

Study of alkaloid extract from *Citrullus colocynthis* fruit and its antimicrobial activity Screening

M. Y. Al-hejjaj¹, Y. A. Alhurba² and S. Ali.Mohamad²

¹⁻ *College of Veterinary Medicine*

²⁻ *College of Pharmacy / Basrah University/ Basrah/ Iraq.*

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Abstract

The antimicrobial activity of alkaloid extracted from *Citrullus colocynthis* were examined against five local isolates bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Bacillus subtilis*, and *Klipsella* sp.) using agar disc diffusion method, beside two traditional antibiotics (Amoxicillin and Cephalosporin). The most active antimicrobial activity of extracted alkaloid were shown against *Strep.* sp, the Cephalosporin showed the most antimicrobial activity. Broth dilution methods were used to determine the minimum inhibitory concentration (MIC) for the extracted alkaloid. The study showed that MIC values of 600 µg/ ml, 3000 µg/ ml, against *Staph. aureus*, and *E. coli* isolates respectively.

Key words: alkaloid, *Citrullus colocynthis*, antimicrobial activity.

Introduction

There is cumulative resistance against antibiotics of many bacteria. Therefore, the development of new antimicrobial agents increase interest [1]. There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, [2]. Many people prefer to take herbal products instead of chemical medicines. One of these traditional medicines is *Citrullus colocynthis*, which is used by diabetic patients as an hypoglycemic agent, [3]. *Citrullus colocynthis* (Cucurbitaceae), commonly known as "bitter apple", "colosynth", "vine-of-Sodom" and "tumba" is a tropical plant that grows abundantly in the Arabian countries and widely in other parts of the world. In the traditional medicine, this plant has been used to

treat constipation [4], Diabetes [5], edema, fever, jaundice leukemia, bacterial infections, cancer and used as an abortifacient [6] and growth of hairs [7]. Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, anti-inflammatory properties but there are only few reports about their antimicrobial properties [8].

The aims of this current study: were to locate, isolate, and evaluate novel bioactive alkaloids from plants traditionally used for medicinal purpose *Citrullus colocynthis*. The plants investigated was the predicted to have antimicrobial activity.

Experimentals

The Plant collection. *Citrullus colocynthis* plant was collected in April 2009 from the desert area near safwan, Basrah, Iraq, and were identified by Prof. Dr. Abdulridha A. Alwan in the Department of Biology at Basrah University, Basrah, Iraq.

Preparation of plant and extraction: Before extraction, fruit of the plants were prepared by air drying at room temperature (27°C) followed by grinding separately in a coffee grinder, 100g of fruit grinding material was mixed with 750ml distilled water and 4% H₂SO₄, the mixture were mixed by hot plat magnetic stirrer for 24 hours at room temperature, the mixture filtrated under pressure and transported to separate faunal, 30ml of chloroform were added for every 10ml of the mixture and shaken well, after several minutes the watery layer transformed to Petri dishes and dried under the room temperature, the organic layer were left, at last dried crystal material were washed by acetone [9].

Alkaloid testing: Both Dragendorff's and Mayer's tests were used to determine the alkaloid after extraction according to [10, 11].

Purity Tests: Paper Chromatography (PC) technique was used to determine the extracted alkaloid purity, alkaloid solution was prepared by dissolving (0.1g) in 5 ml distilled water. 5µl alkaloid solution was spotted approximately 3cm from the end of Whatman #1 (5×15) cm. after drying under an air stream; the plate was placed in a glass jar containing the n-butanol, acetic acid and water (4:1:5) (BAW) solvent system, and developed ascending at room temperature until the solvent front reached the upper end. The plate was dried in air and spots were detected under UV light at (360nm), Then retardation factors (R_f) was calculated according to the present equivalent [12].

The distance moved by solute

$$R_f = \frac{\text{The distance moved by solute}}{\text{The distance moved by solvent front}}$$

The distance moved by solvent front

Antimicrobial activity: the antimicrobial activity of extracted alkaloid was comparative with standard commercial antibiotic, [13]. Cephalosporin (5000µg/ml) and Amoxicillin (5000µg/ml) manufacture by MISRCO. Egypt.

Bacterial strains: five clinical isolated bacteria were obtained from [14] microbiology department at veterinary medicine college, university of Basrah, Iraq. (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp, *Bacillus subtilis* and *Klebsiella* sp.)

Preparation of inoculums: A loopfuls of each of the test microorganisms from NA were aseptically transferred into 5 ml of NB and incubated at 37°C for 18 h at 180 rpm in shaker incubater before use. The suspension was

diluted with sterile distilled water to obtain approximately 10⁶ CFU/ml.

Screening for antimicrobial activities: Antimicrobial tests were carried out by an agar diffusion technique. The concentration of bacterial suspension yield 10⁶ CFU/ml in the plates. The discs of waterman #1 paper (diameter, 6 mm) were each impregnated with alkaloid extracts (5000 µg/ml), dried and then placed on the inoculated agar. The plates were incubated at 37C° for 24 h, diameter of inhibition zones were measured [15].

Determination of minimal inhibitory concentration (MIC): the MIC method using broth dilution method, the dried alkaloid extract was dissolved in sterile distilled water and serially diluted to Nutrient broth (100, 200, 300,

500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3250, 3500 and 4000) µg/ml

in order to observe their activities at lower concentrations [15].

Results:

The characteristics properties of extracted alkaloid from *Citrullus colocynthis* were shown

in table (1), and it was appeared as pure molecule with only one spot on PC technique.

Table (1): alkaloid characteristics properties

Fruit of plant (g)	Alkaloid (g)	Purity test (PC)		Diagnostic tests	
		Spots number	R _f (cm)	Mayers test	Dragendroff test
100	5.3	Only one	6.4	(+)	(+) orange preceptate

Table (2) showed the antimicrobial activity and the minimal inhibitory concentration of the extracted alkaloid against the clinical bacterial

strains comparative with the general used traditional medicine (amoxicillin and cephalosporin).

Table (2): antimicrobial activity of extracted alkaloid and some medicine with the MIC.

Bacterial Isolates	alkaloid extraction		Antibiotics Primary screening IZ (mm)	
	Primary screening IZ (mm)	MIC (µg/ml)	Amo.	Ceph.
<i>E. coli</i>	12	3000	11	13±1
<i>K. sp.</i>	15	1000	13	15
<i>S. ureus</i>	24	600	20±2	25
<i>B. subtilis</i>	13	1500	15±2	15
<i>Strep. sp.</i>	27	700	25	30

Amo: amoxicillin and Ceph: cephalosporin, IZ: Inhibition zones,

Table (2). showed extracted alkaloid activity on five clinical bacterial isolates as a primary screening, the highest antimicrobial activity was recorded at (27mm) on (*Strep. sp*) and the lowest appeared at (12mm) on (*E. coli*) and the other effects on Bacterial strain are located between these results. The MICs were recorded

in the range between (600mcg and 3000mcg) on (*S. aureus* and *E. coli*) respectively. However, the highest primary screening of the traditional medicine appeared at (30mm) for Ceph. against (*Strep. sp*) and the lowest was recorded at (11mm) for Amo. against (*E. coli*).

Discussion:

As the results of the present study showed an ability to extract pure alkaloid from the fruit of *Citrullus colocynthis* (CCT) successfully by acidic water method [9], this results in agreement with [16]. They recorded that, the fruit of CCT contains active substances such as saponins, alkaloids and glycosides. The fruit of CCT plant was only used to extract alkaloid in this study, because it was the part of the CCT plant was used as a herbal medicine [17].

The extracted alkaloid had an antimicrobial activity against both Gram positive and Gram negative clinical isolated bacteria, this result was in line with [18] who reported that the extracted alkaloid had a good bioactive material.

Results of recent study showed that the extracted alkaloid was more effective on Gram positive than on Gram negative bacteria; these results may be due to the nature of bacterial cell membrane. The cell membrane of Gram positive bacteria contains mucopolysaccharides, protein and less amounts of phospholipids, while gram negative bacteria have a huge amounts of phospholipids, and more pores in cell envelope. So, the permeability, entrance and reaction of the most antibiotic and /or antimicrobial agents through cell envelope (the outer and cytoplasmic membrane) are highly efficient for Gram positive bacteria depending on reaction with the

protein layer (mucopolyccharides or peptidoglycanes) [19].

Phytochemicals exert their antimicrobial activity through different mechanisms, tannins for example act by iron deprivation, hydrogen bonding or non specific interactions with vital proteins such as enzymes [20]. In the case of indoloquinoline alkaloids the mechanism remains unclear [21], demonstrated that the main indoloquinoline alkaloid, cryptolepine, causes cell lysis and morphological changes of *S. aureus*, but the antimicrobial effects of the alkaloid may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition [22, 23, 24 and 25].

The results of minimum inhibitory concentration for the extracted alkaloid (table, 2), showed some different with other researchers [8], who found that the MIC of alkaloid (400µg) for (*S. aureus* ATCC 25923) bacterial, this different may be due to the deference between the stander strains (he was used in his study) and the local isolated bacteria (we used in the study) itself, because the local isolated bacteria are more resistant than the stander strain for the antibiotics

Conclusions:

It is able to extract a pure alkaloid from the local plant *Citrullus colocynthis* (fruit).

and the LD₅₀, in addition to detect the type of the alkaloid.

Recommendations

We recommended to identify the extracted alkaloid by GC technique and study its toxicity

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Science, University of Basrah, Iraq) for the identification of the plant.

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دراسة حول استخلاص مادة الالكلويد من ثمار نبات الحنظل *Citrullus colocynthus* والكشف عن فعالية ضد مايكروبية

مرتقب يونس الحجاج¹, يسار عبد الحسين الحربة² وسبأ علي محمد²

1- كلية الطب البيطري

2- كلية الصيدلة/ جامعة البصرة

الخلاصة

اجري في هذه الدراسة عزل مادة الالكلويد من ثمار نبات الحنظل في محافظة البصرة وتم اختبار فعاليته ضد مايكروبية ضد كل من العزلات السريرية التالية *Escherichia coli*, *Bacillus subtilis*, *Streptococcus sp.* *Staphelococcus Klebscilla sp. aureous* بجانب اثنين من المضادات الحيوية التجارية الشائعة الاستخدام (السفالوسبورين و الاموكسلين)، حيث اظهرت بكتريا الـ *E. coli* اعلى مقاومة للالكلويد المستخلص ومضادات الحياة، بينما كانت بكتريا الـ *Streptococcus sp.* اكثر حساسية وكان الاموكسلين اكثر فعالية من المضادات الاخرى. كما بينت نتائج فحص التركيز المثبط الادنا MICs بطريقة التخفيف ان اعلى قيمة بلغت 3000 µg/ml ضد بكتريا الـ *E. coli* واقل قيمة سجلت هي 600 µg/ml ضد بكتريا الـ *Staphylococcus aureous*