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Citrullus Colocynthis a Prospective Antimicrobial and Antifungal Agent

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Abstract This research work studied the potential antimicrobial and antifungal effects of Citrullus colocynthis against some standard pathogens. Ethanol and hexane extracts of fruit and seeds of Citrullus colocynthis were tested and proved as effective some bacteria and fungi. The research concluded that this plant can be a useful source for antimicrobial agents.

Keywords Aljabry, Citrullus colocynthis, Antimicrobial and antifungal

1. Introduction

Plants are known worldwide as a source of traditional medicine and represent the major component of the stuff used in pharmacology; this is well documented by the WHO surveys [1, 2]. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardio-vascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic antipyretic and many other pharmacological effects [3]. Chemically, Citrullus colocynthis contains a wide range of elements; it contains carbohydrate, protein, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardic glycoloids and trace elements. These ingredients were used traditionally as antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, gastrointestinal, reproductive, and protective in addition to many other pharmacological effects [4]. Recently many bacteria and viruses developed resistance against most of the known antibiotics and antiviral drugs [5]; this necitates the search for other potential sources e.g. herbal medicine. Herbal medicine may provide a solution in this regard. Ali Alsnafi et at carried out a systematic review on the chemical constituents and potential pharmacological effects of Citrullus Colocynthis; and concluded that *Citrullus colocynthis* contained carbohydrate, protein, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardic glycoloids, trace elements and many other chemical groups. It possessed antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, gastrointestinal, reproductive, protective and many other pharmacological effects [6]. In another study, Shayamala et al proved that the chloroform and acetone extract of Citrullus colocynthis possess significant antibacterial activity against all the bacteria tested; however, the bacterium pseudomonas aueroginosa, showed maximum zone of inhibition in acetone extract. This is because different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity on the solvent [7]. Belsem Marzouk et al, in their work, they made a Comparative evaluation of the antimicrobial activity of *Citrullus colocynthis* immature fruit and seed organic extracts. Their results proved the idea that plants are known to produce certain toxic chemicals to micro-organism, and a large body of literature has validated the antimicrobial activity of plant extracts, showing a great potential especially against multidrug resistant strains. This is the first report of the antimicrobial activity of organic extracts from *C. colocynthis* seeds as well as from the fruits, against four *Candida* sp. and ten bacteria, including *Vibrio* sp. The results presented in their study indicate that the natural products analyzed seemed to be a good choice for the development of new strategies to treat infections in dermatology, gynecological, gastrointestinal and pulmonary infections. Therefore, the traditional use of this plant as antimicrobial agent is validated by the results obtained in this work. So, it can be used as an alternative to conventional formulations for individuals with an interest in naturally-based products [8]. In another study, Gurudeeban S. et al, studied the Antimicrobial effect of coastal medicinal plant –Citrullus colocynthis against pathogenic microorganisms and concluded that *Citrullus colocynthis*

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showed broad spectrum antimicrobial activity against 16 clinical microorganisms isolated from HIV positive patients, including bacteria Viz., *Bacillus subtilis, Escherichia coli, Klebseilla pneumoniae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes, Salmonella typhi* and *Vibrio cholerae;* and six fungal strains *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor sp., Penicillium sp.* and *Rhizopus sp.* No correlation was observed between susceptibility of the test strains with plant extracts and antibiotic resistance behaviour of the microbial strains. The significant antimicrobial activity of active extracts was compared with the standard tetracycline (30 µg/disc). The results obtained in the present study suggest that *C. colocynthis* can be used in treating diseases caused by the test organisms [9]. Another recent study is that of Ilham Bnyan et al who studied the Antibacterial Activity of Citrullus Colocynthis against different types of bacteria and stated that *Citrullus colocynthis* is a valuable plant source of medicinally useful compound that has been traditionally used for several applications. The plant aerial parts and fruit extracts were being good source for the bioactive compounds that exhibited good antimicrobial properties. However, detailed study is required to find out the specific bioactive compounds responsible for antimicrobial property through various advanced techniques [10]. Gacem M. et al in their work; Antimycotoxigenic and antifungal activities of *Citrullus colocynthis* seeds against *Aspergillus flavus* and *Aspergillus ochraceus* contaminating wheat stored, concluded that the results obtained are encouraging and confirm the value of the use of *C. colocynthis* seeds as an antifungal agent and in biotechnology as a preservative for the fight against toxigenic fungi and their mycotoxins. It is there-fore interesting to continue this study in order to determine the mode of action of extracts on mold [11]. Barrow et al [12].

Research objectives: This research is going to test the antibacterial, antifungal and antiviral activities of Citrullus colocynthis on some standard laboratory pathogens.

2. Materials and Methods

Citrullus colocynthis grows in the arid area of different regions of Sudan. The fruits of this plant resemble the fruits of water melon but of smaller size. It has a very bitter taste; hence it was only used traditionally to cure some diseases.

Methodology:

Preparation of crude extracts: Seeds and Fruits of the *Citrullus colocynthis* plant were air dried and ground to powder using a pestle and mortar. 100 g of powder was extracted sequentially with hexane and ethanol at room temperature for 72 h. Extracts were first filtered through Whatman No. 4 filter paper. After filtration, the extracts were vacuum concentrated.

Culture media:

Nutrient broth: This medium contains peptone, yeast extract and sodium chloride. It was prepared according to (Barrow and Felltham, [12] by dissolving 13 gram of the medium in one litre of distilled water. The pH of the medium was adjusted to 7.4 and the medium was then distributed into screw capped bottles, 5 ml each and sterilized by autoclave at 121°C for 15 minutes.

Mueller Hinton agar: Thirty eight grams of the powder of Mueller Hinton agar were weighed, dissolved in 1 litre of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C, mixed well then poured into sterile Petri dishes.

Sabouraud Dextrose agar: Sixty two grams of the powdered Sabouraud dextrose agar was weighed, dispersed in 1 litre water and allowed to soak for 10 minutes, swirled to mix then sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C, mixed well then poured in to sterile Petri dishes.

Antimicrobial activity:

Test strains and culture media: Standard strains of microorganism were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-negative bacteria; *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 6539) and the Gram-positive bacteria; *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus Niger* (ATCC 9763). Bacteria were grown in Mueller Hinton Agar and fungi were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions were adjusted to 108 cells/mL, and that of fungal suspensions to 107 cells/ml.

Antibacterial assay: Antibacterial activity of extracts was evaluated by the disc diffusion method (Kil *et al.* [13] with some modifications. The concentration of extracts solutions (20, 10 and 5 mg/ml) were prepared by diluting with 5% dimethyl sulfoxide (DMSO). The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 µl of crude extracts followed by drying off. DMSO was used as a negative control, while Ciprofloxacin (5 mcg) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Antifungal assay: The same method described for bacteria was adopted to test the antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus Niger*.

3. Results

Results of the study showed obvious antimicrobial activity of the extracts against tested microorganisms (Table 1). Large zones of inhibition are related to high concentration of the extract. In comparison with the standard antibiotic used, 20mg concentrations of both seed and fruit were found to be the most effective; while low concentrations (5mg) are ineffective against S.aureus and S.typhi. (Figure 1) Regarding the antifungal activity against Candida albicans and S. Niger, the inhibition zone is positively correlated with high concentration of the seed and fruit extracts of *Citrullus colocynthis.* (Figure 2)

Table (1). Antibacterial activity of seed and fruit extracts of *Citrullus colocynthis*

Sample	Extract	Concentration	Bacteria strain (M.D.I.Z *)			
			B.subtilis	***S. aureus***	***E. coli***	***S.typhi***
Seed	**Hexane**	20 mg/ml	14 ± 0.00	10 ± 0.70	12 ± 0.70	18 ± 1.41
		10 mg/ml	14 ±0.70	10 ± 0.00	9 ± 0.70	11 ± 0.70
		5 mg/ml	8 ±0.70	-	7 ± 0.00	-
	Ethanol	20 mg/ml	17 ± 0.00	8 ± 0.70	12 ±0.00	15 ±1.41
		10 mg/ml	15 ±0.70	10 ± 0.00	9 ± 0.00	12 ±0.00
		5 mg/ml	11 ±0.70	8 ± 0.00	7 ± 0.00	9 ± 0.00
Fruit	**Hexane**	20 mg/ml	16 ±0.00	15 ±0.70	15 ± 0.70	12 ±0.00
		10 mg/ml	11 ±0.70	9 ± 0.70	10 ± 0.00	11 ±0.00
		5 mg/ml	7 ± 0.00	-	9 ± 0.00	10 ±0.00
	Ethanol	20 mg/ml	17± 0.00	14±0.00	15 ± 0.00	12 ±0.70
		10 mg/ml	9 ± 0.00	8±0.00	9 ± 0.00	8 ± 0.00
		5 mg/ml	7 ± 0.00	-	8 ± 0.00	-
Antibiotic (Ciprofloxacin)		5 mcg	21	17	23	18

B.s = Bacillus subtilis, *S.a = Staphylococcus aureus*, *E.c = Escherichia coli*, *S.typhi = Salmonella typhi*, *M. D. I. Z = Mean diameter of growth inhibition zone in mm .Interpretation of results: MDIZ* (mm): < 9 mm = Inactive, 9-12 mm = partially active, 13-18mm = Active, >18 mm = Very active.

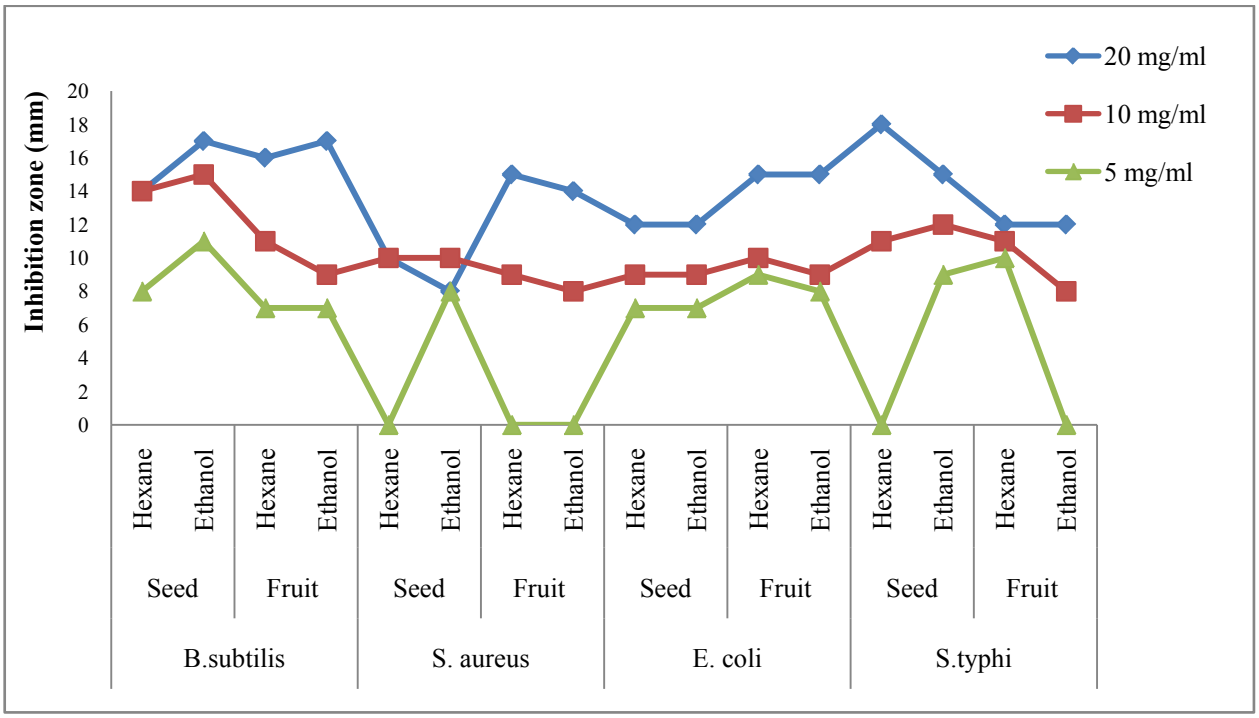

Figure 1

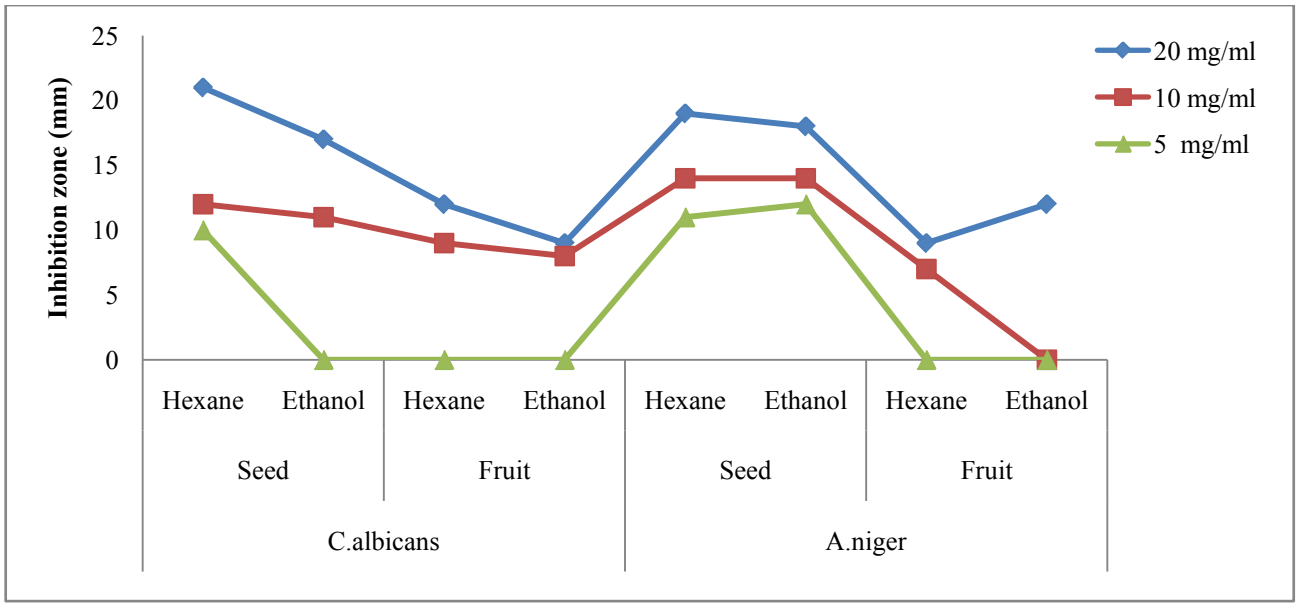

Figure 2

C.a = Candida albicans, *A.n = Aspergillus niger*. *M. D. I. Z = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ* (mm): < 9 mm = Inactive, 9-12 mm = partially active, 13-18mm = Active, >18 mm = Very active.

4. Discussion

This study differs from the previous ones due to the fact that the previous studies utilized only the fruits using only one solvent; while in this study both seeds and fruit were used as substrate and different solvent were used with both (ethanol and hexane) with variable concentrations. The results of the current study demonstrate that *Citrullus Colocynthis* has antibacterial activity against the tested microorganisms (Table 1) as well as antifungal properties (figure 2). This activity is found in both extracts; seeds and fruit. These findings agree with the systematic review of Ali Al-Snafi who reviewed *Citrullus colocynthis* as promising medicinal plant with wide range of pharmacological activities which could be utilized in several medical applications because of its effectiveness and safety [8]. Another study that agrees with current one is that carried out by S.Shayamala et al who concluded that antibacterial sensitivity of leaf extract was observed using the disc diffusion method by measuring the diameter of the inhibition zone. The chloroform and acetone extract of Citrullus Colocynthis showed significant antibacterial activity against all the bacteria tested [9]. The results of Belsem Marzouk et al also concluded that investigation has provided multifaceted results as made obvious by the extraction yields, antibacterial and anticandidal activities of *C. colocynthis* tested parts. The efficiency of extracts against each organism depends on the plant extract which is generally a crude mixture of non-active and active compounds. So, the results proved the idea that plants are known to produce certain toxic chemicals to micro-organism, and a large body of literature has validated the antimicrobial activity of plant extracts, showing a great potential especially against multidrug resistant strains [10]. A recent study by Ilham et al, Antibacterial Activity of Citrullus Colocynthis against different types of bacteria also stated that *Citrullus colocynthis,* is a valuable plant source of medicinally useful compound that has been traditionally used for several applications. The plant aerial parts and fruit extracts were being good source for the bioactive compounds that exhibited good antimicrobial properties [11]. The results the study agrees with the previous studies regarding the antibacterial and antifungal properties of the tested plant.

5. Conclusions

The use of natural stuff in pharmacological industry minimizes the hazards and allergies usually associated with chemicals. With the growing population of the globe, it may be logic to consider sustainable natural resources and how to avail them. The present study concludes that the tested plant has obvious and promising pharmaceutical benefits and can be a potential source of crude stuff for pharmaceutical industry. It is a potential source of cheap natural raw material; the supply of which can be easily maintained due to the ease of growing the plant and the simplicity of its harvesting process.

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