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 activity 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
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 Helvacı 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Ethanol (mg/ml)</th>
<th>Acetone (mg/ml)</th>
<th>Aqueous (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nocardia asteroids</em></td>
<td>&lt;15.62</td>
<td>&lt;15.62</td>
<td>&lt;15.62</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>&lt;15.62</td>
<td>&lt;15.62</td>
<td>62.50</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>&lt;15.62</td>
<td>&lt;15.62</td>
<td>U</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>&lt;15.62</td>
<td>125.00</td>
<td>U</td>
</tr>
</tbody>
</table>

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 antifungal agent by detection active compound of plant. We recommend the phytochemical of plant investigated for new compound.

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**REFERENCES**