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Extraction and characterization of the biological activity of essential oils from *Cotula cinerea* in the region of El Oued (Algeria)

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Abstract:

The valorization of spontaneous medicinal plants, a large part of which is still virgin and requires in-depth studies, then our objective will be an extraction of the essential oils of the aerial part and the flowers of *Cotula cinerea*, identifying physical parameters, pH measurement, density measurement, and refractive index. In parallel, biological activity (Antioxidant and antibacterial) is studied. Regarding the physical properties, we find the essential oils of the flowers and the aerial part have basic characters with the values of pH 8,39 and 8,60, the density is 0.95 and 0.90 and the index of refraction is 1,33350 and 1,33375 respectively. Both antioxidant activities show that both essential oils have IC_{50} 45.98mg/ml and 79.28 mg/ml for the flowers and the aerial part of *Cotula cinerea* respectively. A large ability to trap the DPPH radical is noted with the value of IC_{50} 0.04% for ascorbic acid. In parallel, the antibacterial activity was determined on two bacterial strains : *Escherichia coli* and *Pseudomonas aeruginosa*, the results obtained show that the two strains tested have a low sensitivity with different concentrations of our essential oil (*Escherichia coli* inhibitory zones and *Pseudomonas aeruginosa* is 6 up to 9 mm). On the other hand, these results remain very low compared to the antibiotic used (Amoxyclav: up to 13 mm for *Escherichia coli*, 13 to 15 mm for *Pseudomonas aeruginosa).*

Keywords: Spontaneous medicinal plant, *Cotula cinerea*, essential oils, Antioxidant activity, Antibacterial activity.

Résumé:

La valorisation les plantes spontanées à caractère médicinales dont une grande partie reste encore vierge et nécessite des études approfondis, alors notre objectif sera une extraction des huiles essentielles de la partie aérienne et les fleurs de *Cotula cinerea*, identifier les paramètres physiques, mesure de pH, mesure de la densité et l'indice de réfraction. Par ailleurs, l'étude de l'activité biologique (Antioxydante et Antibactérienne). Concernant les propriétés physiques, on trouve les huiles essentielles des fleurs et de la partie aérienne possèdent des caractères basiques avec la valeur respective de pH 8,39 et 8,60, la densité est de 0.95 et 0.90 respectivement toujours et l'indice de réfraction est 1,33350 pour les fleurs et 1,33375 pour la partie aérienne. Aussi bien l'activité antioxydante, montre que les deux huiles essentielles présentent IC_{50} 45,98mg/ ml et 79,28mg /ml des huiles essentielles des fleurs et la partie aérienne de *Cotula cinerea* respectivement) ont une grande capacité de piéger le radical DPPH dont IC_{50} 0,04% pour l'acide ascorbique. En parallèle, l'activité antimicrobienne a été déterminée sur deux souches bactériennes : *Escherichia coli* et *Pseudomonas aeruginosa*, les résultats obtenus montrent que les deux souches testées possèdent une faible sensibilité avec différentes concentrations de nos huiles essentielle (Zones d'inhibitions est compris entre 6 et 9 mm). Par contre ces résultats restent très faibles par rapport l'antibiotique utilisé (Amoxyclav de telle façon que la zone d'inhibition est entre 10 et 13 mm pour *Escherichia coli* et entre 13 à 15mm pour *Pseudomonas aeruginosa*).

Mots clés : Plante spontanée medicinale, *Cotula cinerea*, huiles essentielles, activité Antioxydante, activité Antibactérienne.

1. Introduction

The evaluation of plant protection, antioxidant and antimicrobial properties remains a very interesting and useful task, especially for plants with a rare or less frequent or unknown use in traditional medicine. These plants represent a new source of active compounds (TEIXEIRA DA SILVA, 2004).

Essential oils are by definition secondary metabolites produced by plants as a defense against phytophagous pests (Cseke et al., 1999).

The activity of volatile oils resides in the hundreds of chemical molecules that constitute it like terpenedia. These give the plant its smell, others are responsible for the perfume (Cowan, 1999).

The field of application of essential oils is diversified despite the arrival on the market of synthetic compounds; it is thus that they find many applications in the chemical industry and the agri-food field (condiments, spices, flavorings,...) and aromatherapy (perfumery, cosmetics, and soap) (Petitjean, 1974).

Our work aims to enhance the medicinal value of essential oils through their use in traditional medicine and to study their biological activity.

2. Materials and methods

2.1. Physical properties

2.1.1. Performance

According to to the French Standards Association, 1987, the yield of essential oils is defined as the ratio between the mass of essential oil obtained after extraction (M') and the dry mass of the plant material used (M). It is given by the following formula:

$$RHE\ \% = (M'/M) \times 100 \qquad \text{(Eq.01)}$$

RHE: yield of essential oil from plant material; M': a mass of essential oil obtained in grams. M: a mass of plant material used in grams.

2.1.2. Determination of pH

The pH indicates the acidity or alkalinity of the medium, it is determined from the number of free hydrogen ions (H) contained in the essential oil (Audigie et al., 1984).

2.1.3. Density

It is the ratio of the mass of a certain volume of oil at 20°C, and the mass of an equal volume of distilled water at the same temperature (Lion, 1995).

$$d_{20}^{20} = \frac{m_2 - m_0}{m_1 - m_0} \qquad \text{(Eq. 02)}$$

With, m_0: Mass of empty pycnometer; m_1: Mass of pycnometer filled with water; m_2: Mass of pycnometer filled with essential oil.

2.1.4. Refractive index

The refractive index nλt is defined as the ratio between the sinus of the refractive angle of the refracted radius in the medium in question. This index is measured at 20°C and related to the D-line of sodium (λ =589nm) (Lion, 1995).

2.2. Antioxidant activity

This method is based on the reduction of a very stable free radical: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in the presence of a donor antioxidant. The latter is reduced to the form of hydrazine (not radical) by accepting a hydrogen atom.

The percentage inhibition is calculated according to this relationship:

$$\% \, Inhibition = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \times 100 \qquad \text{(Eq. 03)}$$

Abs control: White absorbance (containing all reagents except the test compound), and Sample abs: Absorbance of the test compound.

Since there is no absolute measure of the antioxidant capacity of a compound, the results are often compared to a reference antioxidant in our experiment is ascorbic acid.

2.3. Statistical Analysis

The data obtained are treated statistically by the Microsoft Office Excel 2007 software, for the calculation of the concentrations from the calibration curves and the presentation of the results.

3. Resultants and Discussions

3.1. The extraction efficiency of essential oils

The average yield of extraction of essential oils from fresh flowers is 2.54% and 1.07 for the aerial part.

GAD and MORTON (1999) and Ennajar et al., (2009) explained that the yield differs from one botanical family to another, between plants of the same species, from one stage of development to another, and from one season to another.

In parallel, Mahjoub et al., (2012) finds with the same plant the yield of essential oil is very low (0.0801% 0.0117%). It is relatively higher than those obtained by Kether et al., (2012) working on different parts of *Cotula coronopifolia L*. In Tunisia (0.01134% in leaves); 0.03935% for flowers ; 0.00123% in roots and 0.00405% for stems).

3.2. Physical properties

Physical properties such as refractive index, density, acid index, and ester index, are a means of checking and controlling the quality of the essential oil. In the absence of quantity, only the first two characteristics of the essential oils analyzed were determined according to the standards of the French Standards Association (1989).

The results of the calculation of the physical characteristics of the essential oils of *Cotula cinerea* obtained by hydrodistillation are evaluated and recorded in Table 1.

Table 1. Physical characteristics of the essential oils of *Cotula cinerea*.

Characteristics	Flower Species	Aerial Species
Density	0,95	0,90
Ph	8,39	8,60
Refractive index	1,33350	1,33375

The determination of the density of oil tells us about its purity. It depends on the chemical composition of the oils and the temperature (Karleskind, 1992).

It has been noted that the density from the essential oils of the flowers and air part is 0.95 and 0.90 respectively. These density values are close to those recorded by MAHBOUB et al., (2020) and FELLAH et al., (2006) and are in the range of between 0.9 and 2.

According to Tenscher et al., (2005), this parameter is related to the chemical composition of this oil, which is affected by some factors such as phenotype, time of harvest, type of soil, conservation, process, and extraction conditions.

The essential oils of the flowers and the aerial part have the basic character (pH>7). It should be noted that pH plays a decisive role in chemical and biochemical reactions and can influence the stabilizing properties of essential oil.

Therefore, this result can lead to a good stabilizing character against microorganisms; this will allow these essential oils to play the role of preservatives in food products.

The refractive index calculated at 20°C using a refractometer. The value is greater than the refractive index of water at 1.333. The result obtained is higher than that reported by MAHBOUB et al., (2019) ; the refractive index is inversely related to the degree of unsaturation of the oil. The lower the refractive index, the better the essential oil quality (KosiMawuéna et al., 2019).

Denis, (1997) explained that the refractive index varies essentially with the content of monoterpenes and oxygenated derivatives. A high monoterpene content will give a high index. For some authors, the low refractive index of essential oils indicates its low refraction of light which could favor its use in cosmetic products.

3.3. Biological activity

3.3.1. Antioxidant activity

The antiradical activity is carried out by the 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical method which is a frequently used method for its simplicity. This method is based on the reduction of an alcoholic solution of DPPH in the presence of an antioxidant that gives hydrogen or an electron, the non-subject form DPPH-H is formed.

The inhibition of discoloration of the DPPH radical depends on the concentration of the different extracts used (Duen et al., 2016). Note that the free radical inhibition percentage for essential oil is lower than vitamin C for all concentrations used (Figure 1).

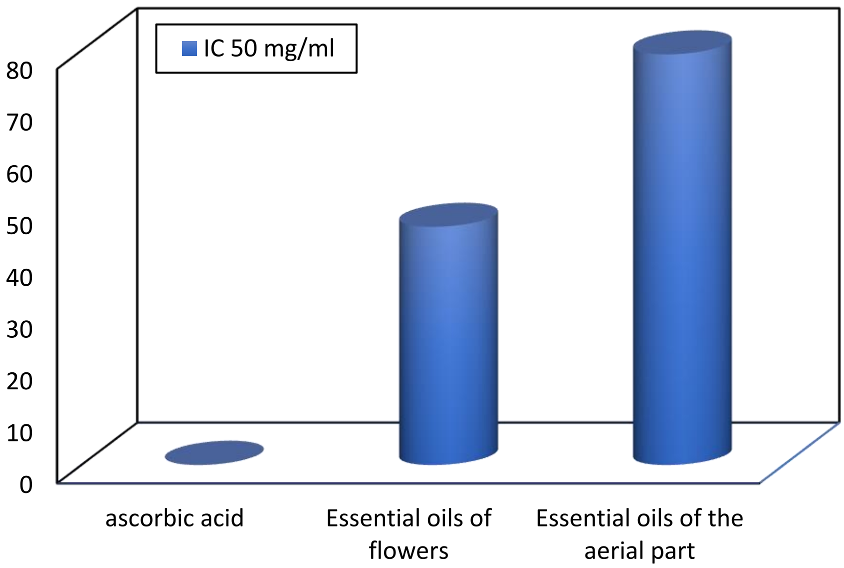

Fig.1. IC_{50} values of ascorbic acid and essential oils of flowers and the aerial part of *Cotula cinerea*.

It was observed that the activity of the essential oils of the flowers of *Cotula cinerea* is greater than that of the aerial part with IC_{50} respectively of 45.98mg/ml and 79.28mg/ml. These results suggest that vitamin C is the most effective antioxidant with an IC_{50} of 0.04 mg/ml compared to the essential oil studied.

These results generally show that the essential oils of *Cotula cinerea* with lower antioxidant activities.

Other work on the same plate by Khallouki et al., (2015) found that IC_{50} of total phenolic compounds determined by analytical HPLC in methanol extracts was 79.23 2.5 mg/g dry matter.

The difference in activity between the sampling of the essential oils from *Cotula cinerea* is explained by the mobility of the hydrogen atom of the hydroxyl group of the phenolic compounds of the essential oil. In the presence of a free radical DPPH, the Hydrogen atom is transferred to the latter then transformed into a stable molecule DPPH, this causes a decrease in free radical concentration and also absorbance over the reaction time until the hydrogen donor antioxidant capacity is exhausted (Villaño et al., 2007).

This plant contains many chemical compounds with therapeutic benefits such as flavonoids (Dendougui et al., 2012).

3.3.2. Antibacterial activity

Two microbial strains: (*Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) gram negative), used to test the antibacterial activity of flowers and the aerial part of *Cotula cinerea* via the diffusion method on a medium of Muller Hinton via the discs impregnated with essential oils (figures 2,3).

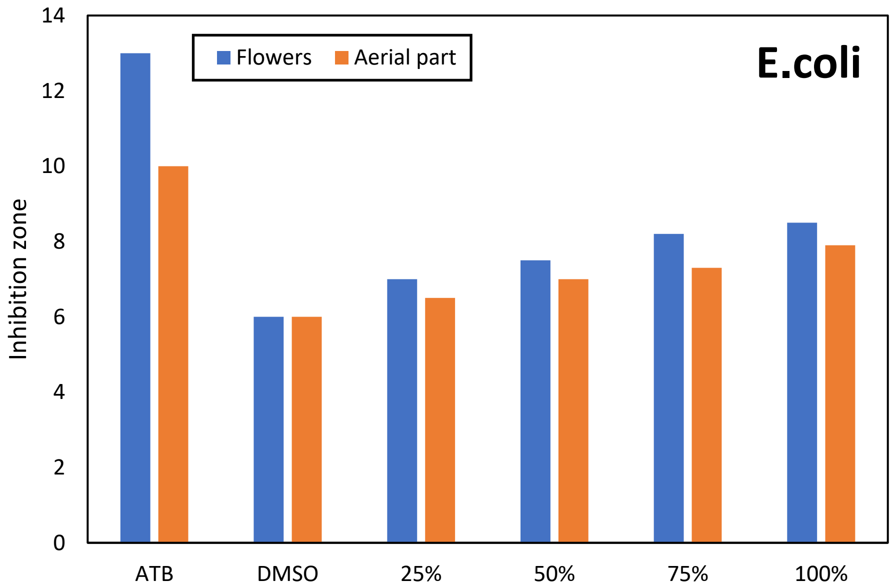

Fig.2. Effect of flower oils and the aerial part of *Cotula cinerea* on *Escherichia coli* depending on the different concentrations of the essential oils.

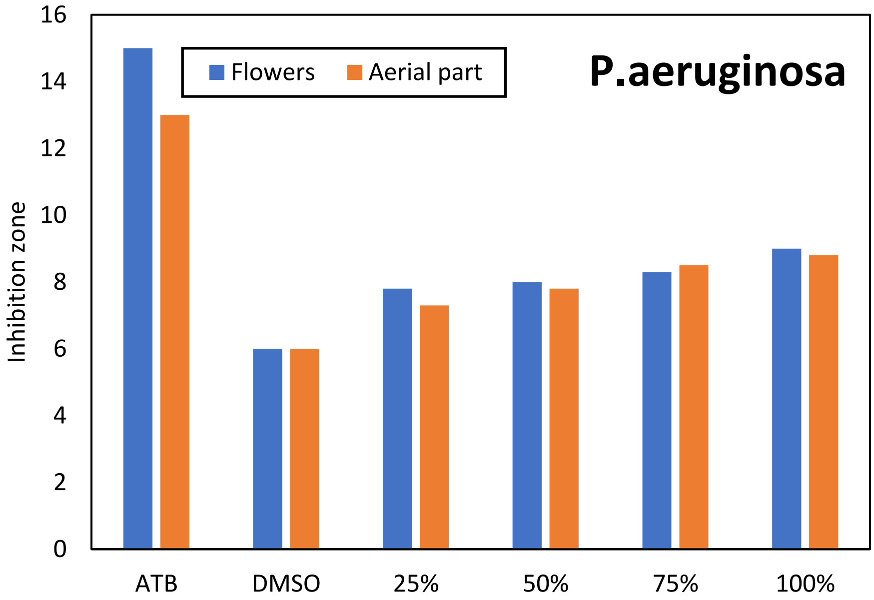

Fig.3. Effect of flower oils and the aerial part of *Cotula cinerea* on *Pseudomonas aeruginosa* depending on the different concentration of the essential oils.

According to the figures (2,3), the results clearly show a low effect of aerial essential oils and *Cotula cinerea* flowers on the two strains studied (*Escherichia coli* and *Pseudomonas aeruginosa*). Showed less antibacterial activity of essential oils on both strains tested with variable inhibition zones (6-9mm) also noted, in both types of bacteria studied the increase in the concentration of the plant's essential oils lead to the increase of inhibition zone (Direct correlation relationship).

The antimicrobial activity of *Cotula cinerea*'s essential oils was achieved by the agar disc diffusion method (Malabadi et al., 2012) Filter paper discs (Whatman No., diameter 6 mm) were sterilized by an autoclave.

The different concentrations of essential oils (1/1: 100%; 1/2: 50%; 1/3: 75%; 1/4: 25%) softening by DMSO (Di Methyl Sulf Oxide); the concentrations tested on a set of pathogenic bacteria: two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*).

The comparison of the results of the study of the extracted essential oils in two parts: the aerial part and the flowers showed no difference in the diameters of the inhibition with all the strains. But with *P.aeruginosa* showed a high resistance to all concentrations of essential oil and increased the intensity of this resistance especially with the concentrations of essential oil of their flowers by contribution recorded a low resistance to all essential oil concentrations with gram negative bacteria *E.coli* and the values of inhibition diameters of the antibiotic used were large by addition to inhibition diameters recorded with both strains. Comparing our results with the results obtained by:

Abdenbi et al, (2014) who noted that inhibition zones obtained by Vincent's method vary between 20 and 55 mm with the essential oil of *Cotula cinerea*, vis-à-vis bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*). Along with the other bacterial strains (*Enterococcus faecalis*, *Salmonnella heidelberg*, *Staphyloccocus aureus* and *Pseudomonas aeruginosa*), there is no inhibition zone.

Moreover, Bouabdelli et al, (2012) which has known several extracts (infusion; decoction; maceration and percolation) on: *E. coli* and *S. aureus* have given diameters of small inhibitors compared obtained where you arrive at the inhibition of greater diameter (20 mm *E. coli* with and 17 mm with *S. aureus*); As with the strain *P. aeruginosa* the results were close (14 mm).

Strains *E. coli*, *M. morganii*, *P. vulgaris*, *S. aureus* and *A. baumannii* showed high sensitivity to concentrations (1/1, 1/2, 1/4, 1/8) Where inhibition diameter ranged from (50 mm to 21 mm), while these strains showed considerable resistance with concentrations (1/16 and 1/32) and high resistance to all concentrations of essential oil and increased the intensity of this resistance especially with concentrations of essential oil in the fructane stage with *P. aeruginosa* noted by Chouikh et al., (2015).

4. Conclusion

Due to the richness of the plant in essential oils, these were extracted using a Clevenger device, the calculation of extraction yield by hydrodistillation showed that the best yield in flowers and the lowest in the aerial part of *Cotula cinerea* were found at rates of 2.54% and 1.07% respectively. This value is lower than the yields obtained in other species belonging to the same genus and higher than in other species.

The determination of the physical characteristics of the essential oils reveals that they conform to the standards established by the various pharmacopoeia and similar to some previous work.

Furthermore, the results of the antioxidant activity of vegetable essential oils using the DDPH test is performed with ascorbic acid as standard, show that IC_{50} of the essential oils of flowers and the aerial part was equal 45,98 mg/ml and 79,28 respectively, while for ascorbic acid was equal 0.04 mg/ml. This confirms that the antioxidant efficacy of the plant is low compared to ascorbic acid.

Regarding antibacterial activity, the results clearly show a low effect of the aerial essential oils and flowers of *Cotula cinerea* on the two strains studied (*Escherichia coli* and *Pseudomonas aeruginosa*). With the presence of a direct correlation relationship between the concentration of the essential oils used and the inhibition zone obtained.

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