

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

Esraa Taher Muslim, Asseel Abdulridha Saeed and Shaimaa Abbas Sabeeh
College of Veterinary Medicine/ University of Al-Qadissiaha

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. Ethanolic 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. notatum* and *A. niger* respectively while the inhibitory observed by the aqueous extract (5-6) mm against *A. niger* for (6-8) mm. Against *P. notatum* 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
e-mail: assel78.saeed@gmail.com.

الفعالية التثبيطية لمستخلصات الزنجبيل والقرفة ضد فطر

Penicillium notatum و *Aspergillus niger*

اسراء طاهر مسلم، اصيل عبد الرضا سعيد وشيماء عباس صبيح

كلية الطب البيطري/ جامعة القادسية

الخلاصة

استخدمت المستخلصات المائية والإيثانولية من الزنجبيل *Zingiber officinale* والقرفة *Cinnamomum zeylanicum* للتحقيق في نشاطها المضاد للفطريات المسببة للأمراض الفطرية وتلف المواد الغذائية. أجريت دراسة التأثير المثبط لهذه المستخلصات بشكل منفصل *Aspergillus niger*, *Penicillium notatum* المعزولة عن (معجون الطماطة، الاجبان)، وتم استزراعها على وسط السابورود سكر العنب (SDA) في درجة الحموضة 5.6 pH ودرجة حرارة 28م° وقد أظهرت النتائج ان كلا المستخلصات النباتية أظهرت نشاطا مضادا للفطريات. عند مقارنة التراكيز 20، 40% من المستخلصات الايثانولية، حيث أظهرت المستخلصات الكحولية *Cinnamomum zeylanicum* أعلى نشاط تثبيطي من مستخلصات *Zingiber officinale* بشكل ملحوظ. (16-22) و (19-25) ملم وضد لكل من *P. notatum* و *A. niger* على التوالي بينما كانت فعالية المستخلص المائي (5-6) ملم وضد *Aspergillus niger* (7-8) ملم. وكان *P. notatum* الأكثر تضررا من لجميع التراكيز بينما *Aspergillus niger*

كان أقل عرضة. وكان هناك فرق معنوي ($P= 0.05$) في الفعالية المضادة للفطريات. وازدادت فعالية المستخلصات الايثانولية مع زيادة في تركيزاتها 20، 40%. إن قدرة هذه المستخلصات على تثبيط نمو الفطريات يجب أن تأخذ في الاعتبار مؤشرا على فعالية التثبيطية ضد الفطريات لكل من القرفة والزنجبيل، مما يجعلها المرشحة لإنتاج عوامل مضادة للفطريات.

الكلمات المفتاحية: الفطريات، استخلاص، الفعالية التثبيطية، مستخلصات الزنجبيل، والقرفة.

Introduction

Numerous scientific researchers have noticed within the field of an effectiveness of antibiotics against 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 essential 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 (m). The extracts were dried using vacuum rotary evaporator at a temperature of 50 °C. The extracts were then weighed and stored at -4 °C (10).
- 2. **Ethanollic extract:** The alcoholic extract was prepared in the same way as the previous except the replacement of water with 70% ethyl alcohol (11) using the Soxhlet apparatus. The final product were extracted and dried using Vaceum rotary evaporator under 50 °C and then storage at 4 °C until use.
- **Sterilization of extract and preparation of stock solution:** According to (12) 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 (1) Phytochemical analysis of alcoholic and aqueous extracts of cinnamon and ginger

Constituents	Ginger		Cinnamon	
	Aqueou extract	Ethanollic extract	Aqueou extract	Ethanollic extract
Flavonoids	+	+	+	+
Phenols	-	+	+	+
Terpenes	-	+	-	+
Alkaloids	+	+	-	+
Tannins	-	+	+	+
Glucose	+	+	+	+

+ 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 (2) the average diameter of inhibition zone (IZ) of cinnamon and Ginger aqueous extract against *P. notatum* and *A. niger*

Fungi	Aqueous extract 20%mg/ml			
	Ginger		Cinnamon	
		IZ (mm)		IZ (mm)
<i>A. niger</i>	5.33±0.33	2.24	9±1	1.66
<i>P. notatum</i>	7±0.57		11.66±0.33	
LSD_{0.05}	2.443			

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*

Fungi	mg/ML %Ethanolic extract							
	Ginger				Cinnamon			
	20%		40%		20%		40%	
<i>A. niger</i>	11.66±0.66	3.91*	17.33±0.66	4.33*	11.66±0.66	3.1*	20.66±0.57	4.1*
<i>P. notatum</i>	15±0.57		21.33±0.66		14.66±0.57		24.66±0.57	
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 mould 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 *P. notatum* compared with that against *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 *P. notatum* (9-15) mm than *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 *Cinnamomum zeylanicum* against the *Penicillium notatum* and *Aspergillus niger* fungi.

References

1. de Boer, H. J.; Kool, A.; Broberg, A.; Mziray, W. R., Hedberg, I. & Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *J. Ethnopharmacol.*, 96(3): 461-469.
2. Akinpelu, D. A. (2001). Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia*, 72(3):286-287.
3. Matasyoh, J. C.; Maiyo, Z. C.; Ngure, R. M. & Chepkorir, R. (2009). Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. *Food Chem.*, 113(2):526-529.
4. Kizil, S. & Sogut, T. (2003). Investigation of antibacterial effects of spices. *Crop Research*, 3: 86-90.
5. He, Z. D.; Qiao, C. F.; Han, Q. B.; Cheng, C. L.; Xu, H. X.; Jiang, R. W.; Put, P. P. & Shaw, P. C. (2005). Authentication and quantitative analysis on the chemical profile of cassia bark (*cortex cinnamomi*) by high-pressure liquid chromatography. *J. Agri. Food Chem.*, 53(7): 2424-2428.
6. Lis-Balchin, M.; Hart, S. L. & Deans, S. G. (2000). Pharmacological and antimicrobial studies on different tea-tree oils (*Melaleuca alternifolia*, *Leptospermum scoparium* or Manuka and *Kunzea ericoides* or Kanuka), originating in Australia and New Zealand. *Phytother. Res.*, 14(8): 623-629.
7. Song, F.; Li, H.; Sun, J. & Wang, S. (2013). Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats. *J. Ethnopharmacol.*, 150 (1): 125-130.
8. Shukla, Y. & Singh, M. (2007). Cancer preventive properties of ginger: a brief review. *Food Chem. Toxicol.*, 45(5):683-690.
9. David, E.; Stephen, D.; Helen, A.; Rosemary, H. & Obyn, B. (2007). Descriptions of Medical Fungi. Second Edition, School of Molecular & Biomedical Science University of Adelaide.
10. Reuter, H. D.; Dock, H. P. & Lawson, D. L. (1996). Therapeutic effects and applications and its preparations in garlic. The Science and Therapeutic applications of *Allium sativum* L. and related species. 2nd Ed., PP. 135- 212.

11. McClure, J. W. (1975). Physiological and Functional of Flavonoids. In: The Flavonoids, ed., Harbone, J. B.; Mabray, T. J. & Marbray, H., Chapter 18, New York, Sanfrancisco.
12. Monks, N. R.; Lerner, C.; Henriques, A. T.; Farias, F. M.; Schapoval, E. E. S.; Suyenaga, E. S.; da Rocha, A. B.; Schwartzmann, G. & Mothes, B. (2002). Anticancer, antichemotactic and antimicrobial activities of marine sponges collected off coast of Santa Catarina, southern Brazil. J. Exp. Mar. Biol. Ecol., 281:1–12.
13. Cary, N. C. (2004). SAS. Statistical Analysis System. User's Guide. Statistical. Version 7th ed. SAS. Inst. Inc. USA.
14. Barkat, M. & Bouguerra, A. (2012). PStudy of the antifungal activity of essential oil extracted from seeds of *Foeniculum vulgare* Mill. for its use as food conservative. Afr. J. Food Sci., 6(9): 239-244.
15. Abad, M. J.; Ansuategui, M. & Bermejo, P. (2007). Active antifungal substances from natural sources. Arkivoc. (VII) PP. 116-145.
16. Owolabi, O. J.; Omogbai, E. K. I. & Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. Afr. J. Biotechnol., 6(14): 1677-1680.
17. Sher, A. (2009). Antimicrobial activity of natural products from medicinal plants. Gomal. J. Med. Sci., 7 (1): 72-78.
18. Irkin, R. & Korukluoglu, M. (2007). Control of *Aspergillus niger* with garlic, onion and leek extracts. Afr. J. Biotechnol., 6(4): 384-387.
19. Vazquez, B. I.; Fente, C.; Franco, C. M.; Vazquez, M. J. & Cepeda, A. (2001). Inhibition effects of eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese. Int. J. Food Microbiol., 67(1-2): 157-163.
20. Zaker, M. & Mosallanejad, H. (2010). Antifungal activity of some plant extracts on *Alternaria alternata*, the causal agent of *Alternaria* leaf spot of Potato. Pak. J. Biol. Sci., 13(21): 1023-1029.
21. McCann, J. (2003). Herbal medicine handbook, 2nd Ed., Philadelphia. Lippincott.
22. Rios, J. L.; Recio, M. C. & Villar, A. (1987). Antimicrobial activity of selected plants employed in the spanish mediterranean Area. J. Ethnopharmacol., 21(2):139-152.