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Full Length Research Paper

Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus*

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***Citrullus colocynthis* (Linn.) Schrad (CCT) is an important medicinal plant belonging to the family of Cucurbitaceae. It is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, antidiabetic and insecticide. In the present investigation, after phytochemical screening of CCT through standard experimental procedure, antibacterial properties of this plant were studied. The antibacterial activity of CCT leaves and fruits extracts (water and ethanolic) 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 CCT revealed the presence of tannins, saponins, alkaloids, flavonoides and glycosides. The ethanolic extract showed inhibitory activity against *S. aureus* more than water 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 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 (Sofowora, 1982). The effective substances of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (Fabricant and Farnsworth, 2001). 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 (Karthikeyan et al., 2009; Lozoya and Lozoya, 1989). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001). Many plants possess antimicrobial activities and are used for the treatment of different diseases (Arora and Kaur, 1999). Medicinal and aromatic plants form a large group of economically important

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plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. *Citrullus colocynthis* (Linn.) Schrad (CCT) is an important medicinal plant belonging to the family of Cucurbitaceae. It is annual or perennial (in wild) herbaceous, stems are angular and rough, leaves rough, 3 to 7 lobed, 5 to 10 cm long, flowers monoecious, solitary, peduncled, axillary, corolla 5-lobed, ovary villous, fruit is a pepo, nearly globular, 4 to 10 cm in diameter with somewhat elliptical fissures, about size of small orange, green and yellow variegated becoming yellow when ripe, with hard rind, pulp light in weight, spongy, easily broken, light yellowish-orange to pale yellow; seeds numerous, smooth, dark brown to light yellowish-orange, borne on parietal placenta. Native to dry areas of North Africa, being common throughout the Sahara, areas of Morocco, Egypt and Sudan, eastward through Iran to India, Pakistan, Afghanistan and other parts of tropical Asia (Dane et al., 2007). It is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, antidiabetic, rheumatism, snakebite, anti-tumor (especially of the abdomen) and insecticide. The chemical confection and ability of CCT as potential sources of antibacterial compounds will be evaluate in this paper. Recent studies have shown that CCT can have an antidiabetic, carcinogenic, antioxidant, antibacterial and toxic effects (Al-Ghaithi et al., 2004; Dehghani et al., 2008; Kumar et al., 2008; Dallak et al., 2009; Memon et al., 2003). 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 (Fujimoto-Nakamura et al., 2005). 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 CCT against standard *S. aureus* strains and hospital isolated strains. In the other hand, we evaluate whether CCT phytochimical components can have a same antibacterial effect in both standard and novobiocin resistance strains.

MATERIALS AND METHODS

Plant Materials

Fresh plants were collected from south-eastern of Iran, especially Sistan region in large quantities. Efforts were made to collect the plant in flowering and fruiting conditions for the correct botanical identification.

Extraction of fresh plant materials for chemical studies

Plant materials were 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 (Harborne, 1973; Sofowora, 1982; Trease and Evans, 1982).

Extraction of fresh plant materials for antimicrobial tests

About 10 g of plant materials (leaf and fruit) were sequentially extracted with ethanol and water extracts 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). 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 virginal swab, blood, skin swab and sputum of patients presenting with symptoms of *S. aureus*-associated diseases (Fluit, 2001). 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 CCT extracts was determined by a tube dilution method.

Table 1. Phytochomical constituents of *C. colocynthis*.

Sr. no.	Tests	Reagents used	Fruits	Leaves
	Water extractives			
1.	Starch	I2-KI	-ve	-ve
2.	Tannins	Acidic $FeCl_3$	+ve	-ve
3.	Saponins	H_2SO_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
		Dragnendorff'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 water and ethanol extract from *C. colocynthis* 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.

Statistical analysis

Values are mean ± 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 water 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 crude extracts (water and ethanol) from *C. colocynthis* 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 CCT extracts (data not showed). The MICs of CCT 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 water 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-*S. aureus* activities of the *C. colocynthis* were confirmed for all the extracts, but with different range. The ethanol

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.

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 CCT, such as hypokalamia, oliguria and oedema, similar to acute nephritis and symptoms resembling Crohn's disease and Addision's Disease, we do not show results from use of high concentration of CCT extracts. It is obviously that higher concentrations of extracts have a more antibacterial and toxic effects in patients.

DISCUSSION

The preliminary qualitative phytochemical screening is reported in this paper. *C. colocynthisis* 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 (Sadipo et al., 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung et al., 1998). 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). It was also found that alkaloids were present in the ethanolic extracts. It will be advisable to extract the leaf of *C. colocynthis* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for this purposes (Trease and Evans, 1982; Zee-cheng, 1997). Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973). It has also been shown that saponins are active antifungal agents (Sadipo et al., 1991). Herbal medicine represents one of the most important fields of traditional medicine all over the world (Hamil et al., 2003). 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 (Cragg et al., 1997). 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 (Farnsworth and Morris, 1976; Service, 1995). The secondary metabolites identified in the *C. colocynthis* could be responsible for antimicrobial activity exhibited by this plant. Results of this investigation offer a scientific basis for the use of *C. colocynthis* 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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