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Antifungal activities of *seeds* oils *Acacia arabica* and *raddiana* from the Hoggar region (southern Algeria)

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ABSTRACT

This work aims to study the antifungal activity of crude oils seeds of *Acacia arabica* and *Acacia raddiana* arid region Hoggar (southern Algeria). At various concentrations, these oils were tested *in vitro* on lots ATCC *Candida albicans* and *Aspergillus niger* by two different techniques the broth dilution and diffusion in solid medium (method well). A multifactorial statistical analysis of data was made of variance ($P \leq 0.05$). Biological tests also show a strong antifungal activity beside *Aspergillus niger* and *Candida albicans* strains. The minimum inhibitory concentrations were recorded in the range $0.079 \leq MIC \leq 0.158$ mg/ml respectively for the two oils of the species *raddiana* and *arabica*. This bioactivity is probably due to antioxidant tocopherols and the other bioactive substances to be identified and characterized by methods of mass spectrometry and NMR. However, an effect of stimulating the development of strain *A. niger* was found in the presence of greater than 0.158 mg/ml concentrations for oils. The stimulating effect of oils can be used in various food and pharmaceutical fields.

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INTRODUCTION

Interest in vegetable oils forestries dryland species in relation to their various properties plays an important role in rural development. Scientific research in the food, pharmaceutical, cosmetics and other industries have been developed for the extraction, identification and quantification of bioactive compounds of some thorny acacias [30, 4, 13, 3, 10, 14, 28]. Vegetable oils come in many cosmetic and therapeutic preparations, they are good thinners certain vitamins (Vit E) and essential oils, which attribute to their different degrees of remarkable properties, which can be used as natural antioxidant agents or antimicrobial in food industries. Both species agroforestry arid Algerian *Acacia arabica* and *Acacia raddiana* are operated in a much more traditional way. The oils from these plants are known for their large biodiverse and their antimicrobial properties and in most cases this bioactivity is due to these active constituents such as polyphenols, monoterpenes, sesquiterpenes, alcohols, sterols and tocopherols. The antibacterial properties of oils and these bioactive compounds are in part related to their hydrophilic and lipophilic character [7, 5, 15, 18]. Indeed, several authors have reported that the accumulation of oils in the level of bacterial and fungal walls and disturb the operation membrane permeability causing the cell wall degradation and damage to the proteins [16, 29].

However, the two acacia species are widely used in traditional African medicine, in particular in southern Algeria by nomadic dens trees. Indeed, in this sense some works have been published by several research teams and worked on organic and aqueous extracts of leaves, bark, roots, bark and pods [1, 8]. They confirmed that these extracts have a significant antimicrobial effect especially against *C. albicans* strain. However, to our knowledge, no work exists on the antifungal effect of oil seeds of *A. arabica* and *A. raddiana* against *Candida albicans* and *Aspergillus niger*. However, this study reports an evaluation of the antifungal activity of the oils by the determination of minimum inhibitory and fungicidal concentrations (MIC and MFC).

MATERIALS AND METHODS

Plant materials:

The seeds of *Acacia arabica* and *raddiana* were harvested in the Hoggar region (Tegnouenen, Tessenaouene and Anfeeg) Tamanrasset wilaya in June 2010-2011. The two species have been identified by the services of the National Institute of Forestry Research and conservation of forests Tamanrasset (INRFT and CFT).

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Fungal material:

Two fungal strains lot ATCC (*Candida albicans* and *Aspergillus niger*) from laboratories microbiologies the Pasteur Institute in Algiers and the University of Tiaret.

Chemical extractions oils:

The seeds were washed dried and sorted by a mechanical grinding mill Retch type, in order to reduce the area of contact with the extraction solvent. The seed oils of powders of different species have been extracted using a solvent hexane by soxhlet [2].

Determination of the minimum inhibitory concentration MFC:

MIC and MFC were determined by the dilution technique followed by plating on solid medium using the technique liquid medium described by Fernanda et *al*., (2007) [11]. The medium used are dextrose broth potato (BDP) and agar potato dextrose (PDA). A series of tubes containing 5 ml broth (DP) of the volumes of oil added at various concentrations (0.079 to 0.316 g/ml) and 0.1 ml of spore suspension 10^7ufc/ml were inoculated into tube each. The tubes at 28 °C were incubated for 48 hours for *C. albicans* and for 7 days *A. niger* [11, 23]. The assessment of the growth will be the naked eye compared with the series of tubes without spore suspension as a witness. Five replicates were performed for each test.

The MIC is the lowest concentration that inhibits visible growth all naked eye after 48 h for *C. albicans* and 7 days for *A. niger*. The CMF was determined by plating 0.1 ml of each concentration greater than or equal to the MIC on solid medium DPA. The CMF also the smallest concentration of CMI that inhibits the growth of germs. According to the report CMF/CMI fungicidal or fungistatic effect was determined.

Determination of the diameters of the zones of inhibition:

The disc diffusion method was used to determine the diameters of inhibition zones oils. Paper discs (6 mm diameter) were impregnated with 10 µl oil at different concentrations (from 0.079 to 0.316 mg/ml; 0.088 to 0.352 mg / ml; 0.105 to 0.420 mg / ml) dissolved in DMSO (dimethyl sulfoxide). These discs are placed on a PDA medium in Petri dishes seeded with 0.1 ml of fungal suspension adjusted to 10^7 cfu/ml. The negative control was prepared using a solvent of DMSO impregnated disk. After incubation at 28 ± 2 ° C for 48 hours (*C. albicans*) and 7 days (*A. niger*) the diameters of zones of inhibition were measured in millimeters [11, 23]. The tests were performed five times. At the end of the percentage of inhibition (I %) was calculated using the following formula: I% = [dc-de/dc]×100

% I: Percentage of inhibition rate;

dc: average diameter of the control cultures;

de: the average diameter of cultures in the presence of oil.

Statistical Analysis:

A multifactorial analysis of variance ANOVA data was conducted using the Newman-Keuls test ($P \leq 0.05$).

Results:

Antifungal activity of the oils:

Multivariate data analysis of antifungal tests by both technique diffusion on liquid and solid medium revealed that there is significant variance between the two oils acacia beside the fungal strain (table 1 and 2).

Table 1: MIC of the antifungal activity of Acacia oils in liquid medium.

Fungal strains	*Oil of Acacia arabica*[b]		*Oil of Acacia raddiana*[a]	
	CMI mg/ml	CMF mg/ml	CMI mg/ml	CMF mg/ml
Candida albicans	0.105 [c]	0.105 [c]	0.088 [b]	0.088 [b]
Aspergillus niger	0.079[a]	0.158 [d]	0.079 [a]	0.158 [d]
MCI : Minimum Concentration inhibitrice ; MCF : Minimum Concentration fongicide; [a, b, c] homogen groups according to Newman-Keuls test at $P < 0.05$				

Table 2: Antifungal activity of oils from two acacia species by the disc method.

Fungal strains / *Oils*	*Oil of Acacia arabica*[b]			*Oil of Acacia raddiana*[a]		
C. albicans [b] ATCC10231	MCI=CMF=0.105 mg/ml[b]	DZI	36.10 [b']	MCI = CMF = 0.088 mg/ml[c]	DZI	37.50[b]
		% TI	55.03[b']		% TI	57.31
A.niger[a] ATCC16404	MCI= 0.079 mg/ml[a] ; CMF= 0.158 mg/ml[c]	DZI	59.5 [a']	MCI=0.079 mg/ml[b] ; CMF=0.158 mg/ml[d]	DZI	68.4[d]
		% TI	66.66[a']		% TI	75.93
MCI : Minimum Concentration inhibitrice ; MCF : Minimum Concentration fongicide; DZI: diamètre de zone d'inhibition en mm; %TI : Pourcentage du taux d'inhibition. [a, b, c] homogen groups according to Newman-Keuls test at $P < 0.05$						

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Discussion

The results obtained on the antifungal effect of the two oil extracts acacia testing by diffusion in liquid media and solid media are almost identical. Multivariate analysis of variance by Newman-Keuls test ($P \leq 0.05$) confirms that there is a significant fungicidal action of the two oils (Table 1 and 2). According to Ponce et *al*. [21], the classification of organisms based on the diameters of the inhibition zones. This quantity can predict susceptibility or resistance of germs beside the tested oils. However, the fungal strains have been extremely sensitive with very high diameters (68.4 ± 0.75; 59.5 ± 1mm) and (37. 5 ± 0.14; 36.1 ± 0.13 mm) respectively for *A. niger* and *C. albicans* in the presence of oils *A. raddiana* and *A. arabica* (Table 2). However, it was reported by Skandamis and Nychas [26], the MIC was defined as the lowest concentration of oil can reduce microbial growth 90%. This percentage *A. niger* is on the order of about 66-75% for *A. arabica* and *A. raddiana* (Tab. 2). According to Caillet and Lacroix [6], the minimum inhibitory concentrations of the oils are not identical towards the target germs and even compared to the desired effect during their use in various fields. This corroborates the results obtained. However, it is not overhear when oils of seeds Acacia species inhibited the growth of *C. albicans* in a low concentration, indeed the mechanism of oils action and their selectivity toward some microorganisms remain unknown. *Candida albicans* belonging to the family of Cryptococcaceae. This is yeast which is present in the mouth in humans, the gastrointestinal hit and the genital track. May become pathogenic under certain conditions, they cause candidiasis or fungal diseases [23]. Indeed, in Algerian arid and some African countries, man has used the seeds in powder form of the two acacia species to treat certain gynecological diseases.

Regarding *Aspergillus niger* is one of the best known of Aspergillus fungi. It appears as black mold. These fungi were very sensitive to oils. It appears that when oils are applied to the fungus at a low concentration, the fungicidal effect is better. However, the fungicidal activity was especially pronounced oil *A. raddiana*. Reverse against and except *albicans* germ when the concentration oils increases beyond 0.158 mg/ml of a good development mycelial growth was observed for this species. In fact *A. niger* is a toxic and pathogenic species. It causes otomycosis humans and also aspergillosis. However, it has active insecticidal toxins properties especially against mosquitoes [19]. Among others, it is not inconsistent as the dose of the oil increases the growth of mycelia of *A. niger* increases. This species is well known for its degrading enzymes and inter-fibrillar proteins also hydrolyze oils and fats free which damages the leather in tanneries industries fatty acids. These fatty acids released will have a source of carbon and energy for the fungus. This makes the environment more conducive to their development. Oils of two Acacia species can present in their chemical compositions tannins. These molecules if present in the culture medium of *A. niger*, they can make it more selective which will enable their good growth. These results appear to be inconsistent with the use of both species in dens industries. In fact, these industries use bark and pods that are low in fat content of about 0.02% [27], but they are rich in tannins. This effect, stimulating the growth of *A. niger* requires further studies to better understand the antifungal activity.

A fact of appreciation could be deduced from these results and relates the two acacia oils. Indeed, these oils were very active towards both fungal strains. It should be noted that the statistical study showed a significant variation ($P<0.05$) between the two oil extracts. It seems to be more appropriate than the oil *A. raddiana* has greater than in vitro antifungal activity oil *A. arabica*. This could be due to the way in which the oils are diffused in the matrices of germs and chemical composition of each oil. However, the composition and the chemical structure of oils determine their antimicrobial modes of action [29]. These oils are generally products of complex composition, containing secondary metabolites represented by active ingredients.

It should be noted that many authors have proved that the two acacia plants their bark, leaves, roots and pods have a variation of very active secondary metabolites such as saponins, triterpenes, alkaloids, flavonoids and tannins. These molecules provide these two plants a certain biological activities to fight against abiotic and biotic attacks [27, 23, 17, 9, 12, 25, 20, 18].

In view of all the existing factors and interactions between the extraction method, substance, microorganism and the culture medium, which can exist we can say that the antimicrobial action is complex as any biological phenomenon.

Conclusion:

Biological tests with two raw acacia oils showed strong antifungal activity *A. niger* and *C. albicans* with MICs of 0.079 and 0.088 mg/ml. The ratio of CMF/CMI recorded does not exceed 2, this indicates that the effect of both oils is a fungicide. Beyond the fungicide concentration effect of the two oils becomes stimulating for the species *Aspergillus niger*. Finally, we can say that this preliminary study provided at least some descriptive data on the antimicrobial effect of the two acacia oils towards fungal strains are more pathogenic to human health. Nevertheless, the biological study of these oils can be followed in the future with other objectives and methods to better identify the pharmacological and therapeutic oils interests of its plants.

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