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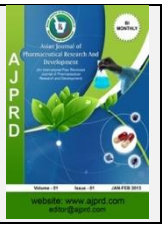

Open Access **Research Article**

Formulation and Evaluation of Antimicrobial Activity of Marine Red Algae Gracilaria Corticata

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A B S T R A C T

Objective: The present study was carried out to investigate the antimicrobial activity of formulated gel containing marine red algae ***Gracilaria Corticata*** belonging to the family **Rhodophyta** by disc diffusion assay method against bacterial and fungal organisms.

Methods: The ethanolic extract of ***Gracilaria Corticata***were subjected to phytochemical and physiochemical analysis and then gel prepared using an ethanolic extract of ***Gracilariacorticata*** were divided into two different concentration such as 250 and 500 µg. The formulated gel was investigated for antimicrobial activity by disc diffusion assay method against *S. aureus, P. aeruginosa, and Candida albicans* and compared with standard drug ketoconazole (30µg) and amikacin (30µg). The zones of inhibition formed against organisms were calculated.

Results: In the present study the formulated gel showed a moderately potent antimicrobial activity. The zone of inhibition of prepared gel against *S. Aureus and P. aeruginosa* was found to be 12 and 10 mm respectively. The zone of inhibition of prepared candida albicans was found to be 12 mm. And the phytochemical analysis shows the presence of active constituents such as flavonoids, sugar, phenol and quinones and physiochemical analysis shows that the antimicrobial activity is due to the presence of active constituents and evaluation of gel proves the stability of the formulation.

Conclusion: The present study shows that the *invitro* anti-microbial activity of the formulated gel at the dose of 500 μg was higher inhibitory against virulent bacteria which cause various infection. From the above studies, it confirms the formulated gel exhibit moderately potent antimicrobial activity**.**

Keywords: Antimicrobial activity, amikacin, ketoconazole, zone of inhibition, *Gracilariacorticata.*

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INTRODUCTION

Bacterial species are responsible for mortality in human population because their infection causes diseases like food borne gastroenteritis, secondary infections, mastitis and upper respiratory complications [1]. Most of the current antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects and their widespread overuse has led to increasing clinical resistance of previously sensitive microorganisms and to the occurrence of uncommon infections [2]. Thus, there arises a search for new antimicrobial agents with high efficacy and no side effect is of the utmost importance [3]. Nowadays, the focus has been targeted to seaweeds which proves to be a promising source of antimicrobials [4]

Algae are photosynthetic organisms which exhibit greater diversity and have gained potential interest as a resource of bioactive compounds. Seaweeds were seen to be eaten for centuries by humans living along the coastlines all around the world. Ireland has a rich tradition of using algae in soups [5]. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds and other marine organisms. The host organisms biosynthesize these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their

environment.There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibacterial, antifungal, antiviral, antitumoral, anticoagulant and antifouling. Gracilariacorticata belongs to the family Rhodophyceae (Red algae). These are highly evolved multicellular forms with well-developed branched thalli. Except for few species they are exclusively marine and vary in size and shape. They are epiphytes, growing as crust on the rocks or shells as a large fleshy, branched or blade like thalli. The thallus is basically filamentous, simple or branched, free or compacted to form pseudoparenchyma with uni or multiaxial construction. The present study was aimed to screen the pharmacological activity of Gracilariacorticata solvent extracts against human pathogenic bacteria and fungi.[6] The marine environment is the habitat of diverse groups of microorganisms. Seaweeds are constantly in contact with potentially dangerous microbes and they have apparently defended against the microbial thread. [8] Hence the present study was designed to study the microbial activity of gel containing marine red algae *Gracilariacorticata*. and evaluation of prepared gel.

MATERIALS AND METHODS

Collection of plant materials:

The algae were collected from the coast of thiruchendur during 2016 were authenticated by the Prof, P.Jayaraman, Ph.D**.** director, plant anatomy research center (PARC), Chennai and the voucher specimen wasdeposited in PARC /2016/3217 for future reference. The sample was collected dried, powdered and stored

Preparation of extract:

The shade dried and finely powdered algae wereextracted in a soxhlet apparatus with ethanol by hot percolation method. After exhaustive extraction, evaporated in an electric water bath and extract was subjected to phytochemical analysis.

Phytochemical analysis [9]:

In the early development of modern medicine biologically active compounds from higher plants have played a vital role in provide medicines to compact pain and diseases the plant species may be considered as biosynthetic laboratory for the synthesis of primary metabolites such as carbohydrates, proteins, fats and secondary metabolites such as alkaloids, terpenoids, flavonoids and glycosides etc which exact certain physiological effects. These physicalcomponents are responsible for the desired therapeutic properties.

For our present study, preliminary phytochemical studies were carried out to characterize the therapeutic active constituent from the extract *Gracilariacorticate.*

Physiochemical analysis [9]:

For the freshly prepared extract, physiochemical analysis is carried out to reveal that various biological activity and therapeutic uses exhibit by them is due to the presence of Phyto-constituents. The physiochemical parameters like ash value, extractive value, loss on drying and thin layer chromatography were carried out.

Formulation [10-12]:

Carbopol gel using ethanolic extract of *Gracilaria corticata* were prepared by dispersing propylene glycol- 2.4%, Carbopol- 0.7%, triethanolamine- 0.1% and methylparaben- 0.1% in water and allowed to stand for 24 hours, finally ethanolic extract of*Gracilaria corticata* 1% added and allowed for stirring at 1000 rpm for 20 minutes and it is homogenized to form a gel.

Evaluation of gel [10-12]:

The prepared gel of *Gracilaria corticata* was evaluated by determining spreadability, homogeneity, measurement of ph and accelerated stability testing.

Pharmacological screening:

IN VITRO ANTIMICROBIAL ACTIVITY

Antibacterial activity

Prepared gel was screened for antimicrobial activity by disc diffusion technique. The sample was screened *in vitro* for their antimicrobial activity against *S. aureus, P. aeruginosa and* are compared with standard drug amikacin (30µg). The zones of inhibition formed for the compounds against organisms were calculated.

Table 1: Detail of the organism used for the study

Grams strain	Name of the organism	Std code
Gram- negative	*Pseudomonas aeruginosa*	(ATCC-2853)
Gram- positive spherical bacteria	*Staphylococcus aureus*	(ATCC-9144)

Disc-diffusion assay [13]

The antibacterial activities for gel were carried out by the disc diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 250 µg and 500 µg /disc.

The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 hrs the suspensions were adjusted to standard subculture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain.

Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in the aseptic chamber. Each concentration was injected into the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug amikacin (30µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 C for 24 h. The diameter of the clear zone aroundthe disc was measured and expressed in millimeters as its anti-microbial activity. anti-bacterial activity of test compound against the *S. aureus, P. aeruginosa.*

Antifungal activity

The prepared gel was screened for antifungal activities by disc diffusion technique. Compounds are screened *in vitro* for

their antifungal activity against *Candida albicans* and compared with standard drug ketoconazole. The zone of inhibition formed for the compounds against organisms was calculated.

Table 2: Detail of the organism used for the study

Name of the organism	Code
Candida albicans	(MTCC-227)

Antifungal assay [14]

Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Potato dextrose broth. The synthesized compounds were applied on the sterile disc. Standard antibiotic (ketoconazole 30µg) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition were observed and measured.

RESULTS AND DISCUSSION

RESULTS

PRELIMINARY PHYTOCHEMICAL TEST:

Preliminary phytochemical analysis of an ethanolic extract of *Gracilaria corticata* shows the presence of following active constituents: Flavonoids, Sugar, Phenol, and Quinones and results are shown in Table 1

Table 1: Physiochemical analysis

S. No	Parameter	Reports / Values % W/W
1	Total Ash	0.9 %
2	Acid Insoluble Ash	0.5 %
3	Water Soluble Ash	0.6 %
4	Sulfated ASH	1.4 %
5	Extractive Value	
I	Water Soluble	0.24% w/w
II	Alcohol Soluble	0.60 % w/w
6	Loss On Drying	12 %

DISCUSSION

Gracilariacorticata was a red alga present in rocks and stones in the sea. It is found throughout the year and occurs in harvestable quantities. It is mainly distributed in the Bay of Bengal.it is used as an antimicrobial, antitumor and anti-obesity agent and also used for agar production. *Gracilariacorticata* was collected, shade dried, milled and extracted, in soxhlet (hot perculation method). The ethanolic extract was found to be 8ml.The preliminary phytochemical studies were done, the active constituent present are flavonoids, sugar, phenol, and quinones.

Physiochemical analysis:

Physiochemical analysis was carried out and results are obtained as shown in table1 These constants are would help to identify the naturally occurring inorganic salts and organic matter added for purpose of adulteration and to standardize the plant and the TLC studies of *Gracilaria corticata* ethanolic extract shows the presence of 5 spots with value 0.2, 0.4,0.6,0.7,0.8

Formulation:

The extract was mixed with Carbopol and another ingredient to form a gel. The formulated evaluated by spreadability, homogeneity, pH and accelerated stability testing which is mentioned in Table 2

Evaluation of gel

Physical Appearance:

Formulated gels were found to be homogeneous brown colored gel preparations.

Measurement of pH:

The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability

The spreadability of the formulation concluded that all the developed formulation showed acceptable spreadability.

Table 2: Evaluation of formulated gel

Formulation	pH	Spreadability	Homogeneity
GCF	6.9	5	Good

Accelerated stability studies

Accelerated stability studies indicated that the physical appearance, rheological properties, extrudability, spreadability in the prepared gel remained unchanged upon storage for 1 month. The pH observed of prepared gel through 1-month storage was in between 6-7. Rheological properties and spreadability were obtained uniformly. Gel formulation was maintaining drug level after 1 month of accelerated stability which is mentioned in Table 3.

Table 3: stability studies of formulated gel

Formulation	Month	Appearance	pH
GCF	0	Clear	6.9
	1	Clear	6.8
	2	Clear	6.9
	3	Clear	7.0

Pharmacological screening:

Antibacterial activity was done by disc diffusion assay formulated drug was compared with a standard drug. The zone of inhibition of formulated gel against staph aureus and Pseudomonas aerugenosa was found to be 12 and 10mm respectively. Antifungal activity was done by disc diffusion assay. The formulated drug was compared with a standard drug. The zone of inhibition of formulated gel against Candida albicans was found to be 12mm. By above studies, the gel was formulated, evaluated and screened for antimicrobial activity. The formulated herbal gel showed moderately potent antimicrobial activity and the result was shown in table 4.

S.No	Plant Extract	Solvent System	No. of Spot	Rf Values
1	Alcoholic Extract	Ethyl Acetate: Petroleum Ether 9:1	5	0.2 0.4 0.6 0.7 0.8

Table 4: In vitro antimicrobial activity

COMPOUNDS	Zone of Inhibitions (mm)					
	S. aureus		P. aerugenosa		C.albicans	
	250µg	500µg	250µg	500µg	250µg	500µg
The gel of *GracilariaCorticata*	-	12	-	10	-	12
Amikacin 30µg	34		36		-	
Ketoconazole 30µg	-		-		32	

ANTI BACTERIAL ACTIVITY

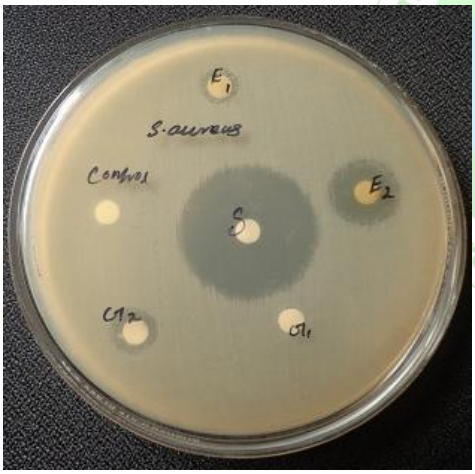

Figure: 1 Gram-Positive Bacteria

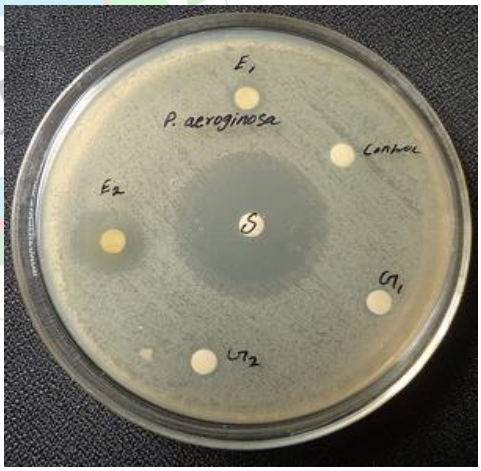

Figure: 2 Gram-Negative Bacteria

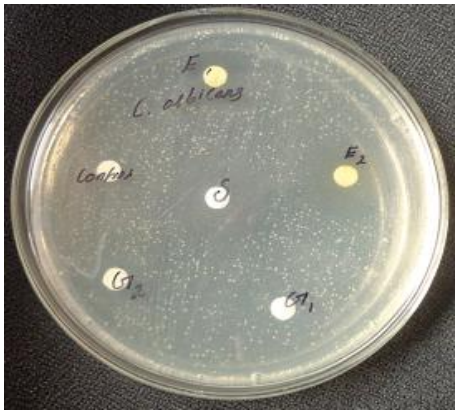

Figure: 3 Antifungal Activities

SUMMARY AND CONCLUSION

The preliminary phytochemical study showed the presence of flavonoids, sugar, phenol and quinones and the physiochemical study also revealed that the various biological activity and therapeutic uses exhibit by them is due to the presence of Phyto-constituents. TLC studies of alcoholic extract show the presence of 5 spots. The *invitro* anti-microbial activity of formulated gel of red algae possesses potent anti-microbial activity by the inhibition of virulent bacteria and fungi. The *invitro* anti-microbial activity of the dose of 500 μg was higher inhibitory against virulent bacteria which cause various infection. From the above studies, it confirms the formulated gel exhibit potent antimicrobial activity.

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