Applied **B**iology in **S**aharan **A**reas *Vol. 1, N. 2, p. 16-22, December 2017*
ISSN: 2571- 9823

Phytochimical and antifungal screening of Algerian *Salvia officinalis*

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Abstract – Medicinal plants constitute an immense source of bioactive molecules, endowed with numerous biological activities.

This study aims at performing phytochemical screening, antifungal and antimycotoxicological effect of aqueous extract of *Salvia officinalis* leaves.

Phytochemical screening revealed reveals the presence of some active substances flavonoids, saponins and steroid, to express the desired activities.

The evaluation of antifungal activity was fined by radial growth technique gave the antifungal indices according to the order of efficacy of aqueous extract on the following recorded antifungal 66.66% and 92% registers for *Aspergillus flavus* and *Aspergillus ochraceus.*

Keywords: Medicinal plant, *Salvia officinalis*, phytochemical, antifungal, antimycotoxicological.

1. Introduction

The Mediterranean region is rich in plant species; there are about 3000 species of which many are considered to have medicinal effects (Saad & *al.,* 2005). The molecules responsible for the therapeutic effect of the so-called natural plants are considered a very important source of drugs; knowing that more than 120 herbal compounds are now used in modern medicine and nearly 75% of them are applied according to their traditional use. Salvia is the largest genus of this family and includes near 900 species. Plants of this genus grow all over the world and the species of *S. officinalis,* it is a perennial round shrub in the family of Labiatae/Lamiaceae and it is native to Middle East and Mediterranean areas (Ghorbani & Esmaeilizadeh, 2017).

Salvia officinalis is known for its wide range of therapeutic activities including antibacterial, antiviral, antifungal and antioxidant effects (Miraj & Kiani, 2016; Fawzi & *al.,* 2017).

In the present study, the phytochemical screening, antifungal and antimycotoxicological effect of aqueous extract from *Salvia officinalis* leaves were tested.

2. Materials and methods

2.1 Plant material

The plant used for the present study was collected in September 2015, from "HASSI 20" located 20 km north west of Béchar; Béchar is located in west of the Algerian Sahara. The leaves were dried during 25 days in the dark at ambient laboratory temperature (20 to 28°C); the leaves were milled into a fine powder in an electrical mill, and stored in the shadow ambient temperature in closed containers until the day of its use.

2.2 Qualitative phytochemical screening

The leaves of *Salvia officinalis* were screened for the presence of key families of phytochemicals (Sakar and Tanker, 1991; Trease and Evan, 1984) using the following reagents and chemicals: alkaloids with Mayer's reagents, Flavonoids with metallic magnesium and hydrochloridric acid, Saponosids for to their ability to produce suds, steroids acetic anhydride and concentrated sulphuric acid, Tanin with ferric chloride.

2.3 Extraction protocol

2.3.1 Aqueous extract

5g of leaves powder of plant were added to 50 ml of distilled water, the mixture was allowed to reflux for 30min. After cooling it was filtered and stored to 4°C prior to analysis (Kadi *&al.*, 2011).

2.4 Antifungal activity

2.4.1 Fungal strains

The fungal strains, *A. flavus*; *A. ochracceus*; were obtained from durum wheat in a biology laboratory from Béchar university (the university mentioned above).

Confirmation of *Aspergillus* genera was realized by micro the culture method described by (Haris, 1989)

Furthermore, the confirmation of *A. Flavus* and *A. ochracceus* species was carried out by a single spore method using three cultures media: Malt Extract Agar (M.E.A) at 25°C; Glycerol Nitrate Agar (G25N) at 25°C and Czapek Yeast Agar (C.Y.A) at 5°C and 37°C. The use of identification key of Pitt (1973) for *Aspergillus*, observation has been made after the first and second week. The fungal strains were stored in tubes of PDA acidified at 4°C.

2.4.2 Mycotoxinogenic Test

A. flavus and A. ochraceus were sowed on Y.E.S (Yeast Extract Sucrose) medium rich on B vitamin complex. After two weeks of incubation at 30 ± 2 °C, the biomass was removed by filtration of Y.E.S. The filtrate was then added to 180 ml of chloroform and stirred for 30min. After decantation, the organic layers were concentrated to 2ml. Aflatoxins and ochratoxins were determined in each extract by spotting samples onto thin layer chromatography plate. Plate is developed with a toluene/Ethyl acetate/ Formic acid (50:40:10, v/v/v) solvent system. Aflatoxin and ochratoxin standard were spotted on the same plate as reference, aflatoxin and ochratoxins spots were identified using 365 nm UV (Asso. of off. Anal. Chem., 1975). The presence of Aflatoxins was provided by appearance of blue fluorescence for AFB and green fluorescence for OTA which have the same Rf as control.

2.4.3 Growth radial technique

The following volumes: 1,2,3 and ml of leaves extract of *Salvia officinalis* were added to 15 ml of PDA_{ac} (Potatos Dextrose Agar acidified), in order to have respectively, the concentrations (4, 8, 12) mg/ml, the latter were transferred into a Petri plats, after the ensemensing of fangal strains with a single spore method, these Petri plats were incubated during 7 days at 25±2°C.A Petri plats containing 15 ml of PDA_{ac} medium without extract is (plural) inoculated to serve as growth controls for each strain and each series of tests. Mycelial radial growth was measured from the third day of incubation (Soro *& al.*, 2010; Kran *& al*., 2009).

The seeding of Petri plats for radial growth evaluation was approved out starting from a previously prepared

sporulation suspension, by counting the number of spores in order to obtain the concentration of 10^5 spores/ml (Serghat & *al*., 2004).

The inhibition percentage of mycelial growth of each extracts was calculated using the following formula of Singh & *al*., (2009):

$$IP = \frac{Dt - D}{Dl} x100$$

Dt: Mean diameter of mycelial growth control.

D: Mean diameter of mycelial growth in treatment.

2.4.4 Determination of mycelial inhibition by biomass technique

Evaluation of biomass liquid medium was achieved by counting the spores' number using malassez hematimeter in order to obtain the concentration of 10^5spores/ml (Serghat & *al*..; 2004). This technique consists of putting different volumes of extract in flasks and completed them with 50 ml of PDBa (Potato Dextrose Broth acidified) in order to obtain the following concentrations (4, 8, 12) mg/ml. These liquid cultures were sowed with 30µl of sporal suspension.

The flasks were incubated for 14 days at 25 ± 2°C (Tubajika, 2006; Hibar & *al*., 2006). After filtration, the filter paper was dried at 60°C during 24hours (Dhandhukia & Thakkar; 2007). Biomass weight formed (P) was determined using the following formula of Imtiaj & Lee (2007) (P = P1 – P0) where P0 is the filter paper weight and P1 is the filter paper and fungal biomass weight after dryness.

2.5 Determination of anti-mycotoxicalogical effect

This filtrate was, then, added to 180 ml of chloroform and stirred for 30min. After decantation, the organic layers were concentrated to 2ml. The organic extracts have been treated with the same technique of mycotoxinogenic Test.

All tests were operated in triplicate.

3. Results

3.1 Qualitative phytochemical screening

Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample.

The phytochemical analysis conducted on *Salvia officinalis* leaves revealed the presence of flavonoids, steroids and saponins (Table 1).

Table 1. Phytochemical Screening of *Salvia officinalis* leaves

Phytochemical constituents	Leaves
_ Alkaloid	-
_ Tannins	+/-
_ Saponins	+
_ Flavonoids	+
_ Unsaturated sterols and terpens	+
_ Sterol and steroid	+

Key: +: present; - : Absent;
+/-: lowpresence

Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological (Nahal & *al.*, 2012).

The extract *gave good result from Salvia officinalis* in the work of kadhim & *al.,* 2016 presented a)positive tests for (glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids, flavonoids, steroids and vitamine C)

similar results are also obtained by other studies. A water-soluble polysaccharides complex from *S. officinalis* composed of galactose, glucose, mannose, xylose, and fructose have shown an immune modulatory activity in the comitogenic thymocyte test, which is interpreted as being an *in vitro* correlate of adjuvant activity in addition to their mitogenic activity (Capek & Hribalova, 2004).

3.2 Growth radial technique

Different concentrations of aqueous extract of *Salvia officinalis* leaves were tested for their efficacy by growth radial technique against fungi of stored food. The histograms (figure1) and (figure.2) show that varying concentrations of flavonoids have diminished the diameters of all the fungal strains, because of inhibition of radial growth of *Aspergillus flavus* and *Aspergillus ochraceus*; The antifungal indexes of fungal strains tested are calculated at each concentration .

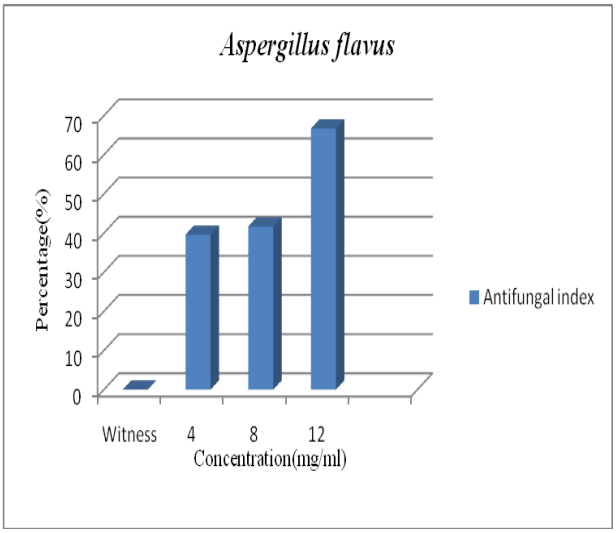

Figure 1. Histograms of radial growth of *Aspergillus flavus* under effect of different concentrations of *Salvia officinalis* extract.

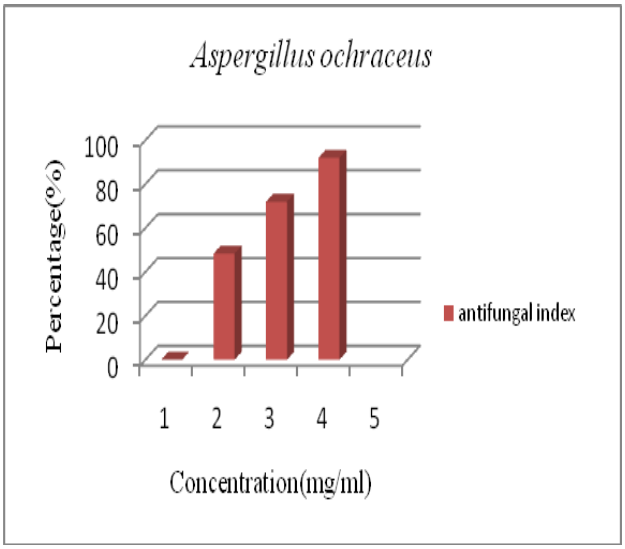

Figure 2. Histograms of radial growth of *Aspergillus ochraceus* under effect of different concentrations of *Salvia officinalis* extract.

3.3 Determination of mycelial inhibition by biomass technique

Results obtained from biomass weight on liquid medium showed different fungal weights which are inferior to controls. These results are represented in figure 3 and figure 4.

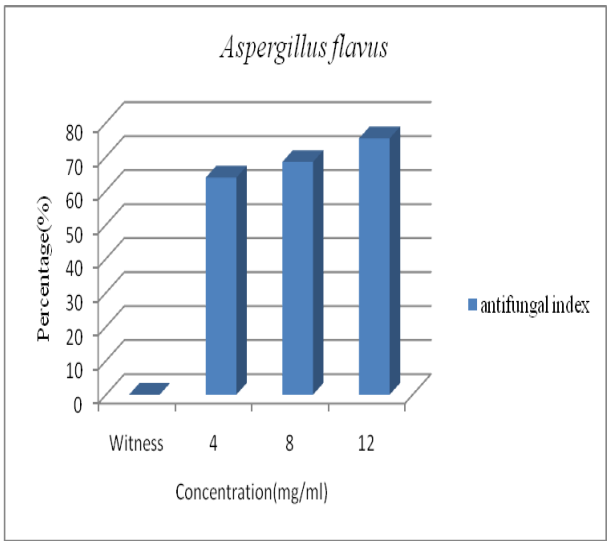

Figure 3. Histograms of biomasse of *Aspergillus flavus* under effect of different concentrations of *Salvia officinalis* extract.

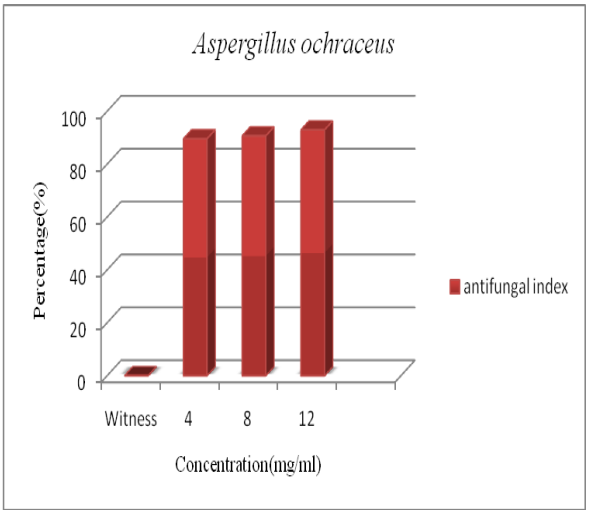

Figure 4. Histograms of biomasse of *Aspergillus ochraceus* under effect of different concentrations of *Salvia officinalis* extract.

The therapeutic effects of many traditional drugs are attributed to this group of compounds because of their inhibitory effect on certain enzymes and antioxydative activity. They have been recommended in order to process antibacterial, antifungal, antiviral and anti-inflammatory activities (Velikhovic *& al.*, 2007). phytochemical test reveals that flavonoids and other phenolic compounds are present in *Salvia officinalis*, which are known to be responsible for the antifungal activity.

According to Beigi Boroujeni & Gholami ,(2017); Ulanowska *& al.*,(2006), the sage has an effect fungistatic and the activity of flavonoids is mainly due to the ability of these molecules to inhibit the expression of DNA and the synthesis of certain enzymes and membrane proteins of microorganisms.

This activity is probably related to their ability to form complex with extracellular and soluble proteins or to form complex with bacterial cell walls (Cowan, 1999).

Hayouni *& al* (2008) and Mitic-Culafic *& al* (2005) support antimicrobial effects of *S. officinalis*. The essential oil and ethanolic extract of *S. officinalis* show strong bactericidal and bacteriostatic effects against both Gram-positive and Gram-negative bacteria.

3.4 Determination of antimycotoxicalogical effect

The mycotoxin separation elaborated by the fungal strains was made on a CCM plate, the spots were revealed under the UV lamp at the wavelength 254nm, the elaboration of Aflatoxin B1 by *A.flavus* and ochratoxin A by *A.ochraceus* in the test and with presence of aqueous extract of *Salvia officinalis* are confirmed with CCM plate illustration in figure 5 and 6.

Figure 5. Photo of fluorecence of Aflatoxin B1 under UV obtained by thin layer chromatography.

Figure 6. Photo of fluorecence of ochratoxine A under UV obtained by thin layer chromatography.

From the results obtained, it can be said that the aqueous extract of *Salvia officinalis* has no reducing effect of the production of mycotoxins in spite of the effectiveness of the latter on the growth of the two fungal strains studied.

4. Conclusion

This study emphasizes antimicrobial properties of aqueous extract of *Salvia officinalis* leaves against two fangs strains isolated from sticky foods. The strains studied are a sensible to the aqueous extract of *Salvia officinalis* leaves in different concentrations towards the characteristics of strains tested *in vitro*.

More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Hence, these extracts and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

The biological activities studied could be an alternative solution to the problem of post-harvest deterioration related to molds as well as a likely path for biomedical research including a solution to antimicrobial resistance with bioactive substances.

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