

Effect of Alcoholic & Water Extracts of *Costusspeciosus* (Koen.) Some Species of *Aspergillus spp.* that Causing of Pulmonary Mycotic Infections (I)

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Abstract

The effect of alcoholic & water extracts of *Costusspeciosus* (Koen.) on some physiological aspects of the most common and the most frequent among mixed fungal infections (Mixed infection) is *A. fumigatus*, *A. niger*, *A. flavus*, *A. terreus* included the vitality and activity of the tested species on the (SDA) and the middle liquid diet (SDB) has also been studying the germination of fungal spores. The study results showed that extracts of plant testing damped growth radiography of the fungi are highly significant when affixed to a center steel, confirmed the test results dry weight of yarn fungi test this effect is down significantly compared to the comparison operators and generally showed transactions plant extracts efficiency inhibition matched perfectly to the anti-fungal Ketoconazole as an anti-fungal standard when All tests and all fungal species

Keywords: *Costusspeciosus* (Koen.), Antifungal activity, *Aspergillus spp.*

Introduction

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in treatment of infectious diseases (Service, 2000). In addition to this, antibiotics are sometimes associated with adverse effects on the hosts like hypersensitivity. Therefore, there is a need to develop alternative antimicrobial material for the treatment of infectious disease from other sources, such as plants (Cordell, 1995). One of these plants are *Costusspeciosus* (Koen.) J.E.Sm., belongs to family Zingiberaceae. It is known as Crepe zinger in English (Choudhury, et al., 2012). Plants have a number of secondary metabolites that serve as molecular of plant defense against predation by microorganisms and at the same time also exhibit medicinal properties for treating several ailments (Mallikharjuna, et al., 2007). Rhizome and roots of this plant is widely used as medicine, it's bitter (Chopra, et al., 2006), useful in treating burning sensation, constipation, leprosy, worm infection, skin diseases (Khare, 2007) fever, antituberculosis (Modamad, et al., 2011), spermatorrhoea (Patterson, et al., 2000), Antifungal (Bandara et al., 1988), antioxidant (Vijayalakshmi & Sarada, 2008), hepatoprotective (Verma & Khosa, 2009) patinflammation and anemia (Tushar Basak et al., 2010). The plant is also used as anti-diabetic agent (Singh et al., 1972) have also been reported.

Aspergillus fungus is one of opportunistic pathogenic fungi that cause disease Aspergillosis and the infection species belonging to the genus *Aspergillus spp.* the phenomenon of cross most frequent among infections fungal mixed (Mixed infection) worldwide and most deteriorating health situation common for patients with weakened immune system (Prescott, et al., 2005). and the number of species potentially unlimited spread on a global scale is widely they are present in tumor soil and water and air (Generally Huang 2007) the term Aspergillosis means infection caused by *Aspergillus fumigatus* in the respiratory tract and can be basis for other types of species such as *A. niger*, *A. flavus* and *A. terreus* (Batra, & Ang, 2006).

Materials and Methods

The research was conducted in the laboratories of the Faculty of Science / Department of Life Sciences / University of Qadisiyah

Plant Materials

Powder of *Costusspeciosus* (Koen ex. Retz.) root was obtained of herbarists store equipped with the consent of the Ministry of Health/ Herbal Medicine Center, and is subject to examination and diagnosis of the health aspects and pharmacological effectiveness. The company has filled in the food business life, Amman – Jordan.

Preparation of Plant Extracts

25g of the dried and powdered form of rhizome of *Costusspeciosus* was extracted successively with methanol 80% (each 250 ml.) using a Soxhlet apparatus. Then collected solution were filtered through Whatman No. 4 filter paper. The extracts were evaporated to dryness under reduced pressure at (50-45°C) by Rotary vacuum evaporator to obtain the respective extracts (Veeramuthu et al., 2012), while the aqueous extract was weight 25g of powder plant, and added to it (250 ml.) of distilled water boiling, then nominated solution through layers of gauze and took the filtrate was dried by electric furnace oven temperature (50-45°C) to while getting the dried powder (Harborne, 1984). and stored in a freeze condition at (4°C) until used for further analysis.

Tested Fungal

The genus *Aspergillus* isolated from sputum samples from patients coming to the clinic advisory Pathogenesis and Respiratory in Diwanayah province and have been diagnosed by study of macrofeatures and microfeatures (De-Hoog, et al., 2000).

Assay antifungal activity

▪ Test the effect of plant extracts in the radial growth of fungi tested :

For the purpose of determining the effectiveness of *Costusspeciosus* extracts on the radial growth of the fungal species tested was the use of technology (poisoned food technique) and by what came way (Tripathy et al., 1976) where the five were tested concentrations are (5, 10, 25, 50.75 %) of each transaction has been added concentration of extracts to the middle diet (SDA) and after intransigence transfer disc diameter (5 mm) from the culture every type of fungal species tested, aged seven days using Cork pore sterile where to put the disc in the middle of the dish and by three replicates for each concentration and type dishes were incubated degree ($28 \pm 2C^\circ$) was the measurement of the growth rate of each type of different concentrations of growth after the arrival to the edge of the dish in the treatment comparison (i.e., after seven days).

▪ Test plant extracts in the dry weight of the tested fungi :

To test the effect of extracts of plant *Costusspeciosus* on the dry weight of yarn fungal species tested in the middle liquid used flasks 250 mL Mode (50) ml of the middle liquid was added to concentrations of extract and as stated in the previous paragraph, and then vaccinated Jugs disc diameter (5 mm) from a farm every type of fungi tested, aged seven days of cuddling under temperature ($28 \pm 2C^\circ$) and after the nomination of fungal growth for each type of filter paper sterile and known weight in an oven temperature ($60 C^\circ$) for a period of (24) hours and by three replicates for each concentration.

▪ Test the effect of plant extracts on the germination of fungal spores test

Used in this test slides concave from the center and put them (0.1 ml) of airborne fungal and mix with (0.1 ml) of extract for each concentration of concentrations of extract water and alcohol using a technique (Slid Spores Germination Technique) (Dixit & Tripathy, 1975), and by three replicates per concentration and put each slice on a plate glass sterile regarding the treatment of comparison has been used a drop of water sterile distilled with a drop of stuck spores without any addition. incubated slides at a temperature $25 \pm 1^\circ C$ for 18-16 hours and then calculated the proportion germination of spores under a microscope by the following equation.

$$\text{The percentage of germination} = \frac{\text{Number of spores germinating}}{\text{The total number of spores}} * 100$$

Statistical Analysis

Moral differences were calculated between equations rates using Duncan test and a probability level of 0.05.

Results

Showed a statistical analysis of the results of the inhibitory effect of extracts of *C. speciosus* to genus *Aspergillus* species tested, all concentrations used, that there are significant differences ($P < 0.05$) between the inhibitory effectiveness of water and alcoholic extracts and standard anti-fungal Ketoconazole rates differing among themselves according to the concentration and species of the fungus. As the rates of fungal colonies diameters were inversely proportional to the concentration of the transaction, as lower rates of growth of colonies diameters greater the concentration of extract and anti-mildew, results have shown significant superiority of the alcoholic extract of plant in vitro inhibition of the radial growth of all types of fungi, Table (1).

The study also confirmed the results of the current table (2) the results of the effect of various transactions in radial growth, has shown once again its ability extracts high inhibitory to the growth of fungi of lower rates of the dry weight of the tested fungi are highly significant in relation to the comparison operators.

In addition, all transactions in different concentrations have significantly affected in the process of germination of spores of all fungal species tested compared with the comparison and treatment at the level of significance (0.05), where the percentage of germination of spores fall increasing the concentration of abstract, Table (3)

Discussion

From the results of the present study clearly shows the sensitivity of all fungal species tested for plant extracts *C. speciosus* and in varying significantly depending on the type of extract concentration and species of fungi, in general, may return the contrast between transactions extracts alcohol and water in their effect counter the growth and germination of spores of fungi test to what it contains chemical compounds basic and secondary schools and the quality of its vehicles effective back to influence the inhibitory, it has been reported that its rhizomes contains diosgenin. (Charkraborty, 2009), prosapogenin B of dioscin, cycloartanol, spirostanol glycoside (steroidal saponins) (Lijuan et al., 2011), β -sitosterol glucoside, gracillin (Khare, 2007), as well as (Gupta et al., 2008) reported number compounds from the rhizomes of *C. speciosus* tetradecyl 13-methylpentadecanoate, tetradecyl-11-methyltridecanoate, 14-oxotricosanoic acid, sitosterol, diosgenin and

other active compounds with antibacterial effect. Generally, the alcoholic extract gave the highest inhibition of aqueous extract of the reason may be due to the polarity of alcohol, which play an important role in extracting some of the active compounds without other solvent which leads to deposition of the largest possible amount of the active compounds during the extraction (Kelmanson, et al., 2000).

Can be explained by the current findings that plant extracts has worked similar to the work of anti-life manufacturers and this is consistent with the (Gayatri&Rajani, 2011), where it was stated that the physiological effects of the compounds effective inhibitory to the growth of fungi may be caused by interference in one of the vital functions of your target and works to neutralize (Bandara et al., 1988). The results of the present study agree with study researchers (Parekh&Chanda, 2008). indicated that Almithanolah extracts of several medicinal plants, including plant *C. speciosus*-fungal activity showed well against three types of fungus *Aspergillus* spp., and consistent with what I said a study researcher Saudi Arabia (Al-Qattan, 2009) proved the effectiveness of the roots of the plant *C. speciosus* Besorth dry against *A. fumigatus* fungi and *A. niger* and yeast *C. albicans* that infect the respiratory tract.

Conclusions

We conclude by the results of the current study that medicinal plants is possible to be a source of products able to control opportunistic pathogenic fungi and of great significance to the development of effective medicines and safe and inexpensive

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Table-1:Effect of *C. speciosus* extract sonthe radial growth of fungitested.

Type of transaction	Concentration (mg / ml)	<i>Aspergillus</i> spp.			
		<i>A.fumigatus</i>	<i>A.niger</i>	<i>A.flavus</i>	<i>A.terreus</i>
		Diameter existence of colonies treatment(mm)			
Alcoholic <i>C. speciosus</i> extract	5	33.5 ^C	35.6 ^D	31.2 ^B	24.7 ^C
	10	25.0 ^D	25.8 ^E	28.5 ^B	22.8 ^C
	25	20.1 ^D	15.8 ^F	19.0 ^C	19.1 ^{CD}
	50	14.8 ^E	11.8 ^F	13.2 ^D	13.1 ^D
	75	10.2 ^E	9.73 ^F	9.73 ^D	8.3 ^E
Aqueous <i>C. speciosus</i> extract	5	57.7 ^B	43.5 ^C	33.7 ^B	33.6 ^B
	10	33.8 ^C	41.1 ^C	29.5 ^B	28.8 ^B
	25	29.4 ^C	37.8 ^D	22.3 ^C	24.4 ^C
	50	21.8 ^D	21.8 ^E	17.0 ^{CD}	23.2 ^C
	75	19.4 ^D	22.1 ^E	15.4 ^D	15.6 ^D
Antifungal Ketoconazole	5	59.2 ^B	67.8 ^B	37.9 ^B	33.6 ^B
	10	54.0 ^B	47.6 ^C	24.4 ^C	30.6 ^B
	25	36.1 ^C	36.1 ^D	23.7 ^C	24.9 ^C
	50	20.3 ^D	29.8 ^{ED}	19.6 ^C	16.4 ^D
	75	12.5 ^E	17.2 ^F	9.56 ^F	0.66 ^E
control	0	90.0 ^A	90.0 ^A	90.0 ^A	90.0 ^A

A-F: Different letters in a columnmeans the existence of significant difference satthe0.05 probability level

Table-2: The effect of *C. speciosus* extract and anti-fungal Ketoconazole in the dry weight of the fungi.

Type of transaction	Concentration (mg / ml)	<i>Aspergillus</i> spp.			
		<i>A.fumigatus</i>	<i>A.niger</i>	<i>A.flavus</i>	<i>A.terreus</i>
		Average dry weight of the yeast mildew (g)			
Alcoholic <i>C. speciosus</i> extract	5	0.38 ^B	0.40 ^B	0.24 ^{BC}	0.37 ^B
	10	0.27 ^C	0.24 ^D	0.19 ^C	0.27 ^C
	25	0.24 ^C	0.21 ^D	0.13 ^C	0.17 ^D
	50	0.12 ^D	0.12 ^E	0.10 ^{CD}	0.11 ^E
	75	0.06 ^E	0.06 ^F	0.05 ^D	0.01 ^F
Aqueous <i>C. speciosus</i> extract	5	0.41 ^B	0.42 ^B	0.27 ^B	0.39 ^B
	10	0.30 ^{BC}	0.33 ^C	0.23 ^{BC}	0.29 ^C
	25	0.25 ^C	0.28 ^C	0.17 ^C	0.20 ^D
	50	0.13 ^D	0.17 ^E	0.12 ^C	0.11 ^E
	75	0.09 ^D	0.12 ^E	0.09 ^D	0.08 ^E
Antifungal Ketoconazole	5	0.34 ^B	0.30 ^C	0.31 ^B	0.30 ^C
	10	0.25 ^C	0.21 ^{DE}	0.26 ^B	0.20 ^D
	25	0.12 ^D	0.16 ^E	0.16 ^C	0.16 ^D
	50	0.01 ^E	0.06 ^F	0.06 ^D	0.06 ^{EF}
	75	0.01 ^E	0.04 ^F	0.06 ^D	0.02 ^F
Control	0	0.86 ^A	0.87 ^A	0.90 ^A	0.85 ^A

A- F: Different letters in a column mean the existence of significant difference at the 0.05 probability level

Table-3: The effect of *C. Speciosus* extract and anti-fungal Ketoconazole in the percentage of germination of spores of fungi tested.

Type of transaction	Concentration (mg / ml)	<i>Aspergillus</i> spp.			
		<i>A.fumigatus</i>	<i>A.niger</i>	<i>A.flavus</i>	<i>A.terreus</i>
		Germination of spores ratio (%)			
Alcoholic <i>C. speciosus</i> extract	5	37.21 ^C	32.99 ^B	28.19 ^B	31.65 ^B
	10	31.55 ^C	29.05 ^B	23.29 ^C	29.25 ^B
	25	25.46 ^D	21.08 ^C	19.04 ^{CD}	22.96 ^C
	50	23.29 ^D	12.77 ^D	17.65 ^D	13.66 ^D
	75	14.90 ^E	10.8 ^D	13.87 ^D	12.86 ^D
Aqueous <i>C. speciosus</i> extract	5	44.29 ^B	40.82 ^B	33.98 ^B	35.25 ^B
	10	40.70 ^B	36.13 ^B	27.63 ^{BC}	29.25 ^B
	25	33.54 ^C	26.73 ^C	23.15 ^C	24.90 ^C
	50	23.27 ^D	21.21 ^C	18.23 ^D	20.56 ^C
	75	14.90 ^E	15.09 ^D	17.58 ^D	16.94 ^D
Antifungal Ketoconazole	5	31.43 ^C	34.06 ^B	31.43 ^B	27.48 ^C
	10	26.64 ^D	24.31 ^C	26.64 ^C	23.15 ^C
	25	22.84 ^D	14.43 ^D	22.84 ^C	14.90 ^D
	50	13.92 ^E	12.77 ^D	13.92 ^D	13.92 ^D
	75	10.58 ^E	11.29 ^D	10.58 ^D	9.58 ^E
Control	0	80.57 ^A	82.30 ^A	82.37 ^A	83.75 ^A

A-E: Different letters in a column mean the existence of significant differences at the 0.05 probability level.

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