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Full Length Research Paper

Antimicrobial effect of coastal medicinal plant – *Citrullus colocynthis* against pathogenic microorganisms

Gurudeeban S, Ramanathan T*, Satyavani K and Dhinesh T

Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, Tamil Nadu, India.

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Citrullus colocynthis **showed broad spectrum antimicrobial activity against 16 clinical microorganisms isolated from HIV positive patients, including bacteria Viz.,** ***Bacillus subtilis, Escherichia coli, Klebseilla pneumoniae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes, Salmonella typhi*** **and** ***Vibrio cholerae;*** **and six fungal strains** ***Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor sp., Penicillium sp.*** **and** ***Rhizopus sp.*** **No correlation was observed between susceptibility of the test strains with plant extracts and antibiotic resistance behaviour of the microbial strains. The significant antimicrobial activity of active extracts was compared with the standard tetracycline (30 µg/disc). The results obtained in the present study suggest that** ***C. colocynthis*** **can be used in treating diseases caused by the test organisms.**

Key words: Antimicrobial activity, *Bacillus subtilis, Citrullus colocynthis*, tetracycline.

INTRODUCTION

Traditional medicine is an important source of potentially useful compounds for the development of phytotherapeutic agents. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases while simultaneously migitigting many of the side effects that are often associated with synthetic antimicrobials (Lwu et al., 1999). The antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. A number of plants from different families of angiosperms have been reported to show antimicrobial activity (Palombo et al., 2001). The spread of multi drug resistant strains of microorganisms necessitates the discovery of new classes of antimicrobial and compounds that inhibit these resistance mechanisms. Natural products continue to play a major role as active substances, model molecules for the discovery and validation of drug targets. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of human kind and there is an ever-increasing demand for more and more drugs from plant sources (Nair et al., 2008).

Egusi (*Citrullus colocynthis* L.) belongs to the melon family of cucurbitaceae and it produces bitter flavoured fruits about the size of cantaloupe and seeds rich in oil and protein. It is a long lived perennial and grows wild in sandy shore under xerophitic conditions. Young fruits are fleshy, mottled with dark green and usually turn yellow when ripe (Tackholm, 1974). The fruit of *Citrullus colocynthis* had been used medicinally since ancient times. Traditionally, fruit of *C. colocynthis* was used for the treatment of diabetes, microbial diseases, ulcer, inflammation, jaundice and urinary diseases in Asian and African countries (Nmila et al., 2000). In our laboratory leaves of *C. colocynthis* had anti-inflammatory (Rajamanickam et al., 2010), antidiabetic (Gurudeeban and Ramanathan, 2010), anti oxidant (Ramanathan et al., 2010) and local anaesthetic (Ramanathan et al., 2010) activities were reported. The purpose of this study was to evaluate the antimicrobial effect of fruits and aerial parts

*Corresponding author. E-mail: drtrcasmb@gmail.com. Tel: 04144-243223, Ext: 213. Fax: 04144-243555.

of *C. colocynthis* against selected pathogens isolated from HIV positive patients.

MATERIALS AND METHODS

Plant material

Arial parts and fruits of *C. colocynthis* were collected early November 2009 during the flowering and fruiting stage from the Southeast coast of Parangipettai, Tamil Nadu, India. The plant was taxonomically identified and authenticated in the Herbaria being maintained at the Centre of Advanced Study in Marine Biology, Annamalai University (Voucher no. CAS 0013) Tamil Nadu, India.

Extraction of the plant material

10 g of powdered fruits and 5 g of aerial parts were taken in 100 ml of ethanol in a conical flask, plugged with cotton wool and then kept on a shaker at 190 to 220 rpm for 24 h. After 24 h, the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Giron et al., 1988) and stored at 4°C in airtight bottles.

Microbial strains

Microorganisms were isolated and obtained from HIV positive patients, in the Rajah Muthiah Medical College and Hospital, Annamalai Nagar, Chidambaram, Tamil Nadu, India. Amongst the ten bacteria investigated were four Gram-positive bacteria were *Bacillus subtilis* and *Staphylococcus aureus*, *Streptococcus faecalis, Streptococcus pyogenes,* while the six Gram-negative bacteria were: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Salmonella typhi* and *Vibrio cholerae*. The six fungi investigated were: *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor* sp., *Penicillium* sp. *and Rhizopus* sp.

Antibacterial activity

The agar diffusion method (Bauer et al., 1966) was followed for the antibacterial susceptibility test. A loopful bacteria was taken from the stock culture and dissolved in 0.1 ml of saline. All the tests were done by placing the disc (6 mm diameter) impregnated with (200 µl) extracts on the Mueller Hinton Agar surface previously inoculated with 10 ml of MHA liquid medium with Gram negative and Gram positive bacteria. Respective solvent without plant extract served as negative control. Standard antibiotics of Tetracycline (30 µg/ disc) were used as positive control.

Antifungal activity

Antifungal activity was determined against six fungi. The stock culture was maintained in glucose peptone yeast and sucrose (GPYS) medium. Fungal inoculum (0.2 ml) of a 48 h old culture was distributed uniformly on to the surface of agar plates containing GPYS medium with the help of a sterile cotton swab. Culture medium was prepared by adding dextrose (20 g/L) peptone (10 g/L) and agar (25 g/L) in distilled water and was sterilized in an autoclave at a pressure of 15 lbs and a temperature of 120°C. At the time of inoculation, the disc impregnated with plant extract (100 µg/disc of 10 mm diameter) was placed the plates were incubated for 48 h at 37°C. For each fungal strain, controls were maintained where pure solvents were used instead of the extract. The fungal activity was measured as the inhibition zone diameter. The experiment was done three times and the mean values are presented (Ramanathan, 2000).

RESULTS AND DISCUSSION

Antimicrobial assays

Table 1 summarizes the microbial growth inhibition by ethanol extracts of *C. colocynthis* the screened plant species. The alcoholic extract of the plant showed high antibacterial activity against *B. subtilis,* and *S. aureus*, *S. pyogenes, S. typhi*, considerably less effect against *E. coli, P. mirabilis, Streptococcus faecalis* and *K. pneumonia, P. vulgaris* and *V. cholerae* did not show any antibacterial activity against the other six species. Table 2 summarizes the fungal growth inhibition of ethanol extracts of the screened plant species. The ethanolic extract showed high antifungal activity against *A. fumigatus, A. flavus* and less effect against *Mucor* sp. There was no effect against *C. albicans, Penicillium* sp., *and Rhizopus* sp., did not show any antifungal activity.

The results showed that *C. colocynthis* (200 µl) extract had antibiological compound having differential potential as an antibacterial and anti fungal activities. The antimicrobial properties were depending on concentration of the extract. It could be attributed to some structural modification of active principle under changed medium of extraction (Singh and Mishra, 2010). In the present study, the microbes activity examined in presence of plant extract could be economically significant. 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 are prevalent in India and Africa (Tortora et al., 1998). Though *E. 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 (Tortora et al., 1998). Since ancient time has been dependent on plants for food, drink, shelter, equipments, dental care and medicine. It has often been said that all plants are potential medicines for many diseases. The preliminary phytochemical investigations carried out on the stem of *S. jamaicensis* showed it consist of secondary metabolities such as saponins, tannins and flavonoids (Idu et al., 2007). Ogbonna et al. (2003) reported positive antimicrobial activity of *Ximenia americana* on *E. coli, S. typhi, B. subtilis* and *P. vulgaris* and it also phytochemical analysis

Table 1. Antibacterial activity of ethanol extracts of *Citrullus colocynthis.*

Microorganism	Ethanol extract inhibition zone (mm)
Bacillus subtilis	11 ± 0.15
Staphylococcus aureus	7 ± 0.25
Streptococcus faecalis	3 ± 0.52
Streptococcus pyogenes	11 ± 0.52
Escherichia coli	3 ± 0.26
Klebseilla pneumoniae	NA
Proteus mirabilis	2 ± 1.50
Proteus vulgaris	NA
Salmonella typhi	12 ± 0.65
Vibrio cholerae	NA

NA- No activity.

Table 2. Antifungal activity of ethanol extracts of *Citrullus colocynthis.*

Name of the microorganisms	Inhibition zone
Aspergillus flavus	6 ± 0.12
Aspergillus fumigatus	10 ± 0.86
Candida albicans	NA
Mucor sp*.,*	5 ± 0.36
Penicillium sp*.,*	NA
Rhizopus sp*,*	NA

NA- No activity.

of the leaf extract of the plant used revealed the presence of alkaloids, saponins, tannins, cardiac glycosides and steroids. Also the preliminary phytochemicals were responsible for antimicrobial properties of leaf extract of *C. colocynthis* (Gurudeeban et al., 2010).

C. colocynthis is a valuable plant source of medicinally useful compounds 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 a detailed study is required to find out the specific bioactive compounds responsible for antimicrobial property through various advanced techniques.

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