

Original Article**Antimicrobial Activity of Five Medicinal Plants on *Candida Albicans***

Fatemeh Masomi, Mehdi Hassanshahian*

Received: 09.04.2016

Accepted: 04.05.2016

ABSTRACT

Background: In recent years, drug resistance to human pathogenic fungi has been increased. Medicinal plants are one way to overcome antibiotic resistance. The aim of this study was to evaluate the antifungal and inhibitory activity of five medicinal plants on the growth of *Candida albicans*.

Methods: This study was done in the Microbiology Lab of Shahid Bahonar University of Kerman, Iran in 2015. Five medicinal plants include: *Trachyspermum ammi* (seed), *Teucrium polium* (leaf), *Piper nigrum* (seed), *Pistachia vera* (skin), *Camelia sinensis* (leaf) were collected. Collected plant materials were extracted by ethanol and methanol solvent with maceration method. Antifungal activity of the ethanolic and methanolic extracts was evaluated by paper disc diffusion and agar well diffusion methods. Besides, MIC and MBC of each extract was determined.

Results: All plant extracts had sufficient inhibitory effect against *C. albicans* but the extracts of *P. vera* had the best inhibitory effect on *C. albicans* (ZOI: 40 mm). The lowest antifungal effect between these five plants related to *Piper nigrum* (ZOI: 13 mm). Besides, the *P. vera* extracts had the best MIC and MBC values (6.25 and 12.5 mg/ml).

Conclusion: This study strongly evidence the maximum antimicrobial activity of medicinal plants against *C. albicans* that this inhibitory effect varies with the different solvent-extract form. A more comprehensive study need to identify the effective compounds that have these antifungal properties.

Keywords: Antibiotic, Antifungal Activity, Extracts, Medicinal Plants, Inhibitory Effect.

IJT 2016 (6): 39-43

INTRODUCTION

Despite extensive progress in scientific knowledge and medical technology, infectious diseases remain a leading cause of worldwide morbidity and mortality. The main chemical agents for eradication of pathogenic fungal and bacteria are antibiotics. For many times antibiotics was gold key for treatment of pathogens. However, in recent years antibiotic resistant microbes was increased and the use of antibiotics for treatment of pathogenic microbes had less efficiency [1]. *Candida albicans* is an oral commensal flora that causes opportunistic local and systemic infections in immune compromised individuals [2]. Due to the development of resistance in known fungal pathogens and the emergence of fungal pathogens intrinsically resistant to the currently available antibiotics, it is important that novel antifungal agents be identified and developed [34]. Natural products are sources of molecules that can be used as antimicrobial agents, an attempt to overcome drug

resistance to old and new antimicrobials used currently in clinical therapy [5]. In addition, it is expected that plant compounds showing target sites other than those currently used by antibiotics will be active against drug-resistant microbial pathogens [6].

Trachyspermum ammi (L.) Sprague (TA) from the family Apiaceae is an old herb with various medical properties. There are medieval and traditional reports on the effects of *T. ammi* oil and hydrosol on neural disorders such as paralysis, tremor, and palsy as well as chronic pains. *T. ammi* was also reported as an aphrodisiac, galactagogue, and diuretic agent. Persian practitioners also reported the use of the fumigation form of *T. ammi* seeds in female genital disorders [7].

The genus *Teucrium*, which belongs to the family Labiatae, includes 300 species widespread all around the world [8, 9]. A large number of known medicinal species belonging to the genus *Teucrium* are used in folk medicine and pharmacy [10].

Black pepper (*Piper nigrum* L.) is a flowering vine of the Piperaceae family cultivated for its fruit, usually dried and used as a spice and seasoning. Pepper is also used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral acetic acid, neuropathy, melanoderma and leprosy due to the presence of volatile compounds, tannins, phenols and other unknown substances [11].

Among the aromatic plants belonging to the family of Anacardiaceae, the genus *Pistacia* is noteworthy for its numerous species and varieties of wild-growing plants. Many of these species are typical of the Mediterranean area. *Pistacia* has an economic value as it is the source of traditional medicinal agent "gum" mastic, an oleoresin exudate from the stem of this plant [12]. It is a traditional natural remedy that has been used by very ancient Mediterranean civilizations like Greeks and Egyptians [13].

Tea is a very popular drink world-wide. It is produced from the plant *Camellia sinensis*, grown in at least 30 countries, and grows best in certain tropical and subtropical regions [14]. Most of the black tea produced is consumed in the United States, Oolong tea is most popular in China and Taiwan, and green tea is most popular in China, Japan, and Korea [15]. Among the health benefits studied using green tea are: as an antioxidant, anti-inflammatory, anticarcinogenic, in cardiovascular health, oral health, and as an antimicrobial. Antioxidant effects come from the ability of green tea to limit the amount of free radicals by binding to reactive oxygen species (ROS) [16].

The aim of this study was evaluation the antifungal and inhibitory activity of these five mentioned medicinal plants on the growth of *C. albicans*.

MATERIAL AND METHODS

Plant Materials

The five plants, namely *T. ammi* (seed), *T. polium* (leaf), *P. nigrum* (seed), *P. vera* (skin), *C. sinensis* (leaf) were collected. The taxonomical identification of the plant was confirmed by Dr. Mirtajadini at the Department of Biological Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.

Preparation of Plant Extracts

Collected plant materials were air dried and pulverized into fine powder. Fifty gr of each

powder of plants was macerated in 500 ml of ethanol and methanol solvents. Different extracts were prepared using the cold maceration process for 72 h at room temperature under constant shaking and filtered with Whatman No.1 filter paper. The residue was further macerated twice with the same solvent overnight and filtered. The filtrates obtained from each extraction were mixed and concentrated under vacuum. The extracts obtained were kept at 4°C for further use [8, 11].

Determination of Antibacterial Activity

Disk Diffusion Method

Antifungal activity of the ethanolic and methanolic extracts was evaluated by paper disc diffusion method. Stock culture of test fungus was grown in PDB (Potato Dextrose Broth) medium at 37 °C for 24 h. Final cell concentrations were adjusted to 10⁸cfu/mL with reference to the McFarland turbidometer. One mL of this inoculum was added on the surface of each plate containing Mueller-Hinton agar (MHA, Oxoid) by sterile cotton swab and allowed to remain in contact for 1 min then, 0.15 mg/ml concentration of each extract prepared from sterile 6mm filter paper discs were placed into each of these concentrations for 1 h. The disc put for 30 minutes at room temperature and transferred to the medium. Disc solvent-free extract used as positive control. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each extract [12, 15].

Agar Well Diffusion Method

Agar well-diffusion method was followed to determine the antifungal activity. Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old-broth culture of *C. albicans*. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts viz. methanol and ethanol. About 40 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 34 °C for 24 h. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates

the readings were taken in three different fixed directions and the average values were recorded [5, 9].

Determination of Minimal Inhibitory Concentration (MIC), and Minimal Fungicidal Concentration (MBC)

The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using 96-well microtiter plates. The different plant extracts viz. Methanol and Ethanol were taken (1 mg/ml) and serial dilution of the extract with potato dextrose broth medium with respective inoculum were used. The microplates were incubated for 24 h at 34 °C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The MBC was determined by spreading 50 µl on PDA plate from the sample showing no visible growth and it was further incubated for 18 h at 37 °C [9, 11].

RESULTS

Evaluation of the Antifungal Activities of Methanolic and Ethanolic Plant Extracts against *C. albicans*

The zone of inhibition (ZOI) for methanolic and ethanolic extracts of five tested plants that assayed by disc diffusion method are shown in Table 1, also the results of agar well diffusion

method of these extracts are illustrated in Table 2. All plant extracts had sufficient inhibitory effect against *C. albicans* but the extracts of *P. vera* have the best inhibitory effect on *C. albicans* (ZOI: 40 mm). The methanolic extracts of these five herbal plants were better than ethanolic extracts. The lowest antifungal effect between these five plants related to *P. nigrum* (ZOI: 13 mm). The ZOI in agar well diffusion was higher than disc diffusion method in all tested plants.

MIC and MBC Values of Five Plant Extracts against *C. albicans*

The results of MIC and MBC values of the five plant extracts were illustrated in Table 3. Approximately all plant extracts exhibited inhibitory effect in lower concentration. However, the *P. vera* extracts had the best MIC and MBC values (6.25 and 12.5 mg/ml). Some plant methanolic extracts such as *T. ammi* and *C. sinensis* had not MIC value and only exhibit MBC value. It means that these plants extracts only kill *C. albicans* and not inhibited this fungus. Considering that these extracts used in broth media in MIC test, in the lower concentration that used in preparing disks (0.156-2.5 mg/ml) and inhibited *C. albicans*, the inhibitory efficiency of these extracts in broth medium is more than solid medium.

Table 1. The inhibitory effect of five medicinal plants extracts against *C. albicans* that assayed by disc diffusion method.

Type of Plant	<i>Candida albicans</i> (ZOI in mm)		
	Ethanolic Extract	Methanolic Extract	Solvent control
<i>Trachyspermum ammi</i>	13 ± 1.1	17 ± 0.8	-
<i>Teucrium polium</i>	11 ± 0.9	15 ± 0.3	-
<i>Piper nigrum</i>	11 ± 0.6	13 ± 1.2	-
<i>Pistachia vera</i>	17 ± 0.7	20 ± 0.8	-
<i>Camelia sinensis</i>	14 ± 1.3	16 ± 0.9	-

Table 2. The anti-*Candida* effects of five medicinal plants extract that assayed by agar well diffusion method.

Type of Plant	<i>Candida albicans</i>			
	Ethanolic Extract		Methanolic Extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Trachyspermum ammi</i>	12.5	25	-	25
<i>Teucrium polium</i>	12.5	50	12.5	25
<i>Piper nigrum</i>	25	50	25	50
<i>Pistachia vera</i>	6.25	12.5	25	50
<i>Camelia sinensis</i>	12.5	25	-	50

Table 3. The MIC and MBC values of methanolic and ethanolic extracts of five medicinal plants against *C. albicans*

Type of Plant	<i>Candida albicans</i> (ZOI in mm)		
	Ethanolic Extract	Methanolic Extract	Solvent control
<i>Trachyspermum ammi</i>	25 ± 0.7	28 ± 0.9	-
<i>Teucrium polium</i>	22 ± 0.1	24 ± 0.3	-
<i>Piper nigrum</i>	18 ± 0.6	21 ± 0.5	-
<i>Pistachia vera</i>	35 ± 0.4	40 ± 0.5	-
<i>Camelia sinensis</i>	30 ± 0.9	35 ± 0.2	-

DISCUSSION

Antibiotic resistant of pathogenic fungi is a global problem. Despite the extensive investigation in the field of systemic fungal and bacterial infections and profound challenges to eliminate such infectious diseases, prevalence of bacterial infection is further increasing.

In this research the antifungal activity of five Iranian plant medicines were examined on the growth of *C. albicans*. The results of this study confirmed that all medicinal plant that used in this research have sufficient antimicrobial activity, although this inhibition was lower in compare to standard antibiotics such as Nystatin. But if we consider the various side effects of antibiotics compare to herbs, herbs was better than antibiotics.

Ghalem and Mohamed investigated the antibacterial activity of *P. vera* against *Escherichia coli*, *Staphylococcus aureus* and *Proteus* spp. Their results showed the best inhibitory effect of this plant is against Gram negative bacteria than Gram positive bacteria [13].

Keller et al. studied the antimicrobial activity of *C. sinensis* against *E. coli* and *Shigella sonnei* and concluded that traditional uses of fuzhuan tea for medicinal and prophylactic properties against enteric pathogen and diarrheal infections [17]. Low concentration range of *T. ammi* essential oil (0.0625 to 1 mL/mL) showed fungistatic activities against *Candida* and *Aspergillus* species [18]. Ghasemi et al., evaluated the anti-candida of nine Iranian medicinal plants and reported that the best anti-*Candida* effect related to *Thymbraspicata* [19].

In this study, the strongest anti-*Candida* activity was shown by methanolic extract of *P. vera*. Similar to previous researches that performed by other researchers these five medicinal plants inhibited *C. albicans* at low concentration. Fungicidal effect of medicinal plants may be due to the lysis of fungal cell wall

and cytoplasmic membrane due the liberation of antimicrobial products and it was also used in reported that plant lytic enzymes act on the fungal cell traditional medicine. The cell wall causing breakage of B-1,3 glycan, B-1,6, glycan and chitin polymer [20].

These results corroborate the importance of ethno-pharmacological surveys in the selection of plants for bioactivity screening. The results obtained represent a worthwhile expressive contribution to the characterization of the anti-*Candida* activity of essential oils and plant extracts of traditional medicinal plants from the Iranian flora. Subsequently, bio-guided fractionation will be conducted on plants showing potential anti-*Candida* activity to identify the active compounds. Evaluations of the essential oils against other important human pathogens are also being conducted.

CONCLUSION

This study strongly evidences the maximum antimicrobial activity of medicinal plants against *C. albicans* that this inhibitory effect varies with the different solvent-extract form. A more comprehensive study need to identify the effective compounds that have these antifungal properties.

ACKNOWLEDGMENTS

This work was supported financially by Shahid Bahonar University of Kerman. The authors declare that there is no conflict of interests.

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