Phytochemical Study and Evaluation of Antimicrobial, Antioxidant and Insecticidal Activity of Essential Oils and Polyphenols of Bitter Orange *(Citrus Aurantium L.)*

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***Abstract*—** In this study, we conducted a phytochemical screening followed by evaluation of the antimicrobial and antioxidant activity of essential oils and polyphenols of *Citrus aurantium*. In parallel, we evaluated the insecticide effect of polyphenolic extract of *Citrus aurantium* L. on the larvae of *Galleria mellonela*, for this, we evaluated the mortality rate, TL_{50} and DL_{50}.

The phytochemical screening has allowed us to highlight the presence of secondary metabolites in the leaves and bark of *Citrus*, like saponins, mucilage, and flavonoids with significant amounts.

Observations on the antibacterial activity show that *Staphylococcus aureus* is the most sensitive strain, followed by *Escherichia coli*, the polar extracts proved devoid of any antifungal activity against *Saccharomyces cerevisiae*, and *Aspergillus* sp.

Two phytopathogenic bacteria were treated with polyphenols of *Citrus aurantium* ; *Pseudomonas savastanoi* and *Pectobacterium spedonicus*. The test of antibacterial activity showed a growth inhibition of these two phyopathogènes bacteria.

The evaluation of the antioxidant, revealed an important activity. Concerning the insecticidal effect of the polyphenols of the bitter orange leaves on the larvae of the wax moth *Galleria mellonela*, very significant results are achieved with 100% mortality recorded 24 hours after treatment at the high dose (30µl / ml).

***Keywords*—** *Citrus aurantium* L., characterization, essential oil, polyphenols, activity.

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I. Introduction

THE essences of plants are much researched because they possess generally interesting biological properties, especially their antifungal, antibacterial, antioxidant and insecticidal activity. The bitter orange (*Citrus aurantium* L.) is part of this category of plants used for medicinal purposes.

It is of the family of *Rutaceae*, probably native of South-East Asia, used for the food, the flavor and in medicinal purposes [1]. On the other hand, pests threaten our environment, our agricultural and forest resources, as well as our health. Since about sixty years, the protection against these devastating was essentially assured by pesticides of synthesis, with reserved results and of heavy environmental and sanitary impacts.

The biological control by the use of extracts of plants, thus establishes a very promising alternative.

It is from this perspective that joins our research work which concerns the evaluation of the antimicrobial and antioxidizing activity of *Citrus aurantium* on one hand and its insecticidal effect on greater wax moth *Galleria mellonela* of others leaves.

II. Material And Methods

A. Phytochemical Screening

The phytochemical screening is a qualitative test which allows determining the various present chemical compounds in the plant by reactions of coloring and haste, such as polyphenols (tannins, flavonoids, anthocyanins, alkaloids, saponosides, etc. These tests can be made either on the powder or on the infused in 20 %. The infused in 20 % of each party of the plant and peels him is prepared by the addition of 20 g of vegetable powder for 100 ml of hot distilled water. Later 15mn of time, the mixture is filtered and the obtained filtrate is fitted to 100 ml by the distilled water.

B. Method of extraction of essential oil by hydrodistilation

Leaves and bark of *Citrus auratium* L. were collected in April 2015 in the region of Annaba (East of Algeria).

Essential oil is extracted by the method of hydro distillation. For that purpose, a quantity of 40g of barks and leaves fresh of the plant are introduced into a ball of a liter containing some distilled water, the set is brought to a boil during several hours

(from 1:30 am till 4 am). Vapors in charge of volatile substances condense upon their arrival at the level of the cooler; they fall again in the form of droplets and train with the water a heterogeneous mixture which we get back in a bulb to be settled.

To extract HE of the water, we add some ether diethylic. Oil after their extraction, is preserved in being placed next temperature 6°C.

The yield in essential oil is defines as being the report enters essential mass of oil obtained and the vegetable mass of used material percent.

C. Evaluation of the antimicrobial activity of essential oil

The antibacterial activity of the essential oil of *C. aurantium* was estimated by tries based on the method of distribution by disk. To be able to obtain various concentrations of the essential oil of *C. aurantium*, we diluted her in the diéthylique ether. Three types of successive tries are realized for Bacteria at the rate of 20 ml in every tube and a single type realized for mushrooms and yeast.

The antimicrobial activity was estimated by measuring the diameter of the growth of the zone of inhibition around the disk, against the trial body. The diameter of the zones of inhibition was measured in millimeters [2].

D. Evaluation of the antioxidant activity of the essential oil

Measure of the power of trapping of the radical DPPH:

The capacity of donation of electrons by essential oil is highlighted by a spectrophotometric method, by following the disappearance of the purple color of a méthanolique solution containing the free radical DPPH + (1,1-Diphenyl-2-picryhydrazyl [3].

We prepare 100ml of a solution of the DPPH in the same type of solvent as that used to prepare the sample and his dilutions (methanol). For it we prepare 100ml of a solution; 2,4mg equivalent in 0,0024g of the DPPH in 100ml of solvent.

The in vitro antioxidizing activity was estimated by the measure of the power of trapping of the radical DPPH (1,1-Diphenyl-2-picryhydrazyl) according to the method described by Ponce *et al.* [2]: 25 µl of each méthanoliques solutions some essential oil and extracts tested in various concentrations (100 µg / ml, 200 µg / ml, 400 µg / ml, 600 µg / ml, 800 µg / ml, 1000 µg / ml) are mixed in tubes placed in the basin of the spectrophotometr with 975 µl of a methanolic solution of DPPH (0.0024 %).

After an incubation time of 30minutes at an ambiant temperature and in the darkness, the absorbance was measured in 517nm.

The experiences are realized in 3 repetitions for every concentration, and the percentage of antioxidant activity (I %) is calculated according to the following formula:

$$I(\%) = (A\ White - A\ Sample) \times 100 / A\ White$$

With:

I (%): Percentage of inhibition

A White: Represent the absorbance of the DPPH

A Sample: Absorbance of the sample tested later 30 min [4]-[5].

E. Evaluation of the insecticidal activity of essential oil

Larvae of last stage *of Galleria mellonella* are individually distributed in jars covered with tulle. The food prepared for these individuals is a mixture constituted by honey and by pollen made in ball. The tested extracts are mixed with the food which is given to larvae during 24 hours. After that, the treated food is replaced by another one untreated

III. Results

A. Results of phytochemical Screening

The phytochemical screening allowed us to highlight the presence of secondary metabolites plant tissues of *Citrus aurantium* L. the results of this study are noted in the table 1.

TABLE 1. Result of screening phytochemical of powder of leaves and bark of Citrus aurantium L.

Compounds search	Results	
	leaves	barks
total tannins	-	+
gallic tannins	+++	+++
catechin tannins	-	-
Anthocyanes	-	-
Saponosides	+++	+++
Mucilages	+++	+++
flavonoids	++	-
Glucosides	+++	+++
alkaloids	-	-
Starch	-	-

- : Negative Test
+ : Slightly positive test.
++ : Testing
+++ : Strongly positive test .

B. Results of the yield of extraction

The extraction yield of essential oils leaves (02 samples) was 3.8 %, for against the bark (01 sample) is much higher with 9.1%. According to bibliographic data, Citrus fruits contain little HEs. So from the results we find that the bark of *C. aurantium* is part of plant that performed well in HE (9.1 %). So the work done by Hosni *et al.* [6] indicate that on the bark of orange varieties; Valencia Late, Navel Thomson, White Maltese and Bigarade; yields HEs extracted by steam distillation were estimated at 2.31 %, 1.89%, 1.49 % and 2.2 % MF. These results are broadly lower compared to our data for the variety of Bitter Orange.

However, the contents HEs of our samples are much higher than those found by Avlessi *et al.* [7] for leaves of *Causena anisata* L. (a medicinal plant of the *Rutaceae* family) (0.14 MF).

C. *Results of antibacterial activity*

Observations of the effect of extracts of *Citrus aurantium* L. on four pathogenic and two phytopathogenic strains show that both strains presente a sensitivity for phynolique *C. aurantium* extract.

So, *Staphylococcus aureus* is the most sensitive strain among pathogenic strains of *C. aurantium* with a 15 mm zone of inhibition , it is followed by *E.coli* and *Klebseila pneumoniae* with an area of 14 mm and 10 mm respectively, and 10 mm for *Salmonella enterica* . These results express a significant sensitivity of bacteria in all samples compared to the control. Gulay Kirbaslar *et al.* [9] studied the microbial activity of oil of several types of *Citrus of* South of Turkey; such as lemon (*Citrus limon* (L.) Burm. F.), The grapefruit (*Citrus paradisi* Macfayden), Bergamot (*Citrus bergamia* Risso and Poit.) , Bitter orange (*Citrus aurantium* L.), The ' sweet orange (*Citrus sinensis* (L.) Osbeck), Mandarin (*Citrus reticulata* Blanco); showed strong antimicrobial activity against Gram (+) and Gram (-). They recorded peel oil Citrus variable activities against bacteria such as *E.coli* applied , *S. aureus* and *K. pneumoniae* have shown a resistance with a diameter of 12 mm for each bacterium , these results are close our results.

With respect to *S. aureus* she displayed sensitivity to this. These results are confirmed by Javid *et al.*[10]., and consistent with the work of De Billerbeck [11] reported that *S. aureus* presented a variable sensitivity to the HES some Citrus species tested , in fact, hes bergamot, lemon and bitter orange small grain showed zones of inhibition of 30 mm, 12 mm and 20 mm , respectively.

In parallel, Two phytopathogenic bacteria were treated with polyphenols of *Citrus aurantium* ; *Pseudomonas savastanoi* and *Pectobacterium spedonicus.* The test of antibacterial activity showed a growth inhibition of these two phyopathogènes bacteria.

Indeed, Knobloch *et al.*[8] noted that these extracts contain phenolic compounds carvacrol and thymol that interfere with membrane proteins of the microorganism thus giving a significant inhibitory activity.

D. *Results of antifungal activity*

The results for the antifungal activity of HES are tested according to the target strains. It turned out that no zone of inhibition around the disks was observed vis-a-vis of *Saccharomyces cerevisiae* and *Aspergillus* sp. The different results observed may suggest that the extract *Citrus aurantium* has no antifungal activity against strains of yeast and fungus tested.

E. *Results of antioxidant activity*

DPPH (C18H12N5O6) is one of the main tests used to explore the use of plant extracts as antioxidants [12]. The results show a change in color of the extract of crude ET in different concentrations using the method of DPPH. The absorbance versus concentrations (mg / ml) of the methanol extract is shown in the following figure:

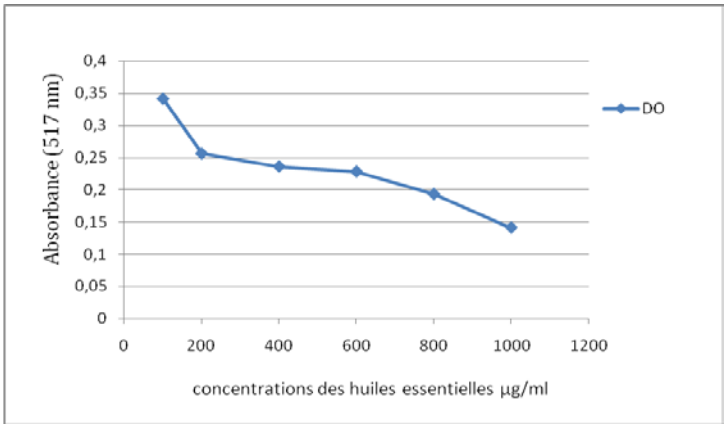

Fig. 1Courbe representing the absorbance of dilutions of HE the bark of Bigarade

The results of percentage inhibition of the free radical DPPH by the methanol extract is shown in Fig. 2

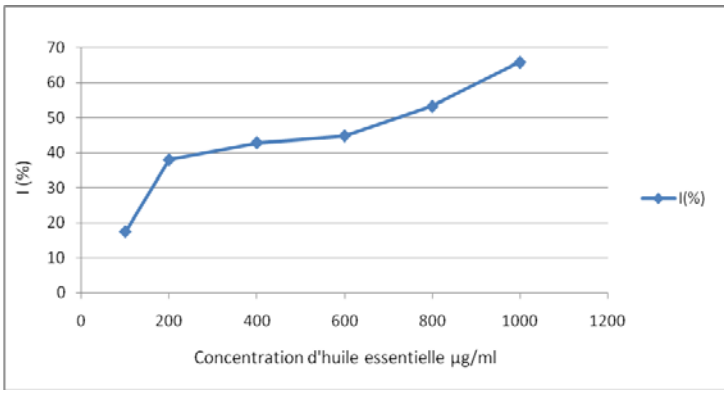

Fig. 2 Courbe percentage inhibition of free radical DPPH of HE the bark of Bigarade

Results showed in the previous figure shows that after 30 min of incubation, the anti -radical activity of the crude methanol extract of *Citrus aurantium* L, 65.86 % is very important for the concentration of 1000µg / ml. In addition, we note that the anti- radical power augment with increasing concentration.

Frassinettis *et al.* [13] report that the UAS orange peel, lemons and tangerines have radical scavenging activity ranging from 20 to 70% .The values of EC (Effective Concentration) are calculated in order to determine the concentrations that reduce 50% essential oil free radicals of *Citrus aurantium* L and standard antioxidant (Vit C). According to this latter, we find that the inhibition values of the HE solution of *Citrus aurantium* L and vitamin C increase with increasing concentrations of these. These EC50 values are determined graphically where the X axis represents the concentration of the extract and the antioxidant activity ordered by percentage. The antioxidant capacity of a compound is even higher than its EC50 is small [14].

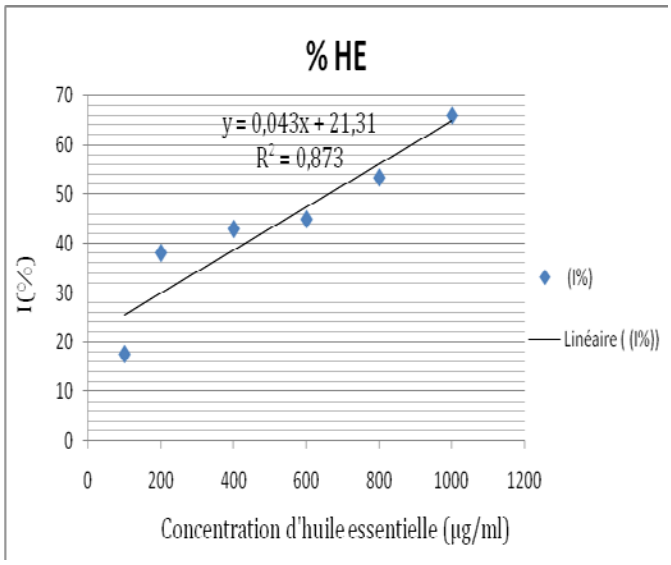

Fig. 3 EC50 calculation for the essential oil of Bitter Orange

For our HE *Citrus aurantium* L, we have the following equation:

Y = 0.043X + 21.31

Therefore, EC50 = 667 209 g / ml

The essential oil of Bitter Orange could bring the stable free radical

2.2 diphenyl -1- picrylhydrazyl (DPPH) and diphenyl picrylhydrazine - yellow - colored with a EC50 667 209 mcg / ml.

F. Results of insecticidal activity

It has been found after treatment with polyphenols that a high dose of our plant extract (30µl / ml) showed a valuable efficacy against *Galleria mellonela* larvae resulting in a mortality rate of 100% from the first day of treatment. By against the larvae of *G. mellonela* showed strong resistance for the low dose (10µl / ml).

In parallel, an LT50 of 2.34 days was obtained after treatment with intermediate dose of 20µl / ml.

For LD50, a value of 20.58 µl/ml was obtained for the first day with 20.08 µl/ml recorded the second day.

It is said that an insect is sensitive to a given insecticide whether it suffices to cause his death, administering to the insect a low dose of insecticide [15].

The two extracts of *Citrus maxima* (essential oil, polyphenols) tested by contact or inhalation showed a toxic effect on the population of *Aphis fabae* [16].

The results of the insecticidal activity of essential oils on *Myzus persicae* revealed that the essential oils of *Eucalyptus globulus* and *Thymus vulgaris* exhibit toxic and repellent effects on *Myzus persicae* [17]. According to Khalfi-Habes and Sellami [18], the insecticidal activity of plants is probably attributed to the semiochemical substances contained in the vegetal.

Finally, certain studies show that many essential oils-based biopesticides are as effective as synthetic products. Likewise, the plants' natural extracts can be aids of choice in the programs aiming to manage the devastators' resistances to pesticides [19].

IV. Conclusion

Our study of the plant extract is based on the extraction of essential oils and polyphenols from the leaves and barks of bitter orange (*Citrus aurantium*) for use in the treatment against certain pathogenic microorganisms and plant pathogens and also against larvae *of Galleria mellonela* .

It turns out from this study that extracts of bitter orange have a very interesting alternative in the pharmaceutical and plant protection area because encouraging results have been obtained. It would be very beneficial to continue this study to deepen research on the mode of action of these biological molecules.

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