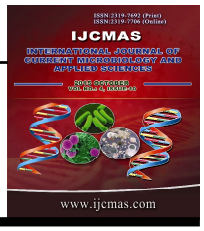
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 60-69

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 60-69**
http://www.ijcmas.com



Original Research Article

In vitro antimicrobial activity of *Citrullus colocynthis* (Linn.) against selected microorganisms

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A B S T R A C T

In the present study the aerial part of *Citrullus colocynthis* was examined for antimicrobial property against gram positive and gram negative bacteria viz. *Bacillus cereus, Brucella canis, Campylobacter sp., Flavobacterium meningosepticum, Vibrio cholera, Staphylococcus aureus, Enterobacter sp., Streptococcus sp., Clostridium perfringens, Escherichia coli, Salmonella enteritidis, Klebsiella sp., Proteus mirabilis, Pseudomonas aeruginosa, Yersinia pestis* and fungal pathogens such as *Aspergillus niger, Microsporum sp., Trichophyton sp., Candida albicans.* and *Penicillium sp.* In the present study revealed that the plant extract exibit wide range of antimicrobial activity against the tested micro-organisms. The antimicrobial activity of *Citrullus colocynthis* was compared with standard antibiotic Cetracyclin. The acetone extract showed the presence of alkaloid, steroidal glycosides, saponin, chlorogenic acid and terpenoid. The phytochemicals jointly or independently cause the antimicrobial property against the selected microorganisms.

Keywords

Citrullus colocynthis, antimicrobial activity, Cetracyclin and phyto-chemicals

Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterials in the last years, resistance to these drugs has increased and has now become a global concern (Adwan and Mhanna, 2008). The global emergence of multi-drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure (Hancock, 2005). Bacterial resistance to chemically unrelated antimicrobial agents is public health concern (Sharma *et al*., 2005) and may be caused by over-expression of MDR efflux pumps (Li and Nikaido, 2004). In Gram-negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms (Lomovskaya and Bostian, 2006).

Among Gram-negative bacteria, many of these MDR efflux pumps belong to the RND (resistance-nodulation-cell division) type family of tripartite efflux pumps.

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds (Hancock *et al.,* 2012). The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez *et al.*, 1996).

In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al*., 1999).

Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease in folk medicine.

The search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents (Bhavnani and Ballow, 2000). Herbal medications in particular have seen a revival of interest (Chariandy *et al*., 1999) due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option.

Use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these plants are still widely used in ethno medicine around the world (Thomson, 1978 and Stockwell, 1988). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller.

Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Gerhartz *et al*., 1985 and Kroschwitz, 1992). Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, atleast 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman *et al*., 2000). Hence in the present study, an attempt has been made with the antimicrobial activity of selected medicinal plant *Citrullus colocynthis* on some human pathogenic bacteria and fungi.

Citrullus colocynthis (L.) a member of

Cucurbitaceae family is a desert plant with a rich history as an important medicinal plant and as a source of valuable oil. It is distributed in African and Arabian countries and India. It is a long lived perennial and grows wild in sandy shone under xerophitic conditions, young fruits are fleshy, mottled with dark green and usually turn yellow when ripe, the fruit of *C. colocynthis* had been used medicinally since ancient times. It is commonly known as bitter apple, colosynth or wild gourd is used as an abortifacient, cathartic, purgative and vermifuse and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, tumour and as an insect repellant (Duke, 2006). The fruit of *C. colocynthis* have been commonly used as a catharsis and antidiabetic agents in traditional Egyptian and Indian Ayurvedic medicines.

A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant (Seger *et al*., 2005). Fruit juice with sugar is a house hold remedy in dropsy (Anonymous, 1970). Root extract is used against jaundice, urinary diseases, rheumatism etc. (Dastur, 1962). Seeds are diuretic (Vohora and Khan, 1981). Fruits are used against tumors of gastrointestinal tract. It is more pronouncedly used in anticancerous drug.

It is effective in leukemia and joint pains. Leaves of *C. colocynthis* had anti-inflammatory (Rajamanickam *et al.,* 2010), antidiabetic (Gurudeeban and Ramanathan, 2010), anti oxidant (Ramanathan *et al*., 2010) and local anesthetic (Ramanathan *et al.,* 2010) activities were reported. The purpose of this study was to evaluate the antimicrobial effect of fruits and aerial parts of *C. colocynthis* against selected pathogens.

Materials and methods

Collection and preparation of plant extract

The whole of *Citrullus colocynthis* were collected from the fields and brought to the laboratory. The leaves were washed with tap water and moisture was removed through filter paper. The washed leaves were chopped into small pieces with the help of a knife and dried under shade at room temperature (27 ± 2^0C) for about 20 days. The completely dried plants were powdered with an electrical blender and sieved to get fine powder. The powders were stored in airtight containers for further analysis. The plant powders were extracted with acetone by using Soxhlet apparatus for 8. The extracts were concentrated using a vacuum evaporator at 45^0C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in separate glass vials at 4^0C in refrigerator for further experiments.

Antibacterial susceptibility testing

The antimicrobial activity of plant extract against different pathogens was determined by Agar Well diffusion method or cork borer method. In the present study gram positive, (*Bacillus cereus, Brucella canis, Campylobacter spp, Flavobacterium meningosepticum, Vibrio cholera, Staphylococcus aureus, Enterobacter sp.* And *Streptococcus sp.*), gram negative (*Clostridium perfringens, Escherichia coli, Salmonella enteritidis, Klebsiella sp, Proteus mirabilis* and *Pseudomonas aeruginosa*) bacterial strains and fungal pathogens such as *Aspergillus niger, Microsporum sp., Trichophyton sp., Candida albicans.* and *Penicillium sp.* were used to estimate the antimicrobial property of *C. colocynthis.*

Agar well diffusion assay

The modified agar well diffusion method of Perez *et al*. (1990) was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts and blanks. The concentration of the extracts employed was 25 µg/ml. The test was carried out by triplicate. The plaques were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated by applying the expression in mm.

Qualitative analysis of phytochemicals

In the present study the acetone extract of *C. colocynthis* subjected to preliminary phytochemical analysis to identify alkaloid, carbohydrate, steroidal glycosides, saponin, tannin, phenol, chlorogenic acid, flavonoids, coumarine, anthocyanin and terpenoid (Harborne, 1973).

Results and Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Govindarajan *et al*., 2006). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations.

In the present study antimicrobial activity of acetone extract of *C. colocynthis* on eight gram positive pathogenic bacteria using agar well diffusion method (Table.1) have showed maximum zone of inhibition (25mm) against *Vibrio cholera*, followed by *Campylobacter sp* (21mm), *Flavobacterium meningosepticum* (20mm), *Bacillus cereus* (19mm), *Staphylococcus aureus* (19mm), *Enterobacter sp.*(16mm), *Brucella canis* (15mm) and *Streptococcus sp.*(14mm). The control Cetracyclin showed maximum zone of inhibition were noticed against *Brucella canis* and *Streptococcus sp.* (19mm) followed by *Campylobacter sp* (18mm), *Enterobacter sp.*(17mm), *Vibrio cholera* (15mm), *Staphylococcus aureus* (15mm), *Flavobacterium meningosepticum* (12mm) and *Bacillus cereus* (11mm).

Table.1 Antibacterial activity of *Citrullus colocynthis* against Gram positive bacterial pathogens by Agar Well Diffusion method

S. No	Name of the microorganism	Zone of inhibition (mm)	
		Control (Cetracyclin)	***C. colocynthis***
1.	*Bacillus cereus*	11	19
2.	*Brucella canis*	19	15
3.	*Campylobacter sp*	18	21
4.	*Flavobacterium meningosepticum*	12	20
5.	*Vibrio cholera*	15	25
6.	*Staphylococcus aureus*	15	19
7.	*Enterobacter sp.*	17	16
8.	*Streptococcus sp.*	19	14

The antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains (Masotti *et al*., 2003; Angioni, 2006). Moreover, different studies found that the type of solvent has an important role in the process of extracting (Al-Zubaydi *et al.*, 2009; Bakht, 2011; Boklari, 2009; Bedi, 2010). Akinpelu (2000) and Ofokansi (2005) that showed strong activities of methanol extract of *Bryophyllum pinnatum* against some Gram-positive organisms. The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active properties of these plants like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial (Cowan, 1999; Okwu and Josiah, 2006).

Table.2 Antibacterial activity of *Citrullus colocynthis* against Gram negative bacterial pathogens by Agar Well Diffusion method

S. No	Name of the microorganism	Zone of inhibition (mm)	
		Control (Cetracyclin)	***C. colocynthis***
1.	*Clostridium perfringens*	14	18
2.	*Escherichia coli*	13	10
3.	*Salmonella enteritidis*	18	14
4.	*Klebsiella sp.*	11	11
5.	*Proteus mirabilis*	10	17
6.	*Pseudomonas aeruginosa*	18	25
7.	*Yersinia pestis*	12	12

In the present investigation the antimicrobial activity of acetone extract of *C. colocynthis* on seven gram negative pathogenic bacteria using agar well diffusion method (Table.2) have showed maximum zone of inhibition (25mm) against *Pseudomonas aeruginosa* followed by *Clostridium perfringens* (18mm), *Proteus mirabilis* (17mm), *Salmonella enteritidis* (14mm), *Yersinia pestis* (12mm), *Klebsiella sp.* (11mm) and *Escherichia coli* (10mm). Similarly the antibiotic has high impact on human pathogenic bacteria such as 18 mm, 18 mm, 14mm, 13mm, 12mm, 11mm and 10mm against *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella sp.*and *Proteus mirabilis. Escherichia coli* inhabit in intestine, normally harmless, but certain strains bind to epithelial wall causes release of toxin that adversely affect the intestine may be gastroenteritis as well (Gurdeeban *et al* ., 2011). Since ancient time has been dependent on plants for food, drink, shelter, equipments, dental care and medicines for many diseases (Idu *et al*., 2007).

The present study revealed that the Gram negative strains to be less sensitive to antibiotics than gram positive bacteria. A crude extract of *Vernonia adensis* was found to have high activity against *E. coli*, a gram negative bacterial strain of clinical importance (Kisangau *et al*., 2007). The leaves of *M. indica* have been reported to possess antibacterial activity against *E. coli* and other bacteria (Doughari and Manzara,

2008). Another different part of the mango plant, the kernel seed was also observed to possess high antibacterial activity against food borne pathogenic bacteria (Kabuki *et al*., 2000). Since ancient time has been dependent on plants for food, drink, shelter, equipment's, dental care and medicine. It has often been said that all plants are potential medicines for many diseases. The extract exerted inhibitory effect on both gram-positive and gram-negative bacteria, the present result agree with the study of (Sokmen *et al*., 2004) which showed that it kills bacteria in respective to their cell wall structure, however other studies showed that gram-positive bacteria is more sensitive than gram negative bacteria or vice versa (Karaman *et al*., 2003).

Table.3. Antifungal activity of *Citrullus colocynthis* against Gram positive bacterial pathogens by Agar Well Diffusion method

S. No	Name of the microorganism	Zone of inhibition (mm)	
		Control (Cetracyclin)	*C. colocynthis*
1.	***Aspergillusniger***	14	10
2.	***Microsporumsp***	9	Nil
3.	***Trichophytonspp***	13	Nil
4.	***Candida albicans***	15	12
5.	***Penicillium sp***	20	11

Acetone extract of *C. colocynthis* were evaluated against the antifungal activity against five fungal pathogen showed maximum zone of inhibition against *Penicillium sp.*(11mm) and *Aspergillus niger* (10mm). Among the fungal species tested *Microsporum sp.* And *Trichophyton sp.* were not exhibit any zone of inhibition against acetone extract of *C. colocynthis*.The control Cetracyclin showed the maximum zone of inhibition 12 mm was noticed against *Candida albicans* followed by *Penicillium sp.* (20mm), *Candida albicans* (15mm), *Aspergillus niger* (14mm), *Trichophyton sp.* (13mm) and by *Microsporum sp.* (9mm). The *Aspergillus* sps are causative agents for several skin diseases, like *A. flavus* releases aflotoxin contaminating food leading to cirrhosis of liver and cancerous growth which is prevalent in India and Africa.

The present study revealed that fungal isolates are not more sensitive than bacterial isolates toward the acetone extract of *C. colocynthis*. The inhibitory action of the extract could be attributed to the presence of active compounds in the extract which are water soluble like glucosides and resins which inhibit enzymatic activity in cytoplasmic membrane (Greulach, 1973). The extract inhibited the growth of fungal isolates, this attributed to the presence of the active compounds like colocynthidin and colocynthin alkaloids which may be disrupt cytoplasmic membrane of the micro-organisms through their action on lipids and protein, furthermore these compounds may penetrated cytoplasmic membrane and competed the active sites of certain enzymes inside the cell that are essential for multiplication of the microorganisms (Al-Jasim and Barakat, 1976).

The preliminary qualitative phytochemical investigation carried out on *C. colocynthesis* showed it consist of secondary metabolites such as alkaloid, steroidal glycosides, flavonoids tannin, saponin, chlorogenic acid. The mechanism of action of highly aromatic

planer quaternary alkaloids is attributed to their ability in intercalate with DNA (Kumar *et al*., 2007). Tannis have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo *et al*., 1991).

Rose Mary *et al*., (2008) and Ayana *et al*. (2008) screened antimicrobial activity of *Citrullus colocynthis* against *Bacillus Subtilis , Escherichia. Coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vilgaris* and *Klebsiella pneumonia*. Flavonoids of *Citrullus colocynthis* considered as microbial inhibitor which are resistant to antibiotics (Linuma *et al.*, 1994). Herbal medicine represents one of the most important fields of traditional medicine all over the world (Hamil *et al*., 2003). To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which folklore reputation in amore intensified way (Cragg *et al*., 1997).

Results of this investigation offer a scientific basis or the use of *Citrullus colocynthis* methanolic extracts to prevention of different types of infections and solved drug resistance problem.

Table.4 Preliminary phytochemical analysis of methanol leaf extract in *C. colocynthis*

S. No	Phytochemical Constituents	Name of the Test	Plant extract
1	**Alkaloid**	Mayer's test Dragendroff's test Wagner Test	+ + +
2	**Carbohydrate**	Molish Test Fehling Test Benedicts Test	- - -
3	**Steroidal Glycosides**	Libermann's test Salkowaski test	+ +
4	**Saponin**	Foam Test	+
5	**Tannin**	Lead Acetate	-
6	**Phenol**	Phenol reagent	-
7	**Chlorogenic acid**	Ammonia test	+
8	**Flavonoids**	Ammonia test	-
9	**Coumarin**	Sodium chloride test	-
10	**Anthocyanin**	H_2So_4 test	-
11	**Terpenoid**	Borntrager's test	+

\+ Present of compounds - Absent of compounds

This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno botanical approach should be considered when investigating antimicrobial properties of plants (Adesanya *et al*., 2005; Iwu, 1993). The implication of the broad spectrum action of some of these extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated (Olukoya

et al., 1993). *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.

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