Research Article

# Study of the Antimicrobial Activity of the Extracts of the *Eucalyptus camaldulensis* and *Eucalyptus globulus* Stemming from the Algerian Northeast

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**ABSTRACT**

The misuse of Organic synthesis plant products, leads to the adverse repercussions on the environment and promote the proliferation of microbial agent resistant to the active ingredients of pesticides used. In response to this scourge and health problems related to the persistence and bioaccumulation of pesticides, a new alternative or complementary protection strategies for the sustainable development of agriculture, such as biopesticides of plant origin that attempt to emerge. The ambition of our work, frame a valorisation of vegetable substances namely *Eucalyptus camaldulensis* and *Eucalyptus globulus* in the northeastern Algerian region. This is to highlight the presence of the different secondary metabolites, extract by solvents (methanol/water) and analyze the content of total phenols from the leaves by the visible UV spectrophotometry method. A determination of the pesticide antibacterial secondary metabolites from the hydro-methanolic extract of *Eucalyptus camaldulensis* and *Eucalyptus globulus* was performed on phyto-pathogenic bacterial strains isolated from agricultural soil. The results obtained indicate that the *Eucalyptus camaldulensis* and *Eucalyptus globulus* contain high concentrations of total phenols (respectively 79, 35 and 105,31 mg GAE/gDM). These polyphenols possess a strong pesticide power or antibacterial power important facing the different bacterial strains tested. The values of inhibition zones are pretty similar if not more important than those obtained in the presence of Vidan fungicide.

**Keywords:** *Eucalyptus camaldulensis*, *Eucalyptusglobulus*, bio-pesticide, polyphenols.

## INTRODUCTION

The use of pesticides has experienced very strong growth over the past decades.

It allowed a huge progress in the matter of food resources and the improvement of public health. So pesticides have become almost indispensable to most agricultural practices. However, they are likely to be a hazard to the environment, ecosystems and man.

A socket to the seriousness of the problems of the environment prompted agencies and research institutions to develop much more biological methods, in its various forms, in order to limit the use of chemical pesticides.

One of its forms is the exploitation of secondary compounds from plants in the fight against insects, fungi, and harmful bacteria[1-2].

The farming community and consumers of agricultural products final, often see pesticides from natural sources, as posing less risk. However, the production of biological, natural and bio-pesticides pesticides remains marginal; it represents less than 1% of the total production of pesticides.

This interest for bio-pesticides, has led us to be interested in two plant species widespread in Algeria, known for their medicinal interest and introduced in 1860[3-4].

It comes from '***Eucalyptus camaldulensis***' and '***Eucalyptus globulus'***.

## MATERIALS AND METHODS

### Experimental Equipment

The leaves of *Eucalyptus camaldulensis* and *Eucalyptus globulus* has been harvested at the level of the forest of Bouguentass Annaba (North - East Algerian), in a period between April and the month of September[5].

They have been dried in the open air and away from light and moisture. The bacterial strains, it was isolated from agricultural soil and from leaves of wheat, their identification is made from the Gram stain.

During this work, a fungicide is used to compare activity biopesticides of the crude ethanol extract of *E.camaldulensis* and *E.globulus.* This fungicide is part of the family of Triazole, its active ingredient is Triadimenol, the latter inhibits the synthesis of sterols.

### Phyto-chemical Screening

The chemical screening is a technique used to determine the chemical groups contained in a plant body by physico-chemical reactions.

Preliminary tests to determine the major chemical groups have been performed according Solfo[6] and Harborne[7].

### Preparation of Methanol Crude Extract

100g powder Ecalyptus globulus and Eucalyptus camaldulensis are setting steeped in 1l of water methanol mixture (7/3 v / v) for 24 hours at room temperature and with magnetic stirring; filter and the solvent is evaporated

to dryness under reduced pressure at 50° C using a rotary evaporator.

### Determination of Total Polyphenols

The total polyphenol content of the extracts was determined by the Folin Ciocalteu method[8]. A quantity of 200 ml of the extract was mixed with 1ml of freshly prepared reagent Folin-Ciocalteu reagent (10 times diluted) and 0.8 ml of sodium carbonate 7.5% ($Na_2CO_3$). The whole is incubated at room temperature for 30 minutes and the reading is taken against a blank using a spectrophotometer at 765nm. The results are expressed in milligrams gallic acid equivalent per g dry plant material.

### Antibacterial Activity

We used the diffusion technique on solid media. This is a method similar to that of the susceptibility of determining the sensitivity of a bacterial strain vis-a-vis one or more products. A sterile disc of filter paper (Whatman No. 1) of 6mm in diameter is soaked in the test products (pure methanol crude extract, crude extract ½, ¼ crude extract), the latter is then placed on the casting agar in boxes Petri standard 4mm thick and previously inoculated with the identified bacterial strains. The inoculum concentration used is on the order of 106 to 108 CFU/ml. The dishes are incubated at a temperature of 37° C for 18 to 24 hours.

If the product is toxic for the species, forms a zone of inhibition or clear halo around the disc. Greater is the area, more species is sensitive.

### Statistical Analysis

The statistical analysis, carried out for the different results of the biopesticide effect is carried out using the MINITAB software Version 13.31 Fr.

The values obtained after 48 hours of incubation were compared pairwise using testing "t" of Student[9].

## RESULTS

### Phyto-chemical Screening of *Eucalyptus camaldulensis* and *Eucalyptus globulus*

**Table 1:** The results of the chemical screening

| Secondary Metabolites | *Eucalyptus camaldulensis* | *Eucalyptus globulus* |
|---|---|---|
| Saponosides | + | + |
| Anthocyanins | + | + |
| Leuco-anthocyanins | - | - |
| Alkaloids | - | - |
| Flavonoids | + | + |
| Tannins | + | + |

+ positive result; - negative result

### Determination of Total Phenols

The determination of the content in total phenolics in the hydro-methanolique of *Eucalyptus camaldulensis* and *Eucalyptus globulus* extract is made by using the colorimetric method of Folin-Ciocalteux, this content has been reported in Gallic acid equivalent mg/g of dry plant material. The results show that alcoholic extracts of two Eucalyptus species have a high content of total phenols (79, 35 and 105, 31 mg GAE/gDM) (Table 2).

**Table 2:** Total Phenolics

| Hydro-methanolic extract | Yield | Total Phenolics |
|---|---|---|
| *Eucalyptus camaldulensis* | 22% | 79,35 mg GAE/gDM |
| *Eucalyptus globulus* | 18% | 105,31 mg GAE/gDM |

### Antibacterial Activity

### Effect of extracts from *Eucalyptus camaldulensis* and *Eucalyptus globulus* on strain B1

The figure 1 represents the effect of various substances tested on the B1 strain. In a comprehensive manner, the hydro-methanolic extracts appear to be effective against bacterial strain B1. The zones of inhibitions are between 6.33 mm and 10 mm for extract of *Eucalyptus camaldulensis* and 4.5 mm and 6.33 mm for *Eucalyptus globulus* extract.

According to the Student's t test, it appears from the significant differences ($P \leq 0.05$) between the activity of the pesticide and the mother of the extract thus solution than the 1/2 of *Eucalyptus camaldulensis* dilution and dilution to 1/4 of *Eucalyptus globulus*. Any time the antibacterial power of extracts of *Eucalyptus camaldulensis* is higher than that of extracts of *Eucalyptus globulus* and the pesticide.

Statistical analysis showed no significant differences ($P > 0.05$) between the activity of the pesticide and the stock solution of the hydroalcoholic extract and dilution to 1/2 of *Eucalyptus globulus*, as well as the dilution to 1/4 of the hydroalcoholic extract of *Eucalyptus camaldulensis*, remains more active that the extract of *Eucalyptus globulus.*

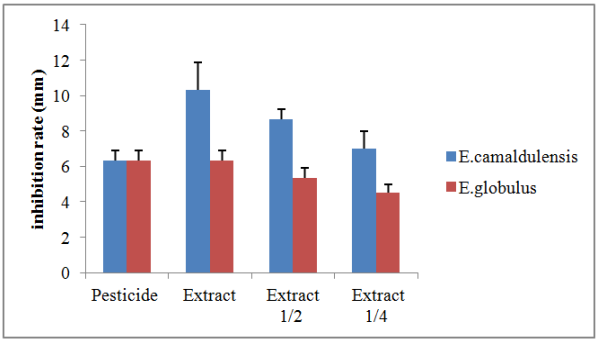


**Figure 1:** Activity of different substance tested on the B1 strain

**Effect of extracts from *Eucalyptus camaldulensis* and *Eucalyptus globulus* on strain B2**

The Figure 2 represents the effect of various substances tested strain B2, the latter seems also be inhibited by all of the tested substances (pesticides, hydroalcoholic extract SM, hydroalcoholic extract 1/2, hydroalcoholic extract 1/4), with different halos whose diameter varies between 5.66 10.83 for *Eucalyptus camaldulensis* and 4.66 mm and 7 mm for *Eucalyptus globulus*.

According to meaning P (Student's t Test), it appears from non significant differences (P ≥ 0.05) between the activity of the pesticide and the extract diluted in 1/2 and 1/4 of *Eucalyptus camaldulensis*, the mother of *Eucalyptus globulus* extract thus solution than dilutions at 1/2 and 1/4.

However, highly significant differences were found (P ≤ 0. 01) between the pesticide and the stock solution of the extract of *Eucalyptus camaldulensis*.

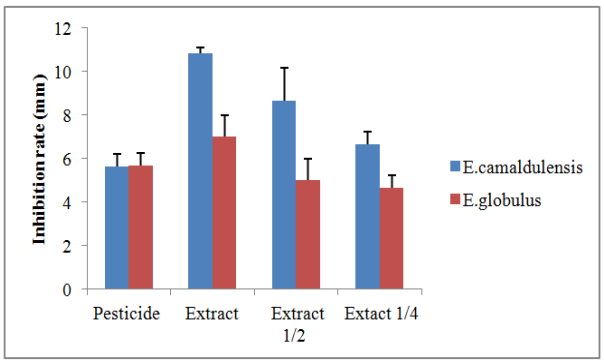


**Figure 2:** Activity of different substance tested strain B2

**Effect of extracts from *Eucalyptuscamaldulensis* and *Eucalyptus globulus* on strain B3**

Natural extracts are a disruption of the development of the bacterial strain B3 (figure3).

According to statistical analysis, there appears to be no significant differences (P ≥ 0.05) between the effect of the fungicide and that of natural extracts they appear to be more effective.

However, we noted significant differences (P ≤ 0.05) between the effect of the pesticide and the effect of the hydro-methanolic extract diluted to 1/2.

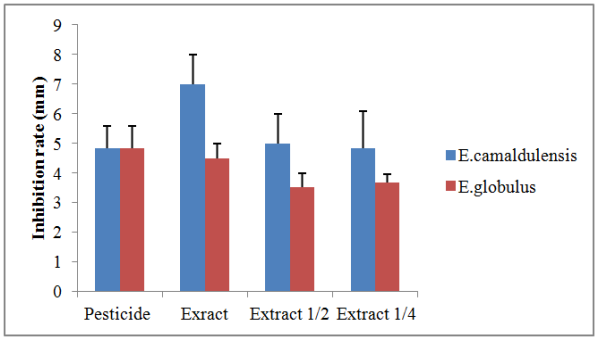


**Figure 3:** Activity of different substance tested on the B3 strain

**Effect of extracts from *Eucalyptus camaldulensis* and *Eucalyptus globulus* on strain B4**

The 3 bacterial strain seems to be inhibited by experienced solutions namely pesticides, extract SM, 1/2 and 1/4, with zones of inhibition between 7.16 and 18 mm for *Eucalyptus camaldulensis* and 7.16 and 12 mm for *Eucalyptus globulus*. (figure4)

Non significant differences (P ≥ 0.05) were observed between the activity of the pesticide and the 1/2 of *Eucalyptus camaldulensis* extract as well as 1/2 and 1/4 of *Eucalyptusglobulus* extracts.

Also, we perceive significant differences between the pesticide and the extract of *Eucalyptus camaldulensis* diluted 1/2.

Nevertheless, we find highly significant differences (P ≤ 0.01) between the activity of the stock solution of the extract of *Eucalyptus globulus* and the pesticide.

However, very highly significant differences (P < 0.001) were noted between the pesticide and the crude extract of *Eucalyptus camaldulensis*.

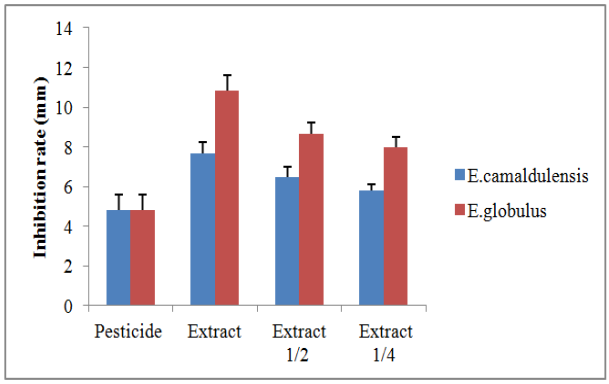


**Figure 4:** Activity of different substance tested on strain B4

It is worth noting that the different extracts of *Eucalyptus camaldulensis*, are much more efficient than the pesticides and the extracts of *Eucalyptus globulus*on strains B1, B2, B3, B4 and they remain less active than this pesticide.

**Effect of extracts from *Eucalyptus camaldulensis* and *Eucalyptus globulus* on strain B5**

The natural substances tested appear to have an inhibitory effect on strain B5 in a comprehensive manner with halos between 4.83 and 7.66 mm for *Eucalyptus camaldulensis* and 4.83 and 10.83 mm for *Eucalyptus globulus* (Figure 5).

The statistical study shows (test student's T-distribution) shows the differences very highly significant (P < 0.01) between the mother solution of extract from *Eucalyptus globulus* and the pesticide.

The T-test reveals highly significant differences (P ≤ 0.1) between the pesticide and the effect of the *Eucalyptus globulus* extract diluted to 1:2 and 1:4.

Significant differences (P ≤ 0.05) were found between the extract concentrated of *Eucalyptus camaldulensis* and pesticide.

Nevertheless, we observe no significant differences (P ≥ 0.05) between the inhibitory effect of extracts of *Eucalyptus camaldulensis* diluted to 1/2 and 1/4 and the effect of the pesticide.

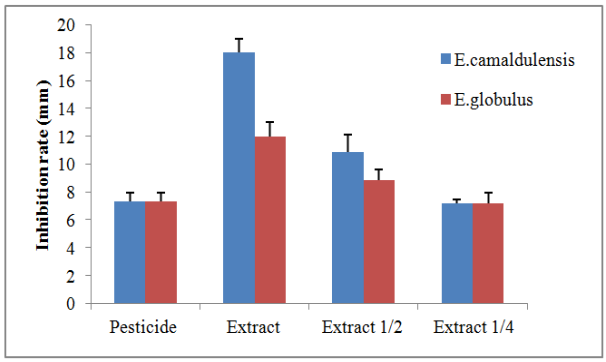


**Figure 5:** Activity of different substance tested on strain B5

## DISCUSSION

Chemical screening has highlighted the presence of some secondary metabolites including anthocyanins, flavonoids and tannins saponins. Other researchers[10-14] have reported the presence of these metabolites in the Myrtaceae; family which *Eucalyptus camaldulensis* and *globulus* belong.

Overall, the testing of the bio-pesticide effect of extracts of *Eucalyptus camaldulensis* show that they have an inhibition activity on the bacterial growth that varies depending on the strain. These inhibitions are greater than those observed with the pesticide.

However the extracts of *Eucalyptus globulus* are less effective on the strains tested except for the bacterial strain B4, where there is a significant inhibition of growth of the latter.

The results obtained confirm the efficiency of natural substances extracted from plants against the development of different microbial agents.

These results seem to be agreed as reported by many authors. A study by Saikhanllate[15] confirms that the essential oils derived from *Citrus medica* have bacteriostatic and bactericidal activities against many bacteria.

Furthermore, investigations carried out by many authors[16-18] affirm that the essential oil of *Eucalyptus camaldulensis* and *Eucalyptus globulus*, has antibacterial power against some bacteria. Many works highlight this antibacterial power, in effect Damjanović-Vratnica[19] and Bachir Raho & Benali[20] indicate that *Eucalyptus globulus* essential oil has antibacterial power that varies from one strain to another and one concentration to another.

Other works carried out by Ayepola & Jacks[11]; Babayi[21] and Enciso-diaz[22] attests that the hydro-methanolic extract of *Eucalyptus camaldulensis* and *Eucalyptus globulus* has an inhibitory effect against several bacterial strains.

## CONCLUSION

In this work, we have tried to contribute to the recovery of the species plant *Eucalyptus camaldulensis* and *Eucalyptus globulus*. Through the study of the antibacterial effect of extracts hydro-methanoliques of these two species with a few strains isolated from agricultural soil, it appears that these substances have strong inhibitory power on tested germs, this inhibition varies depending on the bacterial species and the concentration of the product tested.

Generally, compounds isolated from *Eucalyptus camaldulensis* appear to be effective at all concentrations used, registered inhibitions zones are larger than those caused by the pesticide. Extracts of *Eucalyptus globulus* seem to be less effective than *E.camaldulensis*. However, the diameters of recorded halos are often close than those of the pesticide except for bacterial strain B4 where zones of inhibitions of all concentrations are higher than those recorded by the extracts of *Eucalyptus camaldulensis* and pesticide;

This antibacterial power is due to the richness of extract of *Eucalyptus camaldulensis* and *globulus* in inhibitory substances; it's probably the phenols, which are endowed with strong bactericidal activity.

These results indicate that extracts of *Eucalyptus camaldulensis* and *Eucalyptus globulus* are promising for their power bio-pesticide, phenolic extract raises some phyto-pesticide effect against microbial strains that they may present an interesting alternative to the use of plant protection products.

However, in our opinion, it would be interesting to test other concentrations on a wider panel of fungal and bacterial strains currently become multi-resistants.

**Langue source**

Face à ce fléau et aux problèmes de santé liés à la rémanence et la bioaccumulation des pesticides, de nouvelles stratégies de protection alternatives ou complémentaires pour le développement durable de l'agriculture, tel que les bio-pesticides d'origine végétale qui tentent d'émerger.

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