



## ORIGINAL ARTICLE

# Evaluation of antioxidant and antibacterial activity on *Citrullus colocynthis* seed extract

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### ABSTRACT

*Citrullus colocynthis* Schrad Shows antibacterial and anticandidal properties. The folk medicinal use as a broad-spectrum antimicrobial agent is validated. The present study was carried out to determine the evaluation of antioxidant and antibacterial activity on *Citrullus colocynthis* fruit extract. In this study, the antibacterial activity using 9 Gram-positive and Gram-negative bacterial strains *Streptococcus pyogenes* ATCC® 19615, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratiamarcescens* ATCC 274 and *Staphylococcus aureus* ATCC® 25923 with methods of MIC, MBC were studied and resistance to standard antibiotics, erythromycin, cefixime, ceftazidime, tetracyclin, ampicillin and amikacin were compared. The antioxidant activity using the DPPH assay, the hydroalcoholic extract at concentrations of 25, 50, 75 ml using the positive control BHT (butyl hydroxy toluene) was investigated. The result shows that the highest inhibitory (MIC) for the *C. colocynthis* fruits 100 ppm concentration which 3 strain was inhibited at this concentration. The highest fatality rate (MBC) for the *C. colocynthis* fruits is 200ppm, which the bacteria *Streptococcus pneumoniae* and *Proteus mirabilis* has been lost. While the lowest fatality rate (MBC) for the *C. colocynthis* seed is 50ppm,

**Key Words:** *Citrullus colocynthis*, Antibacterial activity, Antioxidant activity

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### 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 (1). *Citrullus colocynthis* (Family: Cucurbitaceae). This is perennial herbs usually trailing. Commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandal coast. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean Region. The *Citrullus colocynthis* of family Cucurbitaceae is useful against fever, intestinal parasities, hepatic and abdominal diseases, visceral and cerebral congestions. Fruit juice with sugar is a house hold remedy in dropsy (2). Root extract is used against jaundice, urinary diseases, rheumatism etc. (3). Seeds are diuretic (4). Fruits are used against tumors of gastrointestinal tract. It is more pronouncedly used in anticancerous drug. It is effective in leukemia and joint pains. The present study was carried out to determine the evaluation of antioxidant and antibacterial activity on *Citrullus colocynthis* fruit extract.

### MATEREALS AND MTHODS

#### Bacterial strains and culture conditions:

Bacterial strains were obtained from standard laboratory. Evaluate the antibacterial activity of the plant extracts were investigated using strain of bacteria *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus*

*pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, **Acinetobacter baumannii** ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 25923. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4°C until required for study.

#### **Agar disk diffusion assay for antibiotics:**

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (5). The procedure followed is briefly described here. *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, **Acinetobacter baumannii** ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 25923 plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile saline water equivalent to a 0.5 McFarland standard. Suspension (100µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ceftazidim, erythromycin, ceftazidime, ampicillin, amikacin and tetracyclin.

#### **Plant materials:**

The seed of *Citrullus colocynthis* was collection in the region of Iran (Zabol - south-eastern, Iran) and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

#### **Preparation of extracts:**

Plants were properly dried and pulverized into a coarse powder. Each of 40 g grinded powders was soaked in 50 ml ethanol 95 % and 50 ml water, separately for 20 h (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4°C in air tight screw-cap tube.

#### **Minimum Inhibitory Concentration (MIC) and MBC of plant extracts:**

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 12.5ppm to 400 ppm. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10<sup>6</sup> CFU/ml) was added to each well to achieve a concentration of 10<sup>4</sup> CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extracts at which does the incubated microorganism was completely killed.

#### **Study of Antioxidant activity by DPPH Method**

Evaluation of antioxidant activity of hydroalcoholic extract using the stable radical DPPH and the based on Brand-Williams method (6). The was performed, to determine the power of free radical scavenging, 2 ml of extract different concentration to 1/mL 0.004 mol solution free radical DPPH in ethanol was added. Solution uptake after 30 min of incubation in the dark place at 517 nm using a spectrophotometer (Model 6715 UV / VIS Split Double beam model, JENWAY co. England) it was found, BHT or butyl hydroxy toluene was used as a positive control. Percentage of DPPH inhibition by different concentrations of extracts was obtained as follows.

$$: IP (\%) = 100 \times (A \text{ blank} - A \text{ sample}) / A \text{ blank}$$

#### **Statistical analysis:**

The result were expressed as mean and ranked of importance as percent (%). The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17 software. A P-value less than 0.05 were regarded as significant.

## **RESULTS**

The results of the study of plant extract showed the highest inhibitory (MIC) for the *C. colocynthis* seed 100 ppm concentration which 3 strain was inhibited at this concentration. The highest fatality rate (MBC) for the *C. colocynthis* fruits is 200ppm, which the bacteria *Streptococcus pneumoniae* and *Proteus mirabilis* has been lost. While the lowest fatality rate (MBC) for the *C. colocynthis* fruits is 50ppm,

Table1: Antimicrobial susceptibility, MIC extract plant for Standard bacteria seed of *C. colocynthis*

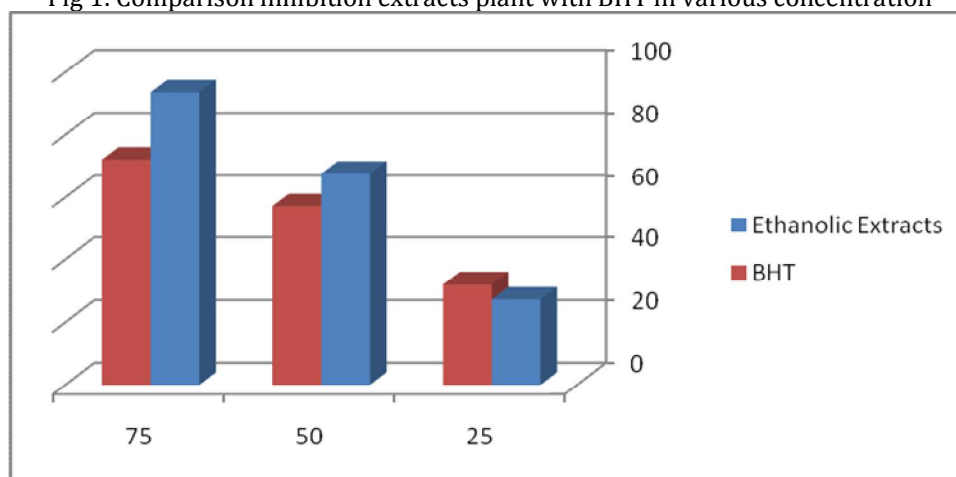
Bacterial	MIC/MBC extract plant	Antibiotic resistance
<i>Staphylococcus aureus</i>	50/100	E,CE,TE,AM
<i>Streptococcus pyogenes</i>	50/100	-
<i>Streptococcus pneumoniae</i>	50/50	E,CE,CF, AM
<i>Hafniaalvei</i>	100/100	E,TE, AM
<i>S. saprophyticus</i>	100/200	E,CF,TE, AM
<i>Acinetobacter. baumannii</i>	50/100	CE,TE
<i>Enterococcus faecalis</i>	50/100	E,CE, AM
<i>Proteus mirabilis</i>	50/50	E,TE, AM
<i>Serratiamarcescens</i>	100/200	CE

E= Erythromycin, CE= Cefixime, CF= Ceftazidime, TE= Tetracyclin, AM=Ampicillin, AN=Amikacin

#### Evaluation of antioxidant activity by DPPH

In this test the ability of hydrogen atoms or electrons with different extracts by the colorless of the purple solution, 2 and 2-diphenyl-Picrilhydrazyl (DPPH) in ethanol was performed. Colorless rate of compound, indicating the degree of power to trap free radicals in extract concentrations. The hydroalcoholic extract at concentrations 25, 50, 75 micrograms per milliliter studied and results were expressed as a percentage, in this study, as shown in Figure 1, with increasing extract concentration, free radical scavenging activity increased, The results showed that the antioxidant activity of hydroalcoholic extract of the seed of *C. colocynthis*. is more than seed, in this study the positive control BHT or butyl hydroxy toluene was used in these test antioxidant activity of the extract was weaker than the synthetic antioxidant BHT.

Fig 1: Comparison inhibition extracts plant with BHT in various concentration



#### DISCUSSION

In the study the results of the study of plant extract showed the highest inhibitory (MIC) for the *C. colocynthis* seed 100 ppm concentration which 3 strain was inhibited at this concentration. The highest fatality rate (MBC) for the *C. colocynthis* fruits is 200ppm, which the bacteria *S. pneumoniae* and *P. mirabilis* has been lost. The study of Marzouk, the highest MICs and MBCs/MFCs were obtained from the fruit aqueous extracts of *Citrullus colocynthis* (MIC 0.10mg/ml against *Candida albicans* and *Candida glabrata*, 0.20mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*), lowest activity from the root extracts [7]. The study of Memon, the result show that ethanollic extracts of fruits, leaves, stems and roots were found to be active against Gram positive bacilli, viz., *Bacillus pumilus* and *Staphylococcus aureus*, while fruit and root extracts in double strength gave positive results against Gram positive bacillus (*Bacillus subtilis*) [8]. Marzouk B and et al assess in vitro antibacterial and Anticandidal activity of aqueous and diluted acetone extracts of *Citrullus colocynthis* Schrad. MIC and MBC/MFC were determined for plant organs at different maturation stages. Aqueous and diluted acetone extracts (from the plant's roots, stems, leaves and three maturation stages of its fruit and seeds) were screened for activity against Gramnegative and Gram-positive bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*)-and various *Candida* spp. (*Candida glabrata*, *Candida albicans*, *Candida parapsilosis* and *Candida kreusei*). All extracts showed activity against all strains. The highest MICs and MBCs/MFCs were obtained from the fruit aqueous extracts (MIC 0.10mg/ml against *Candida albicans* and *Candida glabrata*, 0.20mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*), lowest activity from the root extracts [7]. The study of Rodge, the maximum antimicrobial activity was exhibited by acetone,

ethanol, methanol and distilled water extract of *Citrullus colocynthis* against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella shigella* and *Candida albicans*[9]. Memon et al., (2003) that the ethanolic extract of *Citrullus colocynthis* which is active against gram positive bacteria i.e. *Bacillus pumilus* and *Staphylococcus aureus* whereas it is inactive against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The study of Memon, the crude extract also shows appreciable inhibition of the growth of *Staphylococcus aureus*, *Bacillus pumilus* and *Bacillus subtilis*. The antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* were negative [8]. The results showed that the antioxidant activity of hydroalcoholic extract of the seed of *C. colocynthis* more than seed, in this study the positive control BHT or butyl hydroxy toluene was used in these test antioxidant activity of the extract was weaker than the synthetic antioxidant BHT. Saba and Oridupa isolated Cucurbitacins are triterpenoid steroids. It is efficient antioxidant and this property lies in their ability to scavenge free-radicals such as hydroxyl radical, superoxide anions and singlet oxygen. This broad spectrum radical scavenging capacity surpasses what had been reported for other natural antioxidants such as grape-seed extract, wheat, alfalfa and ginkgo biloba extracts. Reports also show that cucurbitacins adequately inhibit lipid peroxidation and oxidation [10].

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