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Antifungal Activity of the Extracts of Two Medicinal Plants from South Western Algeria: *Ephedra alata* and *Bubonium graveolens*

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ABSTRACT

Background: Medicinal plants constitute an invaluable source of natural substances which can be used in the formulation of new antifungal agents. Among these medicinal plants, the genera *Ephedra alata* and *Bubonium graveolens* are used in traditional medicine. In the search for molecules bioactives, resource exploration natural, especially plants medicinal, appears as a track promising because they constitute, by their biodiversity, a great reserve of active substances. This study aimed to evaluation antifungal activity of medicinal plant extracts *Bubonium graveolens* and *Ephedra alata* against two fungi namely, *Penicillium sp* and *Aspergillus niger*.

Methods: During our work, we prepared the various extracts (aqueous, methanolic and ethyl acetate) for the plants studied, the search for the active compounds of these two species by the extraction method was essentially based. The antifungal tests were investigated by using agar medium methos The tests is estimated by determining the diameters of zone inhibition and growth inhibition rate PI%.

Result: The results obtained show that the aqueous extracts of *Ephedra alata* and *Bubonium graveolens* are inactive against two fungi in our study. However, methanol and ethyl acetate extracts showed very good activity antifungal against the fungal species tested. The evaluation of the antifungal activity of the methanolic and ethyl acetate extracts had a higher activity (99.70±0.240) against all the molds tested compared to the aqueous extract (63.52±0.959). The extracts of *Ephedra alata* and *Bubonium graveolens* significantly inhibited the growth of the two fungi tested. These three extracts showed a good antifungal effect at the laboratory scale. Ours finding demonstrate that *Bubonium graveolens* and *Ephedra alata* extracts possesses antifungal activity that might be a natural potential source antifungal compounds used in pharmaceuticals products, cosmetics and food.

Key words: Antifungal, *Aspergillus niger, Bubonium graveolens, Ephedra alata*, Medicinal plant, *Penicillium sp.*

INTRODUCTION

Medicinal plants and plant derived medicine are widely used in traditional cultures all over the word and they are becoming increasingly popular in modern society as naturel alternatives or supplements to synthetic chemicals (Van *et al*., 2018) Nowadays, a large number of medicinal plants have very important biological properties that find many applications in various fields, The use of plants for medical purposes is experiencing growing success; namely in medicine, pharmacy, cosmetology and agriculture (Badiaga, 2011; Vaou *et al*., 2021; Bendifallah, 2023). The use of chemicals is currently the most widely used technique for controlling harmful molds, due to their efficacy, as well as their easy and convenient applications. However, their use remains a very delicate task because of their very high cost and their harmful side effects on consumers. It is estimated that over 30% of the world's population has ever had a fungal infection (Achimón *et al*., 2022), These various difficulties aroused our interest in the search for other fungitoxic substances of natural origin which could constitute an alternative solution to chemicals. (Balakumar *et al*., 2014; Liu *et al*., 2014).

South Algeria with its rich floral resources and ethnobotanical history is an ideal place of new pharmacological compounds (Berreghioua and Cheriti, 2018). The use of plants by local populations in the treatment of infectious diseases, such as those caused by fungi have been recorded by several studies (Violante *et al*., 2012). The therapeutic value of these plants is due to their secondary metabolites, especially phenolic compounds. Phenolic compounds (mainly flavonoids, phenolic acids and tannins) constitute a wealth widely exploited by the agro-food, cosmetics and pharmaceutical industries. The extraction of active principles from these metabolites is a very important step in their isolation, as well as in their identification (Pacôme *et al*., 2018). The exploration of natural products remains very interesting given the great diversity of antifungal compounds

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identified in certain plant extracts (Angaman *et al*., 2018). Various fungi including *Aspergillus niger* and *Penicillium sp* are involved in these infections.

The genus *Asteriscus* (family Asteraceae) includes eight species. *Asteriscus graveolens* (Forssk.) Less. (syn. *Bubonium graveolens*, *Odontospermum graveolens* or *Nauplius graveolens*), is an endemic aromatic plant mainly distributed in southwestern Algeria. in Sahara folk medicine, this species is used as stomachic, treating fever, gastrointestinal tracts, cephalic pains, bronchitis, and as anti-inflammatory (Said *et al*., 2017; Znini *et al*., 2012) and also previous studies revealed that the extracts from this species have an antifungal effect against *Fusarium oxysporum* (Boulenouar *et al*., 2012), antioxidant (Haddouchi *et al.,* 2016), antimicrobial activity (Ramdane *et al*., 2017).

Ephedra alata belongs to the family *Ephedraceae* which is one of the most widely distributed range plants in Algeria. *Ephedra* is a genus of gymnosperm belonging to the family of *Ephedraceae* (D'Auria *et al*., 2012) *Ephedra* is a medicinal plant that has been used in traditional medicine to treat a variety of ailments, is one of the oldest range and medicinal herbs in the world. The decoction of *Ephedra alata* aerial parts has been reported in folk medicine to relieve nasal decongestion, in the treatment of asthma, digestive system disorders and as a general respiratory decongestant and antifungal properties (Alqarawi *et al*., 2014). The hot broth of the dried green stems is used in traditional medicine, as hot tea, after abortion and to treat bacterial and fungal infections a variety of specific ingredients have been found in *Ephedra* herb, mainly including alkaloids, flavonoids, tannins, polysaccharides, organic acids, volatile oils, and many other active compounds (Shuang-Man *et al*., 2020). As part of the research by our laboratory on the biological activity and phytochemistry of medicinal plants from south western Algeria, it seemed important to us to explore the nature of natural antifungal substances in traditional Saharan medicine. The use of bioactive natural compounds offers a first-choice alternative for plants with antifungal activity in the control of pathogens of several products.

The effect of plant extracts on fungi, for this our study of medicinal plants and Saharan endemic species, by the use of different extraction techniques were carried out on the aerial part of these plants tested against fungi.

The present study was aimed at evaluating the crude extracts of *Ephedra alata* and *Bubonium graveolens* with different solvent for antifungal potential. The antifungal activity was determined on two fungi namely, *Penicillium sp* and *Aspergillus niger.*

MATERIALS AND METHODS

Description of the study area

The current exploratory research was conducted in the South Western Algeria Bechar. Bechar district (31°37′ N latitude, 2°13′ W longitude), is located in the south west of Algeria (Fig 1) covering an area of 161.400 km². The arid climate spreads to the region go through long periods of heat from May to mars, where the temperature reaches sometimes 40°C to 45°C maximum and very low annual rainfall rate (16.9 mm). The flora of Algerian is estimated at more 3000 species belonging to several botanical families. The arid regions constitute an important reservoir of medicinal plants used by people who know the Sahara not only identify the plant but also its cycle of development, its habitat and its use (Sekkoum *et al*., 2011).

Plant material

The plants were collected during the month of February 2022 in the city of Bechar, exactly about 15 to 30 km north of town (near the Lahmer region). The plant parts were dried in the open air, protected from light and moisture for three weeks and then stored until use.

Fungal material

The fungal (*Penicillium sp*, *Aspergillus niger)* used to evaluate the antifungal properties of plants. They belong to the fungus collection of Biology Laboratory of Tahri Mohamed Bechar University. They are cultivated on nutritive medium PDA (potato dextrose agar) during seven days in the darkness and at room temperature 25°C. To prepare spore suspension, the fungi grown spores suspended in 0.85% (w/v) sodium chloride to prepare the homogeneous spore suspension (Danmek *et al*., 2014; Gad *et al*., 2022).

Extraction method

In a 1000 ml flask surmounted by a reflux condenser, 150 g of the plant are placed in the presence of 300 ml of different solvents (Methanol, Ethyl acetate and Distilled water). Extraction under reflux for 2 hours, then filter the evaporated solutions to dryness using a rotavapor.

Antifungal activity

Different crude extracts (Methanolic, ethyl acetate and aqueous) of the plants were dissolved in dimethyl sulfoxide

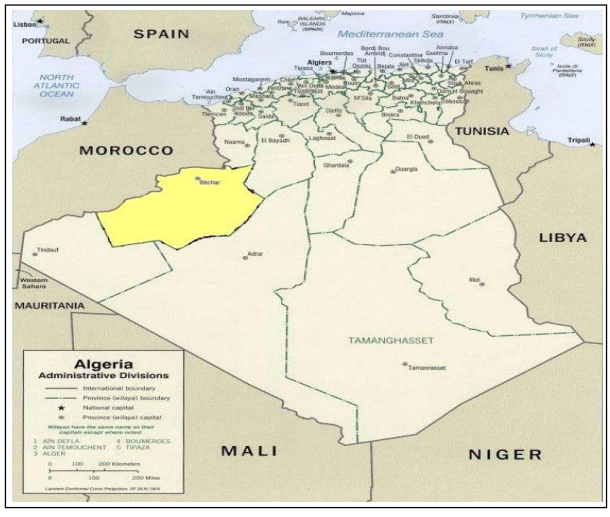

Fig 1. Geographical location of the South Western Algeria (https://www.atlas-monde.net/afrique/algerie).

(DMSO) with the concentration of 1 mg/ml being evaluated for biological potential (Jacinto *et al*., 2011) .

The antifungal activity was determined by Agar tube dilution Method. Two strains of fungus were used such as *Aspergillus niger* and *Penicillium sp* and sample was prepared by dissolving 1 mg/ml in DMSO while 25 ml of media was poured in each Petri plate. Media were allowed to solidify and spores of fungus were applied in the center of the plate (Nisar *et al*., 2010). The crude extract was dissolved in DMSO (1 mg / ml). Sterile Sabouraud's dextrose agar medium (5 ml) was placed in a test tube.

Reading the results

The results were read after seven days of incubation at 25°C by the measurement of the diameter of the zone of fungi growth. In parallel, we determined the diameter of the fungal strains in the absence of the extract plant. The antifungal effect is determined by measuring the percentage diametral growth inhibition using the following formula:

$$\% \text{ Inhibition of growth PI} = \frac{D_T - D_C}{D_T} \times 100$$

DC: Diameter growth and test (mm).
DT: Diameter growth in control (mm).

Statistical analysis

All experiments were spotted three times. Ms Excel 2007 was used to express the values as the mean±deviation.

RESULTS AND DISCUSSION

The following results represent the effect of extracts from the *Ephedra alata* and *Bubonium graveolens* plants on the two fungal strains *Aspergillus niger* and *Penicillium sp*. These results, it is necessary to carry out antifungal tests by the direct contact method in solid medium from the extracts (aqueous, methanolic and ethyl acetate) for the two plants, to examine on two fungal strains generally (*Aspergillus niger*, *Penicillium sp*) (Table 1, 2).

The results obtained showed that this extraits had significant antifungal activity against all the molds tested. The inhibition of *penicillium sp* growth was observed at a concentration of 25 mg/ml and 50 mg/ml for methanol and ethyl acetate extracts of *Bubonium graveolens* 99.7% and 90.29% for extrait ethyl acetate of *Ephedra alata* (Table 3).

The minimum inhibitory and fungicidal concentrations of *Ephedra alata* were in the order of 25 and 50 mg/ml, respectively 40.53% and 41.21% of methanol and ethyl acetate extracts. Indeed, this inhibitory activity is more marked on Aspergillus niger by inhibition its growth from C=25 mg/ml while the methanolic extract for *Bubonium graveolens*. While *Bubonium graveolens* was the most resistant strain with a C= 25 mg/ml and C=50 mg/ml (Table 4). However, *Penicillium sp* was shown to be the most sensitive, it was completely inhibited from the 25 mg/ml concentration of *Bubonium graveolens* of methanol and ethyl acetate extracts. Thus, the concentration of 25 mg/ml of *Ephedra alata* was sufficient to stop the growth of *penicillium sp*. Whereas, the strain that showed some remarkable resistance which resisted up to extraits concentration 25 mg/ml (Table 4).

The results obtained with the different concentrations of *Ephedra alata* show that the inhibitory activity is important of different species tested. The methanol extract of *Bubonium graveolens* is very active against *Penicillium sp* and *Aspergillus niger* with a 100% inhibition rate at the concentration of 25 mg/ml. At the same concentration, inhibition is less significant the aqueous extract. However, these fungal agents are completely inhibited under the action other medicinal plants.

This can be explained by the effect of secondary metabolites contained in the plant tested.

Therefore, the activity of a natural plant is in direct relationship and in correlation with its chemical composition. Several studies have shown the antifungal activity of this extract by studying the antifungal activity of *Bubonium graveolens*. The study of the antifungal activity of the two

Table 1: Inhibition rate (%) of extracts of *Ephedra alata and Bubonium graveolens* against *Aspergillus niger.*

Plants concentration	Inhibition of *Aspergillus niger* growth (%)					
	Ephedra alata			*Bubonium graveolens*		
	25	50	100	25	50	100
Methanol	39.18	29.72	36.48	66.21	59.45	45.95
Ethyl acetate	18.91	5.40	41.89	89.18	60.81	17.56
Aqueous	39.18	29.72	36.48	0	0	18.91

Table 2: Inhibition rate (%) of extracts of *Ephedra alata* and *Bubonium graveolens* against *Penicillium.sp.*

Plants concentration	Inhibition of Penicillium sp growth (%)					
	Ephedra alata			*Bubonium graveolens*		
	25	50	100	25	50	100
Methanol	84.70	89.23	88.23	100	96.47	92.94
Ethyl acetate	87.05	90.58	88.23	100	100	91.76
Aqueous	88.88	85.88	64.7	89.41	87.05	76.47

Table 3: Inhibition rate (%) of the various concentration of the extracts of *Ephedra alata* and *Bubonium graveolens* against *penicillium sp* on the tested molds.

Plants concentration	Inhibition of *penicillium sp* growth (%)					
	Ephedra alata			*Bubonium graveolens*		
	25	50	100	25	50	100
Methanol	84.99 ±0.24	87.64±0.481	87.05±0.950	99.70±0.240	96.76±0.236	92.64±0.240
Ethyl acetate	86.46±0.477	90.29±0.236	86.46±1.441	99.70±0.240	99.41±0.481	91.17±0.481
Aqueous	87.05±0.959	86.46±0.477	63.52±0.959	89.11±0.240	87.64±0.481	75.88±0.481

Table 4: Inhibition rate (%) of the various concentration of the extracts of *Ephedra alata* and *Bubonium graveolens* against *Aspergillus niger* on the tested molds.

Plants concentration	Inhibition of *Aspergillus niger* growth (%)					
	Ephedra alata			*Bubonium graveolens*		
	25	50	100	25	50	100
Methanol	40.53±1.106	31.07±1.106	36.82±0.277	66.88±0.551	59.79±0.277	44.59±1.106
Ethyl acetate	19.59±0.555	4.08±1.077	41.21±0.551	90.53±1.106	60.56±0.204	18.23±0.551
Aqueous	39.86±0.555	29.65±0.053	37.15±0.551	0	1.35 ±1.102	20.26±1.1063

sesquiterpenes of *Asteriscus graveolens subsp odorus* showed better efficacy on the three fungi tested. The results obtained that the essential oil of *Asteriscus graveolens* sub sp. *odorus* may be a new potential source of natural antifungal activity against *Penicillium digitatum*, *Penicillium expansum* (Alilou *et al*., 2014).

Ephedra has long been considered an important source of the alkaloid ephedrine, a vasoconstrictor drug once widely used in the treatment of asthma and as a general respiratory decongestant. 60 Many alkaloids, lignans and phenolic compounds have been isolated from *Ephedra alata* (Mandaville, 2013). The *Ephedra* genus is known for its alkaloids, hence, there are fewer studies about other phytocomponents of its species members, *Ephedra* contains flavonoids (leucodelphinidin, leucopelargonine, leucoanthocyanidin, lucenine, vicenin-1 and vicenin-2), tannins, benzylmethylamine. Tannins, mainly proanthocyanidines, are constituents of many *Ephedra* species (Ibragic and Sofić, 2015 ; Guasch *et al*., 2012).

Biological control through the use of natural alternatives gave a lot of interest in this moment. Many Researchers noted that the possibility of using the extract from plants as an effective natural alternative. A study performed on the essential oils of three plants which Laurus nobilis one of them for 17 fungal species that are: *Aspergillus niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, Alternaria alternata, Aureobasidium pullulans, Penicillium ochrochloron.* The results obtained showed that this plant that is rich in 1,8-Cineole has moderate power over the mushrooms tested (Goudjil *et al*., 2016).

The work by Ebi and Kamalu (2001) showed the activity antifungal extracts dichloromethane, methanolic, ethanolic from *Mitracarpus scaber* (Rubiaceae), on *Aspergillus flavus* and *C. albicans*. They indicated the presence of alkaloids and saponins in these extracts and attributed the activity extracts with saponins which would interact with sterols, proteins and phospholipids fungal cell membranes. Therefore, more in-depth studies can be undertaken for the development of natural products based on extracts to exploit its antifungal properties in the prevention, and treatment of certain fungal infections, and to combat molds resistant to conventional antifungals. The fungal resistance is a major problem in plant protection. There remain few effective antifungal agents against some resistant products. Scientists are looking for new products of natural origin as secondary metabolites of medicinal plants and having an antifungal activity (Hajji *et al*., 2016).

Our results suggest that extracts from this plants *Bubonium graveolens* and *Ephedra alata* could be used as an alternative source of antifungal agents against fungal infections. Further, more in-depth research is more than necessary to discover the true bioactive molecules responsible for antifungal activities.

CONCLUSION

Medicinal plants represent an important source of bioactive natural substances and compounds. The Crude extracts of the specie *Bubonium graveolens* and *Ephedra alinda* showed antifungal activity against investigated by disk diffusion method. The evaluation of the antifungal activity of the methanolic and ethyl acetate extracts have a higher activity against all the molds tested compared to the aqueous extract.

The phytochemical and pharmacological results encourage the application of this medicinal specie for further evaluations of other possible bioactivities and detection of active pure compounds as constituents of drugs.It is necessary to develop the phytochemical and pharmacological aspects of local medicinal plants as a potential source of new active compounds.

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