Acanthus mollis L. Grown in Algeria Exhibits Potent Antioxidant Activity

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Summary

This research aimed to evaluate for the first time the chemical composition and antioxidant potential of volatile fraction and different concentrates obtained from *Acanthus mollis* L.. The volatile components from the aerial parts of *A. mollis* were extracted by hydrodistillation and analyzed by GC-MS. Twenty-four compounds were characterized, representing 94.5% of the oil. The dominant compounds were hexahydrofarnesylacetone (52.4%), pulegone (15.0%) and spathulenol (4.5%), followed by appreciable amounts of menthone (2.8%), borneol (2.7%) and 2-hydroxy-5-methylacetophenone (2.0%). The Folin-Ciocalteu, 2,2-diphenyl-[1]-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays were used to evaluate the total phenolic content and the antioxidant activity of plant extracts, respectively. Quantitative estimation of total phenols content by a colorimetric method showed that extracts were rich in these compounds, with a significant difference between samples. Total phenols varied from 0.0725 to 1.217 (mg GAE g^{-1} dry weight). The percentages of DPPH inhibition were between 60.05 and 91.80%, while ascorbic acid gave 93.54%. The IC_{50} values ranged from 3.066 to 3421 μg.mL^{-1} for DPPH. The water extract of *A. mollis* was the most active (IC_{50} = 3.066 ± 0.02 μg.mL^{-1}) compared to the other extracts. In addition, the volatile fraction of *A. mollis* exhibited a weak antioxidant capacity.

Key words

Acanthus mollis L, volatile fraction, total phenolic content, antioxidant

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Introduction

A large amount of secondary metabolites is responsible for the large use of medicinal plants. These metabolites have repeatedly shown increasing therapeutic use over the past decades. Hence, chemotaxonomy has become an important part of integrative studies that allow for a better comprehension of any plant species.

Various organs of aromatic plants, such as leaves, stems, flowers, seeds and roots are used for the extraction of odorous substances i.e. essential oils (EOs). These volatile liquids are complex mixtures of organic compounds, often endowed with powerful and specific biological activities (Koul et al., 2008; Merghache et al., 2008; Fekih et al., 2016). Recently, many studies have revealed that the essential oils of some aromatic plants have antimicrobial and antioxidant activities (Mimica-Dukić et al., 2003; Kalemba and Kunicka, 2003; Oumzil et al., 2002).

The genus *Acanthus* (Acanthaceae) includes about thirty species originating mainly from Eurasia and Africa. It is composed of perennial herbaceous plants, with white or purplish flowers, widely distributed in tropical and subtropical regions (Quattrocchi Umbert, 2000; Snogerup et al., 2006; Bora et al., 2017). Only two species of this genus live in Algeria, *A. spinosus* L. and *A. mollis* L. (Quézel and Santa, 1963). Both species grow on different soil types, including clayey, dry and sandy ones, and are characterized by few key morphological features, i.e. leaf shape and flower color. Moreover, these plants are grown as ornamental due to their tall inflorescence. *A. mollis*, also named *A. hispanicus* Lou., *A. latifolius* E. Goez., *A. longifolius* Poir., *A. lusitanicus* Auct, *A. niger* Mill. (Bora et al., 2017), is an ethnomedicinal plant used in the treatment of various inflammatory problems (Bader et al., 2015). Moreover, this plant is used in the treatment of asthma, burns, and bronchitis (Bora et al., 2017).

The phytochemical profile of *A. mollis* was previously characterized by estimating its content of glycosides (Řezanka et al., 2008), anthraquinones, flavonoids and phenols (Jara et al., 2017). Other studies reveal that *Acanthus* contains tannins and mucilage, with emollient, demulcent and detergent properties. The study of the ethanol extract of *A. mollis* leaves emphasized the presence of some important chemical compounds, such as phenylpropanoids and benzoxazinoids (Matos et al., 2018).

Recent studies have shown that ethyl acetate and ethanol extracts exhibit excellent antioxidant and anti/inflammatory activities, respectively (Jara et al., 2017; Matos et al., 2019). The content of secondary metabolites causes *Acanthus* to have astringents properties, useful for cleaning and repairing hair. Despite the interesting above-mentioned results, the chemical composition of the essential oil has never been previously reported as a part of a complete characterization of the plant.

The aim of this research is therefore threefold: (*i*) the identification for the first time of the chemical components of the volatile fraction of *A. mollis* using gas chromatography coupled with mass spectrometry (GC/MS), (*ii*) the quantitative estimation of the total content of phenolic compounds and (*iii*) the evaluation of the antioxidant properties of each extract by DPPH and FRAP assays. This comprehensive study should provide a useful basis of information for determining the most suitable harvesting time for both the volatile fraction and other the secondary products.

Materials and Methods

Plant Samples

The aerial parts of wild *A. mollis* were harvested at the flowering stage in July 2017 in Nedroma, located in Tlemcen. The identity of the plant specimen was confirmed by the National Herbarium in Abou Bekr Belkaïd University – Tlemcen (Algeria).

Volatile Fraction Extraction

Dried and crushed plant material (500 g) was introduced in a 6 L round-bottomed flask and covered with 4 L of distilled water. The set was then hydrodistilled in a Clevenger-type apparatus for about 5 h obtaining a yellow oil (European Pharmacopoeia, 1997). The oil was dried over anhydrous Na_2SO_4, filtered and then stored in the dark at + 5 °C until chemical investigation and bioassay testing.

Chemical Analyses

The analysis of the essential oil was performed as previously reported (Chaib et al., 2017; Bechlaghem et al., 2019). GC/EI-MS analyses were conducted using a Varian CP-3800 gas-chromatograph operating with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Injector and transfer line temperatures were set at 220 and 240 °C respectively, while the oven program temperature varied from 60 °C to 240 °C at 3 °C min^{-1}, using helium as carrier gas at 1 mL min^{-1}; split ratio was 1:30. The samples were diluted in hexane (10%) and 0.2 µL were injected.

Compounds were identified by comparison of their retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 2014 and Adams) and MS literature data (Swigar and Silverstein, 1981; Davies, 1990; Adams, 1995; Joulain and König, 1998).

Extracts Preparation

The aerial parts of *A. mollis* were extracted using a 250 mL Soxhlet apparatus. The extraction was carried out using increasing polarity solvents (dichloromethane, ethanol and water). A sample of *A. mollis* (20 g) was extracted with 150 mL of each solvent for 3 h. At the end of the extraction process, the flask content was filtrated and then the solvent was evaporated under reduce pressure. Each extract was weighed and its yield calculated. All the extracts were stored for further experiments.

$$Extract\ yield\ (\%) = (We / Wp) \times 100$$

where *We*: weight of the extract (g); *Wp*: weight of the plant (g)

Total Phenolic Content

The determination of the total content of phenolic compounds was performed using the Folin-Ciocalteu method, as reported by Singleton and Rossi (1965). About 200 µL of herbal extract (0.1 g of extract in 100 mL methanol) were combined with 2.5 mL of Folin-Ciocalteu solution (0.1N) for 5 minutes. Then, 2 mL of a Na_2CO_3 solution with a concentration of 7.5 g per 100 mL were added and incubated at room temperature for 90 min.

To establish the calibration curve, gallic acid was utilized as standard at different concentrations, and absorbance was determined spectrophotometrically (OPTIZEN™ POP) at 765 nm against a blank of distilled water. Results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE g^{-1} d.w.).

Radical Scavenging Activity

2,2-Diphenyl-1-Picrylhydrazyl Hydrate (DPPH) Free Radical Scavenging Potential

The DDPH scavenging activities of the obtained herbal extracts were performed as described by Fekih et al. (2019). 1.0 mL of DDPH 0.135 mmol L^{-1} solution in methanol was combined with 1.0 mL of extract in methanol at various concentrations. After 30 min of incubation at room temperature, the absorbance was determined at 517 nm employing an OPTIZEN™ POP UV/Visible spectrophotometer against a blank. Pure L-ascorbic acid (AA) was used as a reference. Inhibitions of the free radical, DPPH, indicated as % inhibition, were estimated from the equation presented below:

$$\% \ Inhibition = ((Ao - As) / Ao) \times 100$$

where *Ao*: blank absorbance at 517 nm; *As*: sample absorbance at 517 nm.

In the presence of antioxidant components, the color turns from purple to yellow. All tests were performed in triplicate and the average value was recorded. Furthermore, the IC_{50} values (the concentrations of extract that caused 50% inhibitions) were determined.

Ferric-Reducing Antioxidant Power (FRAP) Assay

Ferric reducing/antioxidant power of the herbal extracts was assessed by a method adapted from the literature (Oyaizu, 1986; Benyelles et al., 2016). Hence, 1 mL of different concentrations of the methanol extract were combined with 2.5 mL of phosphate buffered saline (0.2 mol.L^{-1}, pH = 6.6) and 2.5 mL of a 1 g per 100 mL of potassium ferricyanide solution. Then, 2.5 mL of 10g per 100 mL trichloracetic acid were added to the mixture. After centrifugation, 2.5 mL of the supernatant were combined with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (1 g L^{-1}). The intensity of the blue–green color was determined at 700 nm. The positive control was pure L-ascorbic acid.

Results and Discussion

Chemical Composition of *A. mollis*

Hydrodistillation of the aerial parts of *A. mollis* in a Clevenger-type apparatus provided 0.02% (w/w) of yellow oil, based on dry weight. The GC-MS analysis permitted to characterize twenty-four compounds, representing 94.5% of total volatiles. The oil was constituted by a mixture of apocarotenes, oxygenated monoterpenes, non-terpene compounds, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The major chemical class was that of apocarotenes (52.8%), followed by oxygenated monoterpenes (22.0%) and non-terpene derivatives (12.8%). The other classes, such as sesquiterpene hydrocarbons (2.9%) and oxygenated sesquiterpenes (4.5%) were present in very small amounts (Table 1).

Table 1. Chemical composition of the essential oil of *Acanthus mollis* L.

N°	Compounds	RI	Essential Oils [%]	Identification
1	1,3-Butanediol	788	1.9	RI,MS
2	2,3-Butanediol	789	1.1	RI,MS
3	(*Z*)-3-Hexen-1-ol	857	0.6	RI,MS
4	Benzaldehyde	962	0.3	RI,MS
5	1-Octen-3-ol	981	0.4	RI,MS
6	1-Octanol	1071	0.5	RI,MS
7	Nonanal	1102	1.2	RI,MS
8	Menthone	1154	2.8	RI,MS
9	(*E*)-2-Nonenal	1163	0.6	RI,MS
10	Borneol	1168	2.7	RI,MS
11	Decanal	1204	0.4	RI,MS
12	Pulegone	1240	15.0	RI,MS
13	(*E*)-2-Decenal	1263	1.0	RI,MS
14	Carvacrol	1301	1.0	RI,MS
15	2-Hydroxy-5-Methylacetophenone	1317	2.0	RI,MS
16	Piperitenone	1342	0.5	RI,MS
17	2-Methyltetradecane	1462	0.9	RI,MS
18	Germacrene D	1482	0.5	RI,MS
19	(*E*)-*β*-Ionone	1489	0.4	RI,MS
20	Bicyclogermacrene	1498	1.0	RI,MS
21	Cadina-1(10), 6,8-triene	1533	1.4	RI,MS
22	Spathulenol	1576	4.5	RI,MS
23	1-Tetradecanol	1675	1.4	RI,MS
24	Hexahydrofarnesylacetone	1845	52.4	RI,MS
	Total identified %		94.5	
	Oxygenated monoterpenes		22.0	
	Sesquiterpene hydrocarbons		2.90	
	Oxygenated sesquiterpenes		4.50	
	Apocarotenes		52.8	
	Non-terpene derivatives		12.3	

Note: RI - Retention indices; MS - Mass Spectra in electronic impact

The major components of the oil were hexahydrofarnesylacetone (52.4%), pulegone (15.0%) and spathulenol (4.5%), followed by appreciable amounts of menthone (2.8%), borneol (2.7%) and 2-hydroxy-5-methylacetophenone (2.0%). To the best of our knowledge, this is the first report about the chemical composition of the essential oil of *A. mollis*.

Total Phenolic Content and Antioxidant Potential

As showed in Table 2, the aqueous extract (AEAM) yield was 25.25%, by far higher than that of the ethanol extract (EEAM, 17.8%). Additionally, the dichloromethane extract (DMEAM) had the lowest yield (1.8%).

The total content of phenolic compounds in *A. mollis*, measured by the Folin-Ciocalteu is summarized in Table 2. The ethanol and aqueous extracts contained 1.217 and 0.246 mg GAE g^{-1} d.w. of total phenolic, respectively, while the dichloromethane contained only 0.0725 mg GAE g^{-1} d.w. These results are in good agreement with a previous research reported by Jara et al. (2017).

Subsequently, the AEAM, EEAM, DMEAM and the EOAM were subjected to antioxidant activity studies employing two distinct techniques, the DPPH test system and the reducing potential. In the case of the DPPH test, the hydrogen atoms or electron-donating capacity of the related volatile fraction was established from the discoloration of the purple solution of DPPH dissolved in methanol. Methodologically, the DPPH• free radical assay is particularly indicated for molecules containing OH-, NH- and SH- groups (Salah et al., 1995). It is achieved at room temperature, thus eliminating any risk of thermal decomposition (Cai et al., 2006).

Table 2. Total phenols content of *Acanthus mollis* L. extracts

Sample	Yields [%]	Total phenolic content (mg GAE g^{-1} d.w.)
AEAM	25.25	0.246
EEAM	17.80	1.217
DMEAM	1.80	0.0725

Note: AEAM - Aqueous extract; EEAM - Ethanolic extract; DMEAM - Dichloromethane extract

Table 3 reports the DPPH radical scavenging activity as percentages, resulting from different concentrations of volatile fraction and solvent extracts. Water and especially ethanol extracts exhibited a remarkable and dose-dependent antiradical effect (91.80 and 71.40%, respectively at a concentration of 440 $\mu g.mL^{-1}$). On the contrary, the volatile fraction showed a weaker radical scavenging activity (60.05% at 4962 $\mu g.mL^{-1}$).

Order of potency can be useful to establish the structure scavenging activity relationship of the components of *A. mollis* EOs investigated; in fact, several of the identified compounds, among which the main constituents apocarotenoids, are known to possess strong antioxidant and free-radical scavenging activity (Bhatt and Patel, 2020).

Therefore, DPPH scavenging activity was also given as IC_{50} values. Hence, the aqueous extract had the most important activity with an IC_{50} of 3.066 ± 0.02 $\mu g.mL^{-1}$, followed by the ethanol extract (IC_{50} = 27.39 ± 0.05 $\mu g.mL^{-1}$). However, a low anti-radical effect was observed with DMEAM and EOAM extracts, with IC_{50} values higher than 100 $\mu g\ mL^{-1}$. The capacity of the different extracts to reduce the free radicals of DPPH is as follows: AEAM (IC_{50} = 3.066 ± 0.02 $\mu g\ mL^{-1}$) > EEAM (IC_{50} = 27.39 ± 0.05 $\mu g\ mL^{-1}$) > DMEAM (IC_{50} = 271.078 ± 0.05 $\mu g\ mL^{-1}$) > EOAM (IC_{50} = 3421 ± 0.05 $\mu g\ mL^{-1}$).

The extracts revealed a remarkable activity at low values of concentrations. This suggests that these extracts contain compounds that are capable of donating a hydrogen to a free radical in order to remove odd electrons responsible for radical's

Table 3. DPPH Radical Scavenging Activities of different *Acanthus mollis* L. extracts (%)

Samples		Antioxidant Activity					IC_{50} ($\mu g.mL^{-1}$)
AEAM	Conc. ($\mu g\ mL^{-1}$)	0.704	3.52	17.60	88.00	440.0	3.066 ± 0.02
	DPPH (%)	46.70	55.58	51.46	63.39	91.80	
EEAM	Conc. ($\mu g\ mL^{-1}$)	0.704	3.52	17.60	88.00	440.00	27.39 ± 0.05
	DPPH (%)	3.69	9.24	10.25	26.45	71.40	
DCEAM	Conc. ($\mu g\ mL^{-1}$)	0.704	3.52	17.60	88.00	440.00	271.078 ± 0.05
	DPPH (%)	45.92	45.70	49.39	55.91	65.68	
EOAM	Conc. ($\mu g\ mL^{-1}$)	7.936	39.68	198.40	992.00	4962.00	3421.0 ± 0.05
	DPPH (%)	14.09	15.73	19.69	33.37	60.05	
AA	Conc. ($\mu g\ mL^{-1}$)	0.614	3.72	15.00	38.40	96.00	30.00 ± 0.10
	DPPH (%)	42.95	74.39	91.39	90.89	93.54	

Note: AEAM - Aqueous extract; EEAM - Ethanolic extract; DMEAM - Dichloromethane extract; EOAM - Essential oil; AA - Ascorbic acid

reactivity. According to these results, there is a relationship between the chemical composition and the antioxidant activity. Moreover, as reported in literature data, the antioxidant activity of the ethanol extract could be attributed to the following phenolic compounds, namely 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and phenylpropanoids, whose ethanolic extract showed potent antioxidant capacity against DPPH (IC_{50} = 40.00 ± 1.59 µg mL^{-1}) (Řezanka et al., 2008; Jara et al., 2017).

FRAP is an easy, fast and reproducible assay for the evaluation of the antioxidant activity. It is based on the reduction of ferric ions in ferrous ones at low pH values. The rise in UV-visible absorbance corresponds to an increase in reducing power reducer of the tested extracts. The results of the FRAP assay are summarized in Fig. 1. The aqueous extract of *A. mollis* showed a significant reducing power, followed by the ethanolic one. The activity could be correlated with their content of phenolic compounds.

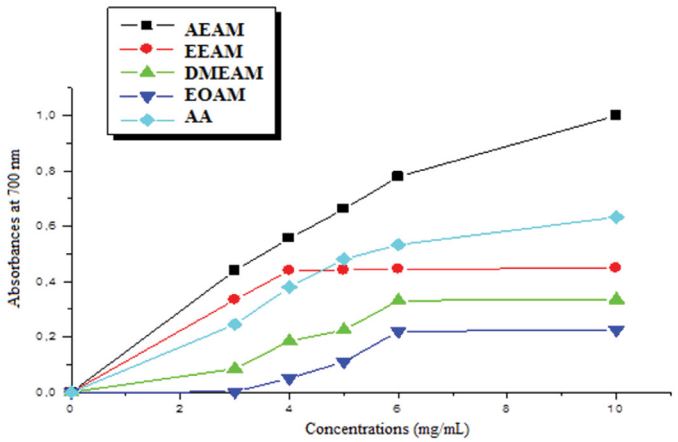

Figure 1. Ferric-Reducing Antioxidant Power Assay of different *Acanthus mollis* L. extracts

Abbreviations: AEAM - Aqueous extract; EEAM - Ethanolic extract; DMEAM - Dichloromethane extract; EOAM - Essential oil; AA - Ascorbic acid

The extracts of *A. mollis* investigated were able to neutralize different charged radical species. It is hence the main source of antioxidants with a very effective potential in the detoxification mechanisms. As stated by Smeriglio et al. (2020), "This direct antioxidant activity against relatively weak primary oxidants can prevent the triggering of secondary oxidative mechanisms, which lead to the formation of very harmful agents such as hydrogen peroxide, and hydroxyl radical". It is generally accepted that monoterpenes bearing activated methylene groups exhibit a good antioxidant activity. Moreover, oxygenated sesquiterpenes show a similar antioxidant activity (Smeriglio et al., 2018). Consequently, we may hypothesize that the variation in H-transfer associated to the observed antioxidant potential could be linked to the variation in the concentration of compounds containing hydroxyl groups.

Conclusion

The volatile fraction of *A. mollis* has not been previously investigated. The current work examined for the first time its chemical profile. The antioxidant properties of different extracts (ethanolic, aqueous, dichloromethane extracts and the essential oil) from Algerian *A. mollis* have shown that the volatile fraction is characterized by large amounts of apocarotenoids (52.8%) and oxygenated monoterpenes (22%). The ethanol and aqueous extracts contain the highest amount of phenols and a remarkable antioxidant potential, whereas the volatile fraction and dichloromethane have the moderate ones. It could be interesting to continue this investigation on the different fractions of the phenolic compounds to identify the individual components as a part of the chemotaxonomical study. Finally, these findings may contribute to the use of this species as a natural antioxidant in the agro-alimentary and pharmaceutical industries.

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