

## Evaluation of Antifungal Activity of *Physalis alkekengi* L. Extracts on *Microsporium canis*, *Candida albicans*, *Trichophyton mentagrophytes* and *Nocardia asteroides*

<sup>1</sup>Parvin Torabzadeh and <sup>2</sup>Pourdad Panahi

<sup>1</sup>Department of Microbiology, Karaj Branch Islamic Azad University, Karaj, Iran

<sup>2</sup>Department of Microbiology Science and Research Branch, Islamic Azad University, Tehran, Iran

**Abstract:** *Physalis alkekengi* L. has been used as anti-infective plant in Iranian traditional medicine. To investigate antifungal activity of the plant extract, wild plant was collected and identified and the aerial parts were air dried and powdered subsequently macerated in solvent. Extracts were concentrated by rotary evaporator at 60 C under reduced pressure. Aqueous, ethanol and methanol extracts were used against *Microsporium canis* (PTCC 5069), *Candida albicans* (ATCC 10231, PTCC 5027), *Trichophyton mentagrophytes* (PTCC 5054) and *Nocardia asteroides* (clinically isolated) in definite concentration to determine minimum inhibition concentration (MIC) of extracts. Antifungal bioassays were carried out by using agar tube dilution method. Aqueous extract had limited spectrum antifungal effect in comparison to other extracts. Ethanol extracts had the strongest effect with MIC= 15.62 for all tested fungi. Acetone extract although had broad spectrum ability as ethanol extract but should be used in higher concentration to fully inhibit *C. albicans*. Isolated *N. asteroides* was the most sensitive fungi in the present study. *C. albicans* was the most resistance fungi compared to the 3 other fungi species.

**Key words:** Antifungal % *Physalis Alkekengi* % Ground Cherry % Herbal Extracts % Phytomedicine

### INTRODUCTION

In the beginning of the last century, the major causes of human death were infectious diseases, but their incidence started to decrease with the improvement of basic sanitation conditions and with the discovery and widespread use of vaccines and antimicrobial agents [1]. Although fungi do not cause outbreaks or pandemics, the incidence of severe systemic fungal infections has increased significantly, mainly because of the explosive growth in the number of patients with compromised immune system. The indiscriminate use of antibiotics also contributes to the worsening of this picture, leading to the installation of fungal infections [1].

For many years, amphotericin B and fluconazole have been the standard therapy for treatment of severe fungal infection. Unfortunately, these established agents suffer from a number of limitations such as nephrotoxicity associated with amphotericin B, limited spectrum activity of fluconazole and development of resistance among fungi [2].

New antimicrobial agents are continually needed because of the following; resistant pathogens are developing, new diseases are evolving, naturally resistant microorganism exist and some of the compounds in use are relatively toxic [3].

Many progresses has been made in using classical approaches to discovering antifungal drugs from natural products, including phytochemical sources which indicated that new antifungals could be developed if systemic and improved strategies are used. Natural products are a rich source of biologically active compounds. Many of today's medicines are either obtained directly from a natural source or developed from a lead compound originally from a natural source. Plants are the largest biochemical and pharmaceutical sources ever known on our planet. These living factories are able to generate endless biochemical compounds [4].

*Physalis alkekengi* L. (*P. alkekengi* or Ground cherry) of the family of Solanaceae is an indigenous herb in Iran and many other countries in the world. In Iranian herbal medicine the plant extracts has been used for treatment of a wide range of diseases including anti

microbial, difficult urination, kidney and bladder stone, febrile diseases, inflammation, constipation, general edema, arthritis and rheumatism. Chemical studies have demonstrated the presence of physalins, citric acid and vitamin C as the major compounds of the extracts of *P. alkekengi* [5].

Antineoplastic and cancer static activity of *P. alkekengi* has been shown [5-7]. antibacterial, anti viral, anti-inflammatory and antipain activity of the plant were reported by Basey [8, 9]. In addition, diuretic, laxative and spleen anti-inflammatory effect of *P. alkekengi* were demonstrated by researchers. Chiang *et al.* [10] reported anti rheumatoid, sedative and anti-inflammatory properties of *P. alkekengi*.

The phytochemical compounds of different aerial parts and root of *P. alkekengi* were screened by many researchers [10-18]. The most important phytochemical components that occur in this plant are physalins that belong to the terpenoid chemical group. Physalins are demonstrated to have many biological effects such as inhibitory effect on leukemia human cell, pain relievers, anti-inflammatory, diuretic and antifever activity, moreover Physalins adjust the natural killer cells in mouse spleen [19-21]. Helvaci *et al.* [22] studied the antimicrobial activity of *P. alkekengi* and demonstrated anti-candida and antibacterial activity in *P. alkekengi* extract [22]. This study conducted to determine the existence of new antifungal materials from *P. alkekengi* extract and further use of them for medical purpose.

## MATERIALS AND METHODS

**Plant Material:** Wild plant *P. alkekengi* was collected during late spring from suburb Mashhad, Iran and identified by Department of Biology Karaj Branch of Islamic Azad University.

**Extraction Method:** Plant aerial parts were air dried and subsequently powdered using a mixer for preparation of ethanol, acetone and aqueous extracts. Air-dried, powdered plant material (30 g) was macerated in 100% solvent (200cc) at the room temperature for 48 hr on a rotary shaker (250 rpm). Aqueous extract was obtained by one hr boiling without soaking. All extracts were filtered through Buchner funnel with Whatmann filter paper No.1. The filtrates obtained extracts were concentrated by rotary evaporator at 60 °C under reduced pressure to a final volume of 20cc (1.5 g/cc) [23].

**Microorganisms:** The microorganisms used for the biological evaluation were, either purchased from Persian type culture (PTCC) or clinical isolates kindly provided by the Mycology Department of Karaj branch of IAU: *Microsporum canis* (PTCC 5069), *Candida albicans* (ATCC 10231, PTCC 5027), *Trichophyton mentagrophytes* (PTCC 5054) and *Nocardia asteroides* (clinical isolate).

**Antifungal Activity Assessments:** Antifungal bioassays were carried out by using agar tube dilution method (macro dilution). On the basis of company instruction the base media was made of Sabouraud's dextrose agar (SDA), in contrast to the instruction we added 80% volume of distilled water (DW) but later the remaining volume of DW was added along with extract [23].

At temperature 25°C 1ml of extract was mixed with 1ml DW to obtain 750 mg/ml extract concentration. On the basis of serial dilution method different concentrations of extracts were archived then 1ml diluted extracts of various concentrations was added to screw capped test tube containing 5ml media before solidification and this mixture was well shaken and allowed to cool down to 50°C. The test tube that contained ethanol or acetone extracts were placed in a water bath and kept at temperature 50°C for 30 min for solvent evaporation. Culture media were inoculated at least 12 hr after preparation to ensure complete solvent evaporation [23]. Culture media without the plant extracts and with solvent and media with solvent and without plant extracts were served as controls.

*T. mentagrophytes* and *M. canis* were stab inoculated into the culture media, supplemented with different concentrations of various plant extracts ('extract-included') or without the plant extracts (controls) and cultures were incubated at 28°C for 14 days. *N. asteroides* and *C. albicans* were streaked inoculated along the surface of slants (with extracts and controls) before incubated at 37°C for 1 week. Cultures were examined daily during incubation. All tests were repeated 4 times to ensure results accuracy [23].

## RESULTS

Result of of the antifungal activity of the 3 extract (ethanol, acetone and aqueous) of *P. alkekengi* against 4 standard and clinically isolated fungi are listed in Table 1.

Table 1: Minimum inhibitory concentration (MIC) of *P. alkekengi* extracts

Microorganism	MIC (Concentration, (mg/ml))		
	Ethanol	Acetone	Aqueous
<i>Nocardia asteroides</i>	<15.62	<15.62	<15.62
<i>Microsporum canis</i>	<15.62	<15.62	62.50
<i>Trichophyton mentagrophytes</i>	<15.62	<15.62	U
<i>Candida albicans</i>	<15.62	125.00	U

U: Undefined fungal growth not fully inhibited by concentrations used in this study

## DISCUSSION

The last two decades have witnessed a remarkable increase in the incidence of deep-seated disseminated mycoses. Opportunistic fungal infections are common among patients who have acquired immunodeficiency syndrome (AIDS) or who have had medical procedures that suppress the immune system, such as organ transplantation and chemotherapy [24]. Hence, fungal infections may become an important cause of human death or at least a significant cause of reduced quality of human living standards. On this basis, it is necessary to have antifungals available for the efficient control of fungal infections.

Owing to a great variety of fungal pathogens, complex clinical manifestations and limited antifungal medications, antifungal drug resistance is an emerging issue in the developing world and problem keeps growing due to the limited availability of drugs. There are relatively few chemical classes and targets represented by existing antifungal drugs. Antifungal drugs cellular targets are limited because of the similarity existing between fungi and hosts, i.e., both are eukaryotic organisms [1].

The increased development of resistance to older antibacterial, antifungal and antitumor drugs has been challenged by following; newly discovered antibiotics from different sources, new semi synthetic versions of old antibiotics, older underutilized antibiotics and new derivatives of previously undeveloped narrow-spectrum antibiotics [25].

Plants have formed the basis for traditional medicine systems, which have been used for thousands of years in countries such as Iran. These plant based systems continue to play an essential role in health care and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care [26]. Plant products also play an important role in the health care systems of the remaining 20 % of the population, mainly residing in developed countries.

According to the present study results *P. alkekensis* extracts had fungicide and fungi-static ability against yeast and filamentous fungi. Aqueous extract had limited spectrum ability antifungal effect in comparison to other extracts. Ethanol extract had the strongest effect. Acetone extract although had broad spectrum ability as ethanol extract but should be used in higher concentration to fully inhibit *C. albicans*. The clinically isolated *N. asteroides* was the most sensitive fungi in the present study. *M. canis* was in the second place in term of sensitivity. *C. albicans* was the most resistance fungi compared to the 3 other fungi species in challenging with different extracts. Aqueous extract was unable to fully inhibit fungi growth in *T. mentagrophytes* and *C. canis* at the concentration used in the present study, showing that polar solvent, had exploited more effective material rather than aqueous extract.

Our findings of antifungal effect of *P. alkekensis* agree with Helvaci *et al.* [22] who demonstrated that *P. alkekengi* has anti-Canidada activity.

The result of this study may form the basis for new antifungal agent by detection of the active compounds of the plant. We recommend phytochemical analysis of the plant to be investigated for new more effective compounds.

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