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# *In-vitro* and *In-vivo* antifungal activity of *Capparis spinosa* against eight storage molds, a causal agent of wheat alteration.

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**ABSTRACT**

This study was designed to assess the antifungal effect of five types of extracts from the aerial part of *Capparis spinosa* grown in southwestern Algeria. The chemical composition was studied using phytochemical screening by conventional techniques. Tannins, saponins and flavonoids, the latter are mostly found from these types of extracts. The antifungal activity of the extracts was tested by the method of diffusion and determination of the zone of inhibition. The results showed that all the types of the extracts examined have an important antifungal activity with regard to the molds tested. There is also a reduction in the fungal flora for the grains coated with aqueous extract in a remarkable manner.
Keywords: *capparis spinosa*; Antifungal activity; mold; soft wheat; storage.

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## INTRODUCTION

Capparis spinosa named locally as "K'bar", It is a small thorny shrub, prostrate, widely replied in Algeria and the Mediterranean basin [1].

Caper is traditionally used for pharmacological reasons [7], as it is used in Mediterranean cuisine various preparations [9]. Several phytochemical studies have shown that Capparis spinosa contains an amalgam of active compounds in its various parts. Polyphenols and flavonoids are also present in the caper [6,12].

## MATERIALS AND METHODS

### Sampling

Capparis spinosa plant samples were harvested at the knadsa sites of Bechar, Algeria, from March to June 2013 and 2014. They were identified at the South West Semi-Arid Zones Plant and Food Security Laboratory Of Algeria, of the University of Bechar. These plants were cleaned with running water, dried in a well ventilated room and then pulverized with an electric grinder to give fine powders.

Sampling is carried out in accordance with ISO 13690, Official Journal of the European Union, and a practical guide for the quality control of cereals and protein crops.

### Phytochemical screening

The collected plants underwent a series of phytochemical studies which consists of identifying the main chemical components shown in Table01.

**Table 1: chemical compounds present in these plants.**

| Chemical groups | Reagents | Positive results |
|---|---|---|
| **Alkaloids** | -mayer (Iodomercurate de potassium)<br>-Dragendroff (Iodobismuthate de potassium) | Precipitate white yellowish<br>Precipitate red aranged |
| **Flavonoids** | - reaction à la cyanidine | Orange, red or violet color |
| **Tanins** | $FeCl_3$ | Dark blue, green or black color |
| **Coumarin** | NH4OH (25%) | Fluorescence under UV light |
| **Quinon** | Bornträger | Purplish-red color |
| **Steroids and Terpenes** | Liebermann-Burchard | Purple (blue or green) |
| **Saponosides** | Determination of the foam index | |

### Preparation of extracts

The extraction under reflux was renewed three times every 2 hours. The extracts were filtered off and evaporated to dryness under reduced pressure. The solvent was chosen according to the polarity gradient: petroleum ether, chloroform, ethyl acetate and methanol

### The strains used

The fungal strains used to test the biological activity of the extracts were isolated from purified and identified French imported soft wheat. These include *Aspergillus flavus, Aspergillus ochraceus, Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Fusarium .sp and Penicillium sp*

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**Biological testing and evaluation of the effect of extracts**

The antimicrobial tests are carried out according to the method reported by Remmal et al. (1993), Farah et al. (2001), Satrani et al. (2001). The extract is emulsified with a 0.2% agar solution in order to disperse the compounds and to improve their contact with the seeds tested. The final concentrations obtained are 1 / 100, 1 / 250, 1/500, 1/1000, 1/5000.

Percentage inhibition is calculated which is determined by the formula [16] :

Percentage inhibition**= (1-Da/Db)*100**
**Da**: The diameter of the growth zone of the test.
**Db**: The diameter of the growth zone of the control

**Tests for the conservation of soft wheat by coating with aqueous extract of the plants studied**

The test is carried out as follows; The wheat is moistened with the aqueous extract of the plants used to coat the grains with substances of the plant. The wheat is then dried and stored in the flasks; Each bottle contains 1 kg of soft wheat.
The effectiveness of these methods is evaluated by post-drying and post-storage analyzes after one year of storage with a one-year period from March 2015 to March 2016

**Analysis and evaluation storage efficiency**

100 randomly selected wheat grains from five samples were placed in 10 sterile petri dishes containing sterile filter paper impregnated with 5 ml of sterile 7.5% aqueous sodium chloride solution [4]. And then compare the contamination rates found.

## RESULTS AND DISCUSSIONS

The phytochemical screening suggests interesting pharmacological activities of the studied plants. The phytochemical screening of plants showed the detection of tannins, saponisides and flavonoids, most of which are found table 02. The results of this screening make it possible to elaborate hypotheses to explain the biological activity of an extract by the presence of a particular chemical family. According to TLILI N et al 2010 showed the richness of capparis spinosa with these compounds, especially phenolic compounds. And Zhou H et al 2010 found The main compounds present in the aqueous extract of capparis spinosa are flavonoids, indoles and phenolic acids.

**Table 2: results of phytochemical screening of *Capparis spinosa***

| Chemical groups | alkaloids | flavonoids | Tanins | coumarin | Quinon | Steroids and Terpenes | Saponosides |
|---|---|---|---|---|---|---|---|
| Results | + | + | + | + | + | +/- | + |
| +: Presence; -: not detected | | | | | | | |

**Evaluation of the antifungal effect of the extracts (in vitro)**

The results presented in the figure 1 The pure extracts are extremely active regardless of the plant and the extraction solvent. On the other hand, our results show a correlation between dilution extraction solvent and antifungal effect. In general, all the sensitivity curves show an increasing trend with slopes of varying degrees depending on the extracts, the mold strains and the concentration. Which is confirmed by Nabavi SF *et al.* 2016; They have proven that Recently, a wide range of evidence has shown that this plant has various biological effects, including antioxidant, anti-cancer and antibacterial effects. Phytochemical analysis shows that *C. spinosa* has large amounts of bioactive constituents, including polyphenolic compounds, which are responsible for its beneficial effects on health.

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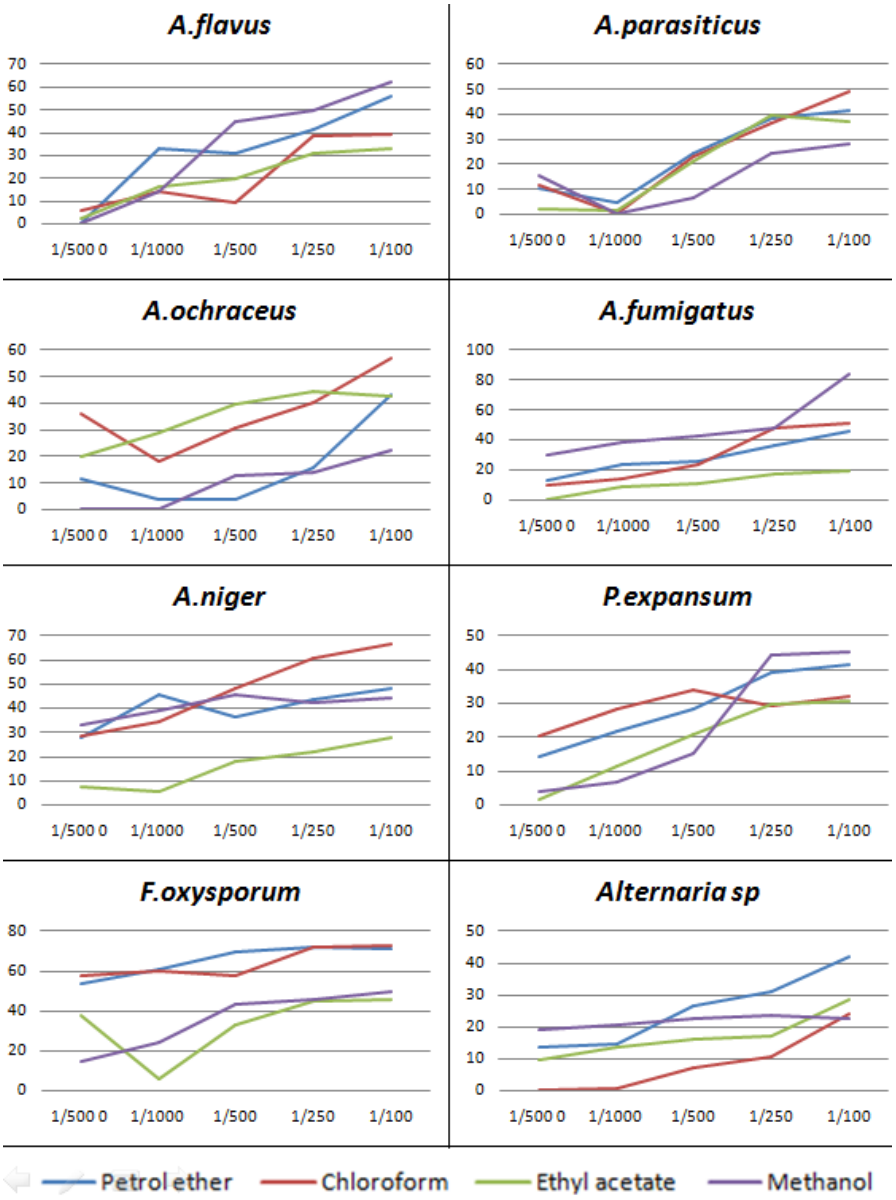


**Figure 1: Results related to the growth of the molds studied subjected to the action of the different concentrations of the extracts tested of Capparis spinosa are illustrated in eight graphs.**

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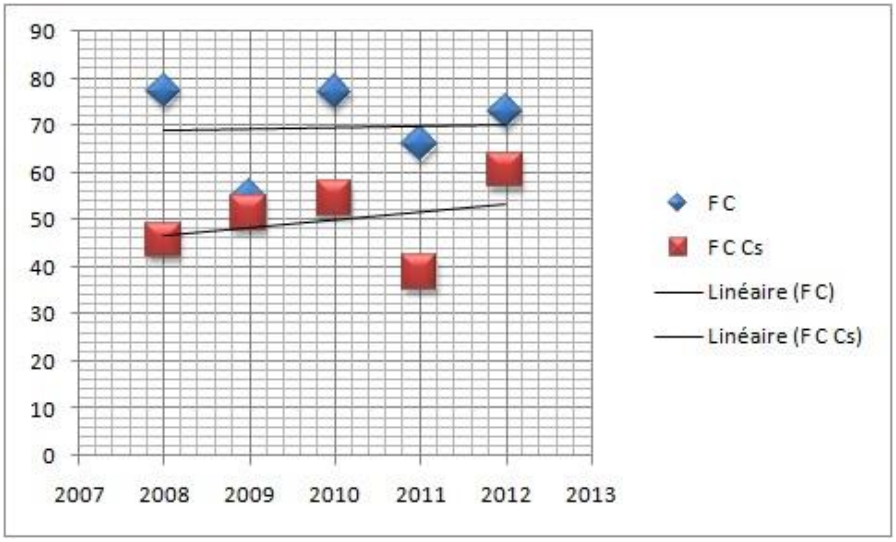


**Figure 2: The results relating to the frequency of contamination of the molds studied subjected to the action of the aqueous extracts of the different plants by the direct method (FC: frequency of contamination without the addition of the control extract, FCCs: frequency of contamination with l Addition of capparis spinosa extract**

**Storage test by the method of coating the grains with the aqueous extract (in vivo)**

After the storage period, reductions in contamination frequencies were observed in an excellent manner in all the samples (figure 2). This is proved by the biological studies of TLILI N *et al* 2011. These studies reveal important antimicrobial, antioxidant, anti-inflammatory, immunomodulatory and antiviral properties. So our plant has a very good antifungal effect there is a variability in inhibition efficiency, it depends on the chemical composition of the extract and the nature of the mold species and the initial contamination rate. The decrease in the level of mold observed in soft wheat samples and the use of Capparis spinosa in the kitchen (no toxic effect) demonstrates the effectiveness of this method of preservation. According to Tesoriere L et al 2007 the Capparis spinosa L. plant is commonly used in Mediterranean cuisine. She is a good candidate to discover new drugs [3], which is confirmed by the work [5].

## CONCLUSION

This study confirmed the efficacy of phytotherapy practiced in our country (Algeria). The antifungal action on mold is remarkable on the extracts of *Capparis spinosa*; This action makes it possible to improve the method of storing soft wheat by using the coating of the grains.

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