FUNGAL TERRITORY
2019, VOL. 2, NO. 2, 6-11

REVIEW ARTICLE

EFFECT OF AQUEOUS EXTRACT OF *EUCALYPTUS* SP., *NERIUM OLEANDER* AND *ALLIUM SATIVUM* ON THE GROWTH OF DERMATOPHYTES

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ABSTRACT

The surface mycosis of skin, hair and nails are common infections in the world. They are due to the several molds and yeasts. The main agent responsible for these affections is the dermatophytes: filamentous fungi which live in depending on the keratin of the cornea layer, the skin and the superficial growths of human and animal. Their distributions vary from one country to another and area to another in the same country, according to the climate of each area and favors factors development of these fungi. The dermatophytes pose serious problems of health. The aim of the present study is to evaluate the inhibitory effect of aqueous extracts of three plants against three dermatophytic species induce dermatophytosis at the area of Annaba "East of Algeria", isolated from patients reached of the dermatophytosis. We have isolated three species (*Trichophyton rubrum, Trichophyton verrucosum* and *Microsporum ferrugeneum*) using Sabouraud Dextrose Agar medium (SDA), that were tested with crude extracts of three plants; *Allium sativum* (garlic), *Nerium oleander* (oleander), and *Eucalyptus sp*. (eucalyptus). The method applied was the solid state dilutions. The obtained results showed that all extracts examined reduced growth of mycelium compared to the controls. It was found that garlic crude extracts proved the highest growth inhibition against the three species (38, 59% to 100%), followed by eucalyptus with 60% to 80%, whereas, oleander reduces the growth with 50% to 60%.

Keywords: Dermatophytes, Dermatophytosis, Antifungal activity, aqueous extracts, Annaba

INTRODUCTION

Skin, hair and subcutaneous tissues in human and animal are subjected to infection by several organisms, including fungi mainly dermatophytes (**Ali and Majid, 2008**); molds which live with depends on the keratin of the cornea layer on the skin and the superficial body growths on the man and the animals (**Liu *et al*., 1997; Fernanda *et al*., 2007, James *et al*., 2012, Ndiaye *et al*., 2013**). A classification currently used is that of Emmons (1934), who distinguish three principal genera of dermatophytes; *Trichophyton, Microsporum* and *Epidermophyton* (**Dominique and Marc, 2008; Jamalian *et al*., 2012; Ranjbariyan *et al*., 2014**). They are responsible mainly of surface mycosis: *Tinea corporis* (skin), *Tinea pedis* (feet), *Tinea capitis* (hair), *Tinea unguinum* (nails) and *Tinea crurus* (groin) (**Gabriela *et al*., 2012; Wisselink et *al*., 2011**). Several factors support the development of dermatophytes in particular the climatic factors; the heat and the moisture which intervene initially (**Del Boz-González, 2012**). Their distribution varies from one country to another and area to another of the same country (**Ilkit et *al*., 2007; Nzenze *et al*., 2009**). Dermatophytosis pose problems of human health **(Balakumar *et al*., 2011; Ali Reza *et al*., 2013**). The treatment of these infections is very expensive and very length. Therefore, the traditional treatment with medicinal plants proved their effectiveness (**Anbin *et al*., 2010**). The purpose of our study was to evaluate *in vitro* antifungal activity of the aqueous extract of three medicinal plants: garlic cloves (*Allium sativum*), oleander leaves (*Nerium oleander*) and eucalyptus leaves (*Eucalyptus sp*.) on the growth of three dermatophytic species: *T.rubrum, T.verrucosum* and *M.ferrugeneum* which are isolated from swabs in patients infected by dermatophytes at Annaba area, East of Algeria.

MATERIAL AND METHODS

Collection of swabs

The affected area was thoroughly cleaned with alcool to avoid the surface contaminants. Petri dish was used for collecting specimens. After disinfection with alcool skin lesions were scalped with a scalpel to collect epidermal scales. From the scalp hair, scales were epilated with sterile grip depilatory and nails sample collected using a sterile nail clip.

Isolation and identification of dermatophytic species

Swabs are cultured on Petri dish of Sabouraud Dextrose Agar (SDA) with chloramphenicol. The Petri dishes were incubated at 27°C for 7 days to one month. After 48 hours of incubation, we examined the dishes for researching of yeasts and other moulds not dermatophytes to reject them. The dermatophytes were observed about the day fifth of incubation, the identification was based on the macroscopic features; so we noted the aspect of the fungi colony (woolly, powdery, downy, plastery… etc.) the color in recto and verso, the diameter, the speed of growth and the diffusion of the pigmentation in the medium, also on the basis of their microscopic features. Whereas, species selected in this study the method applied was the solid state dilutions were isolated and identified by **Ennaghra *et al*. (2016)** at laboratory of biochemistry and applied microbiology, department of biochemistry, faculty of sciences, Badji Mokhtar university, Annaba, Algeria.

Plant extracts

Three plants were collected from Annaba area (garlic cloves, oleander leaves and eucalyptus leaves); they selected to examine the effect of their aqueous extracts on mycelial growth of the three dermatophytic species: *Trichophyton rubrum, Trichophyton verrucosum* and *Microsporum ferrugeneum.* Aqueous extracts is carried out by simple methods according to **Sqalli *et al.* (2007**) and **Eyana (2007**), with some modifications. The plant parts of oleander and eucalyptus were thoroughly washed with 2% aqueous sodium hypochlorite and sterile distilled water (**Abdel-Raouf, 2001**). Aqueous extracts were prepared by adding 10 g of air-dried plant part in 100 ml sterile water and ethanol 96° (v/v). Whereas, the crude aqueous extract of garlic was obtained directly from cloves pulps. The mixture was then filtered and further centrifuged at 6000 rpm for 30 min. According to the method of **Sunaina *et al.* (2013);** the supernatant was filtered through a 0.2-mm pore size Wattman filter paper to remove any impurities. Aliquots were stored at -4°C until required. For the aqueous extract of *Nerium oleander* and *Eucalyptus sp.* we previously evaporated the ethanol, and the filtration is made as garlic extract.

Method of anti-dermatophytic activity

For evaluate in vitro anti-dermatophytic activity of these extracts, we have selected three fungal strains previously identified: *T.rubrum, T.verrucosum* and *M.*

ferrugeneum. The method applied was the solid state dilutions; various aqueous extracts were added to the agar culture medium (S.D.A).

Anti-dermatophytic activity evaluation

Firstly the SDA medium is prepared and autoclaved. For evaluating the aqueous extract of *Eucalyptus sp.* and *Nerium oleander* we used eight concentrations (2.5ml / 100ml, 5ml / 100ml, 7.5ml / 100ml, 10ml / 100ml, 12.5ml / 100ml, 15ml / 100ml, 17ml, 5ml / 100ml and 20ml / 100ml). For the aqueous extract of *Allium sativum*, we used only four concentrations (2.5ml / 100ml, 5ml / ml, 7.5ml / 100ml and 10ml / 100ml). so we have prepared sterile flasks of 200ml, each flask contains 100ml of sterile SDA medium, in a sterile area and around a Bunsen burner, than, we have added the volumes of the extract using a micropipette, we added 2.5ml of the aqueous extract in the first flask, 5ml in the second flask, until the last flask where we added 20ml for eucalyptus and oleander extracts. We have manually shaken the contents of each flask and distributed it in well referenced Petri dishes. Also, Petri dishes were prepared containing S.D.A medium without extract for use as controls for each strain to be tested. After solidification of the medium in the dishes, we put the same quantity of mycelium for each strain (the plates containing the extract and the plates used as controls). According to **Mahboubi and Kazempour (2014),** we have done discs of 5mm diameter in the surface of each mycelial colony, and were aseptically inoculated onto the center of each Petri plate with extract and control. The Petri plates were incubated at 27 °C for 5 days. The experiments were performed in triplicate. After that, we measured the diameter of the different colonies for the control and tested strains. The percentage of mycelial growth inhibition of each concentration was calculated from the mean colony diameter on medium without plant extract (control) and from the mean colony diameter on each concentration (zone of growth). Percentage of inhibition of mycelial growth was determined by using the following formula: %MGI=100(X-Xi) /X **(Eyana, 2007; Dana *et al*., 2019)** Where:

%MGI: refers to % of mycelial growth inhibition.

X: refers to diameter of control colony.

Xi: refers to diameter of tested colony**.**

The results of the experiments carried out are expressed as means ± standard deviation. Statistical evaluation was performed using Student's "t" test. According to the significance level (p), the differences, compared to the controls, are considered as:

- $p \leq 0.05$: significant.
- $p \leq 0.01$: highly significant.
- $p \leq 0.001$: very highly significant.

RESULTS

Effect of Aqueous Extract of *Eucalyptus sp.*

From table 01, fig. 1, fig.2 and fig.3, it appeared that the concentration of 2.5ml / 100ml of *Eucalyptus sp*. did not have a significant effect on the growth of the three species tested, with diameters of 6,2 ± 0,3cm and 22,5±3,75 % MGI; 6,3 ± 0,1cm and 21,25±1,25% MGI; 6 ± 0,6 cm and 25 ± 7,5 % MGI corresponding to *T.rubrum, T.verrucosum and M.ferrugeneum* respectively. while the concentrations 5ml / 100ml, 7.5ml / 100ml, 10ml / 100ml and 12.5ml / 100ml have a significant effect ($p < 0.05$) corresponding to diameters of 5,5 ± 0,3cm and 31,25 ± 3,75 % MGI; 4,7±0,3cm and 41,25 ±3 ,75% MGI; 3,5±0,2cm and 56,25±2,5% MGI, 2,7±0,3cm and 66,25±3,75% MGI respectively on the growth of *T.rubrum*. Also, the concentrations 15ml / 100ml, 17.5ml / 100ml and 20ml / 100ml have a highly significant effect ($p < 0.01$) corresponding to growth diameters of 2,4±0,1cm and 70 ± 1,25 % , 2,1 ± 0,1cm and 73,75 ± 1,25 %, 1,8 ± 0,1cm and 77,5 ± 1,25% respectively. About *T.verrucosum*, it showed that the concentrations 5ml / 100ml, 7.5ml / 100ml, 10ml / 100ml have a significant effect ($p < 0.05$) corresponding to diameters of 5 ± 0,3cm and 37,5 ± 3,75 % MGI; 3,6 ±0,4cm and 55 ± 5% MGI; 3,25 ± 0,55cm and 59,37 ± 6,87% MGI respectively. As well, the concentrations 12.5ml / 100ml, 15ml / 100ml, 17.5ml / 100ml and 20ml / 100ml have a highly significant effect ($p < 0.01$) corresponding to growth diameters of 2,2 ± 0,2cm and 73,75 ± 1,25% ,1,75 ± 0,15cm and 78,12 ± 1,87 %, 1,3 ± 0,2cm and 83,75 ± 2,5%, 0,75 ± 0,05cm and 90,62 ± 0,62% respectively. For *M.ferrugeneum*, all concentrations between 5ml / 100ml until 20ml / 100ml have a significant effect and the highest effect is observed at the last concentration with 2, 5 ± 0, 28 cm and 68, 75±3, 53%.

Effect of Aqueous Extract of *Nerium oleander*

The obtained results about effect of oleander extract, which are mentioned in table 2, and fig. 4, fig. 5 et fig. 6 proved that the concentration of 2.5ml / 100ml and 5ml/100ml of *Nerium oleander extract* didn't have a significant effect on the growth of the three species tested ($p \geq 0, 05$). However all the remainders concentrations had a significant effect ($p < 0, 05$), where the highest effect was observed at the final concentration with diameter of 3, 8 ± 0,2cm and 52, 5 ± 2, 5% MGI, 3 ± 0,2 and 62,5 ± 2,5% MGI, 3,75 ± 0,15 and 53,12 ± 1,87% corresponding to *T.rubrum, T.verrucosum and M.ferrugeneum* respectively.

Effect of Aqueous Extract of *Allium sativum*

The aqueous extract of *Allium sativum* proved the most anti-dermatophytic effect, the results recorded that the concentration of 2, 5 ml/100ml had a significant effect on the growth of *T.rubrum* ($p < 0, 05$), with diameter of 2, 8 ±1, 02 cm and 60, 71±12, 44% MGI (Table3 and Fig.7). Also, the concentration of 5ml/100ml had a highly significant effect ($p < 0, 01$) compared with control, with diameter of 0, 73 ± 0, 57 cm and 89, 67 ± 8, 28% MGI. However, the concentrations of 7,5ml/100ml and 10ml/100ml had a total inhibition. For *T.verrucosum* and *M.ferrugeneum* it showed that the first three concentrations had a significant effect ($p < 0, 05$) compared with control (Table3 and Fig.8 and Fig.9). Whereas the concentration of 10ml/100ml had a highly significant effect ($p < 0, 01$), with diameters of 1 ±0, 4 cm and 85, 71±5, 82% MGI, 1, 45 ±0, 382 cm and 79, 85±5, 96% MGI corresponding to *T.verrucosum* and *M.ferrugeneum* respectively.

Table 1 Effect of crude aqueous extract of *Eucalyptus sp*. on the growth of species tested (expressed in average fungal growth diameters (D) +/- standard deviation).

[E] / Species	Control	2,5	5	7,5	10	12,5	15	17,5	20
T .rubrum	**8±0**	**6,2±0,3**	**5,5±0,3**	**4,7±0,3**	**3,5±0,2**	**2,7±0,3**	**2,4±0,1**	**2,1±0,1**	**1,8±0,1**
T .verrucosum	**8±0**	**6,3±0,1**	**5±0,3**	**3,6±0,4**	**3,25±0,55**	**2,2±0,2**	**1,75±0,15**	**1,3±0,2**	**0,75±0,05**
M.ferrugeneum	**8±0**	**6±0,6**	**5,7±0,3**	**5,1±0,3**	**3,7±0,2**	**3,2±0,2**	**2,8±0,4**	**2,8±0,2**	**2,5±0,28**

[E]: concentration extract (ml/100ml)

Table 2 Effect of crude aqueous extract of *Nerium oleander* on the growth of species tested (expressed in average fungal growth diameters (D) +/- standard deviation).

[E] Species	00	2,5	5	7,5	10	12,5	15	17,5	20
T .rubrum	**8±0**	**6,7±0,3**	**6±0,2**	**5,5±0,3**	**5±0,4**	**4,8±0,3**	**4,5±0,2**	**4±0,2**	**3,8±0,2**
T .verrucosum	**8±0**	**8±0,2**	**8±0,1**	**7,25±0,25**	**6,75±0,25**	**6,5±0,2**	**5,25±0,25**	**5±0,2**	**3±0,2**
M.ferrugeneum	**8±0**	**6,5±0,5**	**6±0,4**	**6±0,3**	**5,5±0,2**	**4,8±0,2**	**4,25±0,25**	**4±0,3**	**3,75±0,15**

[E]: concentration extract (ml/100ml)

Table 3 Effect of crude aqueous extract of *Allium sativum* on the growth of species tested (expressed in average fungal growth diameters (D) +/- standard deviation).

[E] Species	00	2,5	5	7,5	10
T .rubrum	**7±0,5**	**2,8 ±1,02**	**0,73± 0,57**	**00±00**	**00±00**
T .verrucosum	**7±00**	**2,8±0,78**	**2,53±0,85**	**2±0,4**	**1±0,4**
M.ferrugeneum	**7,25±0,25**	**4,51±0,41**	**3,56±0,46**	**2,85±1,18**	**1,45±0,38**

[E]: concentration extract (ml/100ml)

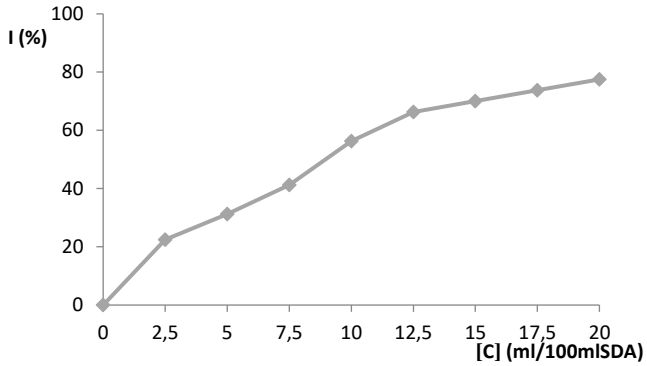

Figure 1 Effect of aqueous extract of *Eucalyptus sp. on* the growth of *T.rubrum.*

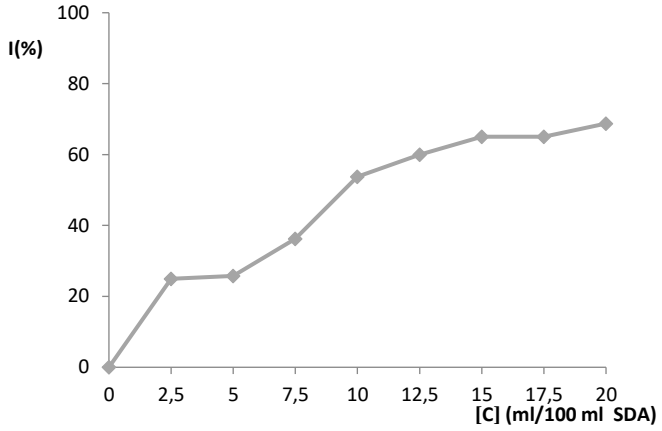

Figure 3 Effect of aqueous extract of *Eucalyptus sp.* on the growth of *M. ferrugeneum.*

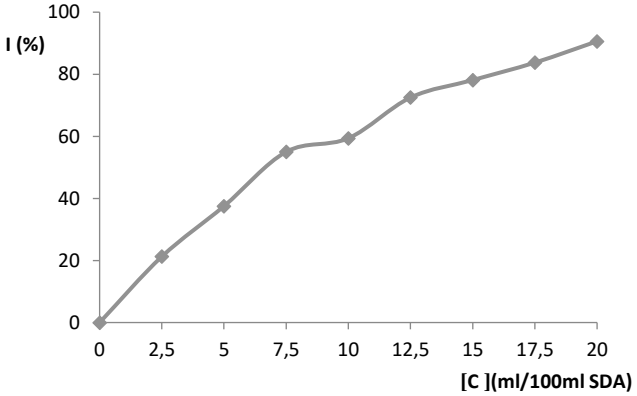

Figure 2 Effect of aqueous extract of *Eucalyptus sp. on* the growth of *T.verrucosum.*

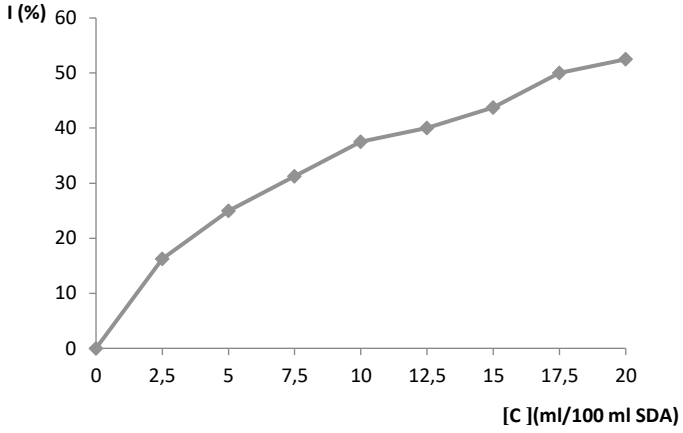

Figure 4 Effect of aqueous extract of *Nerium oleander* on the growth of *T.rubrum.*

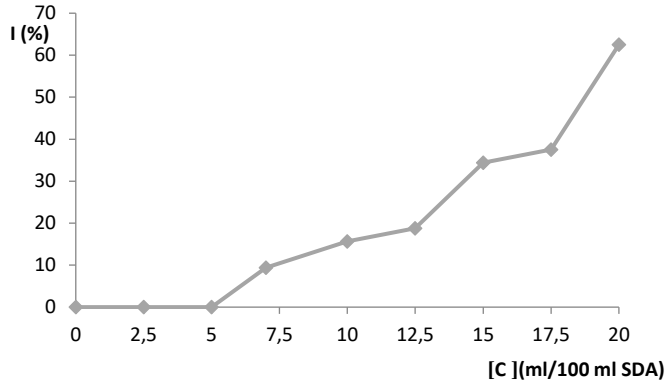

Figure 5 Effect of aqueous extract of *Nerium oleander* on the growth of *T.verrucosum.*

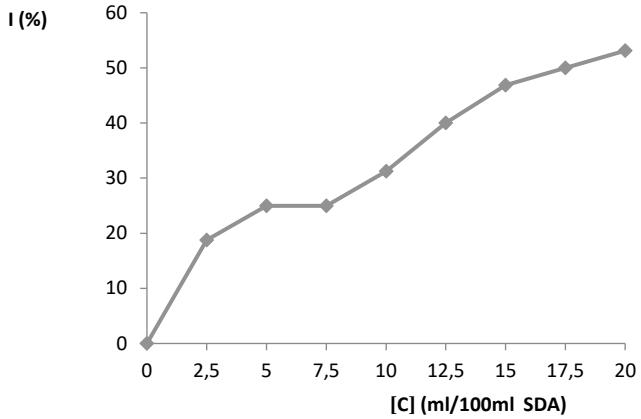

Figure 6 Effect of aqueous extract of *Nerium oleander* on the growth of *M.ferrugeneum.*

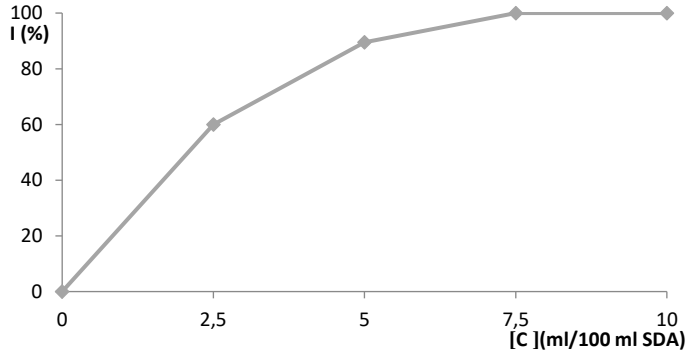

Figure 7 Effect of aqueous extract of *Allium sativum* on the growth of *T.rubrum.*

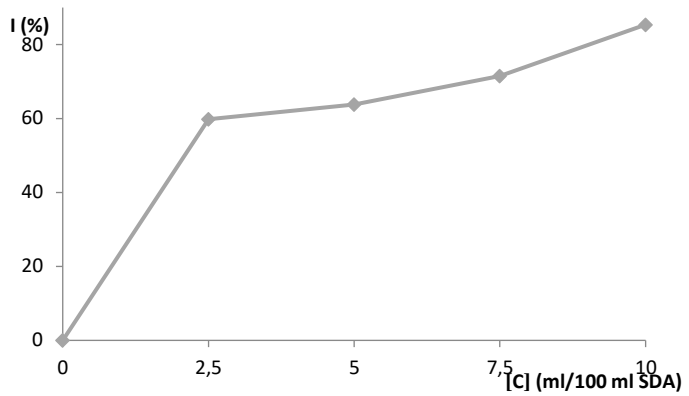

Figure 8 Effect of aqueous extract of *Allium sativum* on the growth of *T.verrucosum.*

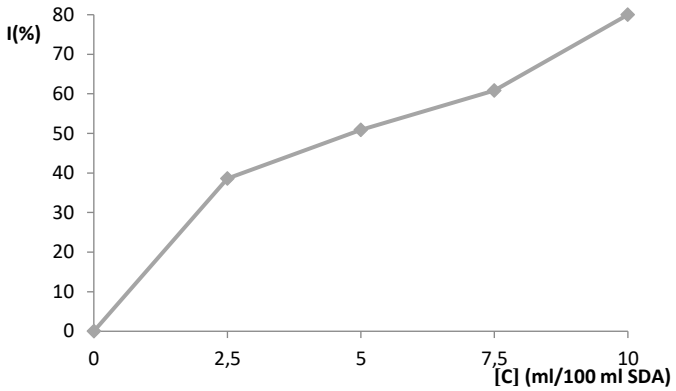

Figure 9 Effect of aqueous extract of *Allium sativum* on the growth of *M.ferrugeneum.*

DISCUSSION

The appearance of antifungal resistance stress of various fungi, drugs such as azoles and griseofulvin are synthetic antifungals, although effective, but the using of these drugs resulting in incidence of resistance to all agents has been reported (**Farzad *et al.*, 2012**). The advantage of using these natural compounds can be reduce the side effects and the cost of treatment, there had increased the interest of the use of medicinal plants to treat skin diseases (**Ali Reza *et al.*, 2013**). Antifungal treatment with medicinal plants is not only used in human pathology, but also for fungi infected animals and plants (**Bokhari, 2009**). Several medicinal plants used to evaluate anti-dermatophytic activity, among which we find: *Allium sativum*, *Eucalyptus sp.* and *Nerium oleander* (**Javed *et al.*, 2015**). In the following, we will discuss the results of the evaluation of the antifungal activity of the three plants mentioned below, on the growth of three dermatophytic species isolated from patients reached with dermatophytosis. The percentage inhibition increases and the diameter of the mycelial growth decreases with increasing concentration of the extract in the medium, this method is already proven by other authors (**Karunyal *et al.*,2001; Masoomeh *et al.*, 2004; Tsopmbeng *et al.*, 2014**). Several studies that were made to evaluate the antifungal activity of garlic extract against molds growth or yeasts, include the work of **Yoshida *et al.*, (1987)** who have proved that garlic extract contains bioactive components (ajoene and allicin), these components inhibit spore germination of some fungi: *Alternaria alternata*, *Colletotrichum spp.*, *Curvularia spp.*, *Candida albicans* and dermatophytes: *T.mentagrophytes, M.canis, M.gypseum* and *E. floccosum* (**Abdel-Raouf, 2001**). Also a study carried out by **Narula and Sareen (2011)** found that *Allium sativum* extract had the best antifungal activity among 14 different plants tested. The inhibitory action of garlic juice is due to the presence of volatile sulfur components; Allicin, which is an inhibitor of the -SH moiety of fungal enzymes, can then bind the cell wall (**Yoshida *et al.*, 1987**). In the context of evaluating the inhibitory effect of *Allium sativum* extract on the growth of dermatophytes, several

studies have been carried out (**Nickel, 1969; Karunyal *et al*., 2001, Pyun and Shin, 2006; Narula and Sareen, 2011**). In our study, the results obtained proved that the total aqueous extract (juice) of garlic has a very important antifungal activity on the growth of the three species tested, particularly against T.rubrum. In order to measure the inhibitory effect of the extract, the results showed that there was a reduction in fungal growth as soon as the first concentration was added for all species, and the percentage of antifungal activity was proportionately increased with the concentration of the extract, so we found that no growth was observed for *T.rubrum* at the concentration of 10ml / 100ml SDA, these results are similar to those obtained by **Karunyal *et al*.(2000)** who found that no mycelial growth was observed by adding 10 g of fresh macerated bulb in 100 ml of SDA medium. Also, there is a study conducted by **Souza *et al.* (1995),** so they examined total aqueous extracts of 38 medicinal plants including *Allium sativum* on the growth of pathogenic microorganisms include *T.rubrum* and *M. ferrugeneum*, and they found that these two species are susceptible to extracts from other plants better than garlic, for the species *T.verrucosum*, beyond there is no research done to evaluate the effect of the extract garlic on its growth.

About the evaluation of eucalyptus antifungal activity, several studies are made at the antecedent. **Abdel-Raouf (2001)** studied the antifungal activity of the aqueous extract of Eucalyptus rostrata on the growth of *Alternaria solani* and *Saprolegniasis parasitica*. **Javad and Tteheh (2010)** evaluated the eucalyptol antifungal activity obtained from the essential oils of *E.largiflorens* and *E.intertexta* leaves on the growth of *candida albicans*, *Aspergillus niger* and 9 bacterial strains. Many studies have indicated the antifungal convenience of aqueous extracts and essential oils against dermatophytes and other molds as well as *candida albicans* **(Sartorelli et *al*., 2007**). The antifungal activity of eucalyptus on the growth of dermatophytes has been proven by **Jack et *al.* (2015)** who demonstrated the inhibitory effect of *E.globulus* on the growth of *T.mentagrophytes*. Thus, the work carried out by **Ameur *et al.* (2012)** has proved the antifungal, antibacterial and antiviral activity of different species of eucalyptus, and their essential oils were examined against five fungal strains that include yeast (*C.albicans*) and molds (*Scopulariopsis brevicaulis)* and three dermatophytic species (*T. rubrum, T. sudanense and M. canis*). In our study, we also proved that the aqueous extract of eucalyptus leaves has a significant antifungal activity on the growth of the three species tested; the inhibitory effect of mycelial growth was proportionally increased with the volume of the extract added. We found that all the species examined were sensitive to the aqueous extract of eucalyptus and the highest inhibition rate was observed on the growth of *T.verrucosum*. The difference between our results and the results obtained by other researchers (**Ameur *et al*., 2012; Jack *et al*., 2015**) may explained by the type of extract, the evaluation method as well as the species of eucalyptus studied and the dermatophytic species tested. However, each species has bioactive molecules, and the percentage of these molecules differs from one species to another.

Few researches are available on the antifungal properties of *N.oleander* which belongs to Apocynaceae family (**Savita *et al*., 2013**). In the context of the evaluation of the anti-dermatophytic activity of *N.oleander*, our study has proved this effectiveness. Our results show that the aqueous extract of *N.oleander* has a considerable effect on the growth of the studied species. These results are similar to the results of **Tawfik *et al*. (2009)** in Iraq, which they indicated the inhibitory effect of the crude extract of *N.oleander* on the growth of dermatophytes (*T. rubrum, M.canis, M.gypseum, E. floccosum, T. mentagrophytes var erinacei, T. verrucosum and T. mentagrophytes var interdigitale*) and other molds such as: *Aspergillus, Alternaria and Geotrichum*. Our results also are resemble to the results of **Bokhari (2009**), who tested the effect of the aqueous extract of *N.oleander* on the growth of the following species (*T.rubrum, T.mentagrophytes, T.verrucosum, M.gypseum and M. canis),* and he found that the inhibition rate of *T.rubrum and T.verrucosum* is 33.3%, 50% respectively. Thus, **Siddiqui *et al*. (2016)** have been shown to have significant effect on the growth of *Fusarium oxysporum, Sclerotium rolfsii and Macrophomina phaseolina* as phytopathogenic agents. In our study, we can say that the anti-dermatophytic activity of the aqueous extract of *N.oleander* due to the presence of phenolic components (tannins and flavonoids) that have been detected, where several studies have highlighted that the inhibitory effect of the aqueous extract of *N. oleander* results in the existence of bioactive molecules in: flavonoids, alkaloids, saponins, tannins and other molecules (**Bhuvaneshwari *et al*., 2007).** Also, **Rajendran (2011)** proved the presence of antifungal agents (myricetin and rutin) in the dry leaves of *N. oleander*.

CONCLUSION

The results obtained demonstrated that the aqueous extracts of the three plants studied: *Allium sativum, Eucalyptus sp.* and *Nerium oleander*, having the capacity of inhibition on the growth of three dermatophytic species tested: *T.rubrum, T.verrucosum, and M. ferrugeneum*. The effect of the aqueous extract of these plants is observed on the diameter of colony mycelial for each species. We have discerned that the growth of these species is influenced by the concentrations used. The inhibitory effect varies from one plant to another and from one species to another for the same concentration of the same plant. From our results, we found that the aqueous extract of *A. sativum* is the most effective for the inhibition of the species tested; however, it gave a total inhibition for *T.rubrum*, followed by extract of *E.sp*., while the aqueous extract of *N.oleander* had a low inhibitory effect against the three species tested.

Acknowledgments: The authors would like to thank the anonymous referees for helpful suggestions and comments. The work was supported by the Algerian Ministry of Higher Education and Scientific Research. The authors declare that they have no conflicts of interest.

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