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Research Article

Effect of *Isatis* spp. Extraction on the Growth of *Aspergillus niger* and *Candida albicans*

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ABSTRACT

In this study, the crude extraction of *Isatis* spp. (*Isatis tinctoria, Isatis busichaina,* and *Isatis lusitanica*) was investigated for their antifungal activity. Each of methanol, ethanol, and H_2O was used as a solvent. Due to find the most effective part of each species, the plant parts were used separately. The aim of this research was to determine the natural products effect on two common pathogenic fungi *Aspergillus niger* and *Candida albicans.* The results show that most of used plants have significant effect on both used fungal species. Extracted each of flower, *I. tinctoria* was showed the best results comparing to the other used species. Extracted leaf and flower of *I. tinctoria* by methanol were showed the best result on *A. niger* and *C. albicans.* The growth zone was around 90 mm for control and 61 mm at 75% concentration of methanolic and ethanolic extraction. The flower was for followed by the stem. Depend on the results, the methanol was showed highest number, then ethanol and the lowest inhibition zone area was for H_2O extraction.

Keywords: Antifungal activity, *Aspergillus niger* and *Candida albicans, Isatis* spp., pathogenic fungi

INTRODUCTION

A latest study presented that tertiary care clinics are consuming antimicrobial and antifungal agents improperly. This rising trend is an ever-increasing threat to the health of humans who are currently at risk of dying from previously fixable infections. Unluckily, drug resistance (adaptation of the drug target site, drug modification, or restricted drug penetration) improves at a pace quicker than new drug development.[1] This sensation is also happening in the pathogenic microorganism *Candida albicans*, an otherwise commensal kinds found in and on the human body.[2] Modern antifungal agents are less active against *C. albicans* now than when they were first introduced because *Candida* species have developed resistance to these agents. In addition, research by the major pharmaceutical companies in the development of antifungal agents has been on the decline due to low coming back on investment.[3]

MATERIALS AND METHODS

Plant Extracts

Three methods were used to extract water, ethanolic, and methanolic, according to several studies, with some modification as needed and as follows:[4]

Aqueous Extraction

Hot water extraction was performed by mixing 40 g of plant form with 160 ml of distilled water twice (i.e., 1:4 weight:volume), heated to 80–90°C, then stirred and left to cool down and mixed together then placed in refrigerator for 24 h, which was then administered through several layers of medical gauze, and again administered by Buchner funnel using filter papers Whatman No.1 to dispose of the non-powdered parts and fibers to obtain the raw liquid extract and then put it in the rotary evaporator device at a temperature not exceeding 40°C, working on the basis of evaporation under pressure and then put the extracted in the shaker incubator at a temperature of 30–35°C. After the drying of the extract,

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it was kept in a sealed containers in the freezer (−4°C) and wrote the information on each until use.

Ethanolic Extraction

Prepare the ethanol extract by mixing 20 g of plant powder in 200 ml of absolute ethanol with stirring (i.e., 1:10 weight:volume) and then leave the mixture in the refrigerator for 24 h. Then, the extract was filtered through several layers of gauze and then filtered for 2nd time using Whatman No.1 filter papers to remove the non-powdered plant parts and the remaining fibers, then put in the rotary evaporator device at 40°C and after evaporation of the alcohol obtained a thick layer of the extract and then placed in the shaker incubator shaker incubator at 25–30°C. After drying the extract, it was kept in a sealed container in a freezer with writing the information on each until use.

Methanolic Extraction

Prepare the methanol extract by mixing 20 g of plant powder in 200 ml of absolute methanol with stirring (i.e., 1:10 weight:volume) and then leave the mixture in the refrigerator for 24 h to soak. Then, the extract was filtered through several layers of gauze and then filtered for 2nd time using Whatman No.1 filter papers to remove the non-powdered plant parts and the remaining fibers, then put in the rotary evaporator device at 40°C and after evaporation of the methanol obtained a thick layer of the extract and then placed in the shaker incubator shaker incubator at 25–30°C. After drying the extract, it was kept in a sealed container in a freezer with writing the information on each until use.

Media Preparation

The above studied media were prepared according to the information on the package by the manufacturer company[5] then sterilized at 121°C and 15 lb/kg^2 for 15 min. The medium was distributed in Petri dishes (diameter 9 cm), and the dishes were left under the temperature of the laboratory until the hardening of the medium in sterilizer hood. A quantity of distilled water was also sterilized for use as a substitute for extracts in control transactions (ddH_2O).

Fungi

Two types of fungus obtained from the media center in Erbil-Iraq for diagnosing the disease with mention the strain type were used in this study.

- *Aspergillus niger* ATCC 16404
- *C. albicans* ATCC 10231

Preparation of Petri dishes to Test Extracts against Fungus

For this study, concentration method was used by mixing with the medium. The fungal isolates were planted in Petri dishes on the potato dextrose agar media, mixed with three different concentrations of plant extracts that mentioned in previously, then incubated the dishes in the incubator at 25 ± 2°C and 37 ± 2°C. For example, *Candida* species need a temperature of between 25 ± 2°C and 37 ± 2°C for optimal growth according to the nature of their growth to fungus or semi-yeast. The process of incubation continued for 10 days. The readings were taken every 3 days according to replicates, influenced by concentrations of plant extracts.[6,7]

Statistical Analysis

GLM-univariate analysis was applied to the arithmetic means obtained in SPSS with 95% confidence interval. If the difference was significant ($P \leq 0.05$), the homogeneity groups were determined by Tukey B test. The analytical test was done separately for each solvent type (hot water, ethanol, and methanol) to show the effect of the samples part (flower, leaf, and stem) and extract concentration (25%, 50%, and 75%).

RESULTS AND DISCUSSION

The antifungal compound is one of the vital substances in pharmaceutical sciences that can find in the natural medicinal plants. This needs to identify the activity of the samples compound by different parameters. In this study, the antifungal activities of extracts obtained with solvents that vary from non-polar to polar were investigated. The results were took after the growth was rich the optimum point of two types of used fungi in optimum incubation condition. The growth of the control Petri dishes was 90 mm for both. The following table is showed the effect of *Isatis tinctoria* on *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 growth. The best results were with flower extraction by methanol with 75% concentration against *A. niger* ATCC 16404, following by the stem with methanolic extraction 75% against *A. niger* ATCC 16404 too. In this table, all of the methanolic extraction had significant effect on both fungi *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 [Table 1]. The second table is showed the effect of *Isatis busichaina* on *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 growth. The best results were with leaf extraction by methanol with 75% concentration against *C. albicans* ATCC 10231, following by the flower with methanolic extraction 75% against *A. niger* ATCC 16404. In this table, all of the methanolic extraction had significant effect on both fungi *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 [Table 2]. However, Table 3 shows the effect of *Isatis lusitanica* on *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 growth. The best results were with flower extraction by methanol with 75% concentration against *A. niger* ATCC 16404, following by the flower with methanolic extraction 75% against *C. albicans ATCC 10231*. In this table, all of the methanolic extractions had significant effect on both fungi *A. niger* ATCC 16404 and *C. albicans* ATCC 10231 [Table 3].

Depend on the results, the significant results were obtained from the extraction of methanol and ethanol for *I. tinctoria* in all concentrations, plant parts, and both types of used fungi. The aqueous extraction has less effect in correlation with ethanol and methanol [Table 4]. The inhibition effects of the extracts of *I. tinctoria* obtained from leaf, stem, and flower parts using different solvents on the two different strains of pathogenic fungi. The results were compared with the each to find the correlation. The aqueous leaf extracts were showed significant results on *A. niger* ATCC 16404, also the flower was showed the significant results by ethanol extraction for *C. albicans* ATCC 10231, although the flower extracts by methanol were showed the significant results for *A. niger* ATCC 16404. Other differences are shown in Table 4.

Table 1: Fungi test results of *I. tinctoria*. (Control=90 mm)

Plant	Plant part	Strains	Growth of fungi (mm)								
			Water concentration			Ethanol concentration			Methanol concentration		
			25%	50%	75%	25%	50%	75%	25%	50%	75%
I. tinctoria	Flower	*C. albicans*	85.75 (0.96)	82.75 (0.50)	80.25 (0.96)	81.00 (0.82)	79.00 (0.82)	72.50 (1.29)	73.00 (1.15)	71.75 (0.50)	62.00 (1.41)
		A. niger	84.00 (0.82)	82.50 (1.29)	79.00 (0.82)	81.25 (0.96)	80.00 (0.82)	78.00 (1.41)	69.75 (0.50)	68.00 (0.82)	61.25 (0.50)
	Leaf	*C. albicans*	84.50 (2.38)	80.75 (0.96)	79.50 (1.29)	79.75 (1.26)	78.75 (0.96)	77.75 (0.96)	70.5 (0.58)	69.25 (0.50)	62.00 (1.41)
		A. niger	84.00 (0.82)	81.00 (0.82)	79.25 (0.96)	80.25 (1.71)	78.75 (0.96)	77.50 (0.58)	70.25 (0.50)	68.50 (1.00)	62.75 (0.50)
	Stem	*C. albicans*	84.75 (0.96)	82.25 (2.06)	80.50 (0.58)	82.00 (0.82)	80.50 (0.58)	73.00 (1.83)	73.50 (1.29)	70.25 (0.50)	63.25 (1.50)
		A. niger	84.50 (1.29)	82.75 (0.96)	80.00 (0.82)	81.25 (0.50)	79.25 (0.96)	78.50 (0.58)	70.75 (0.96)	69.50 (1.29)	61.75 (0.50)

I. tinctoria: Isatis tinctoria, C. albicans: Candida albicans, A. niger: Aspergillus niger

Table 2: Fungi test results of *I. busichaina*. (Control=90 mm)

Plant	Plant part	Strains	Growth of fungi (mm)								
			Water concentration			Ethanol concentration			Methanol concentration		
			25%	50%	75%	25%	50%	75%	25%	50%	75%
I. busichaina	Flower	*C. albicans*	84.75 (0.96)	80.25 (1.26)	79.50 (1.29)	80.00 (0.82)	78.50 (0.58)	76.00 (1.41)	71.00 (0.82)	70.00 (1.63)	63.00 (1.83)
		A. niger	83.50 (1.29)	79.75 (0.96)	77.75 (2.22)	80.00 (0.82)	78.25 (0.50)	76.00 (0.82)	70.75 (0.96)	68.00 (0.82)	61.5 (1.29)
	Leaf	*C. albicans*	83.25 (0.96)	79.50 (1.29)	77.00 (1.83)	80.00 (0.82)	79.00 (0.82)	75.50 (1.29)	71.00 (1.15)	66.75 (2.06)	61.00 (0.82)
		A. niger	83.25 (0.96)	80.50 (0.58)	77.25 (1.71)	80.75 (0.96)	78.50 (0.58)	74.25 (0.50)	70.75 (0.50)	66.25 (0.50)	63.00 (1.83)
	Stem	*C. albicans*	84.00 (0.82)	80.75 (1.50)	77.25 (1.50)	81.75 (0.96)	80.00 (0.82)	78.25 (0.96)	72.5 (2.38)	70.50 (0.58)	68.50 (0.58)
		A. niger	84.75 (1.50)	82.25 (1.26)	79.25 (0.96)	81.75 (0.96)	80.50 (0.58)	79.25 (0.96)	71.75 (0.96)	70.00 (0.82)	69.00 (1.41)

I. busichaina: Isatis busichaina, C. albicans: Candida albicans, A. niger: Aspergillus niger

Table 3: Fungi test results of *I. lusitanica*. (Control=90 mm)

Plant	Plant part	Strains	Growth of fungi (mm)								
			Water concentration			Ethanol concentration			Methanol concentration		
			25%	50%	75%	25%	50%	75%	25%	50%	75%
I. lusitanica	Flower	*C. albicans*	83.25 (0.96)	81.50 (1.29)	79.00 (0.82)	80.50 (0.58)	79.50 (1.29)	78.00 (0.82)	70.00 (0.82)	67.75 (0.50)	64.50 (1.29)
		A. niger	83.25 (0.96)	80.75 (0.96)	79.00 (0.82)	80.50 (0.58)	78.25 (1.71)	76.75 (0.96)	68.50 (1.29)	66.50 (1.00)	63.75 (1.71)
	Leaf	*C. albicans*	82.25 (1.71)	81.75 (1.71)	79.50 (1.29)	80.00 (0.82)	79.00 (0.82)	76.75 (0.96)	69.75 (0.96)	67.50 (0.58)	66.50 (1.00)
		A. niger	82.00 (0.82)	80.25 (0.96)	79.50 (0.58)	80.25 (0.50)	78.25 (1.71)	77.50 (1.29)	68.25 (0.96)	65.75 (0.96)	65.25 (0.50)
	Stem	*C. albicans*	84.50 (0.58)	82.25 (0.50)	80.50 (1.29)	81.50 (1.29)	80.75 (0.96)	79.00 (0.82)	72.00 (0.82)	70.50 (0.58)	69.00 (0.82)
		A. niger	83.50 (1.29)	80.75 (0.96)	80.25 (1.26)	81.50 (0.58)	80.00 (0.82)	79.00 (0.82)	72.00 (0.82)	70.25 (0.96)	69.50 (0.58)

I. lusitanica: Isatis lusitanica, C. albicans: Candida albicans, A. niger: Aspergillus niger

The inhibition effects of extracts of *I. busichaina* obtained from leaf, stem, and flower parts using different solvents on the two different strains of pathogenic fungi. The results were compared with the each to find the correlation. For *C. albicans*

ATCC 10231, each of aqueous leaf extraction, ethanol extraction of flower and leaf, and leaf extraction by methanol was showed the significant result correlations. For *A. niger* ATCC 16404, the aqueous extraction of flower and leaf, the ethanol extraction of flower and leaf, and methanol extraction of flower and leaf too was showed the significant results. The total result statistics shown in Table 5.

The inhibition effects of the extracts of *I. lusitanica* obtained from leaf, stem, and flower parts using different solvents on the two different strains of pathogenic fungi. The results were compared with the each to find the correlation. For *C. albicans* ATCC 10231, each of flower and leaf of aqueous, ethanol, and methanol extraction was showed the significant results. For *A. niger* ATCC 16404, the ethanol and methanol extraction of flower and leaf was showed the significant results. The total result statistics is shown in Table 6. Some factors have to be measured to select a solvent for extracting herbal medicine. About 65–75% methanol extraction at room temperature for 15 h is practical for routine analytical study. Although, it is not fully active to extract other plants with same procedure because of dissimilarities in the biological compositions. This method is appropriate for controlling a large number of fixed samples and needs no special equipment.[8]

The present study proved that methanol and ethanol showed best results than water extracts. Our study was in accordance with several studies.[9] Tryptanthrin, indole-3-acetonitrile, and p-coumaric acid methylester were identified by physical and spectral data (MS, NMR) and by comparison with authentic synthesized samples of these compounds. Tryptanthrin has already been isolated from *I. tinctoria* and characterized as an antidermatophytic.[10] Phenylpropanoids mainly comprised flavonoid and lignan metabolites, have been considered as the primary antiviral constituents, which

Table 4: Fungi arithmetic values and Tukey B statistical analysis results of *I. tinctoria*

	***C. albicans* concentration**				***A. niger* concentration**				**Tukey B**
	Plant part	**25%**	**50%**	**75%**	**Tukey B**	**25%**	**50%**	**75%**	
Water	Flower	85.75 (0.96)	82.75 (0.50)	80.25 (0.96)	A	84.00 (0.82)	82.50 (1.29)	79.00 (0.82)	AB
	Leaf	84.50 (2.38)	80.75 (0.96)	79.50 (1.29)	A	84.00 (0.82)	81.00 (0.82)	79.25 (0.96)	B
	Stem	84.75 (0.96)	82.25 (2.06)	80.50 (0.58)	A	84.50 (1.29)	82.75 (0.96)	80.00 (0.82)	A
	Tukey B	A	B	C		A	B	C	
Ethanol	Flower	81.00 (0.82)	79.00 (0.82)	72.50 (1.29)	B	81.25 (0.96)	80.00 (0.82)	78.00 (1.41)	A
	Leaf	79.75 (1.26)	78.75 (0.96)	77.75 (0.96)	A	80.25 (1.71)	78.75 (0.96)	77.50 (0.58)	A
	Stem	82.00 (0.82)	80.50 (0.58)	73.00 (1.83)	AB	81.25 (0.50)	79.25 (0.96)	78.50 (0.58)	A
	Tukey B	A	B	C		A	B	C	
Methanol	Flower	73.00 (1.15)	71.75 (0.50)	62.00 (1.41)	B	69.75 (0.50)	68.00 (0.82)	61.25 (0.50)	B
	Leaf	70.50 (0.58)	69.25 (0.50)	62.00 (1.41)	C	70.25 (0.50)	68.50 (1.00)	62.75 (0.50)	A
	Stem	73.50 (1.29)	70.25 (0.50)	63.25 (1.50)	A	70.75 (0.96)	69.50 (1.29)	61.75 (0.50)	A
	Tukey B	A	B	C		A	B	C	

I. tinctoria: Isatis tinctoria, C. albicans: Candida albicans, A. niger: Aspergillus niger

Table 5: Fungi arithmetic values and Tukey B statistical analysis results of *I. busichaina*

	Plant part	***C. albicans* concentration**			**Tukey B**	***A. niger* concentration**			**Tukey B**
		25%	**50%**	**75%**		**25%**	**50%**	**75%**	
Water	Flower	84.75 (0.96)	80.25 (1.26)	79.50 (1.29)	A	83.50 (1.29)	79.75 (0.96)	77.75 (2.22)	B
	Leaf	83.25 (0.96)	79.50 (1.29)	77.00 (1.83)	B	83.25 (0.96)	80.50 (0.58)	77.25 (1.71)	B
	Stem	84.00 (0.82)	80.75 (1.50)	77.25 (1.50)	AB	84.75 (1.50)	82.25 (1.26)	79.25 (0.96)	A
	Tukey B	A	B	C		A	B	C	
Ethanol	Flower	80.00 (0.82)	78.50 (0.58)	76.00 (1.41)	B	80.00 (0.82)	78.25 (0.50)	76.00 (0.82)	B
	Leaf	80.00 (0.82)	79.00 (0.82)	75.50 (1.29)	B	80.75 (0.96)	78.50 (0.58)	74.25 (0.50)	B
	Stem	81.75 (0.96)	80.00 (0.82)	78.25 (0.96)	A	81.75 (0.96)	80.50 (0.58)	79.25 (0.96)	A
	Tukey B	A	B	C		A	B	C	
Methanol	Flower	71.00 (0.82)	70.00 (1.63)	63.00 (1.83)	B	70.75 (0.96)	68.00 (0.82)	61.5 (1.29)	B
	Leaf	71.00 (1.15)	66.75 (2.06)	61.00 (0.82)	C	70.75 (0.50)	66.25 (0.50)	63.00 (1.83)	B
	Stem	72.5 (2.38)	70.50 (0.58)	68.50 (0.58)	A	71.75 (0.96)	70.00 (0.82)	69.00 (1.41)	A
	Tukey B	A	B	C		A	B	C	

I. busichaina: Isatis busichaina, C. albicans: Candida albicans, A. niger: Aspergillus niger

Table 6: Fungi arithmetic values and Tukey B statistical analysis results of *I. lusitanica*

	Plant part	*C. albicans* concentration			Tukey B	*A. niger* concentration			Tukey B
		25%	50%	75%		25%	50%	75%	
Water	Flower	83.25 (0.96)	81.50 (1.29)	79.00 (0.82)	B	83.25 (0.96)	80.75 (0.96)	79.00 (0.82)	A
	Leaf	82.25 (1.71)	81.75 (1.71)	79.50 (1.29)	B	82.00 (0.82)	80.25 (0.96)	79.50 (0.58)	A
	Stem	84.50 (0.58)	82.25 (0.50)	80.50 (1.29)	A	83.50 (1.29)	80.75 (0.96)	80.25 (1.26)	A
	Tukey B	A	B	C		A	B	C	
Ethanol	Flower	80.50 (0.58)	79.50 (1.29)	78.00 (0.82)	B	80.50 (0.58)	78.25 (1.71)	76.75 (0.96)	B
	Leaf	80.00 (0.82)	79.00 (0.82)	76.75 (0.96)	B	80.25 (0.50)	78.25 (1.71)	77.50 (1.29)	B
	Stem	81.50 (1.29)	80.75 (0.96)	79.00 (0.82)	A	81.50 (0.58)	80.00 (0.82)	79.00 (0.82)	A
	Tukey B	A	B	C		A	B	C	
Methanol	Flower	70.00 (0.82)	67.75 (0.50)	64.50 (1.29)	B	68.50 (1.29)	66.50 (1.00)	63.75 (1.71)	B
	Leaf	69.75 (0.96)	67.50 (0.58)	66.50 (1.00)	B	68.25 (0.96)	65.75 (0.96)	65.25 (0.50)	B
	Stem	72.00 (0.82)	70.50 (0.58)	69.00 (0.82)	A	72.00 (0.82)	70.25 (0.96)	69.50 (0.58)	A
	Tukey B	A	B	C		A	B	C	

I. lusitanica: Isatis lusitanica, C. albicans: Candida albicans, A. niger: Aspergillus niger

probably contribute to the outstanding pharmacological activity of Radix isatidis.[11,12]

It is distinguished that a solvent system for extraction is selected according to the purpose of extraction such as preparation or analysis, the nature of interested components, the physicochemical properties of the matrix, the availability of reagents and equipment, cost, and safety concerns.[13] The solvent systems included complete ethanol at 25°C by different methods, 95% ethanol, ethanol–water for 10 min,[14] methanol–water, methanol–HCl (1.0 N, 85:15, v/v), and H_2O.

In another study that done by in 2012,[15] using the ethanol under pressure, extraction condition may be a well choice for viable productions, meanwhile, ethanol may be reused and fewer solvent left over is generated through production. However, extraction with ethanol may be a blameless means to make effective extracts for analysis and research from a minor number of samples.

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