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STUDY OF SOME BIOLOGICAL EFFECTS OF THE ESSENTIAL OIL OF *OCIMUM BASILICUM*

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ABSTRACT

This work is part of problematic of enhancing the oily extracts of *Ocimum basilicum* leaves by studying their antifun and antioxidant activities. The extraction of the oil is carried out by hydro distillation, and the yield obtained is 1.06 ± 0 Antifungal test of the essential oil were operated on four fungal strains; *Aspergillus flavus, Aspergillus niger, Penicilli expansum* and *Fusarium oxysporum f.s albidinis* by the method of evaluation of radical growth. The results show that essential oil of *Ocimum basilicum* showed a strong antifungal action against *Fusarium oxysporum f.s albidinis* w antifungal index of 100% at 20µl whereas the three other strains studied were inhibited at 40µl. The results of evaluation of the in vitro antioxidant activity have shown that the reducing power of DPPH is remarkable in the essen oil or the recorded EC_{50} is 2,180 µl / ml.

Keywords: *Ocimum basilicum*, essential oil, antioxidant activity, antifungal activity, antifungal index.

Introduction

Aromatic plants show promise and are a great source of natural antioxidants and antibacterial for the food industry. The presence of antioxidants in food has become essential for food quality and safety. The negative effects of synthetic antioxidants encourage their substitution by natural agents (El Kalamouni, 2010).

Among the families that belong to this category of aromatic and medicinal plants, the Lamiaceae family is of great importance both for its use in the food industry and in perfumery and in therapy. It is one of the families most widely used as a global source of spices and extracts with high antibacterial, antifungal, anti-inflammatory and antioxidant power (Bakkali *et al*., 2008). The space most cited in the literature is: *Ocimum basilicum* (Lee *et al*., 2005).

The use of essential oils turns out to be a relevant choice when faced with a specific risk of contamination or the need to reduce or replace chemical or synthetic preservatives (Lee *et al*., 2005). This led us to study the antifungal activity and the antioxidant activity of the essential oil of *O. basilicum*.

Materials and Methods

This work was carried out within the pedagogical laboratory of biology of the Tahri Mohammed University of Béchar (Algeria), it aims at the valorization of essential oils of *Ocimum basilicum*. We also evaluated the antifungal and antioxidant power of this essential oil.

Preparation of plant material

The samples of *O. basilicum* were harvested during the period from month of February 2017 in the Zauit Kenta region of the wilaya of Adrar. The aerial part of the plant was dried in the shade for fifteen days before use.

Extraction of essential oils

The extraction of the essential oil was carried out by the simple hydro distillation technique.

The extraction of the essential oil of *Ocimum basilicum* was carried out by hydro distillation. The dried plant is introduced by placing 200 g of dry leaves in a flask, and impregnated with water; the whole is boiled for 3 hours. The water vapors charged with essential oil, passing through the cooler, condense and fall into a separator funnel, the water and the oil are separated by the difference in density. The conservation of the essential oil obtained at a temperature of around 4°C, in well-closed and labeled tubes, covered with aluminum foil to preserve it from air and light.

Calculation of the yield of essential oils

According to the AFNOR standard (1986), the essential oil yield (RHE) is defined as being the ratio between the mass of essential oil obtained after extraction (MHE) and the mass of the plant material used (MS). He is. Calculated by the following formula:

$$EoY = \frac{Meo}{Mp}.100$$

EoY: yield of *Ocimum basilicum* essential oil in % .

Meo: mass of essential oil obtained in grams.

Mp: mass in grams of dry plant matter.

Determination of physico-chemical properties

The physico-chemical properties of essential oils (density, refractive index, acid index, ester index) are required for their commercial evaluation.

Refractive index (NFT 75 112, 1977)

The refractive index is the ratio of the speed of a light ray (the 589 nm sodium D line) in vacuum to its speed in the medium. In other words, it is the measurement of the refraction of a given body with respect to the D line of sodium.

The refractive index (RI), at the reference temperature t, is given by the following equation:

$$I_{20} = T_c + 0.00045(t-20)$$

I_{20}: index at 20°C,

T_c: chamber temperature index,

t: measurement temperature.

pH

The pH, expressed by a numerical value, indicates whether a solution is acidic or basic, it also represents the concentration of hydrogen ions in an aqueous solution. The pH is determined using pH paper.

Acid number (NFT 75 103, 1982)

The acid index is the number which expresses in milligrams the quantity of potassium hydroxide necessary for the neutralization of the free acids present in 1 g of substance.

$$Ia = 5.61\left(\frac{V}{m}\right)$$

Ia: acid index

V: volume (ml) of the potassium hydroxide solution used for the titration.

m: mass (g) of the test sample

Ester index (NFT 75 104, 1982)

The ester index (EI) is the number which expresses in milligrams the quantity of potassium hydroxide necessary to neutralize the free acids by the hydrolysis of the esters present in 1 g of substance.

$$Ie = \frac{28.05}{m}(V0 - V1)Ia$$

Ie: Ester index

V0: volume in ml of the hydrochloric acid solution used for the blank test;

V1: volume in ml of the hydrochloric acid solution used in the determination of the IE.

m: mass (g) of the test sample.

Saponification index

The saponification index of a lipid is determined by the mass of potassium in mg necessary to neutralize the free acids and saponify the esterified fatty acids in one gram of fat. According to the European Pharmacopoeia standard (2001), the saponification index is given by the following relationship:

$$Is = Ie - Ia$$

Is: Saponification index

Ie: Ester index

Ia: acid index

Antifungal activity

Fungal material

The strains used in this work are widely found in various foods, they were isolated and purified at the level of the biology research laboratory of the University of Béchar from stored foods (durum wheat, coffee and palm date) (NAHAL, 2016) these are: *Aspergillus flavus, Aspergillus niger, Penicillium expansum and Fusarium oxysporum f.s albidinis*

These fungal species were chosen for the considerable damage they cause to food during storage and even in the field (Manssouri *et al*., 2016).

Identification has for a long time been exclusively based on the observation of the cultural and morphological characteristics of the species.

The microscopic observation is carried out under determined by the cultural characters and microscopic by referring to the manual of Barnett and Hunter (1972)

The identification of *Aspergillus* and *Penicillium* species was carried out according to the single spore method described by Pitt (1973) and Ramirez (1982).

Technique of antifungal activity

Preparation of the spore suspension

The species recovered from a young pure culture of 7 days are dispersed in the form of an agar emulsion (0.2) and a few drops of tween 80 for good dissemination of the spores then the tubes are vortexed (Nahal *et al.,* 2016). The density of each spore suspension was adjusted to 10^5 spores/ml using a Malassez-type hematimetric cell (Mouaragadja and M'Batchi, 1998; Khaldi *et al*., 2015).

Evaluation of radial growth on solid medium

The antifungal activity of the essential oil against the strains was carried out by the radial growth method of Serghat *et al*. (2004).

20, 40, and 60 µl of essential oil were added to 15 ml of PDA a (Potatoes Dextrose Agar acidified) solid medium; after agitation the select solution were transferred into a Petri plats which were incubated for 7 days at 25±2°C after ensemencing the fungal strain in the center. Mycelial radial growth was measured from the third day of incubation. The inhibition percentage of mycelial growth of each extracts was calculated using the following formula (Wang *et al*., 2005; Singh *et al*., 2009):

$$IP = \frac{Dc - D}{Dt} \times 100$$

IP: Inhibition percentage

D: Diameter of the test growth zone.

Dc: Diameter of the control growth zone.

Antioxidant activity

The use of synthetic antioxidant molecules such as butylated hydroxy toluene (BHT), butyl hydroxy anisole (BHA) and tert-butyl hydroquinone (TBHQ) is currently questioned because of the potential toxicological risks (Panichayupakaranant and Kaewsuwan, 2004; Talabi *et al*., 2014).

DPPH reduction method

To study the antioxidant activity of this essential oil, we opted for the DPPH method, according to the protocol described by (Archana *et al*., 2005; Dung *et al.*, 2008).

The methodology is based on the decay of a methanol solution of DPPH following the addition of the antioxidant (Bernardi *et al*., 2007)

One hundred microliters (100µL) of different concentrations of the extracts and methanol were mixed with 2.9 ml of the 0.004% (w/v) DPPH solution in dry test tubes. After 30 min of incubation at room temperature and in the dark, the absorbance was measured at 517 nm, using a UV-visible spectrophotometer.

The negative control is composed of 100ul of methanol and 2.9ml of the DPPH solution, the concentration of the essential oil tested are: 20, 35, 50, 70, 85 and 100µg/ml.

The positive control represented by a solution of a standard antioxidant; the absorbance of ascorbic acid is measured under the same conditions as that of essential oil.

According to Dung et *al*, 2008 and Eyob et *al*., 2008, the reduction power is determined by applying the following formula:

$$Rp = \frac{(Ac - Ae)}{Ac} \times 100$$

Rp: Reduction power %

Ac: absorbance of the DPPH solution in the absence of the extracts.

Ae: absorbance of the DPPH solution in the presence of the extracts.

The variation of the reduction power according to the concentration of essential oils and the vitamin C, allows us to calculate the EC_{50} parameter. The EC_{50} effective concentration is defined as the concentrations of essential oils(or vitamin C) necessary to reduce 50% of the free radicals in the reaction medium. The lower the EC_{50} value, the higher the antiradical activity (Grama et *al*., 2005).

Results and Discussion

Essential oil yield

The yield calculation result obtained during extractions by hydro distillation, of *O. basilicum* essential oil found is 1.062± 0.1g/100g of plant.

O. basilicum shows a yield satisfactory in essential oil of (1.06%), this yield was calculated according to the dry plant matter of the aerial part of the plant used.

Comparing the yield with that of the same plant harvested from other regions; we can see that the latter is better than those of Ourgla, Beskra and Ghardaia, Khemismiliana, El taref and Ougadougou, have relatively low rates: 0.1%, 0.33%, 0.48%, 0.14%, 0.6% and 0.79% respectively.

Kelen and Tepe (2008) assumed that this difference could be explained by the choice of the harvest period because it is essential in terms of yield and quality of essential oil.

Physico-chemical analyzes of essential oils

The essential oil of *O.basilicum* obtained by hydro distillation is a liquid, with a yellowish color and a very aromatic smell. The results of physico-chemical analysis of *O.basilicum* essential oil are reported in table 1.

Table 1: Results of physico-chemical analysis of *O.basilicum* essential oil.

Settings	Results
Refractive index (*RI*)	1.553
pH	5
Acid number (*Ia*)	7.01mgKOH/g
Ester index (*Ie*)	9.8 mg KOH/g
Saponification index (*Is*)	16.83 mg KOH/g

The physicochemical tests of the oil studied indicate an acid pH of 5, the refractive index (I_{20}) is a quantity that allows us to identify the essential oil, also to control its purity, in fact a refractive index varying essentially with the content of monoterpenes and oxygenated derivatives. Boukhatem et *al*., (2010)report that a high monoterpene content will give a high index.

For chemical constants, the acid index (*Ia*) gives an idea of the level of free fatty acids, this parameter can help us to know the quality of our product. The *Ia* of *O. basilicum* essential oil is 7.01 mgKOH/g, a product with a low acidity index is a good quality product.

The value of the ester index (*Ie*) of the *O. Basilicum* essential oil is 9.81; this result is low compared to that recorded by Hamoudi (2012) which is 20.45.

The saponification index of this oil is 16.83 mgKOH/g. the de-saponification index, can tell us about the richness of an oil in long-chain fatty acids for a given weight of triglycerides, about the quality and quantity of carboxylic groups linked, complexed or present in substances likely to form a soap with a base after hydrolysis. This index depends on the molecular weights of the fatty acids entering into the constitution of the triglycerides of the mixture. The more carbon atoms the acid molecules have, the lower the saponification index .

The physicochemical parameters differ depending on the origin of the essential oil. All these parameters are influenced by the edaphic and climatic conditions as well as the plant cultivation conditions; it is logical that their values differ from one place to another of the globe, which is proven in the work published by (Rajeswara *et al*., 1993).

Antifungal activity

The identification of fungal strains is based on the macroscopic (color and appearance of the mycelium) and microscopic (micro culture method) aspects of the colonies for the identification of the genus according to the Barnett technique (1972).

The identification of *Aspergillus* and *Penicillium* species was carried out after cultivation on different media and with different incubation temperatures.

Aspergillus and *Penicillium* species are determined after reading the diameters and colors of the mycelia (Pitt, 1973; Ramirez, 1982).

The evaluation of the diameters of mycelial growth according to the volume of the essential oil added to the culture medium gave a total inhibition of *Aspergillus niger* at a concentration of 40µl parraport has a diameter of 71mm for the control.

The mycelial growth of *Aspergillus flavus* at the 20µm concentration is less than the control, whereas at the 40, 60µm concentration the inhibition is total. For the 20 µl concentration, the effect of the essential oil was effective and it gives a diameter of 10.33mm compared to that of the control which is equal to 32.66mm.

The concentration of 40µl was sufficient to inhibit the growth of *Penicillium expansum*.

There is total inhibition for *Fusarium oxysporum f.s. albidinis* from the concentration of 20 µl compared to the Control which represents a diameter of 81 mm.

The results of the antifungal effect of *O. basilicum* essential oil is illustrated in the histograms of figure 1

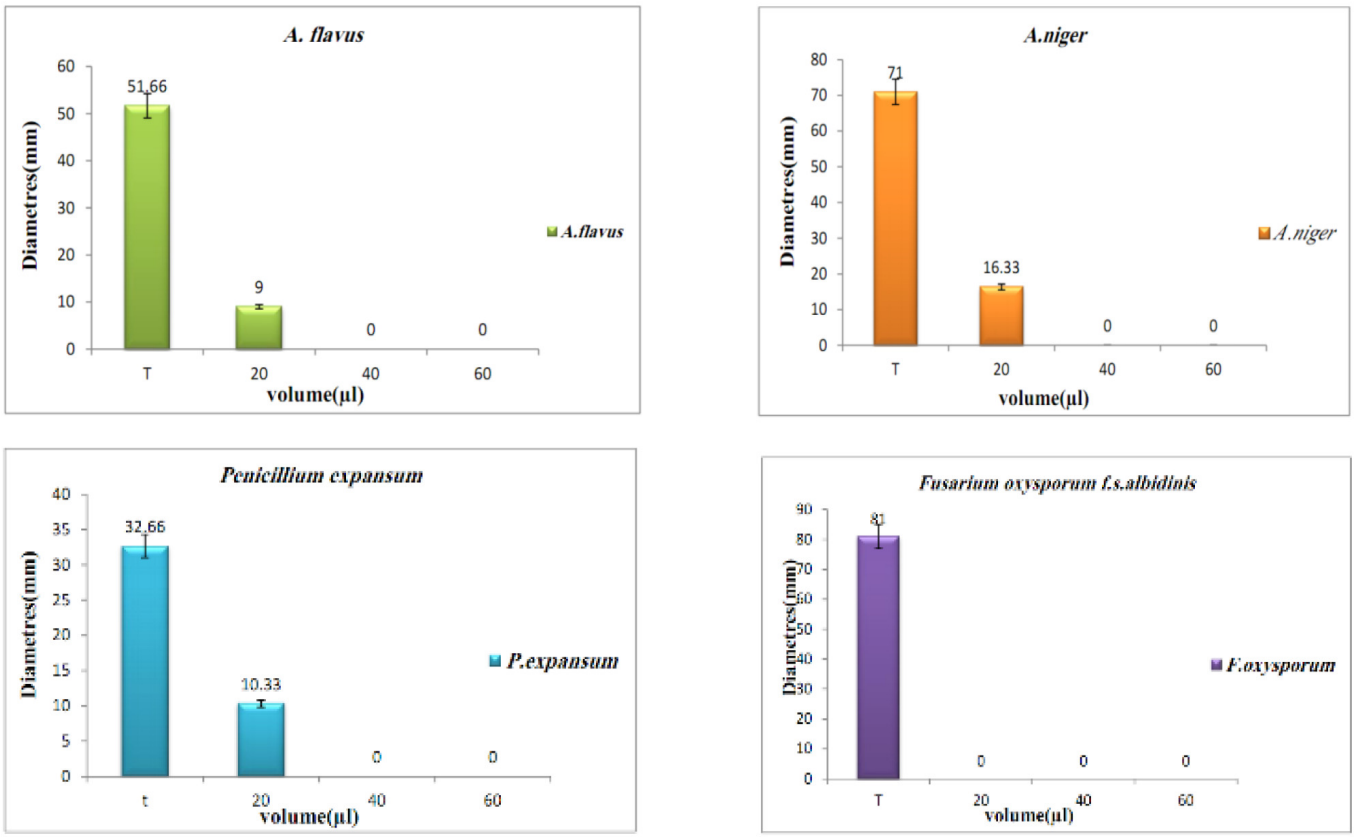

Fig. 1: Results of inhibition diameters of fungal strains under the effect of essential oil of *O. basilicum*

According to the results obtained, it is noted that the inhibition of mycelial growth in the presence of the essential oil of *Ocimum basilicum* is greater compared to the control.

The essential oil of *O. basilicum*, contains a high amount of β-linalool followed by α-Terpineolacetate, and 1,8 cineole. These components are at the origin of the inhibiting power of *O.basilicum* essential oil. Several researches have estimated that the high linalool content is the precursor of antibacterial activity (Sokovic *et al.*, 2009).

The antifungal index of Essential oil of *O. basilicum* for each strain tested is calculated by the formula described by (Wang *et al.*, 2005; Singh *et al.*, 2009).

The antifungal effect was manifested by a delay in growth compared to the control; this growth inhibition was much more marked on *F.oxysporum*. Essential oil of *Ocimum basilicum* exhibited the antifungal index of (100%) compared to the other three fungal strains at 40 µl. The results are reported in Table 2.

Table 2 : Antifungal index of *O. basilicum* essential oil against fungal strains tested.

Strains	***A.flavus* (40µl)**	***A. niger* (40µl)**	***P.expansum* (40µl)**	***F. oxysporum*f.s *aldedinis* (20µl)**
Antifungal index(%)	100%	100%	100%	100%

Antioxidant activity

The result obtained after calculating the reducing power for each concentration of essential oil is presented by the graph: PR%=f (c).

The EC_{50} values determined in mg/ml expressing the effective concentration of the necessary antioxidant extract for entrapment and reduction of 50% moles of DPPH dissolved in methanol.

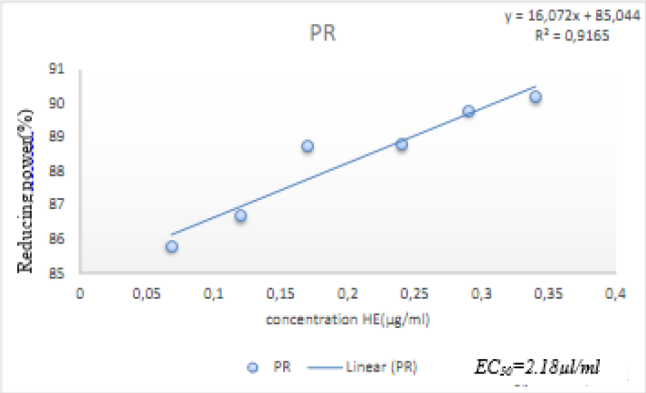

Fig. 2: Linear regression curve of radical inhibition percentages
DPPH according to the different concentrations of *Ocimum basilicum* essential oil

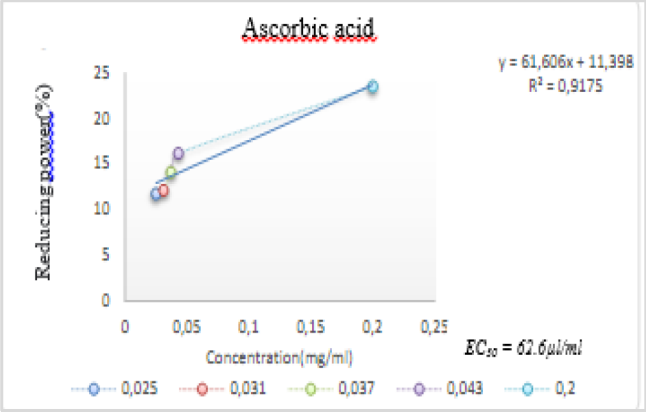

Fig. 3: Linear regression curve of radical inhibition percentages
DPPH according to the different concentrations of ascorbic acid

EC_{50} values were estimated from the linear regression equation of the graphs. The EC_{50} of *O. bacilicum* essential oil is 2.180µl/ml.

The trend line reading of ascorbic acid indicates that the percentage of inhibition or scavenging of DPPH free radicals increases with increasing concentration of ascorbic acid, which was confirmed by NahaL *et al*., 2016.

The essential oil of *O. bacilicum* traps between 86.68% and 90.20% of the DPPH radical with an EC_{50} of 2.180µl/ml. These results are more important than those of ascorbic acid, which is an antioxidant synthetic with a reducing power not exceeding 25% and an EC_{50} of 62.6 µl/ml.

Gramza *et al*. (2005) indicate that the lower the EC_{50} value, the weaker the anti-radical activity.

The results of the antioxidant activity of essential oil are lower compared to other studies, this can be explained by the difference in terms of plant growing conditions, geographical location, climatic conditions, mode and extraction time, as well as the techniques used (Michel, 2011).

Conclusion

Essential oils are aromatic substances with a complex chemical composition, which gives them very interesting antifungal and antioxidant properties.

The extraction of the essential oil of *O.basilicum* is carried out by hydro distillation; the determination of the essential oil yield showed profitability equal to 1.06, this value is better with those obtained in other studies of the same species.

The physico-chemical analyzes carried out on the essential oil show that the five parameters studied (pH, *RI, Ia, Is* and *Ie*) correspond to the references in the literature.

The results obtained show that the essential oils of *O.basilicum* extracted by hydro distillation have biological activities including an antifungal power and a very significant antioxidant activity have been proven in this study. It can therefore be concluded that these essential oils can be considered as an important source in the fight against food spoilage and fungal diseases, and even in the preservation of food against oxidation.

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