

Preliminary Study of Phytochemical Screening and Antibacterial Activity of Physalis Alkekengi Against Staphylococcus Aureus.

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Summary

The increasing rate of development of resistance to commonly use antibiotics have led to search for newer, more effective, affordable and easily available drugs. *Physalis alkekengi* is an important medicinal plant belonging to the family of Solanaceae. It is a well recognized plant in the traditional medicine and was used by people in rural areas. In the present investigation, after phytochemical screening of *Physalis alkekengi* through standard experimental procedure, antibacterial properties of this plant were studied. The antibacterial activity of *Physalis alkekengi* against standard (ATCC 25923) and hospital isolated strains of *Staphylococcus aureus* from novobiocin treatment patients were evaluated using disc diffusion method. The inhibitory effects of this extracts were compared with standard antibiotic, novobiocin. Phytochemical screening of *Physalis alkekengi* revealed the presence of tannins, saponins, alkaloids, flavonoides and glycosides. The ethanolic extract showed inhibitory activity against *S. aureus* more than aqueous extract and this effect was dose dependent manner. Results indicated that 5 mg/mL fruits ethanolic extract have a similar inhibitory effect with novobiocin against standard strain. We suggest one of the chemical components that exist in ethanolic extract such as alkaloids, flavonoides and glycosides can have a powerful antibacterial effect even more than novobiocin, especially against hospital isolated strains. The study scientifically validates the use of plant materials in traditional medicine.

Key words: *Citrullus colocynthis*, *Staphylococcus aureus*, phytochemical screening.

Introduction

The general belief that the advent of antibiotics will bring end to the occurrence of infectious diseases was cut short with the occurrence of resistance to antimicrobial drug. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase. The development of resistance to the newer antibiotics by the microbes causing most of the infectious diseases with debilitating effects made the case worse. The rate of resistance to these drugs is higher in developing countries when compared with developed countries. This may be due to the indiscriminate use of antibiotics and also self medications without prescription by physician. Furthermore, the use of antibiotics in animal feeds may induce resistance. The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes.

These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (1). Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (2, 3). Many plants possess antimicrobial activities and are used for the treatment of different diseases (4). Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. *Physalis alkekengi*, belongs to the family Solanaceae and it has been distributed in Asia (Iran, India, Japan and China) and Europe (Spain, Italy and Turkey). It has a large history of herbal use, and an interesting chemistry but it is seldom used in modern practices (5). Chemical studies have demonstrated the presence of physalin, citric acid and vit C as the major components of *P. alkekengi* extract. Physalin is the most chemical compound with various pharmacological characteristics including, anti bacterial, anti leishmanial and anti tumor (6-8). The whole plant is anti phlogistic, anti pyretic, anti tussive and expectorant (9-11). It is used in treatment of urinary and skin diseases (12). Its extract has been used for treatment of wide range of diseases, including kidney and bladder stone, febrile diseases, inflammation, general edema, arthritis and rheumatism (11, 12). The anti-fertility properties of *P. alkekengi* have been vastly described in the Persian traditional medicine and it has been studied by some researchers (10, 13-15). In recent years there has been a growing awareness of the potentially pathogenic role of *Staphylococcus aureus*. Novobiocin resistance is a strong predictor of the presence of multidrug-resistant. Multiple treatments of *S. aureus* disease with the coumarin antibiotic novobiocin may be result to novobiocin resistant and production of refractory strains. Acquired resistance to novobiocin in staphylococci and bacteria of other genera is predominantly due to the accumulation of point mutations in the gene *gyrB*, encoding the DNA gyrase B subunit GyrB), the target of novobiocin (16). We isolated *S. aureus* strains from hospital patients that previously treatment with novobiocin and had a late response to this antibiotic. The present study aimed to determine the relationship between phytochemical components and antibacterial potential of *Physalis alkekengi* against standard *S. aureus* strains and hospital isolated strains. In the other hand, we evaluate whether *Physalis alkekengi* phytochemical components can have a same antibacterial effect in both standard and novobiocin resistance strains.

Materials and Methods

Plant Materials

Physalis alkekengi was collected from Guilan province, and then was identified by a botanist. Fresh plant was thoroughly washed using deionized water, separated into leaves and fruits, and mopped with tissue paper and air-dried in shade so as to prevent the decomposition of chemical constituents. One gram of the material was ground into fine powder using blender and the crude plant powdered sample was subjected to phytochemical screening, testing for the presence of alkaloids, flavonoids, glycosides, saponins, tannins, protein, starch and carbohydrates using standard experimental procedure (1, 17, 18). About 10 gram of plant materials (leaf and fruit) were sequentially extracted with ethanol and water for an hour. Each plant extract was filtered using vacuum filtration subsequently. The extracts were combined and the solvents were rotary evaporated. The residue from the first extraction was transferred back into the flask and extracted again with additional 50 ml solvents, water/ethanol mixture (80/20, v/v) and pure water.

Extraction efficiency test was completed after the second and third extractions. The dried extracts (1 g) was dissolved in freshly prepared normal saline (0.9%) to a final stock solution (10 mg/ml), which was used later to administer 1, 2.5, 5 and 10 mg/ml of the extract to individual groups.

Antibiotic sensitivity testing

Commercially available novobiocin (5 and 2.5 mg/ml) and normal saline were used as positive and negative controls respectively. The cultures were enriched in sterile nutrients broth for 6 - 8 h at 37°C using sterile cotton swabs; the cultures were aseptically swabbed on the surface of sterile Muller-Hinton Agar (MHA) plates using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over seeded Muller – Hinton Agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 24 h and the diameter of the inhibition zones were measured in mm.

Antibacterial testing

Disc diffusion method was employed for determination of antimicrobial activities of the leaves and fruits, following the method described by Bauer et al. 1966 and Perez et al. 1990 (19,20). The bacterial cultures used were strains of *S. aureus* isolated from patients. One of the *S. aureus* strains used in this study were clinical isolates from urethral swab, seminal fluid, urine, high vaginal swab, blood, skin swab and sputum of patients presenting with symptoms of *S. aureus*-associated diseases (21). The isolated strains were identified by *S. aureus* culture identification test (Gen-Probe Incorporated, San Diego, CA 92121) and *S. aureus* ATCC 25923 was used as a standard strain. The organisms were maintained on agar slope at 4°C and sub-cultured for 24 h before use. Controls were maintained for each test batch. All the tests were done in duplicates and they were incubated at 37°C for 24 h. The diameter of cleared zones was measured in mm. The transparently cleared zones showed bactericidal activity while the cleared zones containing micro colonies showed bacteriostatic activity. The Minimum Inhibitory Concentration (MIC) of *Physalis alkekengi* extracts was determined by a tube dilution method.

Statistical analysis

Values are mean \pm SD (standard deviation) of three replicates. All experiments were performed at least, three times (unless indicated otherwise) and were highly reproducible. Therefore, data from one replicate is presented in the work.

Results

Phytochemical tests were carried out of aqueous extractives for starch, tannins, saponins, proteins, and reducing sugars and on alcoholic extract for alkaloids, glycosides and flavanoids. The detailed of phytochemical screening in the two forms of extract is given in Table 1. Phytochemical screening portrays that most of the natural products tested for were present in the plant material except starch which were not detected in any of the tested fractions. Analysis of saponins, proteins, reducing sugars, alkaloids, glycosides and flavanoids in the leaves and fruits extracts was positive. Fruits extract showed positive results for tannins while the leaves extract showed negative results for tannins. The antibacterial activity of *Physalis alkekengi* extract was observed to be in dose dependent manner that is 5 mg/ml of extracts showed more level of activity than 2.5 mg/ml against *S. aureus* strains (Tables 2 and 3). Lower concentrations of both

extracts (1 mg/mL) did not show any inhibitory effect against *S. aureus* strains but upper concentrations (10 mg/mL) show significant effect about to fold more than 5 mg/mL of *Physalis alkekengi* extracts (data not showed). The MICs of *Physalis alkekengi* ethanolic extract for isolated strains were 1.5 to 3 mg/ml and same with standard strains. Furthermore, MICs of novobiocin for isolated strains were 4 to 5, and for standard strains were 3 to 3.5 mg/ml. The study on *S. aureus* shows that 2.5 mg/ml aqueous extract was not active against the organism and 5 mg/ml is needed for the minimum inhibition of *S. aureus*. Fruits ethanolic extract 5 mg/ml was found to have more activity than leaf ethanol extract 5 mg/ml (Table 2). In vitro anti-bacterial *S. aureus* activities of the *Physalis alkekengi* were confirmed for all the extracts, but with different range extract was most active against standard strain in contrast to *S. aureus* that isolated from patients. The 5 mg/mL fruits ethanol extract have a similar inhibitory effect with novobiocin (Tables 2 and 3). Because observation of toxic effects after chronic use of *Physalis alkekengi*, such as hypokalemia, oliguria and oedema, similar to acute nephritis and symptoms resembling Crohn's disease and Addison's Disease, we do not show results from use of high concentration of *Physalis alkekengi* extracts. It is obviously that higher concentrations of extracts have a more antibacterial and toxic effects in patients.

Table 1: Phytochemical screening of *Physalis alkekengi*

Sr. no.	Tests	Reagents used	Fruits	Leaves
Water extractives				
1.	Starch	I ₂ -KI	-ve	-ve
2.	Tannins	Acidic FeCl ₃	+ve	-ve
3.	Saponins	H ₂ SO ₄ + Acetic anhydride	+ve	+ve
4.	Proteins	Million's test	+ve	+ve
5.	Reducing sugars	Benedict's	+ve	+ve
Alcoholic extractives				
1.	Alkaloids	Mayre's	+ve	+ve
		Wagner's	+ve	+ve
		Dragendorff's	+ve	+ve
2.	Flavonoides	HCl + Mg turnings	+ve	+ve
3.	Glycosides	Benzene + hot ethanol	+ve	+ve

+ ve: Present, -ve: Absent.

Table 2. Antibacterial effect of aqueous and ethanol extract from *Physalis alkekengi* against *S. aureus* standard strain (ATCC 25923) and *S. aureus* isolate from patients.

Inhibitory effect* standard and isolate strain	Plant part used	Extract concentration (water) (mg / ml)
+++ and +++)	Leaves/ Fruits	5
-/- and +/-	Leaves/ Fruits	2.5
Extract concentration (ethanol)		
++++ and +++++	Leaves/ Fruits	5
+++ and ++++	Leaves/ Fruits	2.5

*Diameter of inhibition zone: ++++ 20 mm and more; +++ 12-20 mm; ++ 6- 12mm; + 2-6 mm; - No antibacterial activity.

Table 3. Antibacterial effect of Novobiocin against *S. aureus* standard strain (ATCC 25923) and *S. aureus* isolate from patients.

Inhibitory effect*	Novobiocin concentration (mg / ml)	
++++	5	Standard strain
+++	2.5	
++	5	Isolate strain
+	2.5	

*Diameter of inhibition zone: ++++ 20 mm and more, +++ 12-20 mm, ++ 6- 12mm; + 2-6mm, - No antibacterial activity.

Discussion

The preliminary qualitative phytochemical screening is reported in this work. *Physalis alkekengi* found to contain phytochemicals namely, saponins, tannins, alkaloids, glycosides and flavanoids. The antimicrobial study by agar disc diffusion method shows that the plant has an antimicrobial activity comparable to that of commercial antibiotic novobiocin. Results shown that *S. aureus* isolated from patients are resistance to antibacterial activity of novobiocin in contrast to standard strain. The antimicrobial property is claimed to be conferred by phytochemicals present in the plant. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (22). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (23). Flavonoids display a remarkable array of biochemical and pharmacological actions viz. antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. Flavonoides are also shown to inhibit microbes which are resistant to antibiotics by Linuma et al. 1994 (24). It was also found that alkaloids were present in the ethanolic extracts. It will be advisable to extract the leaf of *Physalis alkekengi* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for this purposes (18, 25). Saponins are a special class of glycosides which have soapy characteristics (26). It has also been shown that saponins are active antifungal agents (22). Herbal medicine represents one of the most important fields of traditional medicine all over the world (27). To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (28). Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (29, 30). The secondary metabolites identified in the *Physalis alkekengi* could be responsible for antimicrobial activity exhibited by this plant. Results of this investigation offer a scientific basis for the use of *Physalis alkekengi* ethanolic extracts to prevention of diseases cause by *S. aureus* and solve drug resistance problem. In conclusion, isolation and purification of the phytochemical followed by a detailed study might result in identification lead compound and thus a potential cure for the diseases caused by the *S. aureus*.

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