herbmed.skums.ac.ir

# Antimicrobial activity of methanol extract of *Citrullus colocynthis* against antibiotic-resistant *Staphylococcus aureus*

Khadije Rezaie Keikhaie[1], Saeedeh Ghorbani[2], Zahra Hosseinzadeh[3], Mehdi Hassanshahian[4*]

[1]*Obstetrics and Gynecology Dept., Maternal and Fetal Health Research Center, Zabol University of Medical Sciences, Zabol, I.R. Iran;* [2]*Biology Dept., Payame noor University, Tehran, I.R. Iran;* [3]*Biology Dept., University of Guilan, Rasht, I.R. Iran;* [4]*Biology Dept., Shahid Bahonar University of Kerman, Kerman, I.R. Iran.*

Received: 17/Apr/2017 Accepted: 21/Jul/2017

## ABSTRACT

**Background and aims:** *Staphylococcus aureus* and anaerobic gram-positive cocci, are medically the most important species in the genus *Staphylococcus*. Sometimes, the bacteria are called *S. aureus*. The constantly growing antibiotic-resistant bacterium is very important in treatments. The aim of this study was to evaluate the antimicrobial activity of ethanol extract from *Citrullus colocynthis* against the *S. aureus* resistant to antibiotics.

**Methods:** *S. aureus* strains were isolated from the city of Zabol and *C.colocynthis* extract was prepared using rotary Department of Biology, Payame noor University, Tehran, Iran devices and the MIC and MBC were determined by microdilution method.

**Results:** The results of this study showed that the extract from *C. colocynthis* inhibits the growth of bacteria in different concentrations, and that despite the relative strength of most species with different concentrations, the highest sensitivity was observed in concentrations of 10 and 20 mg/ml, in which is 100% bacteria were gone. *C. colocynthis* extract at a concentration of approximately 5 mg /ml had the highest inhibitory effect.

**Conclusion:** The results showed high antimicrobial effects of ethanol extract of *C. colocynthis*, which can offer appropriate drug therapy.

**Keywords:** Antimicrobial, Plant extract, *Citrullus colocynthis*, *Staphylococcus.aureus*.

Original article

## INTRODUCTION

*S. aureus* is gram-positive anaerobic coccus which is medicinally regarded as the most important Staphylococcus. It is sometimes called as Golden Staphylococcus. These bacteria might exist as simple flora of skin or nose. It is estimated that 20% of people have long been having the bacteria. *S. aureus* is one of the powerful disease-causing bacteria. It produces a carotenoid natural pigment called Staphylozantine and produces yellow colonies. This pigment causes diseases as it acts as an antioxidant and protects bacteria against free oxygen radicals. These radicals are produced by the host immunity system for killing bacteria *Staphylococcus aureus* causes a wide range of dermal

[*]**Corresponding author:** Mehdi Hassanshahian. Biology Dept., Shahid Bahonar University of Kerman, Kerman, I.R. Iran, Tel: 00989132906971, E-mail: mshahi@uk.ac.ir

infections including dermal infections (such as pimples, pustules, anthrax, sty, and abscess) and life-threatening diseases (such as Penomini, Menangit, Steoilite, endocardite, venomous shock syndrome, and venomous septi). *S. aureus* is a factor causing hospital infections especially surgery infections. An annual number of 500 thousand people are affected with the infections caused by *S. aureus*.

Abujahl water melon, with the scientific name "*C. colocynthis*", is a one-year grassy plant originating from Asia, especially from Turkey. Its ripe fruit contains antrachinon glycosides, Saponine and alkaloid materials, has been traditionally used in Iran and many other Mediterranean countries to treating diabetics. In Iranian traditional medicine, this drug was kept in salty water or vinegar for its toxicity to be reduced.[1] Its fruit was also used as a powerful laxative, menstruation drug, and vermicidal medicine.[2] This paper aims to explore the antimicrobial effects of *Citrullus colocynthis* ethanol extract against *Staphylococcus. aureus*.

## METHODS

The *S. aureus* samples used in this study were collected from urine samples of the patients visiting Zabol hospital and were planted on the specific agar and blood Mannitol salt environments. The pure samples obtained on the artificial plant environment were detected by Catalase test and some other tests. Then, pipe Congolese test was run to recognize agglutination formation and to detect the type of *S. aureus*. The other samples were segregated by different methods.

To prepare microbial suspension, bacteria were transferred from the reservoir to the agar plant environment (German Merc). After growth of bacteria colonies, the plant surface was washed with normal saline solution and thick microbial suspension was obtained. Then, some of the suspension was poured into the sterile pipe containing normal saline and its darkness was measured by spectrophotometer at a wavelength of 630nm. The solution was added normal saline till its darkness equaled that of McFarland and bacterial suspension with a concentration of 1×108 cfu/ml was obtained.

Various *S. aureus* species to the different antibiotics purchased from the Padtan Teb Company were assessed by the Kerbi-Baer disk diffusion standard method. To this end, a half McFarland concentration of all bacteria samples were prepared in Muller Hinton broth and was planted on agar Muller Hinton environment. Antibiotic disks were located on the Muller Hinton environment containing bacteria, near plate brims. Plates were kept in incubator for 24 hours at 37 degrees of temperature. Diameters of inhibition clouds were measured to determine the resistance and sensitivity of species against antibiotics.

The extract was prepared by using maceration method. First, the plant seeds gathered from Sistan and Baluchistan plateaus were grounded. Then, 50 g of the sample was macerated and kept in methanol for 48 hours. The obtained extract was filtered by filter paper and condensed by rotary device.

Determination of the dry weight of the extract: First, the pipe's weight was determined. 1ml of the extracted extract was transferred into the pipe. The extract containing pipe was dried at room temperature. The weight difference of the pipe equaled 1ml of the extract. The mean of three measurements was considered as the dry weight of the extract. Then, the extract was dissolved in DMSO solvent and was kept at 4 degrees of temperature to be used in the study.

Sensitivity of the bacteria samples having multiple resistances to *C. colocynthis* plant extract was explored by using dilution

method in the sink. Seven sinks of microtitre plates were added 100ml of Muller Hinton Broth fluid. The first sink was added 100 ml of the diluted fluid of the extract. After mixture, 100 ml of the first sink was added to the second one, and so on. 100 ml of the broth was taken from the last sink, and 100 ml of the microbial suspension containing 107 unit/ml (0.5 McFarland) was added to all sinks. The obtained mixture was kept at 37 degrees of temperature for 24 hours. The first sink which inhibited the growth of bacteria after being positioned in incubator was considered as MIC. To ensure the precision of the study, 10ml of the bright sinks was transferred to agar Muller Hinton environment. After 24 hours, the first concentration which could remove 99.9% of bacteria was shown as the minimum removal concentration.

## RESULTS

The study results revealed that *C. colocynthis* extract inhibits the growth of bacteria with various concentrations. Despite the relative resistance of most samples in the used concentrations, the highest sensitivity was observed at 10 and 20 mg in which 100% of bacteria were removed (Table 1).

**Table 1:** Sensitivity percentage pattern of bacteria samples to different concentration of *C. colocynthis* extract (%)

| **Concentration (mg/ml)** | 20 | 10 | 5 | 2.5 | 1.25 | 0.62 |
|---|---|---|---|---|---|---|
| **MBC (mg/ml)(%)** | 100 | 100 | 66.66 | 8.3 | 0 | 0 |
| **MIC (mg/ml)(%)** | 0 | 0 | 33.33 | 41.66 | 8.3 | 0 |

Approximately, *C. colocynthis* extract at 5 mg/ml concentration showed the highest inhibition effect. On the other hand, *C. colocynthis* extract at 1.25 mg/ml concentration showed the lowest inhibition effect (Table 2).

**Table2:** Antimicrobial susceptibility (as MIC and MBC) of *C. colocynthis* extract against *S. aureus*

| **Starin bacteria** | **0.62 mg/ml** | **1.25** | **2.5** | **5** | **10** | **20** |
|---|---|---|---|---|---|---|
| 1 | ++ | ++ | + | - | - | - |
| 2 | ++ | ++ | ++ | + | - | - |
| 3 | ++ | ++ | ++ | + | - | - |
| 4 | ++ | + | - | - | - | - |
| 5 | ++ | ++ | + | - | - | - |
| 6 | ++ | ++ | ++ | + | - | - |
| 7 | ++ | ++ | ++ | + | - | - |
| 8 | ++ | ++ | + | - | - | - |
| 9 | ++ | ++ | ++ | + | - | - |
| 10 | ++ | ++ | + | - | - | - |
| 11 | ++ | ++ | ++ | + | - | - |
| 12 | ++ | ++ | + | - | - | - |

*++: Growth; +: Minimum concentration inhibitory; -: Minimum Bactericidal inhibitory.*

The isolated strains showed resistance to 3 antibiotics including penicillin (50%), trimethoprim-sulfamethoxazol (41.66%), and vancomycin (5.3%) (Table 3).

**Table3:** Antibiotic resistance of 12 strains of *S. aureus*

| | SXT | V | P |
|---|---|---|---|
| S | 41.66 | 50 | 25 |
| I | 16.66 | 41.66 | 25 |
| R | 41.66 | 5.33 | 50 |

*SXT:Trimethoprim-sulfamethoxazol, P: Penicillin, V=Vancomycin.*

## DISCUSSION

Natural drugs, especially herbal plants, have long been regarded as the basic, or even the only, treatment method. The basic material found in these plants was used to make drugs.[3] Belief in the medicinal effects of plants is a long-held belief. Some of these natural materials include contents which are usually recognized and used as antimicrobial materials.[4]

The study of Doss revealed that C. colocynthis essential can inhibit bacteria such as *Staphylococcus aureus, Bacilus Sabtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Peroteus Mirabilis*. Yet, its ethanol extract could only inhibit *S. aureus*, *E. Coli*, and *P. aeruginosa*.[5]

Marzouk found that the least inhibitive concentration of *C. colocynthis* extract was for *B. Sabtilis, S. aureus, S. Pyogenes, K. pneumonia*, and *Salmonela typhi* with concentrations of 5+-0.56, 13+-1.52, 2+-0.35, 5+-0.5, and 2+-0.425; respectively.[6,7] Gowrie et al showed that acetone extract of Abujahl is a powerful inhibitor of *Pseudomonas aeruginosa* with an inhibition cloud of 14mm.[8] John peter paul revealed that ethanol extract of *C. colocynthis* could form inhibitions clouds of 7, 5, and 4mm against *E. Coli, P. Mirabilis*, and *S. aureus*; respectively.[9] The other studies showed that could inhibit *Erwinia amylovora* and *Bacillus subtilis* with inhibition cloud diameter of 8.33+-0.14 mm.[10] The study of L. Alkamel showed that *C. colocynthis* aqueous extract with concentration of 128 mg/ml could form inhibition clouds with diameters of 12+_0.5, 11+-0.5, 11+-0.5, and 12+-0.5 mm against *S. aureus, S. Pyogenes, P. aeruginosa* and *E. Coli*; respectively.[11]

The study of Gowri, the results revealed that the crude acetone extract exhibited antibacterial activities against *Pseudomonas aeruginosa* with zones of inhibition measuring 14.0 mm. The chloroform leaf extract exhibited no antibacterial activity against *Staphylococcus aureus*. The minimum inhibitory concentration for the chloroform extract was 4.0 mm for *Escherichia coli*.[12]

The study of Shawkey, the biocidal; antibacterial, antifungal, antiviral and larvicidal activities of greenly synthesized silver nanoparticles (SNPs) in aqueous extracts (AEs) of *Citrullus colocynthis* were investigated. SNPs Formed in *C. colocynthis* AEs were spherical and homogenous and their average mean sizes were 19.267 nm, 16.578 nm, 13.376.nm and 7.398 nm in AEs of fruits, seeds, leaves and roots respectively. The biosynthesized SNPs greatly enhanced the activities of *C. colocynthis* AEs. It showed a significant

inhibitory action against different bacterial species; *Escherichia coli*, *Neisseria gonorrhoea*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*; antifungal activity against Aspergillus fumigatus, Candida albicans, Geotricum candidum and Trichophyton mentagrophytes, with inhibition zones ranging from 15.1 ± 0.44 to 25.2 ± 0.37 mm.[13]

The study of Najafi, 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.[14]

## CONCLUSION

Based on the results of the present study, the leaf extracts of *C. colocynthis*, collected from the mountainous regions of Iran, showed strong antimicrobial activity, although the observed activity was more significant in *C. colocynthis* extracts.

## CONFLICT OF INTEREST

All authors disclose any financial and personal relationships with other people or organizations and the authors declare that there are not any potential conflicts of interest. I indicate here that any color photo in print is required.

## ACKNOWLEDGMENTS

We would like to thank all individuals who collaborated and helped us to complete this project.

## REFERENCES

1. Fallah Hosseini DH, Kianbakht S, Mousavi R, Amnzadeh N. Investigating the effect of processing with vinegar and salty water on toxicity and anti-diabetic effect of hanzal extract in rats. Med Plants Quart. 2011; 4(40): 119-23.
2. Jafarnia S, Khosroshahi S, Ghasemi M. Comprehensive pictorial guide to properties and uses of medicinal plants. Green Agri ped Complex Iran, 2006; 1-179.
3. Cecchini T. Encyclopedie des plantes medicinalis. Paris, 1979. 125-30.
4. Neu HC. The crisis in antibiotic resistance. Science. 1992; 257(5073): 1064-74.
5. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005; 100(1-2): 80-4.
6. Doss A, Vijayasanthi M, Anand SP, Parivuguna V, Venkataswamy R. Screening of Antimicrobial activity of essential oil and methanol extracts of *Citrullus colocynthis* L. Schrad. South As J Biol Sci. 2011; 1(1): 7- 15.
7. Marzouk B, Marzouk Z, Décor R, Edziri H, Haloui E, Fenina N, et al. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. from Medenine. J Ethnopharmacol. 2009; 125(2): 344-9.

8. Gurudeeban S, Rajamanickam E, Ramanathan T, Satyavani K. Antimicrobial activity of *Citrullus colocynthis* in Gulf of Mannar. Int J Curr Res. 2010; 2: 78-81.
9. Priyavardhini S, Vasantha K, Umadevi M. Antibacterial activity on *Citrullus colocynthis* leaf extract. Anc Sci Life. 2009; 29(1): 12-3.
10. Paul J, Peter J. Studies on antimicrobial efficiency of *Citrullus colocynthis* (L.) Schrad: A medicinal plant. J Ethnopharmacol. 2008; 2008(1): 128.
11. Mehni A, Ketabchi S, Bonjar G. Antibacterial activity and polyphenolic content of Citrullus colocynthesis. Int J Biosci. 2014; 4(3): 190-6.
12. Alkamel M. Antimicrobial activity of aqueous extract of *Citrullus colocynthis* L. fruit. Tikrit J Pharmaceut Sci. 2005, 1(2): 9-15.
13. Shawky A, Abdulaal A, Rabeh M, Abdellatif A. Enhanced biocidal activities of *Citrullus colocynthis* aqueous extracts by green nanotechnology. Int J Appl Res Nat Prod. 2014; 7(2): 1-10.
14. Najafi S, Sanadgol N, Nejad BS, Beiragi MA, Sanadgol E. Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (L.) Schrad against *Staphylococcus aureus*. J Med Plant Res. 2010; 4(22): 2321-5.

**How to cite the article:** Rezaie Keikhaie K, Ghorbani S, Hosseinzadeh Z, Hassanshahian M. Antimicrobial activity of methanol extract of Citrullus colocynthis against antibiotic-resistant Staphylococcus aureu. Adv Herb Med. 2017; 3(3): 1-6.