Inhibition activity of Zingiber officinale. and Cinnamomum zeylanicum extract against Aspergillus niger and Penicillium notatum

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Abstract

Aqueous and ethanolic extracts of ginger (Zingiber officinale) and (Cinnamomum zeylanicum) were used to investigate an antifungal activity against food spoilage fungal pathogens. Study of the inhibitory effect of these extract was done separately against Aspergillus niger and Penicillium notatum isolated from (tomato pasts, cheese) the samples were cultured on Sabouraud's dextrose agar (SDA) at pH 5.6 and 28°C. In this study, results had shown that the extracts of Both plant demonstrated antifungal activity. When compare extracts of the two plants, C. zeylanicum extracts showed higher inhibition activity than (Zingiber officinale) extracts significantly. Ethanol extracts of these tow plants gives significantly inhabitation in the mycelia growth of fungi more than their aqueous extracts (16-22), (19-25)mm, P. noitatum and A. niger respectively while the inhibitory observed by the aqueous extract (5-6) mm against A. niger for (6-8) mm. Against P. notatuum was the most affected by all extracts while A. niger was the least susceptible. There was a significant difference (P=0.05) between groups in the antifungal activities of tested fungi, and the effectiveness of ethanolic extracts was increased with increase in their concentrations. The ability of these extracts to inhibit the growth of the two fungi must take into consideration an indication of the antifungal potency of cinnamon and Zingiber officinale, that makes them the candidate for the production of antifungal agents.

Keywords: Antifungal, extracts, inhibition zone, Zingiber officinale, Cinnamomum zeylanicum, phytochemical
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الفعالية التثبيطية لمستخلصات الزنجبيل والقرفة ضد فطر

Penicillium notatum و Aspergillus niger

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الخلاصة

استخدمت المستخلصات المائية والإيثانولية من الزنجبيل (Cinnamomum) والقرفة (Zingiber officinale) للتحقيق في نشاطها مضاد للفطريات المسببة للأمراض الفطرية وتفاوت المواد الغذائية. أجريت دراسة التأثير المثبط لهذه المستخلصات بشكل منفصل عن (Aspergillus niger, Penicillium notatum) (معجون الطماطة, الإجبار)، وتم استزراعها على وسط السبايرو دكتر العنب (SDA) في درجة الحموضة 5.6 (pH). ودرجة حرارة 28°C وقد أظهرت النتائج ان كلا المستخلصات النباتية أظهرت نشاطاً مضاداً للفطريات. عند مقارنة التركيز 20, 40% من المستخلصات الإيثانولية، حيث أظهرت المستخلصات الكحولية بشكل ملحوظ. (16-22) و(19-25) ملم لكل من Zingier officinale و (16-22) و(19-25) ملم لكل من (Aspergillus niger و Penicillium notatum) على التوالي بينما كانت فعالية المستخلص المائي (5-6) ملم وضد Aspergillus niger و (7-8) ملم، وكان P. notatum أكثر تضرراً من لجميع التراكيز بينما P. notatum
Introduction

Numerous scientific researchers have noticed within the field of an effectiveness of antibiotics ageist microorganisms (bacteria and fungi) That utilization of plant extracts as a microorganism is considered to be safer for its side effects for many reasons like low cost and most, Ease of access, and their accessibility (1). Intemperate uses of chemicals compound as preservatives frequently has initiated resistant microorganisms, thereby leading to occurrence of emerging foodborne diseases (2). The extract of spices were recognized as wide spectrum antimicrobial which could be present in food preservation systems as the main antimicrobial agents. The effect of spices and their products had been recognized to stabilize foods against the degradation of microbes. This can be observed when the spice initially shows a high microbial accountability and it is hard that some plants contain biologically active compounds that inhibit the growth of microbes (3). Therefore, continuously, the growth of microbes becomes slow as time progresses and is completely suppressed in the end. Cinnamon and ginger are spices known to contain several bioactive compounds that can be used. (4). Cinnamon extract has been reported continuously to be an antifungal activity. As an antidote to a wide range of microorganisms. This activity is mainly attributed to the most important chemical compounds such as the presence of cinnamaldehyde and Eugenol compounds (5), as well as volatile oils that used as antimicrobial and antifungal agents in creams, toothpaste, and soaps (6). Recently many studies are shown the possible effects of two compounds isolated from C. cassia against myocardial ischemia, aldehyde cinnamic and cinnamic acid (7), indicating that cinnamon also has the adequacy to be used to treat cardiovascular disease. Zingiber Officinal, Family Zingiberasi is one of the most essitional and common spices with a variety of applications in many countries. The health significance of ginger is often attributed to its rich plant constituents, which vary depending on the place of origin and shape of the roots, for example, dry, and new variety. It has various medicinal activities including antioxidants, anti-inflammatory, anticancer activities, Blood diseases and other (8).

Materials and Methods

- Sample collection: The fungal isolates that were used in this study belonged to Aspergillus niger and Penicillium notatum species which were isolated and cultivated in lab from imported canned of tomato paste and cheese samples, All fungi were identified laboratory according to the morphological features as well as relying on microscopic characteristics such as the shape and size of the conidial heads and the nature of their walls, (9) pure isolates were saved on Sabouraud’s dextrose agar (SDA) and kept in the refrigerator until used.
- Preparation of plant extracts: Cinnamon barks and Ginger powder (Zingiber officinale) used in the present study were purchased from the local market of diwaniha city, two types of extracts; such as aqueous, ethanol were prepared separately.
Preparation of extracts:
1. **Aqueous extract**: 20 g of ginger and cinnamon powder mixed with 200 ml of distilled water separately with mixing (shaker) continuous with the blender for five minutes. Then, stopping for three minutes, repeated this process five times. The process was performed in the atmosphere of room 25 °C. The extracts were dried using vacuum rotary evaporator at a temperature of 50 °C. The extracts were then weighed and stored at -4 °C.

2. **Ethanolic extract**: The alcoholic extract was prepared in the same way as the previous except the replacement of water with 70% ethyl alcohol using the Soxhlet apparatus. The final product were extracted and dried using Vacuum rotary evaporator under 50 °C and then storage at 4 °C until use.

Sterilization of extract and preparation of stock solution: According to the preparation and sterilization of stock solution, was done by dissolved 1, 2 ml of stock solution plant extract in 19.38 ml of distilled water. Sterilization by filtration using filter sheets (0.45) micron to remove the bacterial contaminants present in a sterile storage solution. This solution was used as a source of concentrations (20, 40)% mg/ml.

Test the antifungal effectiveness of Aqueous and alcohol extracts: Determination of the sensitivity of fungal isolates to plant extracts under study was performed by using agar well Diffusion method according to (19) by (SDA) Sabouraud's dextrose agar. The effect of both cinnamon barks and ginger against mycelial growth of P. notatum and A. niger are presented in Tables (2, 3). The determined of antifungal activity done by measuring the diameters of inhibition zone produced by these extracts against the two fungi. According to (12) by measurement diameter of inhibition zone (mm).

Statistical Analysis: The Statistical Analysis System- Spss- tow way ANOVA was used to analyze the differences between groups (13). The results contrast statistically to the least significant difference (LSD) to level (0.05).

Results

Phytochemical Test: The produced aqueous- and alcoholic-based extracts of ginger and Cinnamon were evaluated for quality purposes using some phytochemical tests targeting some of the chemical components of these extracts, table (1)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Ginger Aqueous extract</th>
<th>Ginger Ethanolic extract</th>
<th>Cinnamon Aqueous extract</th>
<th>Cinnamon Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ positive, -negative

1. **The inhibitory effect of Aqueous extract**: The aqueous extracts showed moderate inhibitions of the fungal growth in all isolates with a low but significant inhibition that was observed in P. notatum as shown in table (2).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Aqueous extract 20% mg/ml</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ginger IZ (mm)</td>
<td>Cinnamon IZ (mm)</td>
</tr>
<tr>
<td>A. niger</td>
<td>5.33±0.33</td>
<td>9±1</td>
</tr>
<tr>
<td>P. notatum</td>
<td>7±0.57</td>
<td>11.66±0.33</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.443</td>
<td></td>
</tr>
</tbody>
</table>
2. The inhibitory activity of the alcoholic extract: The alcohol extract showed a significant effectiveness with respect to all fungal isolates under study and as indicated.

Table (3) The average diameter of inhibition zone (mm) of cinnamon and Ginger Ethanolic extract against P. notatum and A. niger

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Ginger mg/ML</th>
<th>%Ethanolic extract</th>
<th>Cinnamon mg/ML</th>
<th>%Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>40%</td>
<td>20%</td>
<td>40%</td>
</tr>
<tr>
<td>A. niger</td>
<td>11.66±0.66</td>
<td>3.91*</td>
<td>17.33±0.66</td>
<td>4.33*</td>
</tr>
<tr>
<td>P. notatum</td>
<td>15±0.57</td>
<td>21.33±0.66</td>
<td>24.66±0.57</td>
<td>4.1*</td>
</tr>
</tbody>
</table>

LSD_{0.05} = 2.165

*significant differences between groups

Discussion

Deterioration of Foods which exposed to the fungi could be decreased in its sensory, nutritious and medicinal properties. Several studies have also clarified the fungus's responsibility for the formation of taste and the production of those fungal-made allergenic toxins (14). Spice and its product have an inhibitory effect of on a variety of microorganisms have been described in much scientific research and although there is considerable variation in resistance to various microorganisms to certain spices and microorganisms themselves to different spices as observed (15). Antifungal effects of cinnamon and ginger extracts against the studied fungi may be due to the presence of these bioactive constituents. The results which obtained showed that the phytochemical analysis of these extracts of cinnamon and ginger as shown in Table (1) indicated a wide range of various components and secondary metabolites of plant extracts in both ginger and cinnamon which have the potential of active antifungal agents of P. nutatum and A. niger. Several researchers have recorded these antimicrobial properties, of plant extracts and attributed to the presence of biologically active compounds in parts of this plant (16, 17) in the present research. The results in (tables 1 and 2) show that alcoholic extracts of cinnamon and ginger were more effective than their aqueous extracts in inhibiting the mycelial growth of P. nonatum and A. niger. This finding is in agreement with previous investigation which stated that the extracts of garlic can inhibit mound growth, and the effectiveness of this inhibition is related to the solvent used in the extraction (18). Several investigators revealed that eugenol exerts antifungal activity against Aspergillus spp. and Penicillium spp. that present in various foods (19) of both fungi were inhibited at all concentrations (20, 30)% mg/ml and the zones of inhibition were increased in diameter as the concentrations of both extracts increased. Several investigators showed eugenol to exert antifungal activity against Aspergillus spp. and Penicillium spp. in various foods (20). Table (3) showed that ethanolic extracts exhibited moderate to strong antifungal activity (13-21) mm against both fungi based on the final concentration and, as with cinnamon, the inhibition increased as the concentrations of both extracts increased. Ethanolic extracts of cinnamon exhibited higher inhibition activity against P. noitatum than A. niger at all concentrations. The reason of such activity might have been due to the presence of a major bioactive constituent in the bark of cinnamon which is Cinnamaldehyde. Some reports indicated that cinnamaldehyde killed (80%) of fungi and bacteria (21). In addition to eugenol and cinamic acid flavonoids, alkaloiks, and tannins which recognized by some investigators as antifungal agents (22), whereas the range of diameters of inhibition zone for ethanolic extract against P. noitatum recorded between (8-16) mm and against A. niger recorded (0.0-14) mm. The distinction of the alcohol extract may be due to the fact that the
active substances are soluble in alcohol in water and the ability of the alcohol solvent to pull these active compounds because of its high polarity. Based on the final concentration, ethanolic extract showed a significant reduction in the mycelial growth of \textit{P. notatum} compared with that against \textit{A. niger}. Results showed that there were no inhibition activity at concentrations (10%) mg/l in extracts. The results in a table (2) explained the inhibition activity of the aqueous extracts of the cinnamon and ginger based on the concentration (40%) mg/ml, and it also showed that cinnamon-ethanolic extract exerted higher inhibition activity than that of ginger. On the other hand, aqueous extracts of ginger exhibited slight or no inhibition activity (6-15) mm, but Cinnamon-aqueous extract showed significantly higher inhibition activity against \textit{P. notatum} (9-15) mm than \textit{A. niger} (6-9) mm. The reason may be attributed to the missing of the effective ingredients in the aqueous extract due to lack of the chemical use during the extraction (9) and water unviability to dissolve the plant and release its active materials. The present study showed the potential antifungal activity of the of \textit{Cinnamomum zeylanicum} against the \textit{Penicillium notatum} and \textit{Aspergillus niger} fungi.

References