

In vitro Antifungal Activity of Some Indigenous Medicinal Plant Extracts against Five Isolates of *Aspergillus fumigatus*

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Sharaf *et al.*: Antifungal Activity of Medicinal Plant Extracts against *Aspergillus fumigatus*

There is a constant exposure of humans to *Aspergillus* mold fungus and 95 % of the pathogenic species. It is important to study drugs with new efficacy against infections because of increased prevalence of infections resistant to antibiotics. The present study aims to assess the antifungal activity of aqueous and ethanol plant extracts (cinnamon, garlic, ginger and guava) against five isolates of the opportunistic mold *Aspergillus fumigatus*. The results showed that effect of ethanol and aqueous extracts of cinnamon was same on all the five isolates of *Aspergillus fumigatus*. The recorded diameters of inhibition zone for isolates 1, 2, 3, 4 and 5 exerted by the cinnamon ethanol extracts were 41, 46, 40, 45 and 46 mm respectively. Moreover, ginger was excluded in next experiments because all the isolates showed resistance against the aqueous and ethanol extract of ginger, except for isolate 5. Minimum inhibitory concentration was 2 mg/ml ethanol extract of garlic and cinnamon whereas that of aqueous extract of garlic and cinnamon was 40 mg/ml and 10 mg/ml, respectively. The study concluded that ethanol extract of cinnamon can be used as antimycotic agent against *Aspergillus fumigatus* to treat diseases.

Key words: Antifungal activity, *Aspergillus fumigatus*, extracts, aspergillosis, toxicogenic

Aspergillus fumigatus (*A. fumigatus*) is a known fungus belonging to genus *Aspergillus*. This fungus results in the development of certain disease among the immune compromised individuals and cause allergic reactions^[1]. The infection caused by *A. fumigatus* is called aspergillosis that is a life threatening fungal infection^[2]. Thousands of min grey-green conidia are produced in fungal colonies from the conidiophores that may become airborne^[3]. These spores are present in the atmosphere and can be inhaled. There is increased occurrence of *A. fumigatus* among the poultry flocks causing mild or even severe forms of diseases, which results in greater economic losses. The severity of infection outcome depends on the hygienic conditions and immune system of different animals^[4]. The concentration of fungal spores and bacteria is likely to increase in the atmosphere as a result of warm temperature, degraded litters, poor ventilation, long-term feed storage and excessive ammonia and moisture^[5,6].

The broad spectrum of diseases caused by the members of genus *Aspergillus* are encompassed as aspergillosis, which develop as a result of contaminated intra-hospital

environments, direct inoculation through surgical tools, inhalation of toxic compounds, air conditioners and mechanical ventilation^[7,8]. It has been shown that the antifungal drugs that are used currently are only partially effective in treating the infection^[9]. There is increase in the appearance of *Aspergillus* strains in the hospital settings^[10,11]. There is need to identify new targets and develop new antifungal agents as therapeutic alternatives that would be effective antifungal agents^[12].

The medicinal plants containing active cheap constituents are economic and useful in treating much human-related disease^[13,14]. The oil of four Iranian herbs including, *Thymus daenensis* var *daenensis* Celak, *Zataria multiflora* Boiss, *Thymbra spicata* var. *spicata* L. and *Bunium persicum* (Boiss) for antifungal activity against *A. niger*, *A. fumigatus*, *A. flavus* and

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A. parasiticus was investigated by Pirbalouti *et al.*^[15]. The results showed that the oils from *Thymus daenensis* and *Thymbra spicata* are likely to be used as natural antifungals for preserving food and health of humans. Another study conducted by Mann *et al.*^[16] showed that aqueous root extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* inhibit the growth of the all tested organisms. Moreover, the minimum fungicidal concentration (MFC) ranged between 0.04 and 0.08 µg/ml; while, minimum inhibitory concentration (MIC) of the extracts ranged between 0.03 and 0.07 µg/ml. A large growth inhibition zone was developed by Carmo *et al.*^[17] as he reported strong antifungal activity and growth inhibition for *Cinnamomum zeylanicum* (*C. zeylanicum*) on the growth and morphogenesis of some potentially pathogenic *Aspergillus* species. The decreased condition, loss of pigmentation, cytoplasm leakage and disrupted cell structure are the major morphological changes observed as a result of degradation of the fungal wall. Therefore, Carmo *et al.*^[17] concluded that protection against growth of *Aspergillus* species could be provided by *C. zeylanicum* essential oil as it works as potential antifungal compound.

The health of individuals is significantly affected as a result of increased prevalence of drug resistance among the infectious agents. Doust *et al.*^[18] demonstrated the incidence and spread of drug resistance among various agents including fungi. Nowadays, conducting research on drugs with new efficacy against infections is important because of increased in the infections resistant to antibiotics^[19,20]. Humans are constantly exposed to *Aspergillus* mold fungus and 95 % of the pathogenic species include three species of *Aspergillus* including *A. fumigatus*, *A. flavus* and *A. niger*^[21]. These species may cause local infections in different body organs like lungs and sinuses, no matter how healthy an individual is^[22]. Moreover, this infection can result in severe consequences among individuals with deficient immune systems. None of the previous studies have elucidated effect of the most potent natural antifungal plant extract on some growth criteria of *A. fumigatus*. Therefore, the present study aims to assess the antifungal activity of ethanol plant extracts against the opportunistic mold *A. fumigatus*.

MATERIALS AND METHODS

The fresh plant samples including ginger, garlic, cinnamon sticks and guava leaves used in this study were purchased from Al-Madinah local market.

Five isolates of *A. fumigatus* were obtained from the Microbiological Laboratory of the Department of Biology, Taibah University. *A. fumigatus* isolates were confirmed by Raper & Fennell^[23] and El-Shafie^[24] to belong to genus *Aspergillus*.

Culture medium:

The culture medium that is Czapek Dox's agar medium used in this study comprises of following constituents: 20 g/L sucrose; 3 g/L sodium nitrate (NaNO₃); 1 g/L potassium dihydrogen phosphate (KH₂PO₄); 0.5 g/L magnesium sulfate heptahydrate (MgSO₄·7H₂O); 0.5 g/L potassium chloride (KCl); 0.05 g/L ferrous sulfate pentahydrate (FeSO₄·5H₂O); 20 g/L agar; 1 L distilled water.

Preparing plant extracts:

The fresh plant samples were washed, sterilized, cut and dried at room temperature. The dried form of plant extracts was converted into fine dry powder in electric grinder. The hot aqueous extract was prepared by soaking 20 g of plant powder in 100 ml distilled water and the mixture was left at 60° in water bath, for 30 min. Whereas, ethanol extract was prepared according to Varghese^[25] method by adding 20 g of plant powder to 100 ml of 95 % ethanol and left overnight then filtered. The produced filtrate was of 20 % (w/v) concentration (200 mg/mL). The suspension was centrifuged at 5000 rpm for 10 min and filtered through a Whatman No .1 filter paper. The supernatant fluid was allowed to evaporate under pressure through the rotary evaporator apparatus. The concentrated organic solvent extracts were dissolved in 10 % dimethyl sulfoxide (DMSO) and kept at -20°^[26].

Antifungal activity:

The agar well diffusion method proposed by Hadacek *et al.*^[27] was used to carry out the inhibitory activity of plant extracts. The plates (triplicates) were seeded with 1 ml of fungal homogenous spore suspension of 5 d old culture and after inoculum absorption wells were made using sterile cork borer (9 mm diameter) then 100 µl of the extracts were introduced into each well. The plates were left for 30 min for better absorption. Later, the plates were incubated at 28° for 48 h. Control was conducted using either sterile distilled water or DMSO. Measurement was obtained for the diameter of the produced inhibition zones after subtraction of control value and taken as a criterion for the inhibitory activity.

Extraction using different organic solvents:

Different solvents were prepared^[25] and tested to obtain the best one for extraction, because of increased higher antifungal activity of aqueous and ethanolic extract of the tested plants against isolate 2 of *A. fumigatus*. Antimicrobial activity of the prepared plant extracts was tested against isolate 2 of *A. fumigatus*. Control was conducted with each solvent separately and means were calculated after subtraction of control value (DMSO). Aqueous and ethanol cinnamon and garlic extracts were chosen for the next experiments as they helped in achieving the most potent antimicrobial activity.

Determining minimum inhibitory concentration (MIC):

MIC was determined using different concentrations of either ethanol extract or hot water extract of both cinnamon and garlic as explained by Gayoso *et al.*^[28].

Data analysis:

The data was added and analyzed using Statistical package of social sciences (SPSS). Analysis of variance (ANOVA) was used to determine the significant differences.

RESULTS AND DISCUSSION

Table 1 shows the antifungal activities of four plant extracts selected in the present study. Significant variation of inhibition was exerted by the plant extracts on the growth of five isolates of *A. fumigatus* ($p \leq 0.05$). Isolates 1 and 2 were affected by the ethanol extract of guava leaves, with 36 mm and 30 mm of inhibition zone diameters, respectively. However, isolate 1 was affected by hot water extract with inhibition zone diameter of 40 mm. The effect of ethanol and aqueous

extracts of cinnamon was same on all the five isolates of *A. fumigatus*.

For isolates 1 to 5, the recorded diameters of inhibition zone exerted by the cinnamon ethanol extract were 41, 46, 40, 45 and 46 mm respectively. As compared to the ethanol extract of cinnamon, the aqueous extract of cinnamon was less effective and the diameter of inhibition zone of isolates 1, 2, 3 and 4 were 20, 23, 21 and 19 mm respectively. There was no impact of aqueous extract of cinnamon on isolate 5. Inhibition effect against isolates 2, 3 and 5 was shown in the presence of aqueous extract of garlic; however, other isolates showed resistance against the aqueous extract of garlic. On the contrary, the ethanol extract of garlic was able to exert its effects in isolates 1 and 2 (inhibition diameter was 20 and 44 mm, respectively). In the similar context, ginger was excluded in next experiments because all the isolates showed resistance against the aqueous and ethanol extract of ginger, except for isolate 5. Isolate 5 was weakly affected by aqueous extract of ginger with 15 mm diameter of inhibition zone.

Different solvents were tested for selecting the most effective one because the ethanol extract of all the tested plant extracts was inhibitory to the most sensitive isolate 2 (Table 2), except for ginger. Therefore, the best solvent for extraction was ethanol, as it exerted significant inhibitory activity ($p \leq 0.05$), as compared to methanol, ethyl acetate and chloroform. Moreover, there was significant effectiveness of ethanol extract of garlic and cinnamon, as compared to the guava extracts. However, there was no impact of chloroform extract of guava leaves on isolate 2 of *A. fumigatus*.

The results demonstrate that cinnamon and garlic different solvent extracts showed the prominent antifungal activity against isolate 2 of *A. fumigatus*

TABLE 1: ANTIFUNGAL ACTIVITY OF THE TESTED PLANT EXTRACTS AGAINST *A. fumigatus* ISOLATES EXPRESSED AS DIAMETER OF INHIBITION ZONE (mm)

<i>A. fumigatus</i> isolates	Diameter of inhibition zone (mm)							
	Plant extracts							
	Guava leaves		Cinnamon		Garlic		Ginger	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
I ₁	40	36	20	41	-	20	-	-
I ₂	-	30	23	46	19	44	-	-
I ₃	-	-	21	40	18	-	-	-
I ₄	-	-	19	45	-	-	-	-
I ₅	-	-	-	46	37	-	15	-

(-) means no inhibition; least significant differences (LSD) at 5 % between isolates-3.0; LSD at 5 % between plants extract-5.9

fig. 1A-1D. Therefore, MIC against isolate 2 of *A. fumigatus* was determined using these two plant extracts. Table 3 has illustrated MIC for garlic and cinnamon against isolate 2 of *A. fumigatus*, after incubation of 48 h. The table shows that MIC of 2 mg/ml ethanol extract of garlic and cinnamon with inhibition zones of 17 and 20 mm respectively. Moreover, MIC of aqueous extract of garlic was 40 mg/ml with 18 mm of inhibition diameter; whereas, MIC of aqueous extract of cinnamon was 10 mg/ml with 11 mm of inhibition diameter.

Majority of the dreadful diseases whether animal or plant are controlled using economically essential medicinal plants. The present study has evaluated the inhibitory effect of alcoholic and aqueous extracts of ginger, garlic, cinnamon and guava against the isolates of *A. fumigatus*. There was a difference in the degrees of inhibition among the four plant extracts; however,

TABLE 2: ANTIFUNGAL ACTIVITY OF THE TESTED PLANTS AGAINST ISOLATE 2 OF *A. fumigatus* AFTER EXTRACTION WITH DIFFERENT SOLVENTS

Solvents	Diameter of inhibition zone (mm)		
	Plant extracts		
	Guava leaves	Cinnamon	Garlic
Ethanol	23	47	43
Methanol	17	41	10
Chloroform	-	28	7
Ethylacetate	22	40	30

(-) means no inhibition; LSD at 5 % between plants extract-5.2; LSD at 5 % between solvents-6.0

isolates 1 and 2 were affected by ethanol extract of guava leaves. Resistance was shown by all the isolates against aqueous and ethanol extract of ginger. Antifungal activity is exhibited by all the prepared water and ethanol cinnamon extracts against the isolates of *A. fumigatus*. This finding stated that inhibitory effect of cinnamon ethanol extract for the isolates was in line with the study conducted by Rodriguez *et al.*^[29]. The results demonstrated that antimicrobial activity against *Rhizopus stolonifer* and *Fusarium oxysporum* (*F. oxysporum*) f. sp. *lycopersici* (Sacc) was presented in the presence of various aqueous and alcohol extracts/essential oil distillates.

Previous studies have also exhibited antimicrobial activity of aqueous cinnamon extract for *F. culmorum* Sacc, *F. oxysporum*, *A. niger*, *Alternaria alternata*, along with some hydrolytic enzymes^[30-31]. These hydrolytic enzymes include pectin, protease, lyase and Beta-glucosidase. Similarly, the activity of *Syzygium*, *Cymbopogon*, and *Cinnamomum* species against *Trichophyton rubrum* (*T. rubrum*) and *A. fumigatus* was reported by Khan and Ahmad^[32]. Similar to the present study, cinnamon was proved to be effective against some species of toxicogenic fungi and respiratory tract pathogens.

The results of present study showed that antifungal activity against *A. fumigatus* isolates was exhibited by aqueous and ethanol garlic extracts. These results were consistent with the study conducted by Natheer^[33]. The study exhibited strong inhibitory effect by diethyl ether extract of cinnamon for the tested bacteria and

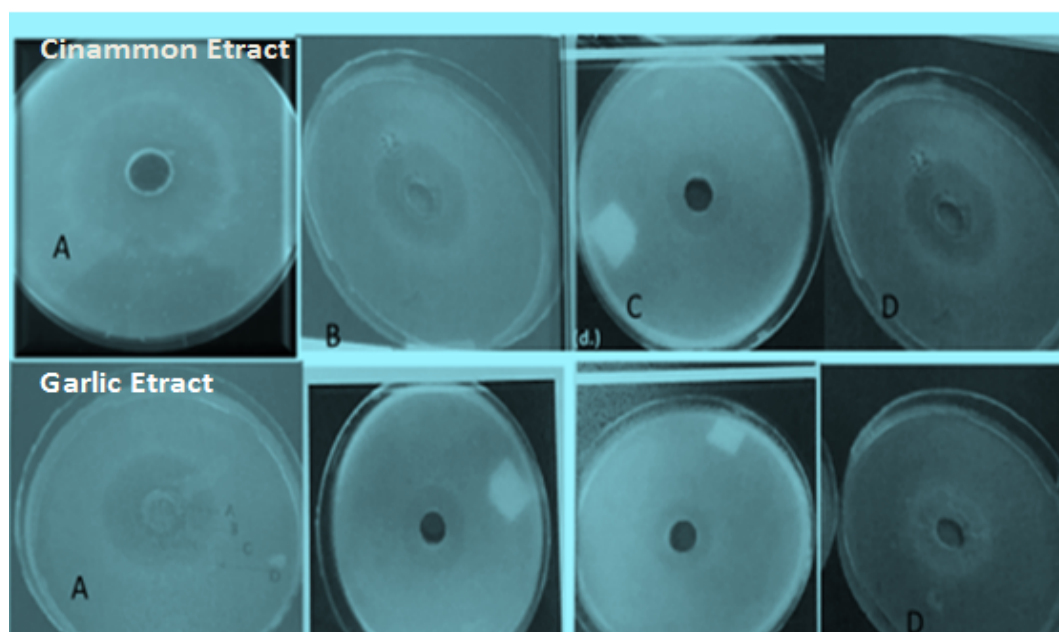


Fig. 1: Inhibitory activity of the different solvent extracts of cinnamon and garlic against *A. fumigatus* I₂ (A) ethanol; (B) methanol; (C) chloroform; (D) ethylacetate

TABLE 3: MIC OF GARLIC AND CINNAMON EXTRACTS AGAINST ISOLATE 2 OF *A. fumigatus*

Plant extracts		Inhibition zone (mm)					
		Concentrations (mg/ml)					
		1	2	6	10	20	40
Cinnamon	Aqueous	-	-	-	11	12	13
	Ethanol	-	20	24	30	32	35
Garlic	Aqueous	-	-	-	-	-	18
	Ethanol	-	17	20	22	23	25

Candida albicans (*C. albicans*). Nevertheless, the antifungal activities of the ethanol extract of *Zingiber officinale*, mycelial dry weight and *Annona muricata*, *Alchornea cordifolia*, *Garcinia cola* and *Allium sativum* against the mycelial elongation of *Rhizopus stolonifer*, *Botrydipodia theobromae*, *A. niger*, *F. solani*, and *F. oxysporum* was reported by Amienyo and Ataga^[34].

Similar to the results presented in the present study, Dhiman *et al.*^[35] showed that inhibition in growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *A. niger* and *C. albicans* was due to the effect of methanolic extract of *Psidium guajava* (*P. guajava*) using paper disc diffusion method. However, Rathish and Sumitra^[36] had stated inactivity of extracts of *P. guajava* against *A. flavus*, *A. niger* and *A. candidus*.

The present study has tested different solvents for selecting the most effective one, along with the ethanolic extract and presented high antifungal activity of cinnamon, garlic and guava ethanolic extract against isolate 2 of *A. fumigatus*. The ethanol extracts were considered to be powerful inhibitor, as compared to the extracts of methanol and chloroform. These results were similar to a previous study conducted by Senhaji *et al.*^[37] showing active antimicrobial potential of ethanolic and hexanic extracts of cinnamon, as compared to other solvent extracts. On the contrary, antimicrobial activity was shown by methanol extract of *C. zeylanicum* towards microorganisms^[38].

The morphological changes taking place within the endomembrane structures and cell membrane of *A. fumigatus* and *T. rubrum* were observed by Khan and Ahmad^[32] using electron light microscope after being treated with cinnamon oil. Cinnamon and other oils exhibited inhibitory effects against the synthesis of cell membrane and cell wall. The cell membrane along with other endomembrane structures of the cell was the primary target of oils; however, synthesis of cell wall was required due to disruption of cell membrane and other associated enzymes. The growth of microorganism is likely to be inhibited by cinnamaldehyde, which is a

highly electronegative compound interfering with the biological processes that involve electron transfer and reaction with nitrogen containing compounds.

This study has determined MIC based on the preparation procedure used by Gayoso *et al.*^[28] undertaking different concentrations of either ethanol extract or hot water extract of both garlic and cinnamon. These concentrations include 1, 2, 6, 10, 20 and 40 mg/ml, the plates were incubated at 28° and after measuring the diameters of inhibition zone and the inhibitory activity was followed. Inhibition zone equal to 10 mm or more was considered for the MIC, which shows the lowest concentration^[39,40].

The present study has investigated the antifungal activity of ethanol and aqueous extract against the opportunistic fungus *A. fumigatus*. The results showed inhibitory effect of ethanol extract of cinnamon, as compared to ginger, garlic and guava extracts. Therefore, it has been concluded that ethanol extracts of cinnamon can be used as antimycotic agent against *A. fumigatus* to treat diseases, rather than using chemical drugs having side effects. The use of natural products for treating various diseases is considered to be cheap, safe and ecofriendly source that reduce the complications and hazards of using chemical drugs. The study has suggested determining the sensitivity of fungal isolate to cinnamon extract before using it as different responses are provided by isolates in a wide range of extracts.

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Conflict of interests:

The authors declared no conflicts of interest.

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