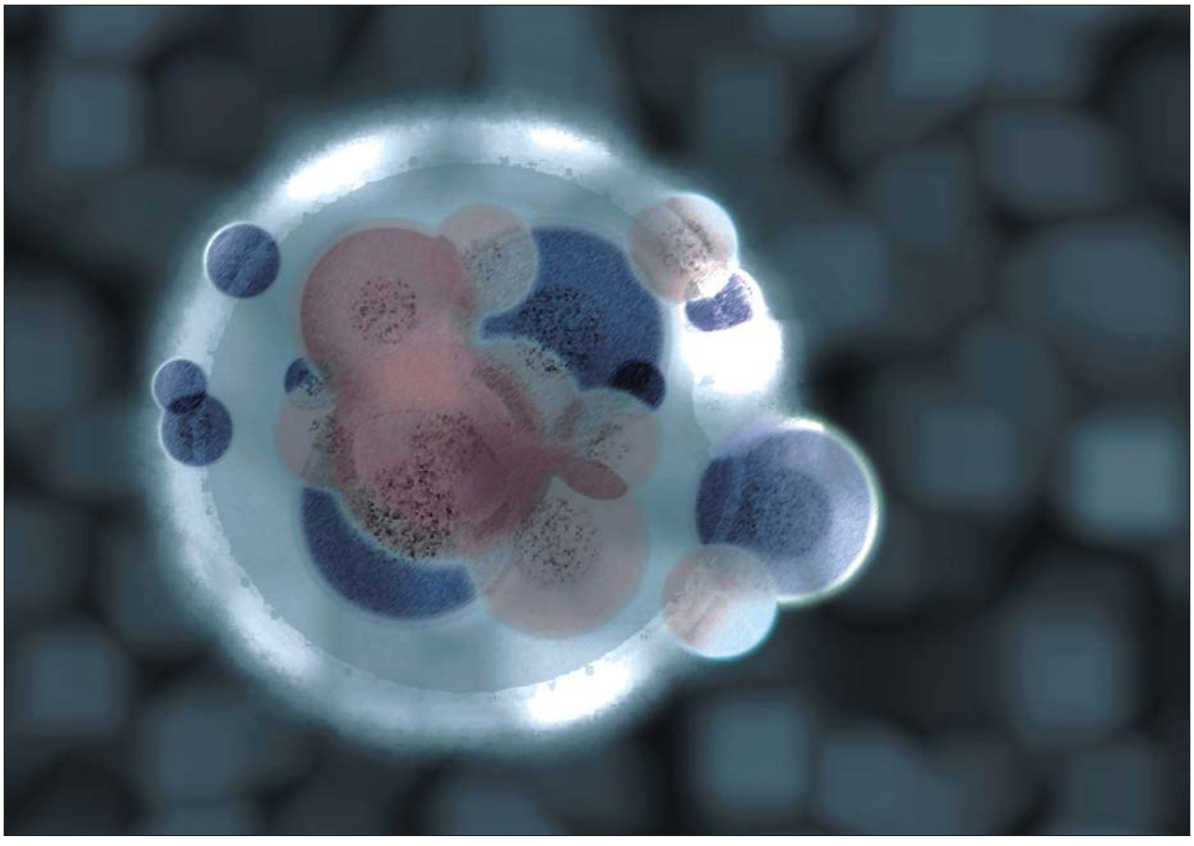

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 10 (7): 682-685, 2010
ISSN 1727-3048

Screening of Anti-microbial Effect in Watermelon (*Citrullus* sp.)

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Abstract: The objective of this study was to determine the potential anti-microbial activity of aqueous ethanolic and chloroform extracts from 3 medicinal plants against bacteria and some fungal strains. The antimicrobial efficacy of *Citrullus colocynthis*, *Citrullus lanatus* and *Citrullus vulgaris*, was evaluated against Gram negative, positive and some fungal strains and including *Esherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus pumilus* some fungal *Candida albicans*, *Aspergillus niger*, *Penicillium chrysogenum* and *Penicillium chrysogenum* using agar well diffusion method at concentration levels (100 mg mL^{-1}). Among the tested plants, *Citrullus colonthis* extract showed the highest antibacterial and anti fungal activity and all bacterial and fungal strains at 100 mg mL^{-1}. In addition, almost all species of plants were found to have activity on at least two microbial strains. However, the diameters of inhibition zone were different according to the kinds and concentrations of plant extracts and bacterial and fungal strains. The ethanol extract of *citrullus colocynthis* was very active against bacterial and fungal at a concentration of 100 mg mL^{-1}. But *Trichosporon begelli* showed that no zone inhibition in *C. valgaries* plants of ethanol and chloroform extracts.

Key words: Anti bacterial, *Cirullus* sp., watermelon, anti fungal, ethanol extract

INTRODUCTION

Watermelon is one of popular fruits consumed all over the world. Beside its juicy texture, watermelon is rich in useful antioxidant (mainly lycopene) which has been demonstrated to inhibit growth of cancer cells (Hall, 2004). Due to its fruits property and size, genetic manipulation of this plant will have a lot of advantages.

Watermelon is a rich source of citrulline, an amino acid that can be metabolized to arginine, a conditionally essential amino acid for humans. Arginine is the nitrogenous substrate used in the synthesis of nitric oxide and plays an essential role in cardiovascular and immune functions (Collins *et al*., 2007). This Cucurbitaceae is widely used in Tunisian folk medicine and it possesses therapeutic activities against a wide range of ailments including inflammatory disorders, arthritis and gout (Marzouk *et al*., 2009). Nevertheless, a human overdose of plant immature fruits is hazard. Intoxication is manifested by colitis, gastro-intestinal irritations, delirium, hypothermia and cerebral congestions (Meyer, 1989; Bellakhdhar, 1999).

Anti-microbial gain interest from both academic research and industry due to their potential to provide quality and safety benefits to many materials. Anti-microbial packaging is the packaging system that is able to kill or inhibit spoilage and pathogenic microorganisms that are contaminating foods (Church and Parsons, 2007; Devlieghere *et al*., 2000). Microbial contamination reduces the shelf life of foods and increases the risk of food-borne illness. The demand for minimally processed, easily prepared and ready to eat ‘‘fresh” food products, poses major challenges for food safety and quality. Application of anti microbial treatment in food packaging is gaining interest from researchers due to its potential to provide quality, safety benefits and to extend the shelf life of the food. Anti-microbial food packaging promotes safety by reducing the rate of growth of specific microorganisms by direct contact of the package with the surface of foods (Coma *et al*., 2001; Ming *et al*., 1997). In the present study, we have selected *Citrullus* sp. like that *Citrullus colocynthis*, *Citrullus lanatus* and *Citrullus vulgaris* is screened against multi drug resistant bacteria like *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Esherichia coli* and some fungal strains like *Candida albicans*, *Aspergillus niger Penicillium chrysogenum* and *Trichosporon begelli*.

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MATERIALS AND METHODS

The leaves of *Citrullus* sp. like *Citrullus colocynthis*, *Citrullus lanatus* and *Citrullus vulgaris* were collected from the sand dune region of Parangipettai on the Northeast coast of India, during November to December in 2009. The work was carried out of Centre of Advanced study in Marine Biology, Annamalai University, Parngipettai, Tamil nadu India.

Preparation of aqueous extracts: The aqueous extract of *Citrullus.* sp. leaves were prepared by squeezing the sand-free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx-1.61 mL cm^{-1}) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4°C in a refrigerator for the further use as crude aqueous extract.

Chloroform extraction: Crude toxin was extracted following the method of Bakus *et al*. (1981) with certain modifications, for chloroform extraction, the *Citrullus* sp. was dried in air for 2 days and after complete drying, 10 g *Citrullus* sp. was put into 200 mL of chloroform, covered and kept standing for 5 h. The solvent was then removed after squeezing *Citrullus* sp. and the filtered through whatman filter paper No 1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45°C in refrigerator for further use as crude chloroform extracts.

Antibacterial activity: Petri dishes with nutrient agar were inoculated with six different species of bacteria *Citrullus* sp. extracts were sterilized by passing each through a 0.22: m Millipore GV filter (Millipore, USA) Round paper discs with a radius of 0.8 cm were dipped into each plants extract and placed in the center on inoculated Petri dishes. Bacterial colonies were allowed to grow overnight at 37°C, then the inhibition zone around the disc was measured.

Antifungal activity: Petri dishes with Potato Dextrose agar were inoculated with three different species of fungus. *Citrullus* sp. extracts were sterilized by passing each through a 0.22: m Millipore GV filter (Millipore, USA) Round paper discs with a radius of 0.8 cm w ere dipped into each *Citrullus* sp. extract and placed in the center on inoculated petri dishes. Fungal colonies were allowed to grow 48 h at 28°C and then the inhibition zone around the disc was measured.

RESULTS AND DISCUSSION

The present study reveals that the leaves extract of *Citrullus colocynthis*, *Citrullus lanatus* and *Citrullus vulgaris* were very effective against bacteria and some fungal strains than the other species. The ethanol extract of the leaves were more antagonistic than the respective aqueous and chloroform extracts both bacterial and fungal tested. The ethanol leaves extracts of showed good antifungal among the 3 plants (Table 4). The ethanol leaves extracts of showed good anti bacterial activity among the 3 plants (Table 1-3). The zone of inhibition in bacterial maximum was found in *Esherichia coil* and minimum in *Staphylococcus aureus* (Table 1-3) and followed by the fungal zone of inhibition was maximum shows in *Candida albicans* and minimum shows in *Trichosporon begelli* for all extracts of aqueous, ethanol and chloroform was good activity showed among the 3 plants (Table 4). In the case of absent of zone inhibition showed that only for *C. valgaries* leaves extract of ethanol and chloroform in *Trichosporon begelli* fungal strains only (Table 4). Minimum zone inhibition was observed in the bacterial strain only for the *C. vulgaris* leaves extracts (Table 3). Maximum zone inhibition was observed in the bacterial strain for the *C. colocynthis*,

Table 1: Anti bacterial activity of *Citrullus colocynthis* leaves extracts and zone of inhibition

	Zone of inhibition (mm)		
Species name	Ethanol extracts	Aqueous extracts	Chloroform extracts
	(100 mg mL^{-1})		
Esherichia coli	7	6	6
Klebsiella pneumoniae	5	4	5
Pseudomonas æruginosa	4	5	5
Bacillus subtilis	5	4	3
Bacillus pumilus	4	3	4
Staphylococcus aureus	2	3	3

Table 2: Anti bacterial activity of *Citrullus lanatus* leaves extracts and zone of inhibition

	Zone of inhibition (mm)		
Species name	Ethanol extracts	Aqueous extracts	Chloroform extracts
	(100 mg mL^{-1})		
Esherichia coli	6	4	4
Klebsiella pneumoniae	4	5	3
Pseudomonas æruginosa	5	4	4
Bacillus subtilis	4	3	3
Bacillus pumilus	4	2	3
Staphylococcus aureus	1	2	2

Table 3: Anti bacterial activity of *Citrullus valgaries* leaves extracts and zone of inhibition

	Zone of inhibition (mm)		
Species name	Ethanol extracts	Aqueous extracts	Chloroform extracts
	(100 mg mL^{-1})		
Esherichia coli	5	4	4
Klebsiella pneumoniae	5	4	3
Pseudomonas æruginosa	4	3	5
Bacillus subtilis	5	4	3
Bacillus pumilus	4	3	2
Staphylococcus aureus	2	2	1

Table 4: Anti fungal activity of *Citrullus* sp. leaves extracts and zone of inhibition

Species name	Ethanol extract	Aqueous extract	Chloroform extract
***Citrullus colocynthis* against fungal strains**			
Candida albicans	5	4	3
Aspergillus niger	3	3	4
Penicillium chrysogenum	4	2	3
Trichosporon begelli	2	1	1
***Citrullus lanatus* against fungal strains**			
Candida albicans	4	3	3
Aspergillus niger	3	2	3
Penicillium chrysogenum	2	3	2
Trichosporon begelli	1	2	1
***Citrullus valgaries* against fungal strains**			
Candida albicans	3	3	2
Aspergillus niger	2	3	1
Penicillium chrysogenum	1	2	2
Trichosporon begelli	0	1	0

C. lanatus (Table 1, 2). The present study reveals that the leaves extracts of *Citrullus* sp. were very effective against bacteria such as *Esherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus pumilusand* and some fungal *Candida albicans*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium chrysogenum* than the other species tested. The ethanol extracts of the leaves were more antagonistic than the respective chloroform and aqueous extracts showed (Table 1). The antibacterial activity may be due to the presence of alkaloids, flavonoids, tannin, polyphenolic and oil as reported by Irobi and Daranola (1994) and Brantner *et al*. (1996). This finding is in agreement with the results of Agatemor (2009) who found that ethanolic extracts of some Nigerian spices were more potent than the aqueous extracts against common food borne microorganisms including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptococcus faecalis*. So, present result indicate the same result in r ethanol extract for more zone inhibition when compare to other like aqueous and chloroform. The volatile oil of *Artimisia afra* has greater activity as an antimycotic than as an antibacterial agent (Graven *et al*., 1992). The present study for all extract of *Citrullus* sp. leaves very good effective in some fungal strains (Tabel 4). The ethanol and chloroform extras of the *citrullus* sp. leaves showed low anti fungal activity against the stains of *Trichosporon begelli* in *C. valgaries* extract Showed (Tabel 4). The maximum zone inhibition was shows in *Candida albicans*, *Aspergillus niger* and *Penicillium chrysogenum* and minimum zone inhibition was shows in *Trichosporon begelli* (Table 4). It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. It also supports the earlier investigation (Banso and Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance.

CONCLUSION

In conclusion, the studied plant could provide some activity against anti bacterial and anti fungal; however, it is not known that which component of the extract is responsible for this effect. Further studies using isolated constituents instead of the whole extract should be carried out.

ACKNOWLEDGMENTS

The authors are grateful Thanks to the Dean, Centre of Advanced study in Marine Biology, Faculty of Marine Sciences, Annamalai University, for providing all support during the study period.

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