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Chemical Composition and Antioxidant Activity of Essential Oil of Sawdust from Moroccan Thuya (*Tetraclinis articulata* (Vahl) Masters

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Abstract: The present study was dedicated to the study of the chemical composition and the antioxidant activity of the essential oils from the sawdust of *Tetraclinis articulata* in Morocco (from Khemisset region). The yield of the essential oil obtained by hydrodistillation from sawdust was 2.19%. The determination of the chemical composition of *Tetraclinis articulata* essential oils (TAEO) was carried out by GC-SM analysis. The results that 22 volatile compounds were identified in the sawdust part of *T. articulata*, representing 87.25% of the total composition. The study of TAEO antioxidant activity was assessed by three different methods: the trapping of free radical DPPH, bleaching assay ABTS, and the reduction of iron (FRAP). The results of DPPH test showed that TAEO (IC_{50}=0.0144 mg/mL) has a very interesting antioxidant activity compared to the ascorbic acid used as reference (IC_{50}=0.0184 mg/mL). These results reveal promising prospects for the future exploitation of TAEO as a potential source of natural antioxidant substances that may be used for future investigations.

Keywords: *Tetraclinis articulata*; essential oils; GC-MS analysis; antioxidant effects.

1. Introduction

Tetraclinis articulata (T. articulata) or Thuya of Berberia is also called Araar in Arabic. It is geographically present in three Maghreb countries (Morocco, Algeria, and Tunisia), in southeastern Spain (Almeria region), and on the island of Malta [1]. In Morocco, *T. articulata* forest has a very important role in the economic and social life of the local populations. Its wood, characterized by its strong resistance to rot, it is used principally in the craft sector for marquetry, cabinet making, and also for heating.

In traditional medicine, various parts are used mainly for the treatment of intestinal and respiratory infections, diabetes, and hypertension [2-7]. Pharmacological investigations reported on TAEO showed antibacterial, antifungal, antidiarrhoeal, anti-ulcerativecytotoxic, anti-Alzheimer, and anti-inflammatory activities [8-16].

Natural substances isolated medicinal plants have multiple interests in the industry, including cosmetics, food, and pharmaceuticals. Among these compounds, essential oils (EOs) or volatile compounds, clearly gain the interest of many investigators. Indeed, EOs have shown several biological effects, such as antimicrobial, anti-inflammatory, antioxidant, and antidiabetic properties [17-21].

On the other hand, oxidative stress is an escalating public health problem. Without adequate control, oxidative stress can lead to premature aging and significantly increase the risk of developing several diseases such as inflammation, diabetes, and cancer [22, 23]. Therefore, reducing oxidative stress with cytoprotective and antioxidating drugs seems to be a promising therapeutic solution [24]. Therefore, the research on a new source of antioxidants is necessary. In this context, a natural antioxidant such as EOs isolated from medicinal plants constitute a promising of these antioxidants. Indeed, current investigations have been oriented towards medicinal plants as a source of antioxidants and is particularly volatile compounds containing in their EOs.

Moroccan Thuya wood industry is dropping approximately tones of wood waste annually in the form sawdust. Due to global ecological and economic challenges, the aspect of this research is the transition of this waste to renewable resources. In this work, we are looking for new uses to be valorized from these wood rejects. Our interest in this present work is to study the chemical composition and the antioxidant activity by using DPPH, ABTS, and FRAP of TAEO collected from the Khemisset region of Morocco in order to develop a new type of disease control alternatives.

2. Materials and Methods

2.1. Essential oil extraction.

Sawdust of Thuya was collected in March 2017 from the industry of wood in Morocco (Khemisset area) and stored in the laboratory at room temperature (25°C). The extraction of Thuya sawdust EOs was conducted by hydrodistillation using a Clevenger type apparatus [25]. This essential oil obtained was dried under anhydrous sodium sulfate and stored at -5°C in the dark before analysis. The yield of the essential oil (%) was calculated using the following formula (EO stands for essential oil):

$$Yield\ \% = \frac{EO\ (g)}{Sawdust\ Material\ (g)} \times 100$$

2.2. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS).

The gas chromatography/mass spectrometry (GC/MS) device is made by Perkin Elmer Clarus TM GC-680 with Q-8 MS. It is equipped with an auto-sampler, which gives access to the automatic injection of samples into the injector and a capillary column type RxiR-5Sil MS traversed by Helium gas. The mass spectrometer is powered by a SMART electronic ionization source. This source can ionize and vaporize the different molecules as well as a quadrupole filter to separate the different ions in their m/z ratio. The GC/MS system is computer-controlled with Turbomass (TM) software, which allows programming of analytical methods as well as qualitative and quantitative identification of detected species.

The analysis parameters are as follows: analysis time: 2 hours, vector gas flow rate: 1 mL/min, ionization energy: 70 eV, injector temperature: 260 °C, oven temperature: 40 °C for 2 minutes, then rise from 10 °C / min to 290 °C, and the injected volume is 0.5 µL.

2.3. Antioxidant activity.

In this study, three methods were used to evaluate the *in vitro* antioxidant activity of TAEO, namely the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, ABTS bleaching assay, and the iron reduction method (FRAP).

2.3.1. DPPH radical scavenging assay.

The activity of the trapping of the radical DPPH was realized according to the protocol described by Lopes-Lutz *et al*. [26]. In test tubes, 2.5 mL of different concentrations are added to 1 mL of the methanol solution of DPPH (0.3 mM). After 30 minutes of incubation in the dark and at room temperature, the absorbances are measured using a spectrophotometer at 517 nm against a blank that contains pure methanol. The negative control was prepared by mixing 1 mL of the DPPH methanol solution (0.3 mM) and 2.5 mL of methanol.

2.3.2. ABTS radical scavenging assay.

A solution of ABTS radical is prepared by mixing 2 mM ABTS with 70 mM potassium persulfate solution. The mixture is allowed to stir for 24 hours in the dark and at room temperature before use. This solution is then diluted with methanol to have an absorbance of 0.700± 0.02 at 734 nm. Then, 2 mL of this solution, 200 μL of essential oil or positive control is added, after 30 min. The absorbance obtained at 734 nm is noted [27].

2.3.3. FRAP iron reduction test.

The reducing power of iron (Fe^{3+}) of TAEO is determined by the method described by Oyaizu [28], with some modifications. Briefly, 1 mL of different concentrations was mixed with 2.5 mL of a phosphate buffer solution 0.2M (pH 6.6) and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%) solution. The mixture is incubated in a water bath at 50°C for 20 minutes. Then 2.5 mL of trichloroacetic acid (10%) is added to stop the reaction, and the tubes are centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant is mixed with 2.5 mL of water and 0.5 mL of aqueous $FeCl_3$ solution (0.1%). The absorbance of the reaction medium was read at 700 nm against a blank using a UV-VIS spectrophotometer. An augmentation in absorbance corresponds to an augmentation in the reducing power of the samples tested [29]**.** For all antioxidants tests, the positive control is represented by a solution of a standard antioxidant (ascorbic acid) prepared under the same conditions as the sample. Each test was carried out in triplicate.

3. Results and Discussion

3.1. Essential oils yield and chemical composition.

The yields of essential oil of sawdust, calculated after 6 hours of extraction is 2.19%. Different yields have been revealed in the literature, particularly in Morocco. The yield of *T. articulata* sawdust collected in the Khemisset region in 2007 was of the order of 1.63% [30]. This yield is lower than ours (2.19%).

The GC-MS analysis of TAEO revealed the presence of 22 volatile compounds. These compounds are belonging to different classes, including oxygenated monoterpenes, (49.2%), Sesquiterpene hydrocarbons (22.38%), oxygenated sesquiterpenes (10.30%), and monoterpene hydrocarbons (2.12%). The major compounds of TAEO are carvacrol (20.59%), acetoveratrone (17.47%), cedrol (10.30%), and α-Cedrene (8.99%) (Table 1).

Our results differ considerably from those reported in the literature. Indeed, Barrero *et al.*, (2005) who identified cedrol, 1,7-di-epi-cedrol, and 1,7-di-epi-isocedrol as the most abundant compounds in sawdust oil [31]. In another study, Zrira *et al.*, have reported a chemical composition of sawdust oil dominated by carvacrol, α-cedrene, cedrol, and terpinen-4-ol [32]. Other authors have identified other major compounds as carvacrol, cedrol, para-methoxythymol, and thymohydroquinone [33]. In the Khemisset sawdust essential oil produced in 2007, Bourkhiss *et al.*, reported the presence of α-acorenol (20.9%), cedrol (17.9%), totarol (8.8%), α-cedrene (8.7%). %) and β-acorenol (7.4%) as major constituents [30]. Satrani cited 2,5dimethoxy-acetophenone (22.5%), β-acorenol (20.4%) and cedrol (12.2%) as majority compounds in sawdust essential oil collected in Morocco [34]. The components of our oil were divided into five classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others. The results show that oxygenated monoterpenes (49.2%) and Sesquiterpene hydrocarbons (22.38), constituted the principal compound classes represented in the essential oils of sawdust, than Sesquiterpene hydrocarbons (10.30%) and oxygenated sesquiterpenes (3.91%), (Table 1). The comparative studies carried out in the literature of the *T. articulata* reveal the heterogeneity of the majority compounds [35, 36]. However, the chemical composition of the essential oil in general and the majority compounds, in particular, vary quantitatively and qualitatively under the effect of several factors such as the ecological conditions, the geographical origin, the analyzed part of the species, the mode of extraction, and the period of harvest [34, 37, 38].

Table 1. Chemical composition of the essential oil from TAEO.

Compounds	**Retention indices**	**% Area**
Terpinene-4-ol	7.745	1.56
2-carene	7.941	0.44
Thymyl methyl ether	8.725	3.86
Thymoquinone	8.836	0.89
Carvenone	8.969	1.21
Carvacrol	9.588	20.59
α-Cedrene	11.309	8.99
α-copaene	11.436	1.87
Acoradiene	11.992	2.65
Acetoveratrone	12.162	17.47
2,5-Dimethoxyacetophenone	12.267	1.86
p-Cresol	12.405	0.69
α-Curcumene	12.564	0.44
γ-Pyronene	12.622	1.02
Cedrol	13.877	10.30
Piperonylamine	14.041	0.8
Cedr-9-ene	14.184	4.88
β-Curcumene	14.232	3.55
allo-Ocimene	15.990	0.66
Isopropylphenol	16.181	1.07
Totarol	21.836	1.28
Carbofuran	22.667	1.83
Monoterpene hydrocarbons		2.12
Oxygenated monoterpenes		49.2
Sesquiterpene hydrocarbons		22.38
Oxygenated sesquiterpenes		10.30
Others		3.91
Total		87.25

3.2. Antioxidant activity of essentials oils.

The results of the antioxidant effect of TAEO are expressed as the percentage of inhibition, as shown in figures 1, 2, and 3. It was noted that the percentages of inhibition antioxidant increase with increasing concentration of TAEO and ascorbic acid used as a reference antioxidant.

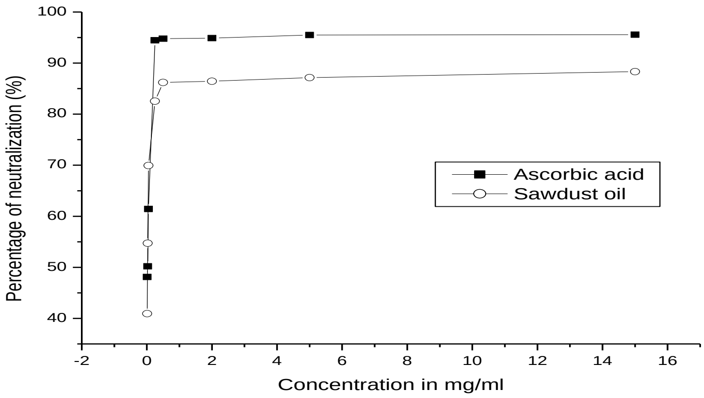

Figure 1. Evolution of percentage neutralization (%) obtained by the DPPH Test for TAEO and ascorbic acid.

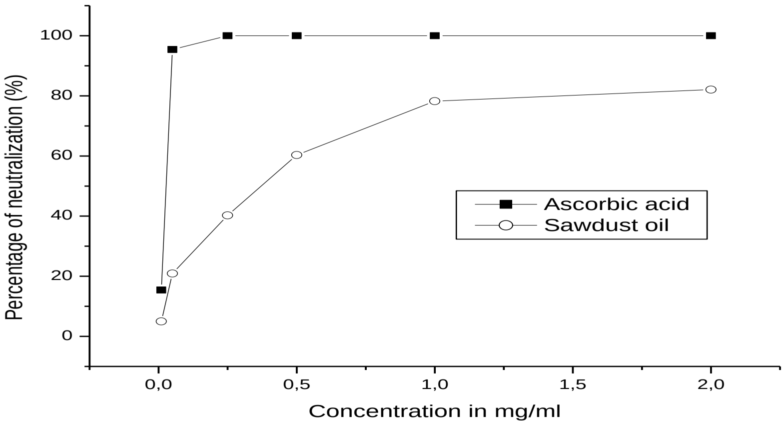

Figure 2. Evolution of percentage neutralization (%) obtained by the ABTS Test for TAEO and ascorbic acid.

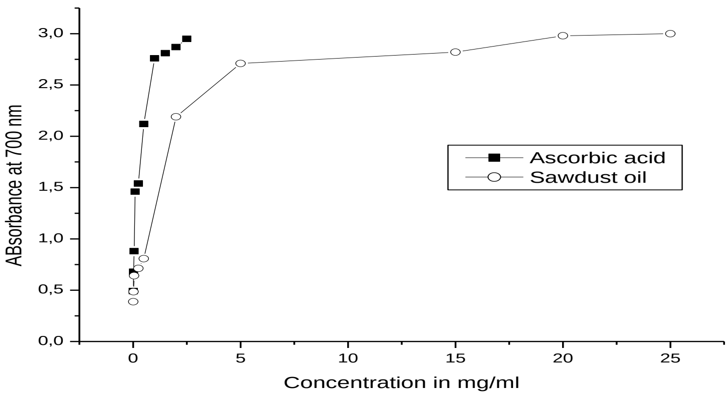

Figure 3. Graph showing the reducing power FRAP of TAEO compared with that of ascorbic acid.

The antioxidant activity of TAEO for DPPH and ABTS is expressed as IC_{50}, which defines the concentration of the tested sample needed to reduce 50% of oxidants. In addition, the more the value of the IC_{50} is small, the more the plant has a strong anti-radical effect. For the FRAP test, the results are expressed as the EC_{50} values, which are defined as the effective concentrations at which the absorbance is equal to 0.5. The exploitation of the results for the FRAP test was carried out following the evolution of the absorbance according to different concentrations of the oil studied. The results of Figure 3 showed that reducing power (FRAP) of TAEO increases with increasing concentration.

The IC_{50} and EC_{50} values are calculated graphically by the linear regression of the graphs plotted previously (Figures 1, 2, and 3). The results are summarized in Table 2.

Table 2. Antioxidant activity of Sawdust essential oil from Thuya and ascorbic acid.

	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	FRAP (EC_{50} mg/mL)
Sawdust oil	0.0144	0.38205	0.02915
Ascorbic acid	0.0184	0.03544	0.01062

The calculated IC_{50} and EC_{50} values for our oil confirmed the reactivity of this samples opposite to the oxidants; this high activity of neutralization of the DPPH radicals in comparison with the reference antioxidant (AA), the EOs obtained from sawdust is more active (IC_{50}=0.01444 μg/mL) than the reference antioxidant (IC_{50}=0.0184 μg/mL) this can be attributed to the higher percentage of carvacrol which represents 18.78% of the total oil, this compound is recognized in the literature by its high antioxidant power [39, 40]. In the FRAP method, the reduction activities revealed for the sawdust oil is active (EC_{50}=0.029 mg/mL) but lower than the reference antioxidant (ascorbic acid EC_{50}=0.01 mg/mL).

In comparison with the literature, the results obtained are superior to those revealed by El Jemli and collaborators who worked on TAEO leaves of the Marrakech region (IC_{50}=12.05±0.24 mg/mL) [4], and those obtained in the three regions of Algeria namely: Zeddine (IC_{50}=113.47±4.19 mg/mL), Mansoura (IC_{50}=125.75±3.33 mg/mL and Tazoult (IC_{50}=252.49±6.14 mg/mL) [10].

These results are considered important, especially in the food industry, because TOEO did not show *in vivo* toxicity on human health and, therefore, can be added to food for conservation [41].

4. Conclusions

The aromatic plants are currently a reliable source of active ingredients recognized by their therapeutic properties, including antioxidant activities. This work was focused on the study of the chemical composition and the antioxidant activity of TAEO. The chemical composition analysis of the essential oils by GPC-MS identified 22 compounds with carvacrol as the major compounds. The results of the antioxidant activity showed that this sawdust oil has a strong antioxidant activity. However, sawdust oil is just waste from wood processing and the manufacture of panels and furniture. This study can find an important application in the pharmaceutical industry and the food industry. However, further investigations should be carried out to determine the antioxidant effects of TAEO bioactive compounds and their applications in the food industry.

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Conflicts of Interest

The authors declare no conflict of interest.

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