Extraction of essential oils and evaluation of the biological activities of *Artemisia herba alba.*Asso and *Artemisia campestris* L in the Tiaret region

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Abstract

The objective of this study is to evaluate the antioxidant activity and antifungal activity of the essential oils extracted from the aerial part of the medicinal plants of the family of asteraceae: *Artemisia herba alba* Asso «chih» and *Artemisia campesiris* L «Dgouft» harvested in the region of Ain Deheb (Tiaret).The extraction of essential oils and hydrolat from the aerial part of the plants studied was carried out by the method of hydrodistillation with a yield of 0.25% and 0.73% respectively. The antioxidant activity of the essential oils of both plants is evaluated by the iron reduction method (FRAP test), shows a low activity of 0.031% of *Artemisia herba alba.*Asso and 0.041% of *Artemisia compestris* L, above all in relation to the percentages obtained from the standards used of 0.96% for gallic acid and 0.42% for ascorbic acid. The antifungal effect of both essential oils was evaluated by the direct contact method (fungistatic or fungicide). The essential oils *of Artemisia herba* alba.Asso*, Artemisia compestris* L have a weak antifungal and antioxidant effect. Overall, the results are promising and open new perspectives for the use of nature.

Keywords: *Artemisia herba alba.*Asso, *Artemisia compestris* L , antifungal, antioxidant, essential oil.

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Introduction

Algeria is one of the Mediterranean countries with a remarkable floristic wealth. The number of taxa of its flora is estimated at around 4000. About 90% of them are in the north of the country **(Sassoui et *al.*, 2020)**. Among the medicinal plants that make up the plant cover, is the genus *Artemisia*, many such species are used in traditional medicine because they contain several molecules with therapeutic activities, among the best known species is "*Artemisia campestris* L and *Artemisia herba alba.*Asso. Approximately 80% of the world's population uses herbal remedies as the main form of health care **(Botrel, 2001).**

The history of natural substances is partly identified with that of pharmacy, of which one discipline, pharmacognosia, studies poisons and natural remedies, or by extension most biologically active substances **(Mohammedi, 2013**). Scientists are currently looking for new antibiotics and natural food additives. Unlike most antibiotics currently used, essential oils and plant extracts are examples of these metabolites that provide new sources of natural alternatives **(Benbelaid et *al.*, 2014).**

In addition, they are known for their anti-cancer, antidiabetic, antiviral, antifungal, antibacterial and antioxidant properties **(Luciardi et *al.*, 2016).** Essential oils are well-known nonphytotoxic compounds with potential activity against microorganisms. Indeed, plants are often characterized by the biosynthesis of olfactory molecules that form so-called essential oils. Essential oils have long been known for their antiseptic and therapeutic effects in traditional medicine **(Selles, 2012).** The therapeutic effects of essential oils are becoming increasingly important. Today, they are in high demand, as they are generally endowed with interesting biological properties.

Essential oils that are considered to be natural bioactives are a good choice in the discovery of new therapeutic molecules, and have attracted interest in several studies because of their considerable number of biological properties. Within the framework of the knowledge and valorization of Algerian natural products mainly of vegetable origin, we are interested in the extraction of essential oils and their hydrolates from medicinal plants: *Artemisia herba-alba.*Asso and *Artemisia campestris* L.

The *Artemisia herba-alba*. Asso, or the white wormwood named in Arabic as the «chih» of the Asteraceae family, usually grows in small tufts. It is a plant with different uses. It is characterized by its richness in essential oil of different composition that led to the definition of several chemotypes; its high forage value and its very important ecological role against erosion and desertification. **(BouzidI , 2016).** *Artemisia campestris* L commonly referred to as "tgouft", "alala" or "tedjouq" **(Sassoui et *al.*, 2020)** is widely used in traditional medicine especially as a decoction for its antivenous, anti-inflammatory, antirheumatic, for the treatment of ulcers, digestive disorders of burns, diarrhea and other diseases. All of these discoveries gave us the idea to study the antioxidant and antifungal activity of the essential oils and hydrolats of these two plants growing in the Wilaya of Tiaret in Algeria. Several studies have been conducted on biological **activity (Mighri et *al*, 2010, Abu Darwish et *al*, 2015).**

The objective of our work is based on the extraction and recovery of essential oils and the hydrolat of plants: *Artemisia herba-alba.*Asso, *Artemisia campestris* L by hydrodistillation on the one hand and to highlight the biological activities of the plants studied.

2. Materials and methods

The samples of both species were collected on the plateau of AIN DHEB located in the central part of the steppe region of the willaya of TIARET, south of the NADHOR massif (Latitude: 34.8445, Longitude: 1.54924 34° 50′ 40″ North, 1° 32′ 57″ East).

The purpose of our study was, on the one hand, to extract the essential oils of *Artemisia herba alba*.Asso and *Artemisia campestris* L by hydrodistillation using a Clevenger apparatus and, on the other hand, to evaluate their antifungal and antioxidant activity.

2.1. Materials

2.1. 1. Plant material:

The species we studied were selected based on their use in local traditional medicine. The aerial parts (leaves, stems) were the focus of our work.

Random collection takes place in a natural location, away from contamination, to eliminate the possibility of changes in the chemical composition of the plant material. The harvest of the two plants, *Artemisia herba alba*.Asso and *Artemisia campestris* L was carried out in February 2020 in the region of Ain Dheb TIARET, the plants were cleaned and dried in the shade, protected from moisture and at room temperature for 15 days and stored in paper bags until use Figure (1).

Figure.1.*Artemisia campestris*.L(**A**) *Artemisia herba alba*.Asso (**B**)

2.1.2 Biological Material

2.1.2.1 Fungal activity:

We used three fungal strains Table (1)

- *Fusarium*
- *Penicillium*
- Type of yeast: *candida*

Table 1: Characteristics of strains used.

Microbialspecies	Characteristics	Diseasescaused	Reference	Provenance
Fusarium	Fusiform pluricellular sports more or less curved	Fusariumheadblight	Scientific website of the ESIAB	Laboratory of microbiology, IbnKhaldoun University Tiaret
Pénicillium	Presence of branched erect conidiophores	Skin and mouth infection	Scientific website of the ESIAB	Laboratory of microbiology, IbnKhaldoun University Tiaret
Candida albicans.	Fungus (unicellular yeast, het Erotrophic	candidiasis	(Assous and al.1999;Bedd or, 2015)	Privatelaboratory MAACHI

2.1.2.2 Antioxidant Activity

The antioxidant power of our oils and hydrolates has been tested using the FRAP Iron Reduction Test method which is often used to study the ability of a substance to give electrons. This property is an important mechanism of antioxidant action **(Ebrahim et *al.*2008).**

2.2 Methods:

2.2. 1 Extraction of essential oils (EO):

The hydrodistillation is carried out using a Clevenger type apparatus by placing 50 g of vegetable material in a 1000 ml bottle containing 500 ml of water. After installation and closing of the assembly, the balloon heater is activated with an optimal adjustment of the heater to allow a stability of the extraction at a constant and well controlled rate. Vapours loaded with essential oils enter the condenser. The total extraction time is estimated at 3 hours. Essential oils differ in density and color of hydrolates. It is separated by decantation. Then harvest, set aside and keep cool (4°C).

2.2.2 Calculation of extraction efficiency:

According to AFNOR (1986), the essential oil yield is estimated by the ratio of the masses of the essential oil and the dried vegetable material. It is expressed in percent (%).

$$Y\ (\%) = MEO / MMV \times 100$$

Y: Essential oil yield in %

MEO: The mass of essential oil in g

MMV: The mass of the vegetable material used in g

2.2.3 Study of antifungal activity:

The methodology followed for the evaluation of the antifungal effect of the essential oil extracted from *Artemisia herba alba*.Asso and *Artemisia campestris* L is the direct contact method that allows the identification of the antifungal activity (fungicide or fungistatic) of these extracts.

The essential oil and hydrolate to be tested are incorporated into the agar culture medium. After solidification, the medium is seeded and incubated.

2.2.3.1 Preparation of culture media containing a concentration of essential oil and hydrolat

2.2.3.1.1 Seeding and Incubation of Petri Dishes

In view of the non miscibility of oils and hydrolates to water and therefore to the culture medium, tween 20 emulsified the latter two in order to obtain in the medium a homogeneous distribution of the compounds in the dispersed state **(Remmal et *al.*, 1993; Satrani , 2001).** The method used is that of **Fandohan (2004).** The selective medium (Mueller Hinton) is cast in Petri dishes Figure (2).

Using a sterile pasteur pipette, we take a volume of 1 ml of fungal culture from a mycelial suspension, and place it in the petri dish and spread it with a glass notch in contact with the agar and rotate the box.

We inhibit the essential oils on the discs and place them sporadically in the petri dish. The Petri dishes are then sealed at room temperature for 48h. Figure (3)

Figure.2. Mueller Hinton in Petri dishes

Figure.3. Petri dishes during incubation

2.2.3.1.2 Assessment of mycelial growth

Mycelial growth kinetics was evaluated after the 48 hours by measuring the diameter of the washer that forms the zone of inhibition.

2.2.4 Study of antioxidant activity

The iron reducing activity of our aqueous extract is determined according to the method described by Oyaizu (1986). Preparation of the different solutions of the protocol the different solutions prepared:

- Phosphate buffer (Ph 6.6)
- Potassium ferrocyanide (K3Fe(CN) 6) at 1
- Trichloroacetic acid (10%)
- Iron chloride (FeCl3) at 0.1

To a test tube containing 1 ml of each sample, 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of potassium hexacyanoferrate (1%) were added. The tube was heated to 50°C for 20 min in a water bath. A volume of 1 ml of trichloroacetic acid (10%) is added to the mixture which is centrifuged at 3000 rpm for 10 min. Finally, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride (0.1%). A control without sample is prepared under the same conditions. The reading is measured at 700 nm. Ascorbic acid and gallicacid are used as positive controls, a prepared solution of 9ml of methanol and 1gm of ascorbic acid. Otherwise a prepared solution of 9 ml of methanol and 1 gm of gallic acid.

3. Results and discussions

3.1. Organoleptic characteristics of essential oils

The essential oil was extracted from the two plants dried in the open air and this by hydro-distillation in an apparatus of type clevenger we obtained an oil of transparent color and accentuated odor, very strong for *Artemisia herba alba.*Asso, *Artemisia campestiris* L gave oil with less accentuated odor.

The essential oils were extracted by hydrodistillation of the dry plant materials. The yields of essential oils were calculated according to the dry plant material of the aerial part of the plant. The samples of *Artemisia campestris* L yielded about 0.73%, which is higher than that obtained from *Artemisia herba alba.*Asso, which represents a low essential oil yield of 0.25%.

According to **Kelen and Tepe, 2008**, this difference in yields between sage birds can be explained by the

choice of harvest period because it is essential in terms of yield and quality of the essential oil. The climate, the geographical area, the genetics of the plant, the organ of the plant used, the degree of freshness, the drying period, the extraction method used. These are factors that can also have a direct impact on essential oil yields. **(Vekiari et *al*, 2002).**

3.2. Antifungal activity

The antifungal power of essential oils and hydrolats of *Artemisia herba alba.*Asso and *Artemisia campestris* L on the three fungal strains (fungi): *Fusarium*, *Penicillium* and *Candida* A used, was studied by the disc diffusion technique Figure (4, 5, 6).

The results of the test of the antifungal activity of the essential oil of *Artemisia herba alba.*Asso *and Artemisia campestris* L from the region of Tiaret are shown in table (2)

Table 2: Antifungal effect of essential oils of ***Artemisia herba alba.***Asso, ***Artemisia campestris*** L

	Artemisia herba alba		*Artemisia campestris*	
	Oil	Hydrolat	Oil	Hydrolat
Fusarium	00%	00%	01.58%	2.05%
Pénicillium	0%	0%	0%	0%
Candida .A	0%	0%	0%	0%

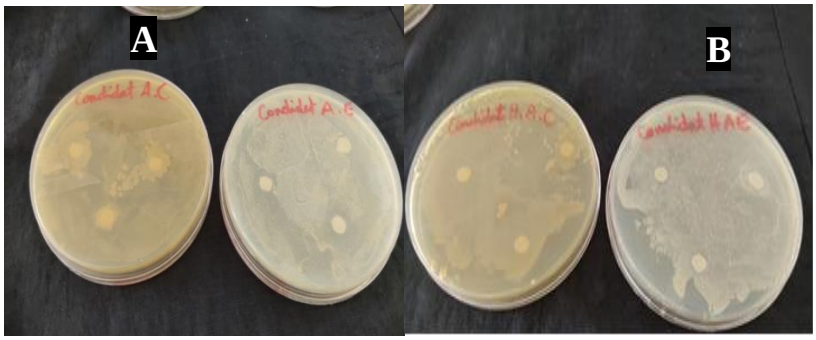

Figure4. Diameters of the inhibition zones (m/m)
Of *Artemisia herba alba* (AH) and *Artemisia campestris* (AC) of Candida
A : oil ; B : hydrolat

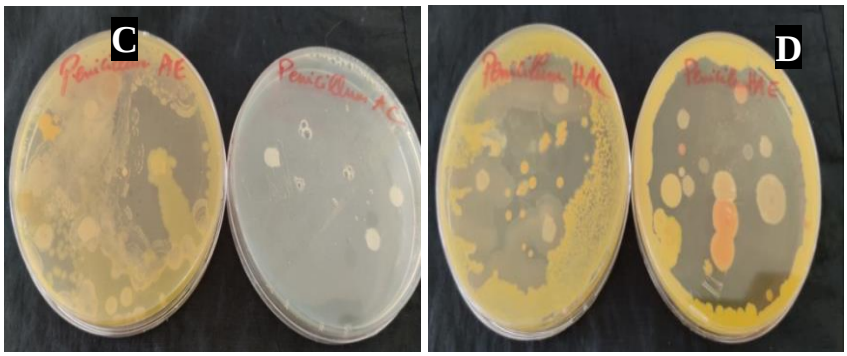

Figure 5. Diameters of the inhibition zones (m/m)
Of *Artemisia herba alba* (AH) and *Artemisia campestris* (AC) of *Penicillium*C: oïl ; D :hydrolat

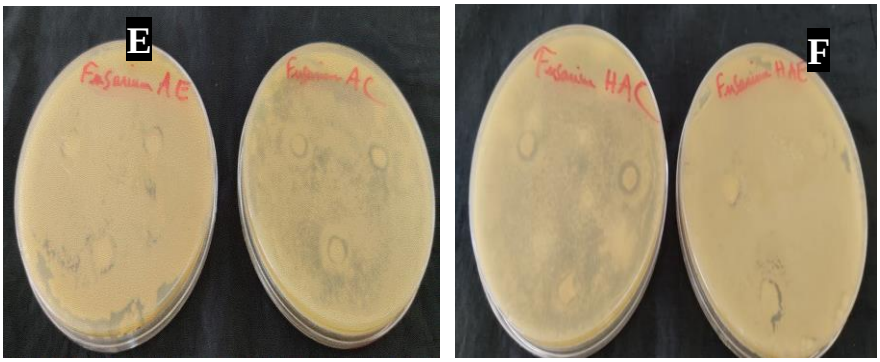

Figure.6. Diameters of the inhibition zones (m/m)
Of *Artemisia herba alba* (AH) and *Artemisia campestris* (AC) of *Fusarium*
E :Oïl ; F : hydrolat

From the results obtained, the antifungal activity of the essential oils and their hydrolats seems to vary from weak to null and no antifungal power, the essential oil of *Artemisia campestris* L shows a very weak antifungal potential on the species *Fusarium* with a diameter of the zone of inhibition of 01.58mm for the essential oil and 2.05mm for the hydrolat, as it appears completely inactive with respect to the remaining fungal samples *Penicillium* and *Candida*. As for the oil and hydrolate of *Artemisia herba alba*.Asso, they appeared with a totally non-existent effect for all fungal samples.

Let's compare our results with recent works, one study showed that *Artemisia campestris* L oil has a weak antifungal activity against the tested fungi and contrary to *Artemisia herba alba*.Asso oil, another study showed that it has a good antifungal activity on the tested molds with a minimal inhibitory concentration of 0.05%. These fungi have a very high resistance potential against the antifungal action of *Artemisia* essential oils.

The weak antifungal activity of the essential oil of *Artemisia campestris* L can be explained by its chemical profile poor in compounds known for their antifungal power such as some monoterpenic alcohols **(Koba et *al*, 2004)** and phenols and rich in terpenic hydrocarbons (68%), including β-pinene and limonene.

The antifungal activity of *Artemisia herba alba*. Asso has been shown to be associated with two major volatile compounds: carvone and piperiton **(Saleh et *al*, 2006).**

These compounds are absent in the oil of *Artemisia herba alba*. Asso tested, which explains the absence of inhibition rate in all tests. In a study of 25 medicinal plants, including *Artemisia herba alba*. Asso, the antifungal activity of *Artemisia herba alba*. Asso essential oil against *Penicillium* was studied. *Artemisia herba alba*. Asso essential oil showed no antifungal activity **(Bochra et *al*, 2003).**

In another study, the antifungal activity of mugwort oil is explained by its richness in oxygenated compounds (chrysontenone, camphor, α-terpin-7-al and trans-β-terpineol). The Oil of White Mugwort of Djelfa is very rich in all sesquiterpenes (74%).

The oils of *Artemisia herba alba.* Asso and *Artemisia campestris* L had no fungal activity against all the fungi tested. These results lead us to conclude that both plants have a weak to completely ineffective effect against the tested fungi.

3.3 Antioxidant activity

The antioxidant activity of the oils of *Artemisia herba alba.*Asso and *Artemisia campestris* L is carried out by the FRAP (Ferric Reducing Antioxidant Power) method. This method is based on the reduction of antioxidants of the Ferro-Tripyridyltriazine complex which based on the chemical reaction of reduction of Fe+3 to Fe+2 this reductive ability can serve as a significant indicator of the potential antioxidant activity of a compound. This reaction creates a very intense navy blue color. The intensity of this staining is measured by spectrophotometry at 700 nm. **(John et *al.*, 2012; Pisoschi et *al.*, 2011).**

The reducing power assay is used to test the ability of *Artemisia* essential oil to convert Potassium Ferricyanide (Fe^{3+}) to Potassium Ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferrous complexes. This property constitutes an important mechanism of antioxidant action.

The increased absorbance of the reaction mixture indicates an increase in the reducing capacity of the tested oil. This capacity can be used as a significant indicator of antioxidant activity **(Chang et *al.*, 2007; Degryse et *al*,. 2008).**

The antioxidant activity of the oils was evaluated by spectrophotometry following the reduction of ferrocyanide which is accompanied by its change from yellow to green colour measurable at 700nm, the results are presented in Table (3) Figure (7, 8).

Table 3: Absorbance values of the two plants and the standards used

	Artemisia herba alba		*Artemisia compestris*		*Ascorbicacid*	*Gallicacid*
	Oil	hydrolat	Oil	hydrolat		
(C)	50	Pur	50	Pur	50	50
DO	0.031	0.004	0.041	0.007	0.422	0.963

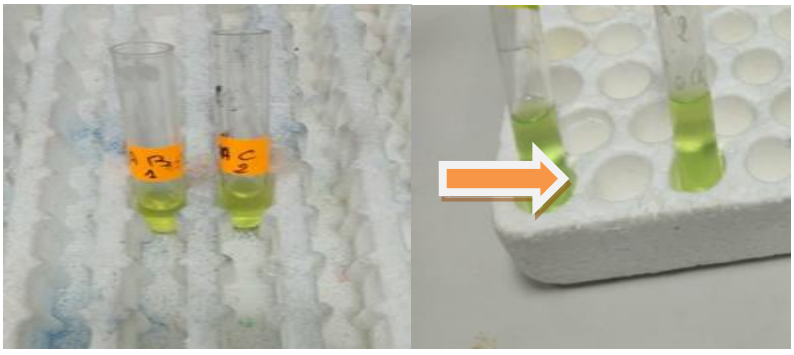

Figure.7. The variation of the color of the essential oils of the two plants

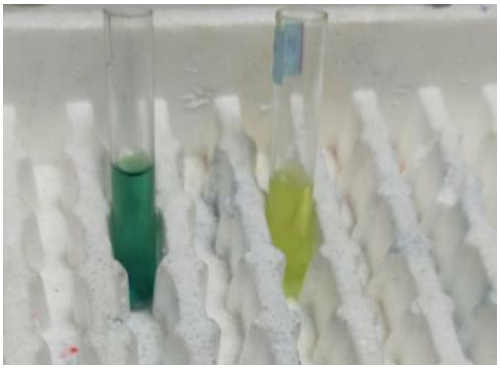

Figure.8: Color change of the two samples each containing one of the standards (Ascorbic Acid, Gallic Acid)

After seeing the results of the color change, they can be taken as preliminary results with the naked eye, which the color of the oil sample is light green, close to the sample of white, a very slight change resulting from a small reduction.

As it is also far from the color of the control, which is dark green, indicating the strength of reduction .We find that both oils have low antioxidant activity before this can be confirmed by optical density measurement.

Evaluating the uniform concentration of the samples tested the value of the corresponding absorbance for each; we have drawn the graph that represents the variation of the optical density as a function of the concentration of 50 mg/ml figure (9).

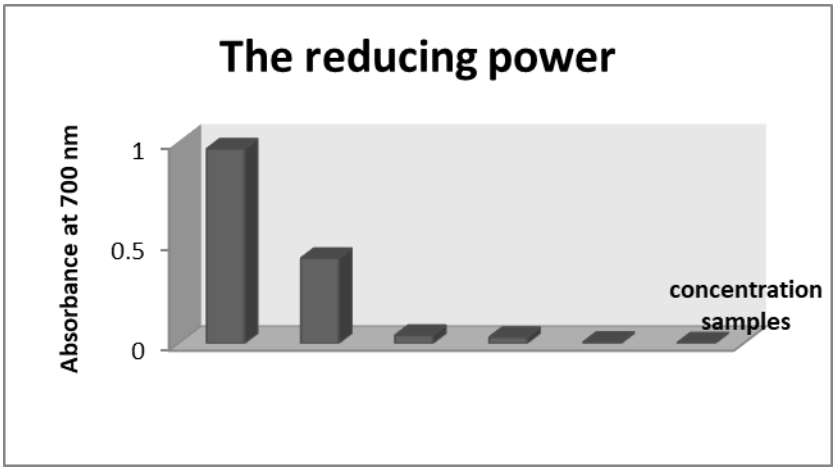

Figure.9: Graph represents absorbance as a function of concentration

Antioxidant activity therefore ranks in the following descending order:

Gallic acid > Vitamin c > *Artemisia herba alba*.Asso> *Artemisia campestris* L> hydrolat d' *Artemisia campestris* L > hydrolat d' *Artemisia herba alba*.Asso.

Concerning the reducing power test, the graph above shows that Artemisia oils and hydrolats have a low antioxidant activity compared to Ascorbic acid and Gallic acid at the same concentration. The reducing power is probably due to the presence of hydroxyl group in the phenolic compounds which can serve as electron donor. Therefore, antioxidants are considered as reducing and inactivating oxidants **(Siddhuraju et *al.*, 2007).**A previous work also shows the same result that *Artemisia herba alba* at low antioxidant activity compared to ascorbic acid

(Kaouan et *al.*, 2017).A recent work also shows the same result as *Artemisia campestris* which has a very low antioxidant power **(Boudjouref, 2011).**

In general, **Lopes-Lutz et *al*, 2008** confirmed in a study made on some *Artemisia* species that the antioxidant activity of these plants is low.

Conclusion

At the end of this work, the study of the antifungal activity was demonstrated by the absence or presence of mycelial growth by the direct contact technique on three fungal strains. The essential oils and hydrolats of the two plants have a weak inhibitory activity towards the tested strains. Our application of antifungal power resulted in low inhibitions compared to controls. The study of antioxidant activity by FRAP method shows that the highest activities were obtained with the two standards: ascorbic acid and gallic acid (0.422%, 0.963%), followed by the oil of *Artemisia herba alba*.Asso (0.031%) and that of *Artemisia compestris* L with (0. 041%) and lastly the hydrolate of both species *Artemisia herba alba*.Asso, *Artemisia compestris* L with 0.004% and 0.007% respectively, noting the previous results, this test showed that both oils and both hydrolates have a low antioxidant power compared to controls used.

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