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Research Article

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Phytochemical screening, antifungal and antimycotoxicological effect of *Acacia raddiana* leaves of south-west Algeria

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ABSTRACT

Although the Sahara is desert country the most unfavourable to the growth of human beings live, port plants planted as Acacia raddiana, thrive in an environment as hostile. The objective of this study is inscribed to the antifongic and antimycotoxicologic evaluation of the effect of aqueous and ethanolic extracts and macerates exits of the leaves of the Acacia raddiana pushing at Bechar against fungic strains toxinogenes eventually Aspergillus flavus- parasiticus and Aspergillus ochraceus. Screening phytochimic shown that the extracts subjects of this study are rich with flavonoïdes, saponosides and tanins. The evaluation of the radial growth on solid medium Potatoes Dextrose Agar reveals that the ethanolic extract and macerate of leaves induced an inhibiting action importance on the two strains. It is noted that the extrcts and the aqueous macerats have proclamation a waek effect antifongic with respect to the strains. The diameter of the fungal growth was 25, 38, 40 and 48 mm respectively for ethanolic extract, aqueous extract, ethanolic macerate and aqueous macerate note that the diameter of the witness is 60 mm for Aspergillus flavus-parasiticus, and 26, 28, 30 and 32 mm respectively for ethanolic extract, ethanolic macerate, aqueous extract and aqueous macerate note that the diameter of the witness is 40 mm Aspergillus ochraceus. The antifongic expertise on the effect on liquid medium Yeast Extract Sucrose revealed a decrease in the weight of fungal biomass formed by comparing the witness. The antimycotoxicologic test of aqueous/ethanolic extracts and aqueous/ethanolic macerate revealed an inhibiting action of production of aflatoxins and Ochratoxin A respectively by A.flavus-parasiticus and A.ochraceus.

Key words : *Acacia raddiana*, aqueous extract/macerat, ethanolic extract/macerat, antifongic effect, *Aspergillus flavus-parasiticus*, *Aspergillus ochraceus*, antimycotoxicologic effect.

INTRODUCTION

The world Organization of health (OMS) estimates that 80% of the world's population employs traditional medicines to meet the needs in primary health care [9], those due use of the richness of plant bioactive substances encompassing various chemical classes [1]. Among the diseases treated by the plants; foodborne illness, the latter are widespread and represent a real threat to health, both in developing countries than in developed countries, their gravities present on the animal health or human (children, the elderly and pregnant women), and on the other hand economic productivity [9]. The presence of the diseases caused by agents able to infect the body through food are the major problems affecting the safety of food [13]. These risks are both microbiological and chemical. Among the chemical risk, commodity supplies Mycotoxin contamination, these molecules are currently a major problem of food safety. The total elimination of mycotoxins is impossible to achieve. However, their reduction necessarily requires a reduction in producing fungal flora of these mycotoxins using the active principles of medicinal plants such as antimicrobial agents [5].

The ambition of this work was to investigate phytochemical and reduction fungal growth flora producing of

mycotoxin. In this context, we have tried to study the antifungal activity of different extracts of the leaves of *Acacia raddiana*, a plant in the region of Bechar, on toxigenic molds, namely *Aspergillus flavus-parasiticus* and *Aspergillus ochraceus*.

EXPERIMENTAL SECTION

Plant Material

The plant was harvested during the month of December 2014 in the region by lane of the wilaya of Béchar located 1150 km southwest of the capital, Algiers. The harvested leaves have been cleaned and free of debris, then spread on in thin layers, returned frequently, paper drying operated out in the open and protected from light at room temperature for 15 days. The dried leaves were crushed and preserved in glass jars tightly closed until their uses.

Qualitative phytochemical screening

One of the main goals of the phytochemical screening is to characterize or identify the main chemical groups of the leaves of the plant according to a suitable extraction method [11].

Preparation of plant extract

Aqueous maceration

Aqueous maceration is performed on 10g of the sample (leaves dried and crushed) with 100 ml of distilled water and placed under shaking for 24 h at room temperature, then aqueous macerate was filtered through filter paper MN 640 of 125mm in diameter, to remove fine particles [8]**.** After filtration, the extract was concentrated in vacuum at 100°C by rotary evaporator (Buchi Rotavapor R-215) [11]. Finaly, the aqueous macerate is kept in a sterile bottle tightly closed and covered with aluminum foil to prevent any penetration of rays indoors. The conservation was made at 4 ° C.

Aqueous extraction

10g of crushed leaves of *Acacia raddiana* was introduced with 100ml of distilled water. All was brought to the boil for 1h under reflux [8]. The extracts was filtered through filter paper MN 640 of 125mm in diameter, to remove fine particles [8] and concentrated in vacuum at 100°C to the rotavapor to eliminate the residual fraction of solvent. The conservation was made at 4 ° C.

Ethanol maceration

10g of the sample (dried and crushed leaves) is mixed with 100 ml of 50% ethanol and placed under shaking for 24 h at room temperature. After filtration with filter paper MN 640 of 125mm in diameter, to remove fine particles, the extract was concentrated in vacuum at 100°C by rotary evaporator (Buchi Rotavapor R-215) [15]. The obtained macerates were collected in sterile flasks and stored at 4°C.

Ethanol extraction

10g of crushed leaves of *Acacia raddiana* was introduced with100 ml of 50% ethanol. All was brought to the boil for 1h under reflux [8]. The extracts was filtered through filter paper MN 640 of 125mm in diameter, to remove fine particles, and concentrated in vacuum at 100°C by rotary evaporator (Buchi Rotavapor R-215) to eliminate the residual fraction of solvent [16]. The conservation was made at 4 ° C.

Origin of fungal strains

To evaluate the antifungal activity of extracts from the leaves of Acacia raddiana, we conducted an in-vitro study of these extracts on growth of fungal strains. Fungal strains selected for this study are *Aspergillus flavus-parasiticus* and *Aspergillus ochraceus* provided by the research laboratory of biology (laboratory of plant resources and the semi-arid food safety) University Tahri Mohammed of Béchar. The choice of strains was based on their frequency of occurrence on foodstuffs, their degree of toxicity by infecting these substrates and their ability to synthesize certain toxic secondary metabolites: mycotoxins. Species of *Aspergillus flavus-parasiticus* and *Aspergillus ochraceus* are identified by the "Single Spore" method described by [10]. A pure culture of 7 days recovered species are inoculated using a Platinum loop in hemolyzed tubes filled with a solid semi solution of 0.2% Agar and a few drops of tween 80. The whole is then stirred with a vortex for homogenization.

Antifungal activity

Evaluation of radial growth on solid media

The technique involves simultaneously place test tubes in different volumes of extracts from the leaves of *Acacia raddiana* 1; 1.25; 1.5; 1.75 and 2 ml of the extract with a volume of liquefied acidified PDA environment completed at 20ml as final volume. After agitation of the tubes by the vortex agitator, the middle is poured in with boxes of

Petri dishes [6]. A loopful of Platinum using a drop of the spore suspension prepared is filed in the center of each box. A box of Petri dishes containing 20ml of medium PDAa without extract was inoculated for the witness [12].

Assessment of biomass on medium liquid

The technique is to put different volume for each snippet in flasks and make up to a volume of 50 ml at the YES middle to late to realize the following concentrations: 3.1 ml; 3.75 ml; 4.37 ml and 5ml. Note that this is a medium of metabolism to test the production of aflatoxins and Ochratoxin A. After shaking the *Aspergillus flavus-parasiticus* and *Aspergillus ochraceus* strains were seeded. A bottle-50 ml of medium YES was seeded by the fungal spores to represent the witness. After mixing, incubation of the vials at 25°C for 14 days was made [7].

Evaluation of antimycotoxicological effect

50ml of the filtrate obtained previously by the method of assessment in the amount of fungal biomass after 14 days of incubation are added up to 180 ml of chloroform (100/50/30). The whole is vigorously shaken for 30 minutes, the homogenized solution is deducted (using a light bulb to count down). The chloroform phase is concentrated by evaporation under vacuum until 2 to 3ml of volume using a rotavapor. The filtrate is kept to 4 ° C in haemolysis tubes well closed to undergo on thin-layer chromatographic separation [2].

RESULTS AND DISCUSSION

Phytochemical screening

Screening has enabled us to characterize the different families of existing chemical compounds at the level of the leaves of *Acacia raddiana*. The results of Phytochemical tests performed on extracts from the leaves reveal the presence of flavonoids, saponins, tannins, sterols, and terpens, while alkaloids are absent. The phytochemical screening results are illustrated in the **Table 1**.

Table 1: Phytochemical screening of *A.raddiana* leaves

Phytoconstituents	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Sterols and terpenes	+

(+) and (-) refer to presence and absent amount, respectively

In the leaves of *Acacia raddiana*, searching of alkaloids proved negative but that flavonoids, tannins, and effective, unsaturated sterols and triterpenes was positive. It is reported that the Saponins are present in small quantities in the plant. These results are consistent with those of the work of [3] that proved that certain species of *Acacia* are very rich in tannins as species *Acacia raddiana*.

Antifungal activity

The comparative study of the extracts after the trials of antifungal activity showed that the aqueous and ethanolic extracts was better than those obtained either by it glycerine aqueous or ethanolic well. On the other hand, obtained either by decoction or maceration ethanolic extracts are more effective than the extraction or aqueous maceration.

Evaluation of radial growth on solid media

The technique of radial growth on solid media is a qualitative technique based on the measurement of inhibition in mm diameters. The results of this methods show that extracts from the leaves of *Acacia raddiana* proved to be active against all strains tested. We note that there is a decrease in radial growth of two strains tested (*A.flavus - parasiticus* and *A.ochraceus*). The **Table 2** shows the results of the action of extracts of the leaves of *Acacia raddiana* on strains of genus *Aspergillus*.

Table 2: Antifungal activity of the aqueous, ethanolic macerate and aqueous, ethanolic extract of the leaves of *Acacia raddiana* depending on the diameter of fungal growth (mm)

Fungal strains	W	A.M	E.M	A.E	E.E
Aspergillus flavus-parasiticus	60	46	40	38	25
Aspergillus ochraceus	40	32	28	30	26

W: witness, A.M: aqueous macerate , E.M: ethanolic macerate, A.E: aqueous exract and E.E: ethanolic extract

Evaluation of antifungal activity on solid medium PDAa shows the efficiency of the ethanolic extract and macerate compared to aqueous extract and macerate from the leaves of the plant *Acacia raddiana* on the inhibition of the growth of the strains tested. This can be explained by the fact that the solvent used (water) has a power of low

extraction for non-polar compounds especially those phenolic of the leaves of *Acacia raddiana*. However, ethanol is a more polar solvent that water has a maximum extraction power of the phytoconstituants of leaves, particularly with respect to the fraction of polyphenols (tannins). [4], reveals that these phenolic compounds are endowed with important and various antimicrobial activities. The investigation of the results of the aqueous extract and macerate showed low activity against two strains compared to the ethanolic extracts and macerate, these offer are similar to those obtained by [17] which has tested the effect of aqueous extracts of some Algerian plants on the germination of the seeds of *lactuca sativa* and *Raphanus sativus*.

Assessment of biomass on medium liquid

The assessment of fungal biomass on the liquid medium containing different concentrations of extract of the leaves, a reveals that ethanolic macerate and extract of the leaves had the best effects of antifungal that the aqueous macerate and extract. The results obtained by this technique are shown in the **Table 3**.

Table 3: Weight formed biomass (A.flavus - parasiticus and A.ochraceus) in the presence of aqueous, ethanolic macerate and aqueous, ethanolic extract of the leaves of *Acacia raddiana* (g)

Fungal strains	W	A.M	E.M	A.E	E.E
Aspergillus flavus-parasiticus	15,67	10,89	7,84	8,32	2,13
Aspergillus ochraceus	6,41	1,71	1,55	5,22	0,99

W: witness, A.M: aqueous macerate , E.M: ethanolic macerate, A.E: aqueous exract and E.E: ethanolic extract

The results of the antifungal activity on liquid medium YES (Yeast Extract Sucrose) are in coordination with those of radial growth in solid media. Indeed the extracts of the leaves of *Acacia raddiana* had a reducing effect on the weight of the fungal biomass formed. The analysis of our results we note that all extracts and aqueous and ethanolic leaf macerates induced fungal biomasses of both strains reduced. This can be explained by the rate of growth of *A.flavus-parasiticus* contribution to the *A.ochraceus.* The [14] say that the *Acacia raddiana* plant has antifungal activities against the strain *Aspergillus niger*.

Antimycotoxicological test

The antimycotoxicologique test results show that the aqueous/ethanolic extract and aqueous/ethanolic macerate have an inhibitory effect on the production of aflatoxin B1 and Ochratoxin A, at a volume of 5ml of each extract. The revelation of aflatoxins and ochratoxin A was examined by UV light at 365 nm. The presence of mycotoxins (aflatoxins, OTA) translates the characteristic fluorescence (blue for AFB1, green for the AFG1 and blue fluorescence for OTA with the same Rf than standard)

CONCLUSION

During this work, we studied a plant species used as medicinal plants in traditional treatment by the population of the south wester Algeria for its therapeutic virtues, this plant is *Acacia raddiana*. The results obtained there is that this species is made active as, elements flavonoids, saponins, tannins and sterols and terpenes. The study of the antifungal effect aqueous and ethanolic extracts/macerate leaves of *Acacia raddiana* extracts reveal an antifungal effect against the strains studied with an inhibition on the production of mycotoxins by the same fungal strains. Additional testing will be required and should be able to confirm performance as the isolation and purification of the phytoconstituants of this plant. And the study regardless of the antifungal effect of each of these components.

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REFERENCES

[1]Alilou, H; Akssira, M; IdrissiHassani, L.M; El Hakmoui, A; Mellouki, F; Rouhi, R ;Boira, H; Blasquez, A and Chebi, B. *Phytopathol Mediterr*, **2008**, 47,3-10.

[2]Amar Belbachir, L. Contribution a une analyse physicochimique, microbiologique et mycotoxicologique des olives et de l'huile d'olive commercialisé dans la région de Tlemcen et recherche de l'aflatoxine b1 par les techniques chromatographiques,Thèse de magistère. Inédite, université de Béchar. Algérie, **200**.

[3]Benbrahim, f ; EL Ghachtouli, N ; Ismaili, M. Les acacias : des plantes fixatrices d'azote prometteuses pour le développement durable des zones arides et semi arides. *International journal of innovation and AppliedStuudies*, ISSN 2028-9324Vol.8 No.1sep.2014,**2014**,46-58.

[4]Cowan, M. *ClinicalMicrobiologyRevie*wes,**1999,**12(4),564-582.

[5]El Ajjouri, M; Strani, B et Ghanmi, M. Activité antifongique des huilles essentielles de Thymus bleicherianusPomel et Thymus capitatus (L.) Hoffm et Link contre les champignons de pourriture du bois d'œuvre .Biotechnologie, Agronomie, Société et Environnement,**2008,**12(4), 346-351.
[6]Hibar, K, Daami-Romadi .N, Jabnoun-Khiareddine .H, Znaidi .I.E, Kra, k.D; Diallo, H.A; Kouadio, Y.J. ''activité antifongiques de l'extrait de ChromolaenaOdorata (L.) King et Robins sur deux isolats de *Fusariumoxusporuim* (E.F.Sm) resonssable du jaunissement mortel des feuilles de *bananier''journale of applied Bioscences*. **2009** ; vol 24, 1488-1496.
[7]Imtiaj, A; Lee, T.S..word Journal of agricultural sciences.**2007** ; vol,3(3),316- 321.
[8]Kadi H ; Moussaoui A ; Benmahdi H ; Lazouni HA ; Benyahia A ; Nahar BN ; *J. Appl. Pharm. Sci*, **2011**, 01 (10), 180-182
[9]OMS. ((Question d'actualités et problème non résolus moyens de les gérer)).conférence européenne sur la sécurités sanitaire et la qualité des aliments. Budapest, Hongrie, 25-28 février **2002**.
[10]Pitt, J.l. **1973** ; *Mycology* 65, 1135-1157.
[11]Sarkar.M ; Tanker.M, phytochemicalanalysis of AnkaraUniversity in the spring of eczacicikfaeulte 10.67. Ankar. Turkey, **1991**.
[12]Soro .S; Quattara. D; Guédé.N. Z; Coffi. K. '*European Journal of scientificResearch*.**2010** ; Vol 39(2),279-288.
[13]Soubra L. Evaluation scientifiques des risques toxiques liés à certaines substances chimique et contaminats. Thèse de doctorat, Institut des sciences et industries du vivant et de l'environnement (agro Paris tech). **2008** ; P198
[14]Tissouras, F ; Larid, M; Lotmani, B. *Advances in Environmental Biology*, 8(10) June **2014**,137-141.
[15]Wei L. S ;Musa.N ; Sengam C. T : WeeWandSchazili ; *Afr.J.Biotechnol*, **2008**, 7,2275-2278
[16]Yasoubi.P ; Barzegar.M ; Sahari.M. A ;Azizi M.H.*J. AgricSciTechnol*, **2007**, 9 :35-42
[17]Zeghada F. Z. Activité allélopathique et Analyse phytochimique. Mémoire de magistère, Université d'Oran Es-Sénia, **2009**,57-65.