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SCREENING OF ARTEMISIA VULGARIS EXTRACTS FOR ANTIOXIDANT ACTIVITY: A COMPARATIVE STUDY ON THREE TESTING METHODS

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ABSTRACT
There is a popularity and scientific interest to screen essential oils and extracts of plants used medicinally in all over the world [1]. The main volatile constituents of the essential oils have been used historically in the pharmaceutical, food and perfume industries because of their antibacterial properties, culinary and fragrance, respectively. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [2].

Keywords:
Antioxidant Activity, Artemisia vulgaris,extracts

INTRODUCTION

There is a popularity and scientific interest to screen essential oils and extracts of plants used medicinally in all over the world [1]. The main volatile constituents of the essential oils have been used historically in the pharmaceutical, food and perfume industries because of their antibacterial properties, culinary and fragrance, respectively. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [2].

With that in mind, Artemisia vulgaris (Asteraceae) might be of interest. This species has long been used in traditional medicine as a remedy for the treatment of various cancerous lesions of the stomach, colon, rectum esophagus and liver. Furthermore, it's have been described in treating hypertention and oedema, and as detoxicant, diuretic, anti-inflammatory, anti-pyretic and anti-purulent agents. It had been reported [3], although the chemical composition and biological activities of *A. vulgaris* have not been fully elucidated.

Here, we report the composition and antioxidant activity of the essential oil and various extracts of *Artemisia vulgaris* from Algeria as well as total phenolics, total flavonoids, condensed tannins, and total anthocyans of this plant.

Artemisia vulgaris

Harvesting of aerial parts (Flower and Leaves) of *A. vulgaris* was carried out in western Algeria; Chorfa region (Mascara, Algeria) in the month of March 2017 located at an altitude of 161 meters, a latitude of 35 ° 25 '55 "North and a longitude of 0 ° 14 '43 "West.

Botanical identification of this species was carried out according to African flowering plants database and by local experts.

The fresh plant was used for the extraction of essential oils, however for the preparation of the various polyphenolic extracts; we have dried in the shade the parts picked from the plant, protected from moisture and at room temperature. The drying is 7 days on average, then kept in paper bags.

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METHODOLOGY

Materials and Methods

Polyphenols extraction

From the remaining aqueous decoction of the hydrodistillation step, a fractionation is carried out in a separating funnel using Hexane, 50 ml solvent per 100 ml of decoction, after a reaction time the two phases are taken separately, one organic phase (hexanic extract) and another aqueous (aqueous extract) this method is based on a solvent extraction principle with increasing polarity [4].

Polyphenols analysis

The total phenolic in extracts content was determined by spectrometry using ‘‘Folin-
Ciocalteu Folin reagent assay [5]. Gallic acid was used as a standard for the calibration curve.
The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Condensed tannin content

Condensed tannins were transformed by the reaction with vanillin to anthocyanidols.
Condensed tannin contents of each organ (three replicates per treatment) were expressed as mg catechin equivalents per gram (mg CE/g) through the calibration curve with catechin.

Total flavonoid content

Total flavonoid content was measured according to [6]. Total flavonoid contents were expressed as mg catechin equivalents per gram (mg CE/g).

Antioxidant activity assays

DPPH scavenging assay

The hydrogen atom donation ability of chemical compounds in leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical [7]. Fifty microliter of various concentrations of the extracts in methanol was added to 1950 µl of a 0.025 g/l methanol solution DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) = $(A_{blanc} - A_{sample}/A_{blanc})$. 100

Where: A_{blanc} is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (EC50) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid methanol solution was used as positive control.

Determination of Ferric Reducing/Antioxidant Power (FRAP)

Different concentrations of the methanolic extract of M. serratulum and its various fractions (10-50 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, gallic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard. Results are expressed in µM Fe (II)/g dry mass [8].

Total antioxidant capacity (TAC)

During this test, hydrogen and electron is transferred from the reducing compound (Antioxidant extract) to the oxidant complex (PPM). This transfer depends on the redox potential of pH of the medium and the structure of the antioxidant compound.

The method comprises introducing 300 .µl of the extract of the leaves mixed with 2.7 ml of a reagent consisting of H_2SO_4 (0.6 M), NaH_2PO_4 (28 mM) and ammonium molybdate (4 mM). The tube is then incubated at 95 ° C for 90 minutes. After being cooled, the absorbance is measured at 695 nm. The control consisted of 300 .µl of methanol mixed with 2.7 ml of the reagent mentioned above; the calibrators, controls and samples are incubated under the same conditions. The results are expressed in mg of gallic acid equivalents per gram of dry material (mg E AG/g Ms) [9].

Statistical analysis

The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor ‘‘r’’. The P-values less than 0.05 were considered statistically significant.

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RESULTS & DISCUSSION

Extraction Yield

From these results, it was noted that the hexane extracts had a relatively low yield compared to the aqueous extracts, the best yield is obtained for the aqueous extract of the leaves (1.9%) followed by the aqueous extract flowers (1.45%).

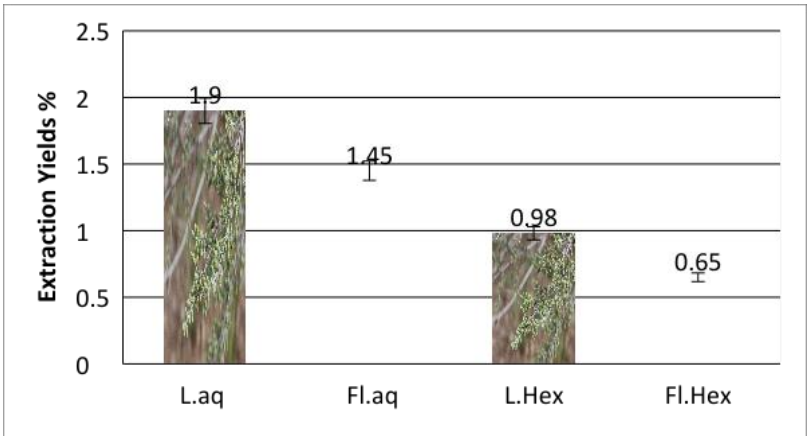

Fig.1: Extraction Yields

This rate is relatively higher than that of the extracts of *Artimisia herba alba*, the ethanolic extract has the lowest yield with (0.48%) [10]. The yield depends on the geographical origin of the plant, the season of harvest, method and conditions of the extraction. It is only relative [5].

Phenolic compound content

The results mentioned in this table indicate that the total polyphenol, total flavonoid and tannin concentrations of the four extracts are classified as follows: Laq> Lhex> Flhex> Flaq. This does not prevent the extracts of the flowers of *Artemisia vulgaris* are also rich in phenolic compounds.

Table1.Total Phenolic, flavonoids and tannins content

		Polyphenols(mg GAE/g PS) [a]	Flavonoids (mg EC/g PS) [b]	Tanins (mg EC/g PS) [c]
Leaves	Aqueous extract	1,631 ±0,601	0,03 ±0,102	4,13 ±0,18
	Hexanic extract	0,659 ±0,628	0,09 ±0,020	5,16 ±0,569
Flowers	Aqueous extract	0,95 ±0,54	0,01 ±0,35	2,95 ±0,32
	Hexanic extract	1,004 ±1,28	0,22 ±0,342	4,28 ±0,1

[a]: mg acid galic equivalent/g dry mass. [b, c] :mg catechin equivalent/g dry mass.

The results of the total polyphenol assay show that the aqueous leaf extract is the richest extract with: 1.631 ± 0.601 (mg GAE / g PS) followed by the hexane flower extract 1.004 ± 1.28 (mg GAE / g PS) and then the aqueous flower extract with 0.95 ± 0.54 (mg GAE / g PS), the hexane leaf extract with 0.659 ± 0.628 (mg GAE / g PS) represents the fraction that contains the lowest grade in polyphenols.

In a study done on *Artemisia campestris* by [6] determined the total polyphenol content of the aerial part of a 70% (v / v) ethanolic extract, they found that the content of total polyphenols is 20.38 mg EAG / g Ps, this content is relatively high compared to our results. This difference in the levels can be explained by the polarity of the solvents used the environmental conditions, climatic and collection period as well as genetic factors and experimental conditions.

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Flavonoids as the most interesting compounds of polyphenols are also determined in this work. The results of this assay show that the content of flavonoids differs from one extract to another. Indeed, the hexane extract of flowers is the richest in flavonoids. (0.22 ± 0.342 mg EC / g PS) followed by the hexane extract of the leaves (0.09 ± 0.020 mg EC / g PS) [11]. found a grade of 131.89 mg EQ / g aqueous extract of leaves of *Artemisia campestris* that appears to be significantly superior to our results. Also, we noticed that the contents of tannins condensed in the extracts are higher than those of the total phenols.

Antioxidant activity

From these results, it is noted that the percentage of inhibition of the free radical increases significantly ($p \leq 0.05$) with the increase of the concentration. The DPPH inhibition rates recorded in the presence of the different extracts of the plant are lower than that of ascorbic acid

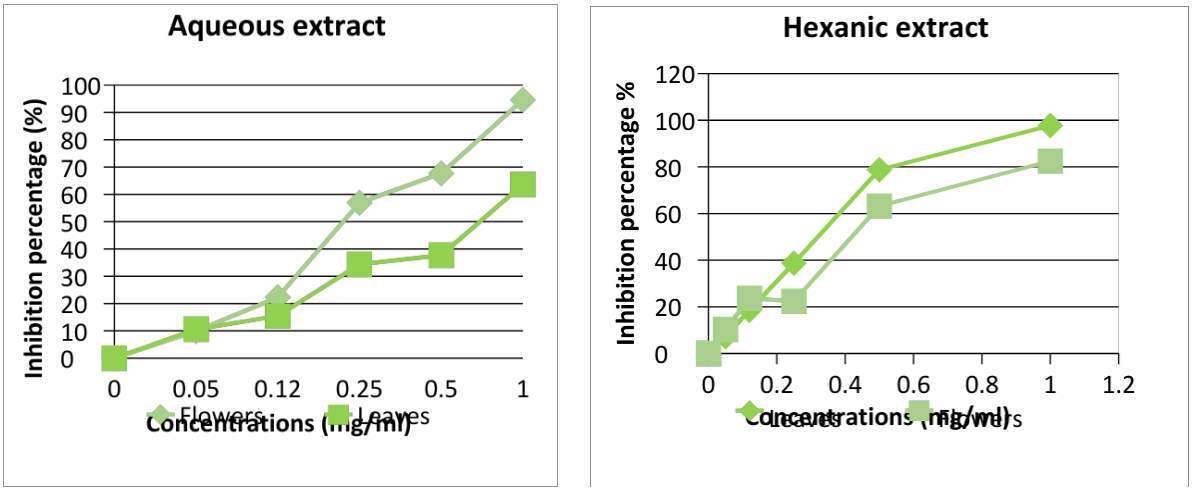

Fig.2: Inhibition percentage of DPPH radical

The aqueous extract of flowersc seems to have a better antioxidant activity than the hexane extract This is probably related to the complexity of the crude aqueous extract in polyphenolic substances including tannins and flavonoids and the synergy between them for a better antioxidant activity [12].

On the other hand, for the extracts of the leaves, we recorded a strong antioxidant activity that that of the aqueous extract that can be explained by the fact that the antioxidant capacity of the extracts of plants is strongly linked to the simple or combined phenolic content of the leaves [13,14,15], this is confirmed by phytochemical screening and TLC results found in hexane extract of *A. vulgaris* leaves. These results remain comparable with those found by [16] who reported that the alcoholic extract of *Artemisia herba alba* showed a strong antioxidant activity but with a much higher IC_{50} than ours which is 20.64 mg / l.

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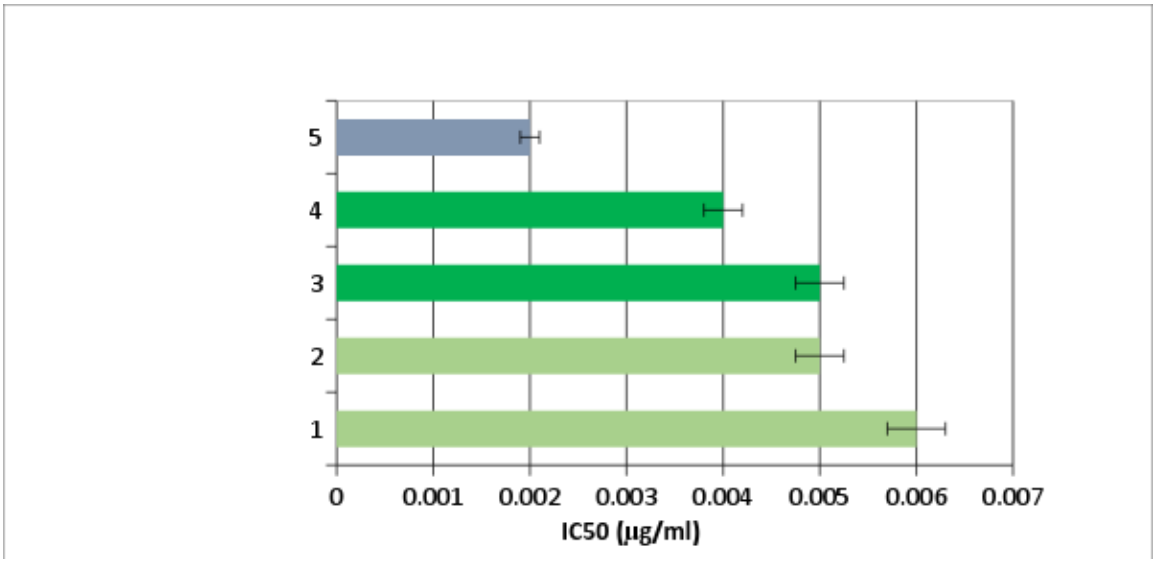


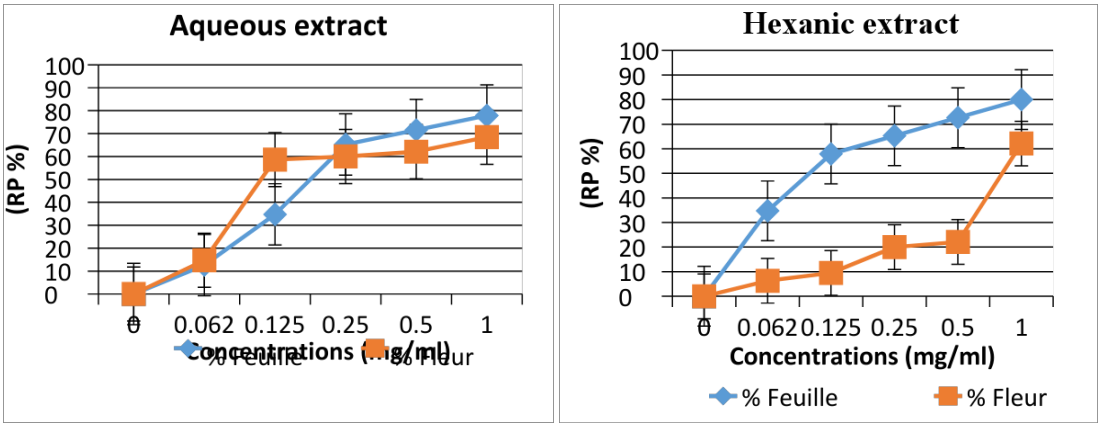
Fig.3: DPPH (IC50, µg/ml)

For the aqueous extract, the results are comparable with those found for the Egyptian *A.vulgaris* species (IC 50 = 11.4µg / ml DPPH) [17] .

[18] studied the antioxidant activity of three extracts of Artemsia campestris, found an IC50 value of 2.053 ± 0.1 mg / ml for the 50% ethanol extract, This result is significantly more important than our results, reflecting the important anti-radical activity of our A.vulgaris extract compared to A.campestris. In general, this antioxidant activity is due to the hydrogen donation ability of the various polyphenols present and bioactive compounds for HE extracts can be ranked in descending order of anti -radical power, as follows:
Vitamin C> Hexanic leaf extract> Aqueous flower extract> Aqueous leaf extract> Hexanic flower extract. This difference between the extracts can be explained that the compositions and the content of phenolic compounds and respectively the antioxidant activity are different for the different organs of the plants [19].

FRAP Method

The results shown in Figures show us that the reduction capacity is proportional to the increase in the concentration of our samples, which has been proven by other authors [20,21,22].



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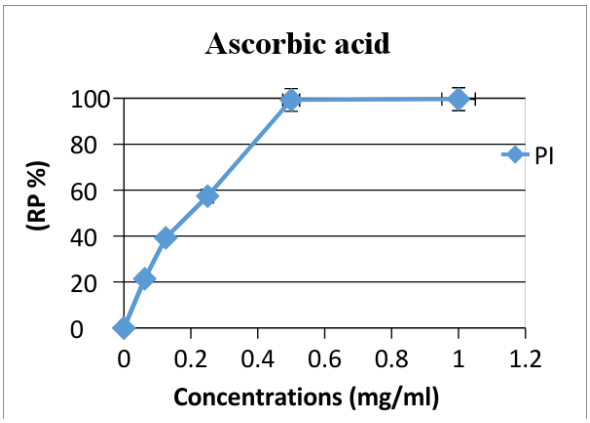

Fig.4: Ferric Reducing Ability

We note in the graphs shown in the figure above, that the ability to reduce iron leaf extracts and flowers is significantly lower than that of ascorbic acid with IC_{50} of the order of 0.003 mg / ml.
The leaf extracts (hexane and aqueous) of *Artemisia vulgaris* appear to have better antioxidant activity than that caused by flower extracts (hexane and aqueous), with maximum reductive activity recorded for the aqueous leaf extract 78.6 ± 1.09%, this remains comparable with the work of [23] on *Artemisia annua* leaves with (RC = 80 ± 1.12%) against 11.82 ± 1.12% for chloroformic and hexanic extracts. And those of [24,25] for other species of Artemisia. On the other hand, we have noticed that the reducing effect is more marked in the aqueous extracts than hexane ones, because one of the recent studies has been able to demonstrate the influence of the polarity of the extraction solvents on the reduction method. iron (FRAP) [26] although the latter may be inadequate for the determination of hydrophilic antioxidants [27].

TAC Method

In the light of these results, we can see that polyphenol extracts (especially hexanic extracts) provide a total antioxidant activity of the order of 0.06 ± 0.02 and 0.028 ± 0, 1 mg EAA / g for Flhex and Lhex respectively.These results are in agreement with [18,28].

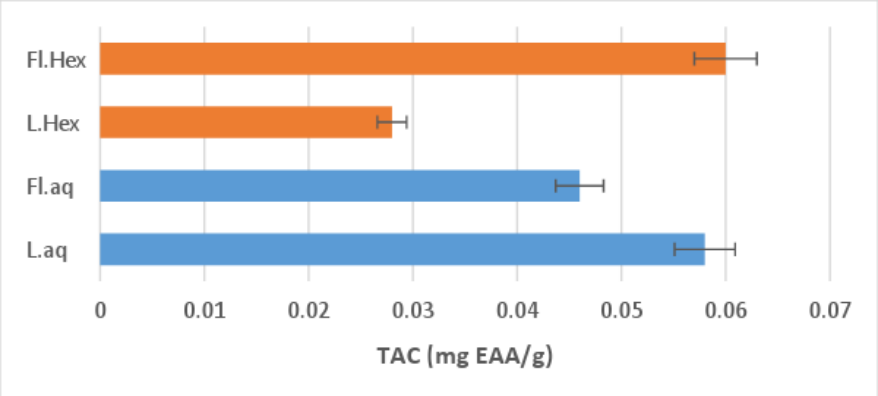

Fig.5: Total Antioxidant Power

The results found during this test (PPM Test) confirm the results previously found in the DPPH test, and the reduction of iron with minimal variability, which can be justified by the variability of the extraction methods, the radicals tested and estimation of antioxidant activity [29].

CONCLUSION

From the results, it can be concluded that the qualitative and quantitative phytochemical study demonstrated a richness of the two parts of Artemisia vulgaris the plant in bioactive compounds whether in their polyphenolic extracts which remains promising for the valorization of these broad-spectrum plants in the field of herbal

medicine. In addition, the extracts examined have an important antiradical activity but which varies from one method to another. These activities were found are probably in relationship with the structure of phenolic compounds , therefore; the antioxidant capacity of plant extracts is largely dependent on the composition of the extracts, but also the handling conditions of the *in vitro* tests .

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