Evaluation of Fungal Infection of Some Medicinal Plants Sold by Herbalists

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In this study, some fungal species were isolated from some parts of the medicinal plants sold by the herbalists. These plant parts are cinnamon bark, ginger rhizome, pomegranate husks and colocynth. These fungi are Fusarium osysporum Schlex Fr. Emend Snyd. & Hans, Aspergillus flavus Link ex Fr., Penicillium chrysogonum Thom., Alternaria alternata (Fr.) Keissl. The results showed that ginger was the most polluted by the fungi. All fungal species were isolated from it except Penicillium chrysogonum. Two fungal species were isolated from both fruits of colocynth and the pomegranate husks. The cinnamon bark was free of fungi and no fungi were isolated from it. The results also showed that the cinnamon bark extract had a great inhibitory effect on the growth of isolated fungal species grown on the PDA, the rate of inhibition was 88.2% in the Penicillium chrysogonum followed by Alternaria alternata 82.6%.

Keywords: Herbalists, Mycotoxins, Cinnamon, Colocynth, Ginger, Pomegranate.

Introduction

The use of pesticides has played a major role against fungi for a long time. However, many problems have emerged in the use of pesticides such as environmental pollution and the emergence of resistant strains, in addition to their impact on human health and others. Therefore, recent studies have focused on the use of medicinal plants and their extracts due to their positive effect an easy to reach. In addition, they are inexpensive and uncontaminated and have no side effects [1].

The last new awakening for the optimal use of medicinal plants through the identification of the active substances by chemical analysis, in order to know the physiological age of these plants, which is the period in which this substance is active with great concentration, the physiological age of plants may be before flowering time or at flowering time or after the maturity of fruits and seeds, this varies from one plant to another. This chemical analysis of the plant determines the part that is abundant in the active ingredient, which in turn helps maximize the benefit of the plant in a timely manner and in its most effective parts.

Herbal medicine is one of the current global trends to eliminate the side effects and dangers of chemotherapy and to open new therapeutic areas through medicinal herbs to cope with traditional and modern diseases without being affected by the dangers of chemicals [2].
But because of the presence of many toxic plants, therefore beware of the use of medicinal plants and use only after the consultation of a doctor, it is also necessary to beware of the storage conditions of medicinal plants in the herbal shops, especially in the absence of control on these shops, as the bad storage conditions may cause growth of some fungi on the plant parts and secretion of mycotoxins, which affect their effectiveness and may result in very dangerous side effects.

The aim of this study is to investigate fungal infection of some medicinal plant sold by herbalists, in order to evaluate the storage conditions of these plants and thus evaluate the validity of their use for medical purposes.

Material and Methods
Samples of cinnamon bark, ginger rhizomes, pomegranate husks, and colocynth fruits were collected from the herbal shops from four locations in Tripoli which are, Souq Al-Gomaa, Tajora, Bab al-Hurriya. The samples were placed in plastic bags and then brought to the laboratory for subsequent laboratory operations.

Preparing For Culture Media
1- Czapek Dox Agar
This medium is suitable for saprophytic fungi and composed of:
Sucrose 30 gr
Sodium nitrate 2 gr
Potassium biphosphate 1 gr
Magnesium sulfate 0.5 gr
Potassium chloride 0.5 gr
Ferrous sulfate 0.01 gr
Agar 15 – 20 %
Prepared by dissolving 4 grams of culture medium powder in 1000 ML distilled water, and heated until boiling in the water bath, then sterilized in the autoclave at 121 °C and 1 bar pressure for 20 minutes. After that cooled and transferred to Petri dishes that every dish contains 15-20 ml culture medium.
2- Potato dextrose agar (PDA)
This medium suitable for fungi isolated from plant parts and composed of:
Potato 200 gr
Dextrose 20 gr
Agar 15 – 20 gr
Distilled water 1 L
The potatoes were sliced into thin slices after peeling, then placed in 1 liter distilled water in a 2000 ML flask, then filtered through a cloth, added the rest ingredients, and then putted in a water bath until the agar melted, and sterilized for 15 minutes under pressure of 10 lbs, at last, poured into Petri dishes with 15 - 20 ml per dish.

Culturing
After the preparing, the culture media, isolation of the fungi was done by taking a part of each plant specimen under study, cut into small pieces and placed in sodium hypochlorite solution, and then inoculated in the culture media, and placed in the incubator for two weeks.

Isolation of pure culture
In order to ensure pure isolates of fungal growth from the studied plant parts, the lateral part of the growing colony was transferred to a petri dish containing culture media and then
incubated for two weeks to allow fungal growth. The pure isolates of growing fungi were examined under a light microscope, the diagnostic characters such as the color of the colony and its diameter, shape, type, and measurements of conidiospores and shape of phialides were examined and recorded. After that, the isolated fungi were identified using taxonomic keys and illustration for the identification of fungi such as [3, 4, 5].

**Effect of Cinnamon Extract on the Growth of Isolated Fungi**

The effect of cinnamon bark extract on the growth of isolated fungi was also studied using PDA and Czapek culture media. The cinnamon extract was added to the culture medium before converting to the solid state, then poured into Petri dishes and left to freeze. A portion of 3 mm diameter from each isolate was taken and inoculated into dishes containing cinnamon bark extract, with four replicates compared with the control which contained only a pure culture medium, the dishes then placed in the incubator at 25 ± 2 ° for two weeks, the fungal growth was recorded every two days.

**Results and Discussion**

The results of the study have shown that four fungal species belonging to four genera were isolated, which are *Fusarium oxysporum* Schles Fr. Emend Snyd. & Hans, *Aspergillus flavus* Link ex Fr., *Penicillium chrysogonum* Thom., *Alternaria alternata* (Fr.) Keissl. All of which are belong to Deuteromycetes.

The results also showed that the rhizomes of Ginger were the most polluted with three fungal species which are *Fusarium oxysporum*, *Aspergillus flavus* and *Alternaria alternata*, followed by colocynth fruits and pomegranate husks with two fungi each, which are *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus flavus*, *Penicillium chrysogonum* consecutively, while cinnamon bark was free of any fungal infection, which encourage us to test the effect of its extract on growth of all isolated fungal species (Table. 1).

<table>
<thead>
<tr>
<th>Studded plant</th>
<th>Isolated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon bark</td>
<td>No fungi</td>
</tr>
<tr>
<td>Colocynth</td>
<td><em>Fusarium oxysporum, Alternaria alternata</em></td>
</tr>
<tr>
<td>Pomegranate</td>
<td><em>Aspergillus flavus, Penicillium chrysogonum</em></td>
</tr>
<tr>
<td>Zinger</td>
<td><em>Fusarium oxysporum, Aspergillus flavus and Alternaria alternata</em></td>
</tr>
</tbody>
</table>

The results showed that the extract of cinnamon bark had a significant inhibitory effect on the longitudinal growth of isolated fungi. It was more effective on *Penicillium chrysogonum*, where the average longitudinal growth was 0.65 cm compared with 5.55 cm in the control, with inhibition rate 88.2 %, followed by *Alternaria alternata* where the average longitudinal growth was 1.1 cm compared with the 6.35 cm in the control, with 82.6 % inhibition rate. *Aspergillus sp* was the least inhibited by 53.8 % (Table. 2). These findings agree with findings of [6].

<table>
<thead>
<tr>
<th>Cinnamon extract</th>
<th>Average of reading 10 days</th>
<th>Percentage of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Extract</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> + Control</td>
<td>5.63</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> + Control</td>
<td>5.1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Alternaria alternata</em> + Control</td>
<td>6.35</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Penicillium chrysogonum</em> + Control</td>
<td>5.55</td>
<td>0.65</td>
</tr>
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Conclusion
We conclude from the results of this study that the method of drying and storage conditions have a great role in preserving the active substances in the plant matter, they also protect the plant matters against fungal infection which secrete mycotoxins that affect the nature of the active substances in the plant matter and then affect human health.

References