ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 2, pp. 078-081, March, 2010

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF *CITRULLUS COLOCYNTHIS* IN GULF OF MANNAR

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ARTICLE INFO

Article History:
Received 10th January, 2010
Received in revised form
15th February, 2010
Accepted 3rd March, 2010
Published online 15th March, 2010

Key words:
Antimicrobial activity,
Citrullus colocynthis,
Disc diffusion,
Plant constituents

ABSTRACT

Studies on the antibacterial activities of the leaf extract of *Citrullus colocynthis* (Cucurbitaceae), a medicinal plant used for the treatment of various ailments was carried out using agar disc diffusion technique. Broad spectrum of an antimicrobial activity against sixteen bacteria and six fungal strains. No correlation was observed between susceptibility of test strains with plant extracts and antibiotic resistance behavior of the microbial strains. Qualitative phytochemical test of active extracts demonstrated the presence of phenols, tannins and flavonoids as active constituents. The significant antimicrobial activity of active extracts was compared with the standard piperacillin (100µg/disc) and Gentamicin (10µg/disc). The results obtained in the present study suggest that *Citrullus colocynthis* can be used in treating diseases caused by the test organisms.

INTRODUCTION

Citrullus colocynthis belongs to the family Cucurbitaceae. Members of this family are generally dioecious herbs which may be prostate or climbing by means of tendrils. Fruit is fleshy and many fruits are used as vegetable or as edible fruits. *Citrullus colocynthis* is a small scarbid perennial creeping herb with prostate or climbing Stem, bearing smooth spherical fruits which are mottled green when young and some what yellow when ripe (Shah & Qadry, 1985). Colocynth was well known to Greeks and Romans, both Dioskurides and Pliny being familiar with it. The drug was equally known to the Arabian Physicians and was produced in Cyprus and Spain during the ages. It is mentioned in Anglosaxon herbal of eleven century (Trease, 1976). It is native of warmer parts of Asia, Syria, Egypt and Martine region of the Mediterranean. It is cultivated to some extent in Spain, Sicily and Morocco for purpose of export. It occurs through the subcontinent and is seen growing wild in the warm and arid sandy tract of northwest, central and south India and on the sea shore of coromandal coast (Anonymous, 1970).

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents. 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). In spite of the millions of chemical structures currently available for screening for therapeutic value, natural products, particularly of plant origin remain most important sources of new drugs (Odugbemi, 2006).

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Staphylococcus aureus is a gram-positive, non spore forming, non motile cocci. They are aerobic and facultative anaerobic. The organism is resistant to many antibiotics. *Salmonella* is a gram-negative, non-spore forming, mostly motile rod. They are aerobic and facultatively anaerobic. They ferment carbohydrates in to acid and gas anaerobically and grown in substrate below pH 4.0 (Uraih, 2004). A fruit of *Citrullus colocynthis* belongs to the family of Cucurbitaceae is traditionally used for the treatment of diabetes, microbial diseases, ulcer, inflammation, jaundice and urinary diseases in Asian and African countries (Nmila *et al.,* 2000). Nevertheless, to date, the scientific scrutiny of *Citrullus colocynthis,* is insufficiently documented and warrants systematic analysis. In particular, the acute effect of aqueous extract of the leaf *in vivo* remains untested. The anti diabetic effects of leaf of *Citrullus colocynthis* was reported (Gurudeeban, 2008). In Southeast coast of India, especially Parangipettai fisher community uses the plant are microbial and diabetic diseases (Ramanathan, 2000). Hence, the present investigation has been aimed to evaluate the antimicrobial effect of *Citrullus colocynthis* against selected pathogens.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plant parts were collected randomly from the coastal region of Gulf of Mannar, Biosphere Reserve and Tamilnadu, India. The taxonomic identification of the study plant was confirmed in the Herbaria being maintained at the Centre of Advanced Study in Marine Biology, Annamalai University. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extract

Citrullus colocynthis leaf was dried in the shade and powdered. The powder was soaked in 1 liter of water (20mg/l) in different glass jars for 72 hours. This was repeated another 24 hours, until the extract became a colorless. The extract were distilled and concentrated under reduced pressure by using Rota evaporator and then lyophilized. This extract was dissolved 10ml of water and used for further studies.

Methanol extraction

10 g of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a shaker at 190 - 220 rpm for 24 h. After 24 hours, 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

In vitro antimicrobial activity was examined for aqueous and methanol extracts. Microorganisms were obtained from the Rajah Muthiah Medical College and Hospital, Annamalai nagar, Chidambaram, Tamilnadu, India. Amongst ten bacteria investigated four Gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus*, *Streptococcus faecalis* and *Streptococcus pyogenes* six Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Salmonella typhi* and *Vibrio cholerae*. Amongst six fungi strain such as *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor* sp*., Penicillium* sp. and *Rhizopus* sp.

Antibiotic resistance of test strains

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Baur *et al* (1966) against a number of antibiotics including two antifungal drugs. *E. coli* B (a sensitive strain) was used to check the potency of the antibiotics discs. Amoxycillin, Cefuroxime, Cefaclor, Chloramphenicol, Doxycycline, Nalidixic acid , Novobiocin , Tetracycline, (30 mg/disc); Cloxacilli, Methicillin, Fluconazole,(10mg/disc); Nystatin, (100 mg/disc) and Nitofurantoin (300 mg/disc). All antibiotic discs were purchased from the Hi-Media (Mumbai).

Media preparation and antibacterial activity

The agar diffusion method (Bauer *et al*., 1966) was followed for antibacterial susceptibility test. Petriplates were prepared by pouring 20 mL of Muller Hinton Agar supplemented with 4% sodium chloride and allowed to solidify. Plates were dried and 0.1 mL of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5 min. About 6 mm paper discs (Whatmann No. 1) were impregnated with 20 µL of the extracts dissolved in 5% dimethyl sulphoxide (DMSO) at the concentration of 50 mg/mL to obtain 500µg/disc. Vancomycin (30µg/disc) and Ciprofloxacin (5µg/disc) were used as positive controls. About 5% DMSO was used as negative control.

Media preparation and 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 48 hr 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 (20g/l) peptone (10g/l) and agar (25g/l) in distilled water and was sterilized in an autoclave at a pressure of 15 lb/in^2 and a temperature of 120°C. At the time of inoculation, the disc impregnated with plant extract (100 g/disc of 10mm dia) was placed the plates were incubated for 48hrs at 37°C. For each fungal strain, controls were maintained where pure solvents were used instead of the extract. The antimicrobial activity was measured in terms of inhibition zone diameter. The experiment was done three times and the mean values were reported (Ramanathan, 2000).

Preliminary phytochemical analysis

Qualitative phytochemical analysis of the crude powder of the plants collected was determined as follows Tannins (200 mg plant material in 10 ml distilled water/Chloroform/ Ethanol and filtered).The filtrate was used for analysis of tannins, (2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins); Alkaloids (2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/ Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids); Saponins (0.5 ml filtrate + 5 ml distilled water frothing persistence indicated presence of saponins); Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4, green-blue color indicated the presence of cardiac glycosides); Steroids (Liebermann-Burchard reaction: 2 ml chloroform filtrate + 2 ml acetic anhydride + conc. H_2SO_4, Blue-green ring indicated the presence of terpenoids); Flavonoids 2 ml ethanol filtrate + conc. HCl + magnesium ,ribbon pink-tomato red color indicated the presence of flavonoids (Harbone,1973).

RESULTS

Antimicrobial assay

The microbial growth inhibition by aqueous and methanol extracts of the screened plant species. The aqueous extract of the plants showed high antibacterial activity against *E.coli* and *Staphylococcus aureus*, considerably less effect against *Klebseilla pneumoniae* and *Bacillus subtilis* and other aqueous extracts did not exhibit any antibacterial activity (Table 1). On the other hand, methanol extracts of the plants Showed high antibacterial activity against *Bacillus subtilis, Streptococcus pyogenes, Salmonella typhi*, considerably less activity against *Streptococcus faecalis and* there was no effect against *Proteus mirabilis, Proteus vulgaris* and *Vibrio cholerae*. The microbial growth inhibition of aqueous and methanol extracts of the screened plant species. The msethanolic extract of the plant showed high antifungal activity against *Aspergillus fumigatus, Mucor* sp*.,* and *Aspergillus flavus, Candida albicans, Penicillium* sp., and *Rhizopus* sp. did not show any antifungal activity (Table 2).

Phytochemical constituent

Preliminary phytochemical analysis revealed that the presence of tannins and flavonoids, was high, cardiac glycosides and alkaloids was moderate and steroids was in trace amount (Table 3). There are differences in the antimicrobial effects of plant species, due to the phytochemical properties which differ among species.

Table. 1 Antibacterial activity of aqueous and methanol extracts of *Citrullus colocynthis*

Human pathogenic bacterial strain	Zone of Inhibition (mm)	
	Aqueous	Methanol
Bacillus subtilis	5±0.56	12±0.15
Staphylococcus aureus	13±1.52	6±0.25
Streptococcus faecalis	-	3±0.52
Streptococcus pyogenes	2±0.35	12±0.52
Escherichia coli	14± 1.25	3±0.26
Klebseilla pneumoniae	5±0.05	NA
Proteus mirabilis	NA	1±1.50
Proteus vulgaris	NA	NA
Salmonella typhi	2±0.42	13±0.65
Vibrio cholerae	-	-

Zone of inhibition were expressed as STD ± SEM (P < 0.05 level in mm)
T – Trace activity; NA – No Activity

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

Fungal strain	Zone of Inhibition (mm)	
	Aqueous extract	Methanol extract
Aspergillus flavus	T	NA
Aspergillus fumigatus	NA	10 ± 0.86
Candida albicans	T	NA
Mucor sp.,	2± 0.16	5 ± 0.36
Penicillium sp.,	4±0.52	NA
Rhizopus sp,	T	NA

T – Trace activity; NA – No Activity

Table.3 Preliminary phytochemical analysis of *Citrullus colocynthis*

Plant Constituents	Present
Alkaloids	+ +
Tannins	+ + +
Flavonoids	+ + +
Steroids	+
Glycosides	+ +

+ low; ++ moderate; +++High content

Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. In conclusion, *Citrullus colocynthis* extracts possesses a broad spectrum of activity against a panel of bacteria and fungi responsible for the most common microbial diseases. This deserves much study to find clinically effective antimicrobial compounds.

DISCUSSION

Since ancient time has been dependent on plants for food, drink, shelter, equipments, dental care and medicine (Sofowora, 1982). It has often been said that all plants are potential medicines for many diseases. The preliminary phytochemical investigations carried out on the stem of *Staphytarpheta 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, Salmonella typhi*, *Bacillus subtilis* and *Proteus vulgaris* and it also Phytochemical analysis of the leaf extract of the plant used revealed the presence of alkaloids, saponins, tannins, cardiac glycosides and steroids. Up till the present time, in spite of the development in chemistry and allied disciplines, some of the widely used drugs of plant origin are still produced by extraction from plants even though their chemical structure is known and method have been developed for their *de novo* synthesis in the laboratory. It would appear in such cases that the product synthesized and stored in the plants by nature is better, easier to access and ultimately cheaper than the one synthesized by man. (Odugbemi, 2006). For our present results against pathogenic microorganisms, *Citrullus colocynthis* serves as effective antibiotics.

CONCLUSION

Efforts in this regard have focused on plants because of their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on plants for the treatment of infections and non infectious diseases (Aibinu, 2006). This present research is a right step in this direction of searching for novel and more effective antimicrobial compounds in plants.

ACKNOWLEDGEMENTS

The authors are gratefully acknowledge to the Director, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, India for providing all support during the study period.

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