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In vitro Antimicrobial Activity of Some Medicinal Plants Found in Saudi Arabia

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The methanolic extracts of *Citrullus colocynthis, Rhazya stricta, Datura stramonium* and *Zygophyllum coccineum* were evaluated for the antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25928, *Streptococcus pyogenes* grp A (clinical isolate), *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231. The extract of different plants tested showed varying degree of inhibitory activity against the tested pathogens. Extract of *C. colocynthis* showed the highest activity followed by *R. stricta,* while *Z. coccineum* exhibited least activity. The gram positive bacteria were observed to be more sensitive to plant extracts as compare to gram negative bacteria. Moreover, all extract were effective against *C. albicans.* While, only the extract of *C. colocynthis* was able to inhibit the growth of *P. aeruginosa* (18.3 mm). The maximum zone of inhibition was observed against *S. aureus* (31.3mm) by the extract of *C. colocynthis* and the MIC against the same bacteria was 0.2 mg/ml. The GCMS analysis of *C. colocynthis* extract showed the presence of several esters, glycosides, alkaloids and flavonoids. However, the main component detected in the extract was l-(+)-Ascorbic acid 2,6-dihexadecanoate; a known hyaluronidase inhibitor. Therefore, the results showed that *C. colocynthis* possesses a broad spectrum of activity against pathogenic bacteria responsible for the most common microbial diseases.

Key words: Antimicrobial activity, medicinal plants, *Citrullus colocynthis,* GC-MS.

The problem of microbial resistance against antibiotics is increasing with every year. The indiscriminate use of antimicrobial drugs for the treatment of diseases is one of the reasons of development of multidrug resistant pathogens. Till now microbial resistance to almost all antibiotics has been reported[1]. Moreover, some antibiotics have side-effects which limit their usage. Therefore, there is a need to discover new spectrum of anti-microbial agents with minimal side effects[2]. Plants have been used by mankind since time immemorial to treat various diseases, including bacterial and fungal infections. Numerous studies on the role of medicinal plants in pharmacology and medicine have been accomplished. A number of plants from different families have been reported to show antimicrobial activity[3]. They constitute a potential source for the production of new medicines and may enhance the effects of conventional antimicrobials, which will probably decrease costs and improve the treatment quality[4].

Most of the plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms. The action mechanisms of natural compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force (PMF), electron flow, active

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transport and coagulation of the cell content. Not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms. In addition to the antimicrobial action of plant extracts and essential oils, a synergism between conventional antimicrobial drugs and products obtained from medicinal plants has also been reported[5]. Possible interactions among medications are frequently observed, which has motivated researchers to test such possibilities.

An important aspect comprises the search for new compounds that have antimicrobial action and synergism with currently available antimicrobial drugs, since bacteria resistant to conventional medicines are increasingly frequent; consequently, medicinal plants constitute an alternative for infection treatment.

Rhazya stricta is an important medicinal plant used in indigenous medicinal herbal drugs to cure various ailments in various countries. It is used in fever, general debility and as curative for chronic rheumatism and tumour. *R. stricta* is used traditionally in Asia for the treatment of different types of diseases such as skin diseases, stomach diseases and antihypertensive. The leaves, flowers and fruit are also used in joint infections and for cancer[6]. *Citrullus colocynthis* is traditionally used as an anti-diabetic medication in tropical and subtropical countries. Its root is given in abdominal enlargements, in coughs and asthmatic attacks[7]. An extract made from the leaves *Datura stramonium* is taken orally for the treatment of asthma and sinus infections and stripped bark are applied externally to treat swellings, burns and ulcers[8]. The fruits of *Zygophyllum coccineum* are used in the treatment of rheumatism, gout, asthma, hypertension, as a diuretic and an anti-diabetic[9].

The present study was aim to evaluate the antimicrobial activity of *C. colocynthis, R. stricta, D. stramonium, Z. coccineum* extracts against human pathogenic bacteria and fungus.

MATERIALS AND METHODS

Collection and Storage of Plant Samples

Plants of *C. colocynthis, R. stricta, D. stramonium, Z. coccineum* were collected from the desert of Riyadh, Saudi Arabia. The plants were identified by the taxonomist Dr. Mona Alwahibi, Department of Botany and Microbiology, King Saud University. Collected fresh plant materials were examined and the old, insect- and fungus-infected leaves were removed. Plant parts were washed with tap water and were surface sterilized by dipping them in 0.1% sodium hypochlorite solution for one minute. After that plant parts were washed with distilled water and were dried at room temperature (25 °C) for about a week on a laboratory desk. Samples were covered with clean sheets of paper to avoid any deposition of dust. The dried material was ground to a fine powder using a grinding mill and stored in airtight bottles in the dark until extraction was done.

Preparation of Plant Extract

The powdered plant material (50 g of each) was extracted with 400 ml methanol (CH_3OH) by Soxhlet extraction for 8 hours. The obtained methanolic extracts were filtered and evaporated by using a rotary evaporator. The dried extracts were stored at -20° C until used.

Determination of Antimicrobial Activity of Plant Extracts

Four human pathogenic bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25928, *Streptococcus pyogenes* grp A (clinical isolate) and *Bacillus subtilis* ATCC 6633) and a human pathogenic fungus (*Candida albicans* ATCC 10231) were procured from the Department of Botany and Microbiology, King Saud University. Stock cultures of bacterial strains were maintained on the slants of nutrient agar whereas; *C. albicans* on slants of potato dextrose agar, all stock cultures were stored at 4°C.

Antimicrobial Assay

Determination of antimicrobial activities by agar well diffusion method

The crude extracts were screened against various human pathogens by agar well diffusion[10]. In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, Germany) was inoculated with the bacterial pathogens and incubated at 37°C for 24 h.

Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the nutrient agar plate to obtain uniform inoculums. Four wells per plate were made with the reverse side of the sterilized micropipette tips. Crude extract (50 ¼l) was poured in respective wells with the help of micropipette. Ampicillin and

Ketoconazole were used as positive control for bacteria and fungus respectively. Methanol solvent was used as the negative control. Each extract was analysed in triplicate. All the plates were incubated for 24 h at 37°C. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest (mm) as observed from the clear zone surrounding the well. For the fungal pathogen *C. albicans* the protocol mentioned above was followed except the culture media used was Potato dextrose broth and Potato dextrose agar (Scharlau Chemie, Spain).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by the streak method[11]. To determine the MIC, extracts were dissolved in distilled water and serially diluted in eppendorf tubes under a laminar flow cabinet. The same volume of an actively growing culture of the tested pathogen was added to the different eppendorf tubes and cultures were grown overnight in an incubator at 37°C. The following morning, streaking was done from all samples on nutrient agar plates. MIC was rated by the lowest concentration of the test solution that inhibited growth.

GC-MS Analysis of Crude Extract

The plant extract which showed strong antimicrobial activity against large number of pathogens tested was analysed for its chemical composition. The analysis was done by using Perkin Elmer (Clarus 500, USA) gas chromatography coupled with (Clarus 500, USA) mass spectrometer (MS) equipped with RTx-5 column (30x0.32nm). The oven temperature was initially held at 75°C for 2 min, then increased to 75 to 175°C at a rate of 50°C per min and finally held at 175°C for 7 min. Helium (3 ml/min) was used as a carrier gas. Neither internal, nor external chemical standards were used in this chromatographic analysis. Interpretation of the resultant mass spectra were made using a computerized library-searching program (NIST database) and by studying the fragmentation pattern of such compound resulted from mass spectrometry analysis. Concentration of compound was calculated by the following formula:

Compound concentration percentage= [P1/P2] x 100

Where, P1 is the peak area of the compound and P2 is whole peak areas in the fractionated extracts.

RESULTS AND DISCUSSION

In the present investigation, the antimicrobial activity of methanolic extracts of *C. colocynthis, R. stricta, D. stramonium, Z. coccineum* were evaluated against *P. aeruginosa, E. coli, B. subtilis, S. aureus, S. pyogenes* grp A, and *C. albicans.* The results obtained showed that methanolic extracts of all the tested plants had inhibitory effects (8.0 to 31.3 mm) on most of the tested pathogens as represented in Table 1. It is evident from the results that the gram positive bacteria were more sensitive to plant extracts compared to gram negative bacteria. Further, it was noted that all the plant extracts were effective against *C. albicans.*

Extract of *C. colocynthis* was found to be effective against all pathogens with the exception *B. subtilis.* The maximum zone of inhibition was

Table 1. *In vitro* antimicrobial activity of methanolic extract of medicinal plants against human pathogenic bacteria and a fungus

Plant name	Pathogen					
	P. aeruginosa	*E. coli*	*S. aureus*	*S. pyogenes* grp A	*B. subtilis*	*C. albicans*
	Zone of Inhibition (mm)					
R. stricta	NA	8.0±1.0	15.3±0.5	20.0±0.0	NA	19.3±0.58
D. stramonium	NA	NA	22.7±0.58	NA	28.3±0.58	24.7±0.58
Z. coccineum	NA	NA	NA	NA	NA	21.3±0.58
C. colocynthis	18.3±0.58	10.0±1	31.3±0.58	21.7±0	NA	20.0±0
Antibiotic*	40.3±0.58	15.3±0.58	23.7±0.58	41.3±0,58	40.7±1.15	34.3±0.58**

Zone: mean ±SD for N = 3; NA: no activity; *antibiotic: Ampicillin 40 µg/ml; **antibiotic Ketoconazole 10 µg/ml

observed against *S. aureus* (31.3 mm). Further, it was observed that only *C. colocynthis* extract was able to inhibit the growth of *P. aeruginosa* (18.3 mm). The methanolic extract of *R. stricta* was observed to be highly effective against *S. pyogenes* grp A (20 mm). Extract of *C. colocynthis* and *R. stricta* exhibited weak zone of inhibition against *E. coli* (10.0 and 8.0 mm, respectively). *D. stramonium* extract was found to be effective against *B. subtilis* (28.3 mm), *C. albicans* (24.7 mm) and *S. aureus* (22.7 mm). The methanolic extract of *Z. coccineum* was found to be effective only against *C. albicans* (21.3 mm). This data is in agreement with previous reports elsewhere using the same plants[12-13]. Methanolic, chloroform and aqueous extract of 26 medicinal plants used in folklore medicine in Saudi Arabia were screened for *in vitro* antimicrobial activity. The highest activity was observed from extract of *Withania somnifera* followed by *D. stramonium*, while, *Z. portulacoides* exhibited the least activity[14]. The chloroform and methanol extracts of the roots of *R. stricta*, showed antimicrobial and antifungal activities against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Aspergillus terreus*, *A. flavus* and *C. albicans*. Tetrahydrosecamine, isolated from the plant showed broad spectrum antimicrobial activity. Similarly, another active component, Strictanol, was also found to be most active against *E. coli* and *P. aeruginosa*[15]. The methanol extracts of *D. stromonium* and *D. inoxia* showed activity against gram positive bacteria in a dose dependent manner. Little or no antimicrobial activity was found against *E. coli* and *P. aeruginosa*[16]. Several studies have reported that *C. colocynthis* have an anti-diabetic, carcinogenic, antioxidant, antibacterial and toxic effects[17-21].

Table 2 represents the data regarding the plant parts of the medicinal plants used for the extraction, the total yield of extract obtained, total per cent of pathogens inhibited by plant extract and minimum inhibitory concentration (MIC) of the extract. The highest total yield of the extract was obtained from the leaves and stems of *R. stricta* (18.27%) and the least yield was obtained from the leaves of *Z. coccineum* (5.18%). The MIC of *C. colocynthis* extract against *S. aureus* was 0.2mg/ml, whereas, MIC of *R. stricta* extract against *S. pyogenes* grp A was 0.25mg/ml. Of all the extract tested only the extract of *D. stramonium* was observed to render the growth of *B. subtilis* and the MIC was 0.6 mg/ml. The data of Table 2 clearly showed that the extract of *C. colocynthis* was highly effective in inhibiting the growth of maximum number of pathogen tested (83.3%). Thus analysis was carried out to determine the chemical composition of methanolic extract of *C. colocynthis*. Gas chromatography coupled with mass spectrometer (GC-MS) revealed that the main components in the extract was l-(+)-Ascorbic acid 2,6-dihexadecanoate; a known hyaluronidase inhibitor[22] besides that esters, glycosides, alkaloids and flavonoids were also detected (Fig. 1). The compounds reported earlier in *C. colocynthis* are glycosides (Estrols classified to groups A, B, K, L, and E curcurbitacins), alkaloids, and flavonoids[23].

Table 2. Plant parts used for the extraction, total yield of extract, total per cent of pathogens inhibited by plant extract and minimum inhibitory concentration (MIC) of the extract

Plant name	Part of the plant used for extraction	Yield of the extract (%)	Pathogens inhibited (%)	MIC (mg/ml)
R. stricta	Leaves and stems	18.27	66.7	0.25*S. pyogenes* grp A
D. stramonium	Leaves and stems	5.24	50.0	0.6*B. subtilis*
C. colocynthis	leaves	15.5	83.3	0.2*S. aureus*
Z. coccineum	Leaves and stems	5.18	16.7	1.0*C. albicans*

Medicinal plants have enormous therapeutic potential as they have wide range of natural antibiotic agents/chemicals that can serve the purpose with lesser side effects. The plants in this study were selected on the basis of their ethno-medical application in the treatment of diseases. Therefore, our investigation further confirms the traditional medicinal values of these plants as the plants tested showed the ability to combat one or

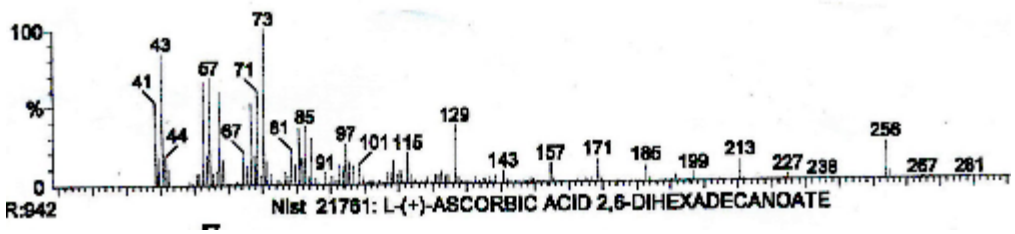

Fig. 1. Mass spectrum of l-(+)-Ascorbic acid 2, 6-dihexadecanoate detected in the methanol extract of *C. colocynthis*

more than one pathogen. *C. colocynthis* possesses a broad spectrum of activity against pathogenic bacteria and fungus responsible for the most common microbial diseases. Thus this plant can be explored further to find out clinically effective antimicrobial compounds.

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