

The Investigation of the phytochemical compounds and the antibacterial effect of Algerian –*Citrulluscolocynthis* Schard

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ABSTRACT

The Resistances of the current antibacterial drugs are growing to global concerns. This study is to identify the phytochemicals from the fruits, root and leaves of *Citrulluscolocynthis* and also for studying the antibacterial effect of aqueous and hydromethanolic extracts of the leaves, roots and fruits. The Phytochemical screening was revealed the presence of some active substances including flavonoids, the saponins and steroid. The Aqueous and hydromethanolic extracts of the fruits, leaves and roots of *Citrulluscolocynthis* Schard. That were examined for their antibacterial potentials against the Gram positive and negative bacteria. All extracts are showed an activity against all bacteria strains, the least minimum inhibitory concentration (MIC) was obtained from the hydromethanolic root extract with 5.6mg/ml against *Klebsiellapneumonia* and 6 mg/ml against *Bacillus stearothermophilus* and *Staphylococcus aureus*.

KEY WORDS: The *Citrulluscolocynthis* Schard, the phytochemical screening, the antibacterial effect, the aqueous extract, the hydromethanolic extracts.

INTRODUCTION

The Citrulluscolocynthis Schard is a member of the gourd family (Cucurbitaceae), It is originally from the tropical part of Asia and Africa; it is widely distributed now in the Saharan-Arabian phytogeographic region in Africa and the Mediterranean pool [6]. It is a small scaped perennial creeping herb with prostrate or climbing stem. The bearing smooth spherical fruits are mottled green when they are young and some white yellow when they are ripe [17].

In moderate doses a drastic hydrogogue, cathartic and diuretic; in large doses emetic and gastro-intestinal irritant; in small dose it is expectorant and alterative. The Physician is used this drug extensively as a drastic purgative in ascites and jaundice in various uterine conditions, especially in amenorrhea. The Colocynthis in its form of the solid extract is entered into many of the purgative pills of the modern pharmacy. It is used a complete form in the biliousness, the fever, the intestinal parasites, the constipation, the hepatic and abdominal, the visceral and cerebral congestions, the dropsy, etc. The fruit Juice is mixed with sugar is a house-hold remedy in the dropsy [19].

The *Citrulluscolocynthis* has a very high medicinal value; the plant contains three antitumor ingredients: cucurbitacin B, Cucurbitacin E and the D-glycoside of Beta-sitosterol [15]. In the present study, the purpose is to investigate the phytochemical compound and the antibacterial activities of the fruit (Bark and pulp), the roots and the leaves extracts of *C. The colocynthis* against Gram-positive and negative bacteria. The selected bacteria is an antibiotic resistant or a multi-resistant human pathogens.

MATERIALS AND METHODS

The Plant material

The plants that are used for the present study were collected in September 2011, from Béchar's valley, Béchar is a city in the south-western Saharian Department in Algeria. The leaves and roots were dried for 20 days in the dark at ambient laboratory temperature (20 to 28°C); they were dried for three months in the same conditions, The grains were embarrased, The different parts of the fruits were milled to a fine powder in an electrical mill, and stored in the dark room with the temperature enclosed containers until they are required.

The Qualitative phytochemical screening

Each organ of plant (leaves, roots and fruits) was screened for the presence of the key families of the Phytochemicals [16,18]. Using the following reagents and chemicals: Alkaloids with Mayer's reagents, the

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Flavonoids with the Metallic Magnesium and Hydrochloridric Acid, the Saponosids for their ability to produce Suds, the Steroids Acetic anhydride and concentrated Sulphuric Acid, Tanin with Ferric Chloride.

The Extraction protocol

Aqueous extract

A total of 5 g of the different powdered plant parts were added to 50 ml of the distilled water, the mixture was allowed to reflux for 30 min. After cooling, it was filtered and stored to 4°C without concentration [9].

The Hydromethanolic Extract

A 5 g of the different organs powder of the plant was added to 50 ml of the methanol: using the distilled water (v/v), the mixture was allowed to reflux for 30 min. After cooling, it was filtered. The filtrate was passed in a rotary evaporator at 65°C to vapor the methanol; the crude extract was stored to 4°C prior to analysis.

The Bacterial Strains and Media

The antibacterial activity of the different part of the extracts of *C. (colocynthis)* were) was evaluated the using of the following strains of the bacteria,

Gram-positive: *Listeria monocytogenes* (ATCC19115); *Bacillus stearothermophilus* (ATCC11778); *Staphylococcus aureus* (ATCC25923); *Enterococcus faecalis* (ATCC29212).

Gram-negative bacteria are: *Klebsiella pneumonia* (ATCC4352); *Pseudomonas aeruginosa* (ATCC27853); *Escherichia coli* (ATCC25922).

These bacterial strains were obtained from the Pasteur Institute, Algiers, Algeria. All strains were identified by the using of the biochemical profiles according to the recommendations of the manual of the clinical microbiology[11]. All the organisms were maintained in brain-heart infusion (BHI medium) containing 30% (v/v) glycerol at -20°C. Before the testing, the suspensions were transferred to trypticase soy agar supplemented with 5% of the sheep blood and aerobically grown overnight at 35°C. The Individual Colonies were isolated and suspended in 5ml of 0.9% NaCl solution. The inoculate were prepared by adjusting the turbidity of the suspension to match the 0.5 Mcfarland that is standard and diluted in the CAMHB (Cation –adjusted Muller Hinton broth) in order to achieve the adequate inoculum in each case.

The cell number in CAMHB was estimated that used a serial dilution technique[13]for each assay.

The Antibacterial Activity

The Disk Diffusion Method

The Petri dishes were prepared with 20 ml of a base layer of Muller Hinton gelose medium and inoculated with 100 µl of each bacterial suspension (106 UFC) [20].

After drying in a sterile hood, 6 mm diameter disks soaked with different extracts were placed at 35°C for 24 h. The antibacterial activity was expressed as the mean of the inhibition diameters (mm) produced.

The MIC Determinations

The minimal inhibitory concentration (MIC) is preventing a visible bacterial growth that was measured by the different concentrations of the extract of the Muller Hinton agar media. The Different volume of extract was prepared and added to 20 ml of the Muller Hinton Agar media; after agitation, the select solution was transferred into a Petri plates that were incubated at 35°C for 24 h[1].

THE RESULTS AND DISCUSSION

The Qualitative Phytochemical Screening

The Phytochemical screening is usually carried out to the screen and in order to characterized the constituents that is available in a given plant sample. All the phytochemical constituents which were tested are identified in *C. colocynthis* fruits, leaves and roots as shown in Table 1.

Table 1: Phytochemical Screening of *Citrullus colocynthis* fruits, leaves and roots

Phytochemical constituents	Fruits	Leaves	Roots
– Alkaloid	+	+/-	+/-
– Tannins	+	+/-	+
– Saponins	+	+/-	+
– Flavonoids	+	+/-	+
– Unsaturated sterols and terpens	+	+	+
– Sterol and steroid	+	+	+

Key: +: present; -: Absent; +/-: low presence

The traditional use of the plants as medicines is provided the basis for indicating the essential oils and the plant extract maybe useful for the specific medical conditions [8,10].

The present investigation has explored the using of one, (*C. colocynthis* Schard endemic in the southwestern of Algeria), and for testing the phytochemical compound and the antimicrobial activity of this endemic plant.

Generally, in the phytochemical screening of any plant -there is one normally identifies as a secondary metabolites that have accumulated to some extent at the specific organ of the plant. These metabolites are mainly used by the plant for protection against herbivores which may have the pharmacological activity when they are tested on animals[12].

The Result of the phytochemical screening of the *Citrulluscolocynthis* Schard fruits leaves and roots are showed the presence of the saponins, sterols, steroid, terpen, flavonoids, tannin and alkaloids in different proportions in the xx tree part of the plant.

This result is in agreement with the findings of the Belsem et al. (2009) which proved that alkaloids were found in all extracts except the roots, the flavonoids were presented only in seeds; gallic tannin and coumarins only in the leaves, and all of them are contained steroids.

Ambi et al. (2007) confirmed that three phytochemical constituents were identified in *Citrulluscolocynthis* Schard seeds extracts as alkaloid, steroid, glycosides and flavonoids.

Extraction of the secondary metabolites in highly depends on the using of the extractor techniques that depend on the chemical properties of these compounds, the soluble water compounds and proteins can be extracted in water or polar solvents whereas water insoluble compounds can be extracted with organic solvents [5].

The Antibacterial Activity

The Disk diffusion method

The disk result diffusion method is indicated that the inhibition diameters of aqueous and hydromethanolic extract of *C. colocynthis* roots are the broadest comparing to the leaves and fruit extract. (**Results are presented in Table 2.**)

MIC determination

As Table 3 shows, the MICs are depending on the bacterial strains tested. The hydromethanolic extract of the root shows the best antibacterial activity screw all the bacterial strains tested.

Table 2: Antibacterial Activity of the Aqueous and hydromethanolic Extract of *Citrulluscolocynthis* fruits; leaves and roots by the Disc Diffusion Method.

Bacterial strains	Inhibition Diameters(mm)					
	Fruits		Leaves		Roots	
	Aq	Hyd	Aq	Hyd	Aq	Hyd
- <i>Klebsiella pneumonia</i> (ATCC4352)	9.0	9.4	10.5	10.2	10.4	11.0
- <i>Listeria monocytogen</i> (ATCC19115)	9.9	10.0	10.8	11.9	11.4	10.2
- <i>Pseudomonas aeruginosa</i> (ATCC27853)	9.8	10.4	10.2	10.7	11.0	10.8
- <i>Escherichia coli</i> (ATCC25922)	9.2	10.0	8.9	10.9	10.4	11.1
- <i>Bacillus sterothromphillus</i> (ATCC11778)	9.8	9.2	9.8	9.7	10.4	11.2
- <i>Staphylococcus aureus</i> (ATCC25923)	9.6	10.0	10.6	10.3	10.6	10.6
- <i>Enterococcus faecalis</i> (ATCC29212)	9.4	8.6	10.5	9.7	10.7	10.7

Aq: Aqueous Hyd: Hydromethanolic

Table 3: The MICs of the Aqueous and Hydromethanolic Extract of the *Citrulluscolocynthis* fruits, leaves and roots.

Bacterial strains	MIC (mg/ml)					
	Fruits		Leaves		Roots	
	Aq	Hyd	Aq	Hyd	Aq	Hyd
- <i>Klebsiella pneumonia</i> (ATCC4352)	9.8	13.93	17.1	10.8	18.9	5.6
- <i>Listeria monocytogen</i> (ATCC19115)	9.8	10.85	17.1	10.8	18.9	5.6
- <i>Pseudomonas aeruginosa</i> (ATCC27853)	9.8	13.93	25.2	10.8	20.92	5.6
- <i>Escherichia coli</i> (ATCC25922)	9.45	13.93	17.1	08.1	15.52	6.0
- <i>Bacillus sterothromphillus</i> (ATCC11778)	9.45	10.85	25.2	10.8	21.6	6.0
- <i>Staphylococcus aureus</i> (ATCC25923)	9.8	10.85	25.2	10.8	21.6	6.0
- <i>Enterococcus faecalis</i> (ATCC29212)	9.8	12.40	25.2	10.8	15.52	6.0

Aq: Aqueous Hyd: Hydromethanolic

This study is confirmed the efficacy of the aqueous and hydromethanolic extract of the fruits, leaves and the roots of *Citrulluscolocynthis*Schard by the diffusion method to measure the diameters of the inhibition and the method of the MIC (minimum inhibitory concentration).

Generally, the hydromethanolic extract of the tree parts is efficient overlooked the bacteria tested contribution to the aqueous extracts. The strongest antibacterial activity with inhibition zone is remarked with hydromethanolic root extract or the MIC obtained that is 5.6 mg/ml for *Klebsiella pneumonia*, *Listeria*

monocytogen and *Pseudomonas aeruginosa*. These results are not in the agreement with those ones that were reported by Belsem et al. 2009 who found that the plant organs with the highest antibacterial properties that were immature fruits and immature seeds, and the lowest activity was observed for the root extracts. The strongest inhibitions were obtained against *E. coli* with hydromethanolic root extract which is consisted with the results of Belsem et al. (2009).

Usman et al. (2003) found that the inhibition zone of *S. aureus* with ethyl alcohol extract of the root was 13.2mm. *Pseudomonas aeruginosa* is the leading cause of nosocomial infection and has developed mechanisms of resistance to common antibiotic classes [4], Since it must always seek a new anti-*Pseudomonas aeruginosa*, These results are suggested that the inhibitory effect exhibited by the extract of *C. colocynthis* Schard may be attributable to the secondary metabolites like phenolic compound and saponins. No activity can be imputed to one family of phytochemical only; alkaloids are commonly found to have antimicrobial properties [14]. Flavonoids are known to be synthesized by plants in response to the microbial infection [7].

CONCLUSION

The obtained results might be considered the adequate to be demonstrated that *Citrullus colocynthis* Schard extract can be considered a good antibacterial agent; it can be used to an antibacterial overcoat against the strain that a major problem of resistance in the hospitals.

However, the results are only the first steps of the antibacterial activity; further studies on the isolation and identification of the active principal and on the evaluation of the possible synergism among the extract component for their antibacterial activity that are needed. The Investigations are in progress to determine the degree of toxicity of these extracts.

Conflict of interests

None.

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