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Evaluation of Antifungal Activity of *Physalis alkekengi* L. Extracts on *Microsporum canis*, *Candida albicans*, *Trichophyton mentagrophytes* and *Nocardia asteroids*

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

Key words: Antifungal · *Physalis alkekengi* · Winter 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
- 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

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either obtained directly from a natural source or were 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 wide range of diseases including anti infection, 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 Vit C as the major compounds of the extracts of *P. alkekengi.*

Antineoplastic and cancer static activity of *p. alkekengi* has been shown [5-7]. antibacterial, anti viral, anti-inflammatory and antipain activity of the plant reported by Basey [8, 9]. In addition diuretic, laxative and spleen anti- inflammatory effect of *P. alkekengi* demonstrated by researcher. chiang reported anti Rheumatoid, sedative, anti- inflammatory properties of physalin [10].

The phytochemical compound of different aerial part and root of *P. alkekengi* screened by researchers [11-19]. most important phytochemical component occurred in this plant are Physalins that belong to the terpenoid chemical group.

Physalins 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 cell in mouse spleen [10, 20, 21].as far as we study only one study of antimicrobial activity conducted by Helvaci *et al*. in *P. alkekengi* and demonstrated anti-candida and antibacterial activity in *P.alkekengi* extract [21].

MATERIAL 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: Aerial plant parts were air dried and subsequently powdered using a mixer to preparation ethanol, acetone and aqueous extracts. Air-dried, powdered plant material (30 g) was macerated in 100% solvent (200cc) at the room temperature for 48 h on a rotary shaker (250Rpm). Aqueous extracts obtained by 1 hours boiling without soaking. All extracts were filter through Buchner funnel through Whatman filter paper No.1. The filtrates obtained extracts was concentrated by rotary evaporator at 60°C under reduced pressure to final volume 20cc (1.5 g/cc) [21].

Microorganism: Following microorganism used for the biological evaluation were, either purchased from Persian type culture (PTCC) or was clinical isolated kindly provided by mycology department of Karaj branch of IAU: *Microsporum canis* (PTCC 5069), *Candida albicans* (ATCC 10231, PTCC 5027), *Trichophyton mentagrophytes* (PTCC 5054) and *Nocardia asteroids* (clinically isolated).

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 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 the temperature 25°C 1ml of extracts mixed with 1ml DW to obtain 750 mg/ml extracts concentration. on the basis of serial dilution method different concentration of extracts were archived then 1ml diluted extracts in various concentration were added to screw capped test tube containing 5ml media were autoclaved 121°C for 15 minute. before solidification this mixture well shaken and allowed to cool down to 50°C. the test tube that contain ethanol or acetone extracts were placed in water bath and kept at temperature 50 for 30 minute to solvent evaporation. Then tube containing medium left to solidify agar at room temperature on slanting position. Culture media inoculated at least 12 hours after preparation to ensure complete solvent evaporation [23]. Culture media without the plant extracts and solvent and medium with solvent without plant extracts served as control.

T. mentagrophytes and *M. canis* were stab inoculated in 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. asteroids* and *C. albicans* streaked inoculated along the surface of slant medium (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].

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

	Extracts Concentration (mg/ml)		
Microorganism	Ethanol	Acetone	Aqueous
Nocardia asteroids	<15.62	<15.62	<15.62
Microsporum canis	<15.62	<15.62	62.50
Trichophyton mentagrophytes	<15.62	<15.62	U
Candida albicans	<15.62	125.00	U

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

RESULTS

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

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

Our finding of antifungal effect of *P.alkekegensis* agreed with Helvaci that demonstrated *P.alkekgeni* has anti-Canidada activity.

The result of this study may form the basis for new anfungal agent by detection active compound of plant. We recommend the phytochemical of plant investigated for new compound.

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