Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2017; 9(2): 55-63

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Phytochemical Analysis, Antibacterial and Antifungal Activities of Different Crude Extracts of Marine Red Alga *Gracilaria corticata* From The Gulf of Mannar South Coast, Navaladi, South India

Gnanaprakasam Adaikala Raj, Manivachagam Chandrasekaran*, Sakthivel Jegan, Venugopalan Venkatesalu

Department of Botany, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India

ABSTRACT

To investigate the antibacterial and antifungal activity of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Gracilaria corticata* J. Ag against bacterial and fungal strains *viz., Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, Proteus mirabilis* and *Proteus vulgaris.* Fungal strains *Candida albicans*, *Candida krusei*, *Candida guilliermondi*, *Candida parapsilosis*, *Candida tropicalis, Candida glabrata,* four dermatophytes *viz., Trichophyton rubrum*, *Trichophyton mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum*. The extracts of *G. corticata* were extracted with different solvents *viz.,* hexane, chloroform, ethyl acetate and methanol against bacterial and fungal strains by using disc diffusion method, MIC, MBC and MFC were determined. The ethyl acetate extract of *G. corticata* showed the highest antibacterial and antifungal activity against all the bacterial and fungal strains tested than the other extracts. The mean zones of inhibition produced by the extracts in agar disc diffusion assays were from 7.1 to 16.0 mm. The Minimum Inhibitory Concentrations (MIC) was between 125 and 500μg/ml, while the Minimum Bactericidal Concentrations and Minimum Fungicidal Concentrations (MFC) were between 250 and 500μg/ml. The highest mean zone of inhibition (16.0 mm) was observed in ethyl acetate extract of *G. corticata* against *B. subtilis.* The lowest MIC (125μg/ml), MBC and MFC (250μg/ml) values was observed in ethyl acetate extract of *G. corticata* against *B. subtilis*. The results suggest that the effective ethyl acetate crude extract of *G. corticata* showed the presence of phytochemical, terpenoids, tannins and phenolic compounds strongly than the other solvent extracts. These finding suggest that ethyl acetate crude extract of *G. corticata* have potential antimicrobial activity are under going further analysis to identify the active compounds currently progress.

Keywords: *Gracilaria corticata,* Antimicrobial activity, phytochemical analyses, MIC, MBC.

INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. [1] Bacterial infection causes high rate of mortality in human population and aquaculture organisms. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. [2] During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population

***Corresponding author: Dr. M. Chandrasekaran,**
Department of Botany, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India; **Tel.:** +91-9487022100; **E-mail:** adaikalamvsp@gmail.com
Received: 29 November, 2016; **Accepted:** 15 March, 2017

such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs [3] has been the reason for an extended search for newer drugs to treat opportunistic fungal infections. [4]

Nowadays the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alteration. [5] Many antifungal drugs, including imidazoles, butenafine and terbinafine, have been used clinically for the topical treatment of dermathophytosis. [6] Triazoles, griseofulvin and terbinafine are used as oral antifungal drugs for systemic therapy of severe dermatophytosys [7], but the prolonged duration of treatment, drug toxicity and interactions, fungal resistance and high costs are encountered difficulties. [8] These factors render the development of new more efficient and safe antifungal drugs a requirement.

Secondary metabolites produced by plants constitute a major source of bioactive substances. The scientific interest in these metabolites has increased today with the search of new therapeutic agents from plant source, due to the increasing development of the resistance pattern of microorganisms to most currently used antimicrobial drugs. According to World Health Report of infectious diseases 2000, overcoming antibiotic resistance is one of the major issues of the WHO for the present millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management. [9] Algae appear to be an interesting source for ethno medicinal and phytochemical studies. The power of algal resources has been sought for thousands of years for their ability to prevent disease and prolong life. Algae contain minerals, an abundance of vitamins, variety of trace elements and have shown high potential in controlling antimicrobial, antitumor, anticoagulant and cytotoxic activity. [10]

Gacilaria corticata J. Ag (*Gracilaria*, Rhodophyta) is a red marine alga which is widely distributed in the Indian Ocean and in the Pacific Ocean. *Gacilaria corticata* belongs to the family Rhodophyceae, plants growing in dense tufts, several growing together from a firm and hard hold-fast on rocky substrata. Thallus teaching 10-15 cm high, rigid, cartilaginous for greater part except for the extremities of the ramuli, repeatedly branched, tri-partite; width or segments 1-2 mm or 2-3 mm ; in some cases even up to 3-4 mm. *Apices of segments* acute or obtuse. Some plants, however, having narrow almost linear thallus, tapering a little towards extremities, regularly and sub-dichotomously divided or irregularly divided with cuneate elongated segments, thickness of frond more or less uniform. *Gracilaria* species are a major source of agar, particularly the agar used by the food industry and approximately 60% of all agar is produced from this alga. [11-12] Gracilarioids are farmed on a large scale in several countries. [13] Polysaccharides from *Gracilaria* genus are mainly composed of alternating 3-linked β-D-galactopyranosyl residues (A-units) and 4-linked α-L-galactopyranosyl (or 3, 6-anhydrogalactopyranosyl) residues (B-units). This backbone is further modified by different substitutions. [14] Gracilaria genus has shown potential for synthesis of new natural medicines. [15] Most identified active antimicrobial compounds are water insoluble and thus organic solvent extracts have been found more potent. [16] Hence, the present work was made to evaluvate the antibacterial and antifungal activity of different extracts of *G. corticata* against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis* and *P. vulgaris, Candida albicans*, *C. krusei*, *C. guilliermondi*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, Four dermatophytes *viz.*, *Trichophyton rubrum*, *T. mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum.*

MATERIALS AND METHODS

Algae sample collection

Gracilaria corticata J. Ag. Marine red alga were collected by hand picking from the submerged marine rocks at Manappad village, (Lat. 8°30'N; Long. 78°8'E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. Seaweeds collections were made from the month of during December 2012. The algae was identified by Dr. R. Selvaraj, Former Professor, Department of Botany, Annamalai University and the museum specimens are deposited in the Department of Botany, Annamalai University, Annamalainagar Tamilnadu, India.

Preparation of extracts

The alga sample species were handpicked during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal castings and adhering sand particles etc., morphologically distinct thallus of alga were placed separately in new polythene bags and were kept in a ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. Then the seaweeds were shade dried under room temperature and kept in a hot air oven for 50°C for half an hour. After that the material was ground by using electric blender. The powdered materials were stored in air tight container. Five hundred gram of seaweed materials was packed inside a Soxhlet apparatus and successive extraction was carried out using solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 hours. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4°C until further assay.

Phytochemical analysis

The phytochemicals like terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds and coumarins were carried analysed according to the method described by Harborne [17] and Trease and Evans. [18]

Microorganisms

The bacterial strains *viz., Bacillus subtilis* (MTCC 2063), *Streptococcus pyogenes* (MTCC 442), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Shigella flexneri* (MTCC 1457), *Salmonella typhimurium* (MTCC 98), *Vibrio chloerae* (MTCC 3906) and five yeast *viz., Candida albicans* (MTCC 3017), *C. glabrata* (MTCC 3019), *C. tropicalis* (MTCC 184), *C. krusei* (MTCC 9215), *C. parapsilosis* (MTCC 2509), *C. guilliermondii* (NCIM 3126) four dermatophytes *viz., Trichophyton rubrum* (MTCC 296), *T. mentagrophytes* (MTCC 8476), *Microsporum gypseum* (MTCC 2819) and *Epidermophyton flocossum* (MTCC 7880) were used in this study. These standard bacterial and fungal strains were obtained from Microbial Type Culture Collection and Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Gene Bank, Biochemical Sciences Division, National Chemical Laboratory, Pune, India. The stock cultures were maintained on Nutrient Agar for bacteria and Sabouraud Dextrose Agar for fungi at 4°C.

In vitro antibacterial activity was determined by using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB). *In vitro* antifungal activity was determined by using Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) (for mycelial fungi) and Yeast Nitrogen Base (YNB) (for yeast) and they were obtained from Himedia Ltd., Mumbai.

Disc diffusion method

The disc diffusion method Bauer *et al.* [19] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of MHA for bacteria and SDA for yeast and filamentous fungi. Then the plates were allowed to solidify and used in susceptibility test. The standard inoculum using bacterial suspensions containing 10^8 colony forming units (CFU) per ml, yeast suspensions containing 10^6 colony forming units (CFU) per ml and fungal suspensions containing 10^4 colony forming units (CFU) per ml were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. The methanol extract was dissolved in 10 per cent Dimethyl sulfoxide (DMSO) and under aseptic conditions, sterile discs were impregnated with 20 µl of different concentrations. The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ampicillin (10µg/disc) for bacteria and Amphotericin-B (100 units/disc) for Yeast and Ketoconazole (5µg/disc) for dermatophytes were used as positive control and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37°C for 24 h for all bacterial strains and plates were incubated at 28°C for 24 h for yeast and 30°C for 4-7 days for dermatophytes. The zone of inhibitions was observed and measured in millimeters. The assay in this experiment was repeated three times.

Microdilution broth assay

Determination of the Minimum Inhibitory Concentration (MIC) for bacteria

The determined for the *G. corticata* crude extracts, a modified reaszurin microtitre plate assay was carried out according to methods of Sarker *et al.* [20] 50µl of Sterile MHB were transferred in to each well of a sterile 96-well micro titer plate. The *G. corticata* extracts was dissolved in 10 per cent DMSO to obtain 1000µg/ml stock solution. 50µl of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth 50µl of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625µg/ml of the extract in each well. To each well, 10µL of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Finally, 10µl of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/mL. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10µl of MHB instead and a column with 10% DMSO solution as a negative control. The plates were incubated at 37°C for 24 h for all the bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of *G. corticata* extracts that exhibited the growth of the organisms the values by visual reading.

Determination of the Minimum Inhibitory Concentration (MIC) for fungi

The MIC of each crude extract was determined by using broth micro dilution technique as recommended by CLSI M27-A3 [21] and M38-A2 [22] for yeasts and filamentous fungi, respectively. The MIC values were determined in RPMI-1640 (Himedia, Mumbai) with L – glutamine without sodium bicarbonate, pH 7.0 with Morpholine propane sulfonic acid (MOPS). 20µl of a stock solution (50 mg/ml) of each algae extracts in 10% DMSO was dissolved with 980µl of RPMI-1640 medium solution 1000µl (1 mg/ml). From that, the two fold serial dilutions in the range from 500 to 15.7µg/ml were prepared. 200µl of solution was poured into first well of 96 well microtitre plates and then, 100µl were transferred to the next well containing 100µl of RPMI-1640. The same procedure was performed for all wells. Finally, 100µl of standardized inoculum suspension was transferred to each well to achieve a concentration of approximately 1–2 × 10^3 cells/mL for yeasts and 1–2

× 10^4 cells/mL for filamentous fungi. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated at 28°C for 24-48 hours for yeast and 4-7 days with dermatophytes. MIC of the extracts was recorded as the lowest concentration of extracts that inhibited the growth of the *Candida* and dermatophytic strains when compared to that of control.

Determination of the Minimum bactericidal concentration (MBC) and the Minimum Fungicidal Concentration (MFC)

MBC and MFC were determined by plating a loop full sample from each MIC assay well with growth inhibition in to freshly prepared MHA for bacteria and SDA for fungal strains. The plates were incubated at 37°C for 24 h for all bacterial strains, 28°C for 24 hours for yeasts and 30°C for 4 -7 days for dermatophytes. The MBC and MFC were recorded as the lowest concentration of the extracts that did not permit any visible bacterial and fungal growth after the period of incubation.

Table 1: Phytochemical analysis of different extracts from the *Gracilaria corticata*

S. No	Secondary metabolites	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
1	Terpenoids	+	++	+++	+	+
2	Tannin	+	+	++	+	+
3	Cardic glycosides	-	-	-	-	-
4	Steroids	-	+	+++	-	-
5	Alkaloids	-	+	+	-	-
6	Phenolic compound	+	+	++	+	+
7	Coumarins	-	-	-	-	-

- = Absence, + = weak, ++ = medium, +++ = strong

RESULTS

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. corticata* were used to analyses the phytochemicals, terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds and coumarins and results are presented in Table 1. The terpenoids, tannins and phenolic compounds were present in the all the extracts of *G. corticata*. Alkaloids were present only in the chloroform and ethyl acetate extracts of *G. corticata*. Steroids were present chloroform and ethyl acetate extracts. Among the tested phytochemicals, coumarins and cardiac glycosides were absent in all the extracts of *G. corticata*.

In the present study, the different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. corticata* were studied against *B. subtilis, S. pyogenes*, *E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. flexneri* and *V. cholerae* and *C. albicans*, *C. krusei*, *C. guilliermondi*, *C. Parapsilosis*, *C. tropicalis, C. glabrata*, *T. rubrum*, *T. mentagrophytes, M. gypseum* and *E. flocossum*. The highest mean zone of inhibition was observed by ethyl acetate extract of *G. corticata* against *B. substilis* (the mean zones of inhibition, 16.0 mm) followed by *S. pyogenes* (14.5 mm), *C. Parapsilosis* (14.3 mm) and *C. albicans* (14.0 mm). All the extracts of marine macro algae possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. The mean values are presented in Tables 2 and 3. When the different extracts were assayed against the test bacteria and fungal by agar disc diffusion assays, the mean zones of inhibition obtained were between 7.1 and 16.5 mm. Ampicillin (10µg/disc), antibacterial positive control produced mean zones of inhibition ranged from 7.0 to 10.8 mm. The Amphotericin-B (100 units/disc) anticandidal positive control produced zones of inhibition that ranged from 12.5 to 15.6 mm. Ketoconazole (10µg/disc) anti dermatophytic positive control produced zones of inhibition that ranged from 14.5 to 19.3 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The MIC values of the different extracts of *G. corticata* ranged between 125 and 500µg/ml, while the MBC and MFC values were between 250 and 1000µg/ml.

DISCUSSION

Marine macro algae are eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products. [23] They contain compounds ranging from sterols, terpenoids to brominated phenolic, which shows bioactivity against microorganisms. [24] Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations. [25]

In the present investigation different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. corticata* possessed antibacterial and antifungal activity against all the standard microbial strains tested. The ethyl acetate extract of *G. corticata* showed the highest antibacterial activity than other extracts against *B. subtilis, S. pyogenes*, *E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. flexneri* and *V. cholerae* and *Candida albicans*, *Candida krusei*, *Candida guilliermondi*, *Candida parapsilosis*, *Candida tropicalis, Candida glabrata* , four dermatophytes *viz., Trichophyton rubrum*, *Trichophyton mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum*. The highest mean zones of inhibition (16.0 mm) followed by *S. pyogenes* (14.5 mm), *C. Parapsilosis* (14.3 mm) and *C. albicans* (14.0 mm) was observed in ethyl acetate extracts of *G. corticata* against *B. subtilis*.

Table 2: Antibacterial activity of different extracts of *Gracilaria corticata*

Bacterial strains\ Seaweed prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc) 500	250	125	Ampicillin (10µg/disc)	MIC (µg/ml)	MBC (µg/ml)
Bacillus subtilis						
Hexane	12 .3 ± 0.57	10.5 ± 0.50	8.3 ± 0.57	8.1 ± 0.28	500	500
Chloroform	13 .5 ± 0.50	10.8 ± 0.76	9.0 ± 0.50	9.3 ± 0.57	250	500
Ethyl acetate	16.0 ± 0.50**	13.1 ± 0.28	9.8 ± 0.76	8.6 ± 0.76	125	250
Acetone	11.8 ± 0.76	9.5 ± 0.50	8.3 ± 0.57	7.8 ± 0.76	500	1000
Methanol	11.3 ± 0.57	9.0 ± 0.50	7.5 ± 0.28	7.5 ± 0.50	500	1000
Streptococcus pyogenes						
Hexane	12.6 ± 0.76	10.1 ± 0.28	7.6 ± 0.76	10.0± 0.50	500	1000
Chloroform	13.0 ± 0.50	10.5 ± 0.50	7.8 ± 0.76	10.1 ± 0.28	250	1000
Ethyl acetate	14.5 ± 0.50**	11.8 ± 0.76	8.8 ± 0.76	9.3 ± 0.57	250	500
Acetone	11.5 ± 0.50	9.6 ± 0.76	7.5 ± 0.50	10.0± 0.50	500	1000
Methanol	11.1 ± 0.28	9.5± 0.50	7.3`± 0.57	9.8 ± 0.76	500	1000
Escherichia coli						
Hexane	12.1 ± 0.28	9.8 ± 0.76	7.8 ± 0.76	9.3± 0.57	500	1000
Chloroform	12.8 ± 0.76	10.5 ± 0.50	8.3 ± 0.57	8.1 ± 0.28	250	1000
Ethyl acetate	13.1 ± 0.28	10.8 ± 0.76	8.5 ± 0.50	9.5 ± 0.50	250	500
Acetone	11.5 ± 0.50	9.6 ± 0.76	7.6 ± 0.28	10.1 ± 0.28	500	1000
Methanol	10.3± 0.57	9.1± 0.28	7.3± 0.57	9.8 ± 0.76	500	1000
Klebsiella pneumoniae						
Hexane	12.0 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	9.3 ± 0.57	500	1000
Chloroform	12.6± 0.76	10.1 ± 0.28	8.3 ± 0.57	10.3 ± 0.57	500	1000
Ethyl acetate	13.3 ± 0.57	10.6± 0.76	8.6 ± 0.76	8.1 ± 0.28	250	500
Acetone	11.5 ± 0.50	10.0 ± 0.50	7.8 ± 0.76	9.6 ± 0.76	500	1000
Methanol	10.3± 0.50	9.3 ±0.57	7.3±0. 57	9.8 ± 0.76	500	1000
Proteus mirabilis						
Hexane	12.0 ± 0.50	10.0 ± 0.50	7.3 ± 0.57	8.8 ± 0.76	500	1000
Chloroform	13.1 ± 0.28	10.6 ± 0.76	8.0 ± 0.50	9.5 ± 0.50	250	1000
Ethyl acetate	13.5 ± 0.50	11.1 ± 0.28	8.1 ± 0.28	10.1 ± 0.28	250	500
Acetone	11.5 ± 0.50	9.8± 0.76	7.5 ± 0.50	8.5 ± 0.50	500	1000
Methanol	10.3 ± 0.57	9.0 ± 0.50	7.3 ± 0.57	9.3 ± 0.57	500	1000
Proteus vulgaris						
Hexane	11.1 ± 0.28	9.8 ± 0.76	8.0 ± 0.50	10.6 ± 0.76	500	1000
Chloroform	11.8 ± 0.76	10.6 ± 0.76	8.3 ± 0.57	9.1 ± 0.28	500	500
Ethyl acetate	12.6 ± 0.76	11.1 ± 0.28	8.5 ± 0.50	8.0 ± 0.50	250	500
Acetone	10.5 ± 0.50	9.5 ± 0.50	7.8 ± 0.76	9 .1 ± 0.28	500	1000
Methanol	9.8± 0.76	8.1 ± 0.28	7.5 ± 0.50	9.5 ± 0.50	500	1000
Pseudomonas aeruginosa						
Hexane	11.6 ± 0.76	9.8 ± 0.76	8.1 ± 0.28	8.3 ± 0.57	500	1000
Chloroform	12.6 ± 0.76	10.5 ± 0.50	8.6 ± 0.76	9.6 ± 0.76	500	1000
Ethyl acetate	13.0 ± 0.57	11.1 ± 0.28	9.0 ± 0.50	9.1 ± 0.28	250	500
Acetone	10.5 ± 0.50	9.5 ± 0.50	7.8 ± 0.76	7.5 ± 0.50	500	1000
Methanol	9.3 ± 0.57	8.1 ± 0.28	7.3 ± 0.57	9.8 ± 0.76	500	1000
Salmonella typhimurium						
Hexane	11.8 ± 0.76	9.5 ± 0.50	7.8 ± 0.76	9.3 ± 0.57	500	1000
Chloroform	12.5 ± 0.50	10.6± 0.76	8.3 ± 0.57	8.5 ± 0.50	250	250
Ethyl acetate	13.6 ± 0.76	11.0 ± 0.50	9.0 ± 0.50	9.0 ± 0.50	250	500
Acetone	11.3± 0.57	9.5 ± 0.50	7.6 ± 0.76	7.5 ± 0.50	500	1000
Methanol	10.1 ± 0.28	9.3 ± 0.57	7.3 ± 0.57	8.5 ± 0.50	500	1000
Shigella flexneri						
Hexane	10.1 ± 0.28	9.5 ± 0.50	7.6 ± 0.76	9.3 ± 0.57	500	1000
Chloroform	11.5 ± 0.50	9.8 ± 0.76	8.0 ± 0.50	8.6 ± 0.76	500	1000
Ethyl acetate	13.1 ± 0.28	11.3± 0.57	8.6 ± 0.76	9.0 ± 0.50	250	500
Acetone	10.8± 0.76	9.3 ± 0.57	7.5 ± 0.50	8.6 ± 0.76	500	1000
Methanol	10.0± 0.50	8.6 ± 0.50	7.1 ± 0.28	10.8 ± 0.76	500	1000
Vibrio cholera						
Hexane	10.8 ± 0.76	9.6 ± 0.76	7.8 ± 0.76	8.0 ± 0.50	500	1000
Chloroform	12.3 ± 0.57	10.5 ± 0.50	8.1 ± 0.28	8.6 ± 0.76	500	1000
Ethyl acetate	14.1 ± 0.28	11.5 ± 0.50	8.5 ± 0.50	9.6 ± 0.76	250	500
Acetone	10.5 ± 0.50	9.0± 0.50	7.3 ± 0.57	10.1 ± 0.28	500	1000
Methanol	9.8 ± 0.76	8.3± 0.57	7.1 ± 0.28	10.5 ± 0.50	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation; ** significant at $p < 0.05$

The MIC values of the different extracts of *G. corticata* ranged between 125 and 500µg/ml, while the MBC & MFC values were between 250 and 1000µg/ml. Kolanjinathan and Stella [26] reported that the antibacterial activity of five different solvents *viz*., methanol, acetone, chloroform, hexane and ethyl acetate extracts of *G. corticata* was evaluated against *Staphylococcus aureus, S. pyogenes, S. epidermis, B. subtilis* and *B. cereus*, *K. pneumoniae*, *Enterobacter aerogens Aspergillus flavus* followed by *A. fumigatus*, *A. niger*, *C. albicans*, *C. glabrata* and *Saccharomyces cerevisiae*. The ethyl acetate extracts of *Ulva lactuca* and *Gracilaria verrucosa* showed the highest antimicrobial activity against *E. coli*, *K. pneumoniae*, MRSA and *B. subtilis* and also identified the presence of myristic and palmitic acid, linoleic acid, oleic acid, lauric, stearic and myristic acid, from ethylacetate extracts. [27]

Table 3: Antifungal activity of different extracts of *Gracilaria corticata*

Fungal strains\ Seaweed prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000	500	250	Amphotericin-B (100 units/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	12.3 ± 0.57	10.3 ± 0.57	8.0 ± 0.50	13.5 ± 0.50	500	1000
Chloroform	12.8 ± 0.76	10.5 ± 0.50	8.8 ± 0.76	14.1 ± 0.28	500	1000
Ethyl acetate	14.0 ± 0.50**	11.5 ± 0.50	9.0 ± 0.50	13.8 ± 0.76	250	500
Acetone	11.5 ± 0.50	9.8 ± 0.76	7.8 ± 0.76	14.6 ± 0.76	500	1000
Methanol	10.3 ± 0.57	9.3 ± 0.57	7.5 ± 0.50	13.1 ± 0.28	500	1000
Candida krusei						
Hexane	11.8 ± 0.76	10.1 ± 0.28	7.8 ± 0.76	14.3 ± 0.57	500	1000
Chloroform	12.1 ± 0.28	10.6 ± 0.76	8.1 ± 0.28	15.6 ± 0.76	500	1000
Ethyl acetate	13.5 ± 0.50	11.0 ± 0.50	9.3 ± 0.57	14.1 ± 0.28	250	500
Acetone	11.0 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	15.5 ± 0.50	500	1000
Methanol	NA	NA	NA	13.6 ± 0.76	NT	NT
Candida guilliermondi						
Hexane	11.6 ± 0.76	9.8 ± 0.76	7.6 ± 0.76	14.3 ± 0.57	500	1000
Chloroform	12.5 ± 0.50	10.1 ± 0.28	8.3 ± 0.57	15.5 ± 0.50	500	1000
Ethyl acetate	13.3 ± 0.57	10.5 ± 0.50	8.8 ± 0.76	13.3 ± 0.57	250	500
Acetone	10.5 ± 0.50	9.6 ± 0.76	7.5 ± 0.50	14.5 ± 0.50	500	1000
Methanol	NA	NA	NA	10.6 ± 0.76	NT	NT
Candida glabrata						
Hexane	11.3 ± 0.57	9.6 ± 0.76	7.3 ± 0.20	14.0 ± 0.50	500	1000
Chloroform	12.5 ± 0.50	10.1 ± 0.28	7.6 ± 0.76	15.3 ± 0.57	500	1000
Ethyl acetate	13.1 ± 0.28	10.8 ± 0.76	8.0 ± 0.50	13.6 ± 0.76	250	500
Acetone	10.8 ± 0.76	9.5 ± 0.50	7.1 ± 0.28	14.1 ± 0.28	500	1000
Methanol	NA	NA	NA	15.5 ± 0.50	NT	NT
Candida parapsilosis						
Hexane	12.8 ± 0.76	9.5 ± 0.50	8.3 ± 0.57	13.6 ± 0.76	500	1000
Chloroform	13.0 ± 0.50	10.5 ± 0.50	9.0 ± 0.50	14.1 ± 0.28	500	1000
Ethyl acetate	14.3 ± 0.57**	11.5 ± 0.50	9.3 ± 0.57	15.0 ± 0.50	250	500
Acetone	11.5 ± 0.50	9.3 ± 0.50	8.0 ± 0.50	13.1 ± 0.28	500	1000
Methanol	11.8 ± 0.76	9.0 ± 0.50	7.6 ± 0.76	14.5 ± 0.50	500	1000

Fungal strains\ Seaweed prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000	500	250	Amphotericin-B (100units/disc)/ Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida tropicalis						
Hexane	NA	NA	NA	14.0 ± 0.50	NT	NT
Chloroform	11.5 ± 0.50	9.3 ± 0.57	8.1 ± 0.28	12.8 ± 0.76	500	500
Ethyl acetate	10.8 ± 0.76	9.0 ± 0.50	7.3 ± 0.57	12.5 ± 0.50	500	1000
Acetone	NA	NA	NA	13.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	14.8 ± 0.76	NT	NT
T. rubrum						
Hexane	12.1 ± 0.28	9.8 ± 0.76	7.5 ± 0.50	17.1 ± 0.28	500	1000
Chloroform	12.8 ± 0.76	10.1 ± 0.28	8.3 ± 0.57	18.3 ± 0.57	500	1000
Ethyl acetate	13.8 ± 0.76	10.5 ± 0.50	8.8 ± 0.76	15.6 ± 0.76	250	500
Acetone	10.5 ± 0.50	9.3 ± 0.50	7.3 ± 0.57	16.8 ± 0.76	500	1000
Methanol	10.1 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	17.5 ± 0.50	500	1000
T.mentagrophytes						
Hexane	NA	NA	NA	16.1 ± 0.28	NT	NT
Chloroform	11.8 ± 0.76	10.0 ± 0.50	7.3 ± 0.50	17.0 ± 0.50	500	1000
Ethyl acetate	12.8 ± 0.76	10.6 ± 0.76	7.8 ± 0.76	18.3 ± 0.57	500	1000
Acetone	NA	NA	NA	18.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	16.8 ± 0.76	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	16.0 ± 0.50	NT	NT
Chloroform	11.3 ± 0.57	9.5 ± 0.50	7.5 ± 0.50	17.1 ± 0.28	500	1000
Ethyl acetate	13.0 ± 0.50	10.6 ± 0.76	8.6 ± 0.76	18.3 ± 0.57	500	1000
Acetone	NA	NA	NA	16.5 ± 0.50	250	500
Methanol	NA	NA	NA	16 .0 ± 0.50	NT	NT
Microsporum gypseum						
Hexane	NA	NA	NA	15.6 ± 0.76	NT	NT
Chloroform	10.5 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	14.5 ± 0.50	250	500

Ethyl acetate	13.1 ± 0.28	10.5 ± 0.50	9.8 ± 0.76	16.1 ± 0.28	500	1000
Acetone	NA	NA	NA	19.3 ± 0.57	NT	NT
Methanol	NA	NA	NA	18.5 ± 0.50	NT	NT

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm [b]-mean of three assays; ± - standard deviation ** significant at $p < 0.05$
NA-No activity; NT-Not Tested.

Subba Rangaiah *et al.* [28] reported that the *Sargassum ilicifolium, Padina tetrastromatica,* of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol extracts and minimum with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. Antifungal activity of all the crude extractions of *G. corticata* showed maximum activity against *Rhizopus stolonifer.* Mansuya *et al.* [29] reported the aqueous and methanolic extract of *U. lactuca, U. reticulata, Cladophora glomerata, G. corticata, Kappaphycus alvarezii* and *Sargassum wightii* against *E. coli, P. aeruginosa, S. typhi, Staphylococcus epidermis* and *S. pyogenes.* The antibacterial activity from methanol, ethanol, dichloromethane and hexane extracts of *Gracilaria fisheri* and *Ulva intestinalis* was tested against *S. aureus, Listeria monocytogenes*. Methicillin-resistant *S. aureus, Enterobacter faecalis, V. alginolyticus, V. parahaemolyticus, V. harveyi, E. coli, P. aeruginosa, K. pneumoniae, Salmonella typhi* and *P. mirabilis.* [30] Chandrasekaran *et al.* [31] showed the antibacterial activity from *U. fasciata* against multi-drug resistant bacterial strains of *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis* and *P. vulgaris.*

In the present study different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. corticata* antibacterial activity and antifungal activity. Hediat *et al.* [32] reported that different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. In this present study also supported that optimizes their antibacterial activity by selecting the best solvent to extract the active compound from seaweeds. So this suggests that seaweeds should be extracted in different solvent systems in order to optimize their antibacterial activity by selecting the best solvent system. Seaweed extracts in different solvents exhibited different antimicrobial activities. [33] The high and low effect of organic extract against microorganisms could be related to the presence of bioactive metabolites, which can be soluble in solvents. [34]

In the present study, different extracts of *G. corticata* possessed antibacterial activity against all the bacterial strains. Chandrasekaran *et al.* [35] showed the antibacterial activity from *Sargassum wightii* different extracts against multi-drug resistant strains of *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, S. dysentriae, P. mirabilis* and *P. vulgaris.* The antibacterial activity of aqueous, ethanol and methanol crude extracts of *Sargassum longifolium* and *G. corticata* against *Aeromonas hydrophilia, P. aeruginosa, V. cholerae, V. harveyi, and V. parahaemolyticus.* [36] The methanol and aqueous extracts of *G. verrucosa, Gracilaria ferugusoni, G. verrucosa* var *Hypnea musciformis, Enatiocladia prolifera,* and *Gelidium* species against *B. substilis, E. coli, P. aeruginosa, S. typhi, Streptococcus aureus* and *Candida albicans.* [37] Radhika *et al.* [38] reported that the antibacterial activity of seaweeds *Ulva fasciata, Sargassum wightii* and *Gracilaria corticata* against *Bacillus cereus, Vibrio cholerae* classical, *V.cholerae* 0139, *E.coli, Pseudomonas aerogenosa*, *Aeromonas hydrophila, Salmonella typhii* and *Shigella flexneri*

Phytochemicals are compounds from food and medicine to protect and maintain human health. These have antioxidant or hormone-like effect which helps to fight against diseases like cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues. It is reported earlier that seaweeds are also rich in polysaccharides such as alginates, fucans, and laminarans which possess medicinal values. [39] In the present work, the ethyl acetate extract of *G. corticata* showed the antibacterial activity due to the presence of phytochemicals, terpenoids, tannins, phenolic compounds and steroids. Krishnaveni and Johnson [40] reported that phytochemical analysis of various solvents extracts revealed that the presence of alkaloids, glycosides, saponins, steroids, phenol and tannins in *G. corticata*. Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores. [41] Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids are reported to have anti-inflammatory effects. [42] Tannins play a major role as antihaemorrhagic agent and showed to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties. [43] Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities. [44] Steroids, saponins and triterpenoids showed the analgesic properties. [45] Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration. [46]

The present study derived from *G. corticata* often show considerable activity against gram positive bacteria were more sensitivity than the gram negative bacteria. The resistance of Gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside. [47] However, the Gram positive bacteria do not possess such outer membrane and cell wall structures. [47] In the present study, almost all crude extracts tested have shown strong antibacterial potential against pathogenic bacteria.

The different solvent extracts of *G. corticata* used in the present study found to be the most effective antibacterial agents. Finally it can be conclude that ethyl acetate extract of *G. corticata* showing good antibacterial activity is currently underway in an effort to identify the active constituents currently progress.

ACKNOWLEDGEMENTS

The authors acknowledge greatfully the financial support given by the University Grants Commission, New Delhi under Major Research Project Programme (F.No.: 40-312/2011(SR) Dated 30.06.2011). The authors are thankful to Dr. K. Arumugam, Professor and Head, Department of Botany, Annamalai University for providing laboratory facilities.

REFERENCE

1. Mandal FB. The Changing Ecology of Infectious Diseases in Human. World Environ. 2011; 1(1): 14-19.
2. Kandhasamy M, Arunachalam KD. Evaluation of in *vitro* antibacterial property of seaweeds of southeast coast of India. African J Biotech. 2008; 7(12): 1958-1961.
3. Giordani R, Trebaux J, Masi M, Regli P. Enhanced antifungal activity of ketoconazole by *Euphorbia characias* latex against *Candida albicans*. J Ethnopharmacol. 2001; 78: 1-5.
4. Fostel J, Lartey P. Emerging novel antifungal agents. Drug Discovery Today. 2000; 5: 25-32.
5. Claudio D, Miranda. Raul Zemelman Bacterial resistance to oxytetracycline in Chilean salmon farming. Aquacul. 2002; 212: 31-47.
6. Watanabe S. Present state and future direction of topical antifungals Japaneses. J Med Mycol. 1999; 40: 151-155.
7. Lesher JL. Oral therapy of common superficial fungal infections of the skin. J Ame Acade Dermatol. 1999; 40: S31-S34.
8. Bennett ML, Fleischer Jr AB, Loveless JW, Fledman SR. Oral griseofulvin remains the treatment of choice for tinea capitis in children. Pediatric Dermatol. 2000; 17: 304-309.
9. Prashanth D, Asha MK, Amit A. Antibacterial activity of *Punica granatum*. Fitoterapia. 2001; 72: 171-173.
10. Kolanjinathan K, Ganesh P, Saranraj P. Pharmacological Importance of Seaweeds: A Rev World J Fish Mar Sci. 2014; 6 (1): 01-15.
11. Meena R, Prasad K, Ganesan M, Siddhanta AK. Superior quality agar from Gracilaria species (Gracilariales, Rhodophyta) collected from the Gulf of Mannar, India. J Appl Phycol. 2008; 20: 397-402.
12. Villanueva RD, Sousa AMM, Goncalves MP, Nilsson M, Hilliou L. Production and properties of agar from the invasive marine alga, Gracilaria vermiculophylla (Gracilariales, Rhodophyta). J Appl Phycol. 2010; 22: 211-220.
13. Skriptsova AV, Nabivailo YV. Comparison of three gracilarioids: growth rate, agar content and quality. J Appl Phycol. 2009; 21: 443-450.
14. Maciel JS, Chaves LS, Souza BWS, Teixeira DIA, Freitas ALP, Feitosa JAP, *et al.* Structural characterization of cold extracted fraction of soluble sulfated polysaccharides from red seaweed *Gracilaria birdiae*. Carbohydr Res. 2008; 71(4): 559-565.
15. Almeida CLF, Falcão HS, Lima GRM, Montenegro CA, Lira NS, Athayde-Filho PF *et al.* Bioactivities from Marine Algae of the Genus Gracilaria. Int J Mol Sci. 2011: 12: 4550-4573.
16. Villanueva RD, Sousa AMM, Gonçalves MP, Nilsson M, Hilliou L. Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). J Appl Phycol. 2010; 22: 211-220.
17. Harborne IB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York, 1973, pp. 88-185.
18. Trease GE, Evans WC. Text book of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London, 1983, pp. 343-383.
19. Bauer AW, Kirby WMM, Scherris J, Turck C. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45: 493-496.
20. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007; 42: 321-324.
21. Clinical and Laboratory Standards Institute, 2008a. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, third ed. CLSI Document M27-A3. Clinical Laboratory Standards Institute, Wayne, PA, USA.
22. Clinical and Laboratory Standards Institute, 2008b. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard, second ed. CLSI Document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
23. Michael TM, John MM, Jack. P. Brock Microbiology of Microorganisms. 11thEdition, New Jersey. ISBN: 2005. 13-978-0226701479.
24. Perry NB, Blunt JW, Munro, MHA. Cytotoxic and antifungal 1,4-naphthoquinone and related compounds from a New Zealand brown algae.. *Landsburgia quercifolia*. J Nat Prod. 1991; 54 (4): 978-985.
25. Elena M, Francisco Y, Erickson KL. "Mailiohydrin, a Cytotoxic Chamigrene Dibromohydrin from a Phillippine *Laurencia* Species," J Nat Prod. 2003; 64 (6): 790-791.
26. Kolanjinathan K and Stella D. Pharmacological Effect of *Gracilaria corticata* Solvent Extracts against Human Pathogenic Bacteria and Fungi. Int J Pharmac Biol Arcs. 2011; 2(6):1722-1728.
27. Ravikumar S, Ramanathan G, Subhakaran M, Jacob Inbaneson S. Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int J Med Sci. 2009; 1(5): 184-191.
28. Rodriguez-Bernaldo de Quiros A, Lage-Yusty MA, Lopez-Hernandez J. Determination of phenolic compounds in macroalgae for human consumption. Food Chem. 2010; 121: 634-638.
29. Mansuya P, Aruna P, Sridhar S, Suresh Kumar J , Babu S. Antibacterial Activity and Qualitative Phytochemical Analysis of Selected Seaweeds from Gulf of Mannar Region. J Exper Sci. 2010; 8: 23-26.
30. Srikong W, Mittraparp-arthorn P, Rattanaporn O, Bovornreungroj N Bovornreungroj P. Antimicrobial activity of seaweed extracts from Pattani, Southeast coast of Thailand. Food Appl Biosci J. 2015; 3 (1): 39–49.
31. Chandrasekaran M, Venkatesalu V, Adaikala Raj G, Krishnamoorthy S. Antibacterial activity of *Ulva fasciata* against Multidrug Resistant Bacterial Strains. Int Lett Nat Sci. 2014a; 14: 40 – 51.
32. Hediat MH. Salama, Najat Marraiki. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L.Polygonaceae), naturally growing in Egypt. Saudi J Biol Sci. 2010; 17: 57-63.
33. Rangaiaha GS, Lakshmi PA, Sruthikeerthia K. The antimicrobial activity of the crude extracts of Chlorophycean seaweeds Ulva, Caulerpa and *Spongomorpha* spp. against clinical and phytopathogens. Drug Invent Today. 2010; 2: 311-314.

34. Manivannan K, Karthikai Davy G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vidal coarse waters, Gulf of Mannar. Asian Pac J Trop Biomed. 2011; 1: 117-123.
35. Chandrasekaran M, Venkatesalu V, Adaikala Raj G, Krishnamoorthy S. Antibacterial properties of various extracts of *Sargassum wightii* against Multidrug Resistant Bacterial Strains. Phykos. 2014b; 44(2): 17-28.
36. Sangeetha S, Dhayanithi NB, Sivakumar N. Antibacterial activity of Sargassum longifolium and Gracilaria corticata From Gulf of Mannar against selected Common Shrimp Pathogens Int J Pharm Bio Sci. 2014 ; 5(2): 76-82.
37. Adaikala Raj G, Patric Raja D, Johnson M, Janakiraman N, Babu A. Antibacterial potential of selected red seaweeds from Manapad coastal areas, Thoothukudi, Tamil Nadu, India. Asian Pacific J Trop Biomed. 2012; S1077-S1080.
38. Radhika D, Veerabahu C, Vijayalakshmi M, Priya R. Antibacterial Effect of some species of seaweed from different station of the gulf of mannar coast. International J Biol Pharmace Res. 2013; 4(11): 783-787.
39. Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol. 2004; 16: 245-262.
40. Krishnaveni E, Johnson M. Preliminary Phytochemical, UV-VIS, HPLC and Anti-bacterial Studies on *Gracilaria corticata* J. Ag. Asian Pac J Trop Biomed. 2012; S568-S574.
41. Dhar ML, Dhar MM, Dhawan BN, Ray C. Screening of Indian plants for biological activity. Part-I, Indian J Biol. 1979; 6: 232-234.
42. Price KR, Johnson TI, Fenwick GR, The chemistry and biological significance of saponins in food and feeding stuffs. Rev Food Sci Nutr. 1987; 26: 22-48.
43. Cherian S, Augusti KT Insulin sparing action of leucopelargonidin derivative isolated from *Ficus bengalesis* L, Indian J Exper Biol. 1995; 33: 608-611.
44. Miliauskasa G, Venskutonisa PR. Beekb van TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004; 85: 231–237.
45. Reguant C, Bordons Arola A, Roze LN. Influence of phenolic compounds on the physiology of *Oenococcus oeni*. J Appl Microbiol. 2000; 88: 065-1071.
46. Shan B, Cai Y-Z, Brooks JD, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extract. Int J Food Microbiol. 2007; 117: 112-119.
47. Tajkarimi MM, Ibrahima SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010; 21: 1199-1218.

Source of Support: Nil, Conflict of Interest: None declared.