Biological activities (antioxidant and antimicrobial activity) of the aqueous extracts and essential oil of *Ammoides verticillata* (Nounkha)

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Abstract

The phytochemical review conducted on the aerial part *Ammoides verticillata* has revealed the presence of six chemical families: flavonoids, tannins; gallic tannins, anthocyanins and the coumarins, cateichic tannins. The aqueous extract of the *Ammoides verticillata* could bring back the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-colored diphenylpicrylhydrazine with an IC50 of 0.020 $mg.mL^{-1}$. It is gifted with antioxidant activity, however it was less effective than vitamin C (0.001 mL^{-1}). The essential oil of the *Ammoides verticillata* has presented a strong antimicrobial activity against Gram negatif germs targets of original clinical action**:** *E. coli* (ESBL) (37 mm), *E. coli* (39.5 mm), *Klebsiella pneumoniae* (36.5 mm) and especially with the yeast: *Candida albicans* ATCC 10231 (47 mm) and *Saccharomyces cerevisie* ATCC (42 mm), which are highly sensitive to the oil inhibitory action. However, the oil has presented a low activity against the bacteria *P. aeruginosa* (12.5 mm).

Keywords: *Ammoides verticillata* , essential oil, aqueous extract, organic activities.

INTRODUCTION

The aromatic plants are not only endowed perfuming and culinary qualities, but also with various medicinal properties due to the different active ingredients they contain: alkaloids, flavonoids, tannins, saponins and essential oils (Tefiani *et al.*, 2016).

The Apiaceae family is one of the richest in essential oils. It includes vegetables (carrot, fennel, celery) and condiments (caraway, coriander, cumin, parsley) (Kambouche, 2000). The present study of *Ammoides verticillata*(Fig.1) has had for objectives:

- The chemical components identification of the aqueous extract and the evaluation of its antioxidant activity with respect to free radicals using the DPPH method;
- The essential oils extraction and the evaluation of their antimicrobial activity.

I- MATERIALS AND METHODS

I-1 Biological material

Plant material: The work focused on the plant *Ammoides verticillata*, collected at the Terny station (south of Tlemcen, Algeria) (Fig.1).

Bacteria: The antimicrobial potency of the essential oils of *Ammoides verticillata* has been tested using 2 reference strains and bacterial strains of clinical origin isolated from three different pathological products (urine, pus and stool).

Animals: The anti-inflammatory activity of *Ammoides vertivillata* focused on 18 Albino mice, NMRI race (Naval Medical Research Institute), male and female, weight ranging from 19 to 23 g. These mice were fed with granules and tap water. The hosting conditions meeted the local standard temperature (20-24°C), photoperiod (10 hours per day), humidity rate (≈50%).

Figure 1. Original photo of the dry plant in bouque(*Ammoides verticillata)* (2016).

I-2- METHODS

Samples collect of *Ammoides verticillata*: In the selected plots, all the feet of healthy, well-supplied and ungrazed *Ammoides verticillata* were cut. Their number inside a surface of 1 m^2 varied from 1 to 3. A sample of 100 feet on 10x10 m^2 was collected during a month, then the samples were placed in well-ventilated bags and spread on paper in the shade and protected from moisture at room temperature until they become completely dry.

Extraction of essentiel oil from *Ammoides verticillata*: In this study, the extraction method used is hydrodistillation in Clevenger type apparatus. This method is interesting on the one hand for its optimality in the essentiel oil yield and on the other hand for advantages in several parameters such as the plant material quantity and state, the quantity of water introduced or the duration of extraction.

Experimental protocol: A well-weighed cup of the dry and possibly milled plant is introduced into a one liter glass flask with 3 necks, impregnated with distilled water, placed over a balloon heater and surmounted by a glass column, connected to a refrigerant which communicates directly to a separating funnel for the distillate recovery. The bulb is connected to the balloon by a plastic pipe which allows the return of evaporated and condensed water to the balloon. The average duration of extraction is about 3 hours.

After decantation, the essential oil is recovered by aspiration using a Pasteur pipette and stored in glass tubes, hermetically closed, in the dark and at a temperature between 4 and 6°C.

Performance calculation: The essential oil yield is defined as the ratio of the obtained mass of essential oil by the dry mass of the treated plant material. The yield, expressed as a percentage, is calculated by the following formula:

$$Rd = (m/m)\times 100$$

Rd is the essential oil yield expressed as a percent, m the essential oil mass in grams and m_0 the mass of the dry vegetable matter in grams.

A-Preparation of aqueous extract

The preparation of the 10% aqueous extract of plant is carried out by adding 10 g of powder of the aerial part to 100 mL boiled distilled water, then left for 30 min in infusion with stirring from time to time. The aqueous extract obtained is then centrifuged at 1000 rpm for 10 min to get rid of plant debris and filtered on Wattman n° 3 filter paper. The filtrate is finally put in small glass vials.

Phytochemical screening

According to Harborne (1983), depending on turbidity, medium color and precipitate intensity, the phytochemical results are classified as follows: very positive reaction (+++), moderately positive reaction (++), shady reaction (+), negative reaction (-).

A-Flavonoids

The presence or absence of flavonoids in an extract can be demonstrated by a simple and rapid test: the magnesium test. A few drops of concentrated HCl (2N) and 0.5 g of Mg are added in 5 mL of the methanolic extract. This is allowed

to act for 3 min. Orange or red color appearance indicates a flavonoids presence.

B-Tannins

One mL of 10% aqueous extract is mixed with 1 mL of distilled water and 1 to 2 drops of 10% diluted $FeCl_3$ solution. The test is considered positive by dark green or blue-green color appearance.

C-Galenic tannins

Add 2 g of sodium acetate to 15 mL of the infused, then few drops of a 5% $FeCl_3$. A dark blue color indicates a gall tannins presence.

D-Catechetical tannins

Add 7 mL of Stiasny reagent to 15 mL of the infused. A red color indicates a catechetical tannins presence.

E-Free quinones

Add 2 g of the powder to 2 mL of hydrochloric acid (97%), leave in contact for 3 hours in chloroform. After filtration, add few drops of ammonia(0.5%). The presence of free quinones is shown by a red coloring.

F-Coumarins

Put 2 g of powder in 20 mL of absolute ethanol. Boil for 15 min under reflux then cool and filter. Add 10 drops of KOH and a few drops of concentrated HCl (37%), diluted at 10% in 2 to 3 mL of filtrate diluted in ethanol (10%). The test is considered positive by the appearance of a red color.

Principle of evaluation of the antioxidant activity of aqueous extracts (*in vitro*)

The antioxidant activity of *Artemisia judaïca* L. was highlighted with the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The antioxidant capacity is measured using more stable free radicals. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is a very stable free radical in the crystalline state and in solution, of violet coloring. By this method, it is considered that the antioxidant activity is none other than the ability of antioxidants to act as a scavenger of free radicals. They act by transferring a hydrogen atom, which leads to the DPPH disappearance during the reaction and to a color change (yellow) in the initial solution **(**Boulanouar *et al.*, 2013).

$$\text{Percentage of antioxidant activity} = I\% = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/(\text{Absorbance}_{\text{control}})]\text{x}100.$$

A-IC50

IC50 (50% inhibitory concentration), also called EC50 (efficient concentration 50), is the concentration of the tested sample, required to reduce 50% of the DPPH radical. It is inversely related to the antioxidant capacity. The IC50 are graphically determined by percent inhibition based on different concentrations of the tested extracts (El Ouariachi *et al.*, 2011).

Evaluation of antimicrobial activity

A-Preparation of the inoculum

• the bacterial inoculum

A well isolated colony of the germ to be studied is taken from a pure bacterial culture in a Petri dish, then introduced into a hemolysis tube containing 1 mL of nutrient broth. From the suspension obtained, dilutions are made in order to obtain a dense culture: density equivalent to 0.5 Mac Farland standard (108 $CFU.mL^{-1}$, the suspension containing from 10^6 to 10^7 bacteria. mL^{-1}).

• yeast inoculum

From a pure culture, a well isolated colony of the yeast is collected and then seeded on a liquid Sabouraud medium incubated at 30°C for 18 h. Dilutions are then carried out until obtention of 10^5 $cells.mL^{-1}$. The number of cells is counted with the Mallassez cell.

B-Confrontation essential oil-bacteria and essential oil-yeast

• Flood seeding

One mL of the bacterial inoculum or yeast is deposited then spread on Muëller-Hinton or Sabouraud medium. Excess liquid is aspirated and the can is allowed to dry for 15 min at 35°C.

• Deposition of disks impregnated with essential oil and incubation

Three to five sterile discs of Whatman n° 3 paper, 6 mm in diameter, are impregnated with 2.5 µL of different essential oil extracts from plant sites. They are deposited at the surface of Müeller-Hinton medium or Sabouraud agar. The disks are arranged in such a way that the inhibition zones do not overlap.

After the essential oil diffusion into the medium for 15 min at 30°C, the dishes are incubated at 37°C for the bacteria and at 30°C for the yeast. The reading is carried out after 24 h of incubation for the bacteria and 48 h for the yeast by measuring the inhibition zone diameter of the target germ.

The inhibition zone diameters (mm) are measured, including the disks diameters. These measurements are transcribed into different symbols proportional to the activity (Abdelouahid and Bekhchi, 2004). All tests were performed in triplicate. Imipenem (10 µg) and Vancomycin (30 µg) served as positive controls.

II-RESULTS

II-1-Organoleptic characteristics

The organoleptic characteristics of the *Ammoides verticillata* essential oil obtained by hydro distillation are liquid appearance, light yellow color and characterized by a strong pungent odor.

II-2- Essential oils extraction by hydro distillation

II-2-1-Determination of the vegetable quantity necessary for extraction

The yield of essential oil was calculated according to the dry plant matter of the aerial part of the plant. The results have shown a yield of essential oil which varies depending on the plant material mass. It has reached its maximum (1.6%) with 100 g of the dry plant. It has rapidly decreased to about 1.4% for 200 g of the dry plant and then continued to decrease more slowly.

These variations in the yield are probably related to the degree of unsuitable settlement (insufficient or excessive) that forces steam to take preferential paths.

As a result, in some places, the steam does not come into contact with the plant material with consequently decreasing in the yield. The ideal weight for the essential oils extraction seems to be 100 g of vegetable matter.

The *Ammoides verticillata* essential oil was obtained with a relatively average yield (1.60% on a dry weight basis). Some differences in yield were observed in samples of *Ammoides verticillata* from different origins: Algeria, 4.41% (Kambouche and Abed, 2003), Morocco, 2% (El Ouariachi *et al.*, 2011).

II-3-Phytochemical Screening

Phytochemical screening allowed us to highlight the presence of secondary metabolites in the plant tissues. Phytochemical examination carried out on the aerial part of *Ammoides verticillata* revealed the presence of 6 chemical families: flavonoids, tannins, gallic tannins, catechin tannins, anthocyanins and coumarins (Boulanouar *et al*., 2013). However, there is a lack of free quinones in our extract. Flavonoids and gallic tannins were present in larger quantities compared to other chemical families.

These chemical families, detected in our study, confirm earlier work of Toubal *et al.* (2012) on the phytochemical tests of the aqueous extract of *Ammoides verticillata* which certified the presence of tannins, anthocyanins, coumarins and especially flavonoids in significant quantities. It is noted that this plant contains a high amount of tannins and confirms works of Oumessaad *et al.* (2011). In addition, the existence of tannins explains the woody form of this shrub.

II-4-Antioxidant activity

The antioxidant activity is dependent on the hydrogen atom mobility of the hydroxyl group of the essential oil phenolic compounds. In the presence of a free radical DPPH, the H atom is transferred on the latter thus transformed into a stable molecule DPPH, this causes a decrease in the concentration of the free radical and also the absorbance during the reaction time to depletion of the hydrogen donor antioxidant capacity.

The DPPH has a dark purple color but when trapped by antioxidant substances its color turns to pale yellow. The turn towards this coloring and its intensity depends on the nature, the concentration and the potency of the anti-radical substance.

II-4-1-Determination of percentage inhibition

The test results have shown that the inhibition percentage of free radical increases with the concentration increase for either the control product of ascorbic acid (vitamin C) or aqueous extract of *Ammoides verticillata* . It is noted that the antioxidant effectiveness increases with the the aqueous extract concentration. However, the free radical inhibition percentage for the extract is slightly lower than that of ascorbic acid for all the used concentrations. At the concentration of 100 $mg.mL^{-1}$, the aqueous extract of *Ammoides verticillata* revealed a percentage inhibition of DPPH at 93.58% while that of vitamin C was 94.77%.

II-4-2- Determination of IC50

IC50 is inversely related to the antioxidant capacity of a compound. It expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The lower the IC50 value,

Table 1. Transcription of Inhibition Diameter (ID) values

Inhibition	Transcription	Sensibility
D<8 mm	-	Resistant
9 mm≤D≤14 mm	+	Sensitive
15 mm≤D≤19 mm	+ +	Quiet sensitive
D≥20 mm	+ + +	Very sensitive

the greater the antioxidant activity of a compound. The aqueous extract of *Ammoides verticillata* could bring the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to yellow-colored diphenylpicrylhydrazine with an IC50 at 0.020 $mg.mL^{-1}$. It exhibits lower antioxidant activity than vitamin C (0.001 mL^{-1}).

According to the results found, it appears that the aqueous extract of *Ammoides verticillata* has an antioxidant activity, but less effective than that of vitamin C. Moreover, by comparing the IC50 of our extract (0.020 $mg.mL^{-1}$) with that of essential oil of *Ammoides verticillata* (0.10 $mg.mL^{-1}$) studied by Merzougui and Tadj (2012), our extract is unveiled with more antioxidant power than the essential oil. However, some authors have detected a remarkable antioxidant effect (IC50=0.007 $mg.mL^{-1}$) in organic extracts of another species of the same genus *Ammoides atlantica*. However these IC50 results were performed with other methods of antioxidant activity. On the other hand, it turns out that this antioxidant activity is related to phenolic compounds presence in extracts and essential oils. They are known as potent antioxidants and as free radical reducers (Valnet**,** 2010). In addition, phenolic compounds are very important components in the extracts and their free radical scavenging ability is due to their hydroxyl groups.

II-5-Antimicrobial activity

The antibiogram is an effective biological method to directly determine the antimicrobial effect of the *Ammoides verticillata* essential oil on the micro organisms tested. For this, the sensitivity of the strains tested was estimated by measuring the inhibition zones diameters of in both perpendicular directions around the disks impregnated with the *Ammoides verticillata* essential oil. The inhibition zones diameters (mm) were measured, including the disks diameter. These measurements were transcribed in different symbols proportional to the activity. All tests were performed in triplicate.

According to several authors, essential oils are considered active if they produce inhibition diameters equal or greater than 20 mm. As a result, the *Ammoides verticillata* essential oil has a strong antibacterial action against the Gram- target germs of clinical origin: *E. coli* (ESBL), *E. coli* and *Klebsiella pneumonia* which are highly sensitive to the oil inhibitory action with respective inhibition diameters: 37, 39.5 and 36.5 mm. However, the oil is endowed with a lower activity against the bacterium *P. aeruginosa* (12.5 mm).

The *Ammoides verticillata* essential oil has also a strong activity against Gram+ bacteria: *Staphylococcus aureus*, *Faecal Streptococci* and *Bacillus spp.* which are extremely sensitive to the oil inhibiting action with 25% diameters respective inhibitions: 39, 33 and 34 mm. The yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisia* ATCC are also very sensitive towards the very important inhibiting power of the *Ammoides verticillata* essential oil since they are inhibited with the respective inhibition diameters: 47 and 42 mm. The present study have shown that the antibacterial activity of the volatile oil was more pronounced against Gram- bacteria than *P. aeruginosa*. This result is in agreement with numerous studies carried out on other plant species. This bacterium is also known for its resistance to many antibiotics, it is an opportunistic pathogen in immunocompromised persons where it is responsible in frequent and serious nosocomial infections in patients (Caillet and Lacroix, 2007)**.**

The antibacterial activity of essential oils as well as their mode of action are directly influenced by the nature and the proportion of

the constituents entering in their composition. The majority compounds are often responsible for the observed antibacterial activity; thus, the important inhibitory power of the *Ammoides verticillata* essential oil against the target germs could be attributed to the high content of tymol and carvacrol already cited by several works. These phenolic aromatic compounds, in fact, are known to their antimicrobial properties.

The antimicrobial activity of the essential oil can be explained by the lipophilic nature of the monoterpene contained therein. Monoterpene act by disrupting the microbial cytoplasmic membrane, which causes a destabilization of the structure and an increase in membrane permeability. These changes result in ion leakage and intracellular compounds. If the material loss is too important to the bacterial survival, this causes cell death. The *Ammoides verticillata* essential oil has a strong antibacterial activity against the bacteria of clinical origin and especially with respect to the yeasts. Similar observations about extracts and essential oils from the same species have also been reported by several researchers (Bakchiche *et al.*, 2013).

CONCLUSION

The main idea of our study was to extract *Ammoides verticillata* essential oil (said *Nounkha*) from the Terny region (wilaya de Tlemcen), to determine its physicochemical properties and to evaluate *in vitro* its antimicrobial properties towards different microbial species, as well as its antioxidant activity. From the literature review, it appears that this plant, belonging to the family of Apiaceae carries many plant synonyms. Hydrodistillation, the method of choice for the essential oils extraction, allowed us to show that the *Nounkha* plant is rich in essence. The estimated yield is 1.6%. Phytochemical examination carried out on the aerial part of *Ammoides verticillata* revealed the presence of chemical compounds especially flavonoids and tannins. The biological activity tests, carried out *in vitro*, allowed to assess the presence of important inhibitions zones, indicating that the essential oil has a biological activity on all the microorganisms tested. These experiments have revealed a high sensitivity of yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisia* ATCC opposite gasoline compared to other germs. In addition, the results of the evaluation of the antioxidant activity have revealed that the *Ammoides verticilata* essence has a high antioxidant power. Finally, it is recommended for this plant species the study of the chemical composition variability by the joint implementation of chromatographic techniques (TLC, HPLC and CG/MS) and spectroscopic methods, taking into account the the plant age, and if possible, the time and place of harvest. This will allow to observe the different changes in terms of quality and quantity of essential oils in order to estimate under what conditions or in what period these essential oils could have an interesting activity.

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