Assessment of artemisinin and antioxidant activities of three wild Artemisia species of Algeria

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ABSTRACT
Artemisinin, a natural product, has received considerable attention in the last few years as a potent antimalarial drug. This study reports the presence of Artemisinin in three Algerian wild Artemisia species assessed by HPLC method: A. herba-alba (AH), A. campestris subsp. glutinosa (AC), and A. judaica subsp sahariensis (AJ). The HPLC analysis of the hexane extracts showed a difference in artemisinin content in studied species with a yield of 0.64%, 0.34% and 0.04% for AC, AH and AJ, respectively. Moreover, the level of artemisinin obtained in A. campestris was better than those found in A. sieberi and A. annua. This rate has been reported for the first time. Furthermore, the antiradical activities of methanolic extracts of plants were also tested. There was a remarkable antioxidant capacity found in all Artemisia methanolic extracts analysed.

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1. Introduction

Natural products have been used for the treatment of a variety of diseases for many years. Among them artimisinin, isolated from the aerial parts of Artemisia annua, is a high value endoperoxide sesquiterpene lactone with proven effects even against multidrug resistant strains of the malaria parasite (Woerdenbag et al. 1992; Cheong et al. 2020). These properties of artemisinin and its derivatives received considerable attention from the scientific community (Kavak et al. 2021; Ozok et al. 2021).

Different methods and processes are used for the extraction of artimisinin in particular, Soxhlet extraction, maceration, hydrodistillation and sonication (Wang and Weller 2006; Chemat et al. 2017). However, several methods have been developed for their identification, mainly thin layer chromatography, gas chromatography, high performance liquid chromatography (HPLC) with UV as well as electrochemical detection, radioimmunoassay, enzyme immunoassay and HPLC/MS method (Ivanescu et al. 2011).

For the methods developed so far, HPLC with UV detection was the most suitable method to determine the artemisinin content in crude plant extracts (Qian et al. 2005; Reale et al. 2008).

In this context and in order to enhance our diverse and rich national heritage, the present study aimed to determine the amount of artemisinin contained in three species of wild Algerian Artemisia (A), namely: A. herba-alba (AH), A. campestris subsp. glutinoso (AC), and A. judaica subsp. sahariensis (AJ) and to evaluate the antioxidant effect of their methanolic extracts.
2. Results and discussion

The phytochemical profile of *Artemisia* genus has shown abundance in flavonoids, phenolic acids, coumarins, isocoumarins and fatty acid. However, the pharmacological activities span a wide range of potentials uses such as antioxidant, antifungal, insecticidal, antibacterial, antitumor and antihypertensive (Ramezani et al. 2004; Dib et al. 2017; Mohamed et al. 2021). The results of this study were compared to those previously published. In a recent study, he showed that the *Artemisia annua* also repellent activity against two storage pests (Liu et al. 2021).

2.1. Total phenolic (TPC), total flavonoids (TFC) and tannin contents (TC)

The results of TPC, TFC and TC reported in Table S1 indicate that *A. judaica* and *A. herba-alba* represent a rich source of phenolic compounds (692.82 mg GAE/g and 480.41 mg GAE/g) and also for flavonoid components (132.87 mg QE/g and 260.03 mg QE/g) compared to *A. campestris* which can be considered as the poorest source in such elements (189.49 mg GAE/g and 73.3 mg QE/g). The highest amount of tannins content was found in *A. judaica* (74.81 mg CE/g) followed by *A. herba-alba* and *A. campestris* with an amount estimated at 62.15 mg CE/g and 52.53 mg CE/g respectively.

TPC, TFC and TC displayed in all *Artemisia* extracts appeared to be significantly higher than those reported in other studies (Djeridane et al. 2006). Therefore, this richness in phenolic compounds was confirmed in recent studies (Allam et al. 2019; Mohammed et al. 2021). According to Laboukhi-Khorsi et Chemat et al. (2017), this wealth increases as and when the polarity of extraction solvents used increases (Laboukhi-Khorsi et al. 2017). In other reports, several factors, namely climatic, geographic conditions and ontogeny of collected plants may severely affect their composition, and their biological properties, as well as nurturing (Schlaepfer et al. 2014; Zouari et al. 2014).

2.2. Antioxidant activities

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. Therefore, in many studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants (Kähkönen et al. 1999). Comparison with Svetlana V. Zhigzhitzhapova, data showed that two samples exhibited the high antiradical properties, whereas the activity of the essential oil from the mixture of flowers, leaves was 1.4 times higher than that from the whole aerial part (Zhigzhitzhapova et al. 2020).

The antioxidant activity of phenolics is mainly due to their redox properties, which make them, act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential (Rice-Evans et al. 1995).

The antiradical activity of all *Artemisia* extracts was assessed using four methods and the results are given in Table S2 as a half-inhibitory concentration IC\textsubscript{50} and half absorbance A\textsubscript{0.5} values.

According to the obtained results, there was statistically a remarkable antioxidant capacity found in all *Artemisia* extracts tested by all methods. In the DPPH assay, *A.
judaica has exhibited the highest antioxidant effect (21.92 μg/mL) followed by A. campestris and A. herba-alba with a half-inhibitory concentration IC50 estimated at 40 μg/mL and 72.07 μg/mL, respectively. AH can be considered as the specie with a lowest effect then those expressed by two others.

The second antioxidant test assessed using ABTS showed once more that all studied extracts presented a high activity with an IC50 value extending from 11.01 μg/mL to 27.19 μg/mL. Furthermore, the greatest activity for this test was displayed by A. judaica extract when compared to the reference compounds BHA and BHT with IC50 values of 5.98 μg/mL and 1.68 μg/mL, respectively. These results concord with those reported in a recent study (Allam et al. 2019) carried also on an Algerian A. judaica which showed that its hydromethanolic extract of its aerial parts exhibited a high DPPH and ABTS activities (10.23 μg/mL and 15.07 μg/mL, respectively). It should be noted that the antiradical effect of the extracts is attributed to the phenolic compounds. Therefore, the low IC50 recorded for hydromethanolic extract indicated that it contained a high amount of phenolic compounds and flavonoids (El-Massry et al. 2002; Kordali et al. 2005; Djeridane et al. 2006; Al-Mustafa and Al-Thunibat 2008).

In the β-carotene/linoleic acid model system, the absence of an antioxidant produce a discoloration of β-carotene. This assay is widely used to evaluate the antioxidant activity of plant samples. Table S2 suggests that the antioxidant activity tested in A. campestris using β-carotene showed the strongest inhibition effect with an IC50 value less than 12.5 μg/mL. This effect was lowest using AJ and AH extracts (45.22 μg/mL and 58.64 μg/mL, respectively). However, the high activity recorded can be explained by the presence of kaempferol (Luo et al. 2004) and apigenin (Cavin et al. 1998) in high amounts in this extract. These two phenolic compounds were reported to inhibit the oxidation of β-carotene (Škerget et al. 2005; Sharififar et al. 2009).

The results obtained using the latest assay (CUPRAC) were also compared to those of BHA and BHT and the half absorbance was calculated (A0.5). This time, all extract showed approximately the same activity extended from 15.03 μg/mL to 28.66 μg/mL. Very little reports have been given on antioxidant activity of hydromethanolic extract of these Algerian species where the most are concentrated on the chemical composition analysed by Gas chromatography GC-MS.

All extracts in this study showed antioxidant effects. Although the antioxidant activity is generally related to the phenolic compounds present in the plant (El-Massry et al. 2002; Kordali et al. 2005; Djeridane et al. 2006; Al-Mustafa and Al-Thunibat 2008). In addition, based on previous research, the efficiency of phenolic compounds as antiradicals and antioxidants depends on many factors. One major factor is the number of hydroxyl groups directly bonded to the aromatic rings (Sroka and Cisowski 2003). However, the difference in the antioxidant activities may also be attributed to the structural diversity as well as to the interactions in the extracts of the phenolic compounds (Allam et al. 2019).

### 2.3. Artemisinin content assessment

Literature reports indicated that extraction of artemisinin (Figure S3) can be carried out by different extraction methods: traditional solvent extraction, microwave-assisted...
extraction, ultrasound-aided extraction, and supercritical fluid extraction method using CO as a solvent (Bayarmaa and De Zorzi 2011; Briars and Paniwnyk 2012) (Bayarmaa and De Zorzi 2011; Briars and Paniwnyk 2013). n-hexane (Ivanescu et al. 2011; Badshah et al. 2018), toluene (Zhang et al. 2018), chloroform (Efferth 2017), petroleum ether (Ferreira and Gonzalez 2009), acetone, and ethanol (Huter et al. 2018) were the solvents most widely used for artemisinin extraction from Artemisia species.

It should be noted that hexane was the best extraction solvent, giving 0.21% artemisinin in the case of Artemisia annua Romanian, followed by chloroform and dichloromethane with the same artemisinin concentration. Methanol extracted almost the same amount of artemisinin as chloroform and dichloromethane, probably due to the fact that the methanolic extract was subjected to ultrasonication for 30 min, and not only to maceration as in the case of the other extracts (Ivanescu et al. 2011).

The most common method for analysis of artemisinin is based on high performance liquid chromatography (HPLC). In our study, quantification of the artemisinin was performed using a linear calibration graph with increasing amounts of artemisinin and their peak area response with UV detection (220 nm) (Table S3). This calibration curve was obtained by injection of different concentrations of artemisinin standard solution (0.125–5 mg/mL) into the HPLC system, run at least three times for each concentration.

Using the regression line equation, the content of artemisinin per dry weight of Artemisia species was ranged from 0.04 to 0.65%. The highest content of artemisinin was observed in A. campestris extract with a yield estimated at 0.65% which was higher even then that obtained in A. annua (0.11%-0.45%), considered as the highest content of artemisinin (Hao et al. 2002; Ivanescu et al. 2011; Numonov et al. 2019). In the same context, A. herba-alba presented a yield less than campestris (0.34%) while A. judaica showed the lowest yield of artemisinin 0.04% in this study. The HPLC chromatograms of the analysed artemisia samples are showed in Figures S4–S6.

HPLC chromatograms of the plant samples showed many resolved peaks. The peaks were identified by comparison of their retention times to that of standard artemisinin (Table S3). Linear regression was used to establish the calibration curve. The good linearity of artemisinin was found within the range of 0.125–2.5 mg/mL ($r^2 = 0.99854$). The regression equation and correlation coefficient were determined as Formula: $y = mx + b$.

Previous works have reported that artemisinin concentration varied due to differences in methods of artemisinin extraction as well as the solvents used (Efferth 2017; Zhang et al. 2018). In others, the content of artemisinin, found in the plant, is affected by some factors such as growth conditions, seasonal and geographical variations as well as breeding (Pavarini et al. 2012).

According to our results, ultrasound-aided extraction and n-hexane as a solvent for artemisinin extraction were suitable for the extraction of artemisinin from Artemisia species. Our experiments are in agreement with previous reports that the yield of artemisinin extraction is enhanced by ultrasound-aided extraction when compared to comparable conventional extraction processes (Briars and Paniwnyk 2013).
2.4. Artemisinin crystallisation

The extracts obtained from plant material using organic solvent extraction are very complex, and have several unwanted components such as chlorophylls and other coloured organic molecules from the feed material. Removal of the contaminants from the extracts has been performed with charcoal and clays (Patil et al. 2012; Chemat-Djenni and Sakhri 2013).

In the present work, the crystallization of artemisinin molecule was tested on *A. herba-alba*. This method should be preceded by a purification of the hexane extract by using activated charcoal and silica gel as adsorbent to eliminate all pigments and impurities which can help in the enrichment of artemisinin.

Crystallization was clearly marked under 4°C for 24 h for treated extract with adsorbents as reported in other studies (Liu et al. 2011). After treatment using adsorbents, the total peaks in the chromatograms decreased from 16 to 8 (Figure S8).

Using silica gel compared to an adsorbent with specific ligands was less effective. However, silica gel is a cheap alternative that can be used for primary treatment of crude artemisinin extracts from the feed material (Numonov et al. 2019).

Crystallization under the cited conditions shows a marked difference in crystalline form for crude extracts compared to those treated with adsorbents. The untreated extracts give low crystal yield with yellowish colour.

However, HPLC profiles of artemisinin standard, ultrasound crude extract at 40°C and the purification show clearly the disappearance of some of the interfering peaks on purified extract indicating a success in eliminating most co-metabolites, which is believed to be a good argument for an easier crystallisation of artemisinin (Chemat et al. 2017).

3. Experimental section

See Supplementary materials.

4. Conclusion

This article was the subject of an identification and quantification of artemisinin in samples of three plants of Artemisia sp from the native Algerian flora by HPLC method. The artemisinin content of these three artemisia species is also reported for the first time. The level of artemisinin in the studied plants varied between 0.04 and 0.65%, the extraction of each was assisted by ultrasound and hexane was used as solvent. However, the artemisinin content of these Algerian wild plants mainly in *A. campestris* subsp. glutinosa (0.65%) is much higher than that found in wild *A. annua* Romanian (0.17 to 0.22% of artemisinin in the leaves) (Ivanescu et al. 2011) and lower than that contained in cultivated *A. annua* (0.88-1.49% of artemisinin in aerial parts) (Bhakuni et al. 2001).

Our samples exhibited a remarkable antioxidant capacity, which is found in all the methanolic extracts of Artemisia tested, in particular *Artemisia judaica* which proved to be the most promising. The variability of antioxidant activities between the three plants can be attributed to the different chemical profiles of each of them.
Upon this study, we can state that the obtained result could be used further to develop a powerful method for extraction of artemisinin from the selected species to be applied in various pharmaceutical applications. It can be also noted that Artemisia is considered as good sources of natural antioxidants that holds a great potential for human health and its therapeutic effects should be more strictly and intensively analysed.

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