25.00 ± 1.13 mm. Moghaddam *et al.* [54] reported that IZ of Iranian *A. absinthium* EO was around 32 ± 0.016 for *S. aureus* and 30 ± 0.11 mm for *E. coli*. It has also been demonstrated that Tunisian *A. absinthium* EO of Kasserine region was active against *E. coli* (IZ = 14 mm) and *P. aeruginosa (*IZ = 18 mm) [25]. However, Juteau *et al.* [54] mentioned that French *A. absinthium* EO had no antibacterial activity against *E. hirae*. Accordingly, to Kheyar et al. [56] and Saada *et al.* [57], the variability of the antibacterial activity of the EOs against the two types Gram-positive and Gram-negative bacteria strains is due to a difference in the ability of penetration of the active compounds present in EOs. In fact, it is difficult to attribute the antibacterial activity of a complex mixture to a single or constituent; also, possible synergistic and antagonistic effect of compounds in the EOs should be taken into consideration [40].

It has been conclusively shown that *A. absinthium* EOs were active against both Gram-positive and Gram-negative bacterial strains. In our present research, it could be deduced that the antibacterial effect of *A. absinthium* EO was region dependent. Indeed, the variability of the chemical composition and the different geographical parameters of the studied regions had an important role on the antibacterial activity of the EOs.

Conclusion

In conclusion, the present study showed that *A. absinthium* is characterized by an important antioxidant activity thanks to its richness in bioactive components which were highly influenced by the regional factor. As it was reported, considerable qualitative and quantitative differences existed between wormwood samples. It's noteworthy to mention that this research is the first to evaluate the antibacterial activity of EO extracted from Tunisian wormwood against *B. licheniformis*, *S. marcescens* and *E. hirae* bacteria strains. Indeed, *A. absinthium* EO presented an interesting antibacterial effect against both Gram-positive and Gram-negative bacteria. According to our findings, *A. absinthium* EO presented a promising potential application for industries and could be used in several treatments particularly those of bacterial infections.

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Table 5. ANOVA analysis and antibacterial activity (IZ in mm) of the different essential oil of *Artemisia absinthium* collected from five different regions of Tunisia.

Bacteria strain	**Collection region**					***d.l***	***F***	***P***	***Streptomycin* (1 mg/mL)**
	Bizerte	**Zaghouan**	**Kasserine**	**Gabes**	**Tozeur**				
Gram-negative									
Pseudomonas aeruginosa	8.00 ± 1.13^{b}	na	8.30 ± 0.65^{a}	na	na	4	225.25	0.000***	17.66 ± 0.65
Escherichia coli	28.70 ± 2.61^{b}	17.30 ± 2.85^{e}	31.00 ± 1.96^{a}	27.30 ± 2.36^{c}	24.70 ± 0.65^{d}	4	21.44	0.000***	18.33 ± 1.3
Serratia marcescens	17.00 ± 0.00^{c}	19.70 ± 0.65^{a}	16.30 ± 0.65^{d}	18.00 ± 1.13^{b}	14.00 ± 1.13^{e}	4	24.68	0.000***	14.00 ± 0.01
Gram-positive									
Bacillus licheniformis	20.70 ± 2.36^{c}	25.00 ± 1.13^{b}	19.30 ± 1.31^{d}	26.30 ± 1.73^{a}	11.30 ± 1.31^{e}	4	50.69	0.000***	16.00 ± 0.02
Staphylococcus aureus	23.30 ± 3.27^{a}	17.30 ± 2.36^{d}	20.30 ± 1.73^{c}	21.70 ± 3.27^{b}	10.00 ± 1.23^{e}	4	17.73	0.000***	15.00 ± 0.05
Enterococcus hirae	20.30 ± 2.85^{b}	23.30 ± 3.27^{a}	13.00 ± 1.96^{c}	20.00 ± 1.58^{b}	20.00 ± 1.48^{b}	4	12.31	0.000***	13.00 ± 1.13

na: not active. Values of inhibition zones represent the average of three replicates ($n=3$). Letters (a-e) indicate significant differences at $P< 0.05$.
*** Significant effect at % 0.001.