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Research Article

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Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-west Algeria

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ABSTRACT

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

Key words: *Citrulluscolocynthis* Schard, phytochemical screening, antibacterial, extract.

INTRODUCTION

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

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

*C. colocynthis*has very high medicinal value; the plant contains three antitumor ingredients: cucurbitacin B, Cucurbitacin E and the D-glcoside of Beta- sitosterol[15].

The purpose of the present study was to investigate phytochemical compound and the antibacterial activities of fruit (Bark and pulp), roots and leaves extracts of *C. colocynthis*against Gram- positive and Gram- negative bacteria.

The selected bacteriaare antibiotic resistant or multi- resistant human pathogens.

EXPERIMENTAL SECTION

Plant material

The plants used for the present study were collected in September 2011, from Owed Béchar, Béchar, a city in West Sahara Department, Algeria. Theleaves and roots were dried for 20 days in the dark at ambient laboratory temperature (20 to 28°C); the fruits were dried for three months at the same conditions, the grains were debarrassed, the different part were milled to a fine powder in an electrical mill, and stored in the dark room temperature in closed containers until required.

Qualitative phytochemical screening

Each organ of plant (leaves, roots and fruits) was screened for the presence of key families of phytochemicals[16,18]. Using the following reagents and chemicals: alkaloids with Mayer's reagents, flavonoids with metallic magnesium and hydrochloridric acid, saponosids for their ability to produce suds, steroids acetic anhydride and concentrated sulphuric acid, Tanin with ferric chloride.

Extraction protocol

Aqueous extract

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

Hydromethanolic extract

A 5 g of different organs powder of plant were added to 50 ml of methanol: 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.

Bacterial strains and media

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

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

Gram-negative bacteria were: *Klebsiella pneumonia (*ATCC4352); *Pseudomonas aeruginosa*(ATCC27853); E*scherichia coli* (ATCC25922).

These bacterial strains were obtained from the Pasteur Institute, Algiers, Algeria.

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

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

Antibacterial activity

Disk diffusion method

Petri dishes were prepared with 20 ml of 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 extract were placed at 35°C for 24 h. The antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

MIC determinations

The minimal inhibitory concentration (MIC) preventing visible bacterial growth measured by the different concentrations of extract of Muller Hinton agar media.

Different volume of extract were prepared and added to 20 ml of Muller Hinton Agar media; after agitation , the select solution were transferred into a Petri plates which were incubated at 35°C for 24 h[1].

RESULTS AND DISCUSSION

Qualitative phytochemical screening

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

Table 1:Phytochemical Screening of *Citrulluscolocynthis* 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 plants as medicines provide the basis for indicating which essential oils and plant extract may be useful for specific medical conditions[8,10].

The present investigation has explored the use of one such plant, *C. colocynthis*Schard endemic in the south west of Algeria, for testing phytochemical compound and the antimicrobial activity of this endemic plant.

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

Result of phytochemical screening of *C. colocynthis*Schard fruits leaves and roots of showed the presence of saponins, sterols, steroid, terpen, flavonoids, tannin and alkaloids in different proportion in the tree part of plant.

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

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

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

Antibacterial activity

Disk diffusion method

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

MIC determination

As Table 3 shows, the MICs are more or less important depending on the type of bacteria studied. The hydromethanolic extract of root shows the best antibacterial activity screw all bacterial strains tested.

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

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

Aq: Aqueous *Hyd: Hydromethanolic*

Table 3: The MICs of Aqueous and hydrometanolic extract of *Citrulluscolocynthis* fruits, leaves and roots

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

Aq: Aqueous *Hyd: Hydromethanolic*

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

Generally, the hydrometanolic extract of the tree parts are efficacy overlooked the bacteria tested contribution to aqueous extracts.

The strongest antibacterial activity with inhibition zone is remarked with hydromethanolic root extract or the MIC obtained is 5.6 mg/ml for *Klebsiella pneumonia*, *Listeria monocytogen*and *Pseudomonas aeruginosa*. These results are not in agreement with those reported by Belsem et al. 2009 who found that the plant organs with the highest antibacterial properties were immature fruits and immature seeds, and the lowest activity was observed for root extracts.

The strongest inhibitions were obtained against *E. coli* with hydromethanolic root extract which is consistent 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 root was 13.2 mm.

*P. aeruginosa*is the leading cause of nosocomial infection and has developed mechanisms of resistance to common antibiotic classes[4]Because of its must always seek a new anti-*Pseudomonas aeruginosa*.

These results suggest that the inhibitory effect exhibited by the extract of *C. colocynthis*Schard may be attributable to the secondary metabolites like phenolic compounds and saponins.

Activity cannot 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 microbial infection[7].

CONCLUSION

The obtained results might be considered adequate to demonstrate that *Citrulluscolocynthis*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 hospitals.

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

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REFERENCES

[1]Abdel-Massih R; Abdou E; Baydoun E; Daoud Z, *J. Bot.* Article ID 464087, **2010**,:1-8.
[2]Ambi AA; Abdurrahman EM; Sule MI; Pateh UU; Abdurrahman YR; Ibrahim NDJ, *J. Pharm. Sci.* ,**2007**, 6(2): 7-12.
[3]Belsem M; Marzouk Z; Décor R;Edziri H, *J. Ethnopharmacol*, **2009**, 125: 344-349.
[4] Carmeli Y ; Troillet N ; Eliopoulos G M ;Samore M H, *Antimicrob.Chemother. Agents*, **1999,** 43: 1397-1382.
[5] Cseke L; Setzer W; Vogler B; Kirakosyan A; Kaufman P, Traditional, analytical and preparative separation of natural products. Natural products from plants.CRC press/ Taylor and Francis. Boca Raton. Fla. USA.**2006**, pp. 263-318.
[6] Feinbrun-Dothan N , Flora Palaestina- Part III. Jerusalem. The Israeli.Academy of Sciences and Humanities, **2006**, p. 380.
[7] Fogliani B;Raharivelomanana P;Bianchini JP; Bouraima-Madjébi S;Hnawia E, *Phytochemistry*, **2005,** 66: 241-247.
[8] Hoffman DL, The herb user's guide wellingborough. UK: Thomsons publishing group, **1987**.
[9] Kadi H; Moussaoui A; Benmahdi H; Lazouni HA; Benyahia A;Nahal BN, *J. Appl. Pharm. Sci* ,**2011**, 01(10): 180-182.
[10] Lawless J ,The illustrated encyclopedia of essential oils. Shaftesbury. UK. Element books Ltd, **1995.**.
[11] Murray PR; Baron EJ; Pfaller MA; Tenover FC;Yolke RH, Manual of clinical microbiology. ASM. Washington, **1999**, 6: 51-59.
[12] Nahal BN; Kadi H; Moghte S;Meddah B; Moussaoui A , *Open Conf. Proc*, **2012**, J. 3: 66-69.
[13]National Committee for Clinical Laboratory Standards (NCCLS) ,Performance standards for antimicrobial susceptibility testing. Twelfth informational supplement. M100-S12 (M7). NCCLS. Wayne. Pa, **2002**.
[14] Omulokoli E; Khan B;Chhabra S,. *J. Ethnopharmacol*, **2007**, 56: 133-137.
[15] Rajkiran C;Urvija G; Take RK, *J. Chem*, **2011**, 8(1): 85-90.
[16] Sarkar M; Tanker M, Phytochemical analysis of Ankara University in the spring of eczacicikfaeulte 10.67.Ankar. Turkey, **1991**..
[17] Shah C S;Qadry J S, Text book of pharmacognosy. 5th ed. B. S. Shah, Prakashan, Pankore Naka, Ahmed abed, Inndia, **1985**, p.284.
[18] Trease GE; Evans CW,Pharmacognos. 12th ed. BalliereTindall. London. UK. London, **1984**..
[19]Usman M; Abdul hakeem B; Syed Waseemuddin A; Iqubal A;Husam B, *J. Pharm. Sci*, **2003**, 16(1):1-6.
[20]Velickovic DT; Randjelovic NV; Ristic MS;Velickovic AS;Melcerovic AA, *J. Serb. Chem. Soc*,**2003**, 68(1): 17-24.