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Antibacterial efficacies of brown sea weed *Sargassum Wightii* on Periodontal Pathogens: An In-Vitro Microbiological Analysis

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ABSTRACT

Objective: *Sargassum wightii* is brown algae available across the south east coast of India which can produce many secondary metabolites and are opulent sources of potential plant derived by-products with antiviral, antihelminthic, antifungal and antibacterial activities. The objective of the current study was to assess minimal inhibitory concentration MIC and minimum bactericidal concentration MBC of whole leaf extract of *Sargassum wightii* on *P.Gingivalis (Pg), F.Nucleatum (Fn), T.Forsythia (Tf) and A. Actinomycetemcomitans (Aa)*. **Methods:** The method executed out by serial dilutions of the test drug with thioglycollate broth nine times. Cultures of chosen pathogens were added to obtain culture suspension up to 10^{-9}. Their turbidity has monitored to assess MIC after incubating in oxygen independent container. The First 3 or 5 dilutions tubes which were MIC sensitive were removed and incubated one complete day. To determine whether there was bacterio-static or bactericidal effect of the extract on selected organisms, colony numbers were assessed the day after. **Results:** Outcome for MIC was in the span of 0.2µg/ml to 0.4µg/ml and for MBC from 0.2µg/ml to 3.12µg/ml. **Conclusion:** Positive pharmacological effect does exists against tested microorganisms of whole leaf extract of *Sargassum Wighitti* & demands more extensive research for treating multi-factorial periodontal disease.

Key words: *Sargassum wightii, antimicrobial activity, natural derivative, pharmacological effect, periodontal pathogens.*

INTRODUCTION

Periodontitis is one among the main diseases of oral cavity endorsing universal concern of constant diseases which strokes human populations worldwide at high prevalence rates depicting a major public health problem.[1] It occurs owing to infection with sub-gingival plaque-forming bacteria, followed by host immune response.[2] Although there is surfeit of mixed organisms present as sub-gingival colonies only few contribute to pathogenicity like *P.gingivalis(Pg), F.nucleatum(Fn) T.forsythia(Tf) and Aggregatibacter actinomycetemcomitans(Aa)* and have been strongly associated with periodontitis.[3]

Bothe the inceptive & maintenance phase of treatment commonly employed for periodontal therapy to remove deposits ranging from bio-film to calculus on tooth surfaces is Scaling and root planning.

Teeth anatomy aren't straight forward and offensive behaviour of microbes into connective tissues makes scaling and root planning a challenging task leading to incomplete wipe out of etiological factors and progression of disease in subjects. Such cases demands

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for enhanced visibility and accessibility paving way to surgical therapy beside chemotherapeutic adjunctive for effective results.[4] Merging scaling and root planning with careful selection of pharmacological medicine provides an additional anti-microbial effect that allow a greater chances of disease controll.[5] Many synthetic chemical therapeutic agents like systemic antimicrobials have been used effectively for more than 3 decades to treat diseases of microbial origin like periodontitis and have exhibited a wide range of antimicrobial activity along with potency & substantivity.[6] but systemic antimicrobial administration is accompanied by many potential after effects like gastric disturbances, vomiting, anorexia; a more serious problem in systemic therapy is building resistant strains and superinfections because of the low achievable concentrations.[7]

Massive active compounds from various marine algae, including as kainite, autocoids of arachidonic acid, carotenoids, oxygen, brominated heterocyclics, amino acids, phenolics, polysaccharides, arginine, guanidine derivatives, dibenzo annulated pyrazine, biogenic amines, phytosterols, prostaglandins and fucoxanthin, have served as the basis for the creation of novel primary chemicals for medicines.[8] The need for harmless, affordable, and efficacious substitutes for synthetic antimicrobials has led to our interest in natural resources like seaweed marine algae.

Seaweeds are the photosynthetic microalgae classified as brown, green and red algae derived from their pigment constituents. Secondary metabolite components of these algae have diverse functions and their earlier reports evaluated the use of seaweed by many pharmaceutical industries in drug development and attention has been given to the antibacterial activities of marine algae.[9]

Brown macroalga *Sargassum wightii* of family Sargassaceae and order Fucales includes its own different kinds, showing round the world apart in extreme temperature and torrid zones. It dwells in depthless waters and marine invertebrate sessile polyps on parts of Coastal Asia and India. Abundant flavonoids & terpenoids take it to its greater anti-oxidant activity. To fight against drug resistant bacteria it exhibits potent cellular signal transduction interactions and synthesizing different pharmacophores with good nutritional values.[10] Sulfated polysaccharides called fucoidans are the main constituents giving their other potential bioactive benefits for man like anticoagulant, anti-Neoplastic, anti-swelling, and antibacterial effect.[11]

In 18th century A.D East Asian's used *S.wightii* as heritage to treat goitre. Presence of high amount of terpenoids and their potential antioxidant and cholinesterase inhibitory activity showed the anti-Alzheimer potential as well.[12] Isolated Fucoidan has expressed eminent α-d-glucosidase repressive effect on postprandial hyperglycaemic patient by inhibiting the carbohydrate uptake from gut to reduce glycemic levels after taking food making it feasible medicine treatment option for type ii diabeties mellitus.[13] Proliferation, migration and initiating apoptosis in cancer breast cells by SWP1 fraction of *S.wightii* extract macromolecules guide to excess generation of ROS (reactive oxygen species) damaging its nucleus and mitochondrial membrane denote its anticancer activity in-vitro.[14] Extracted fucoxanthin from *S.Wighitti* revealed inhibition of Angiotensin I - Converting Enzyme in vertebrates through non-competitive mixed inhibition mode making way for its application against long term hypertension.[15]

For making new medicaments, most suitable and secure way of apprising antimicrobial potency is through microbiological assays capable of determining least concentrations of extract required to inhibit and kill the cultured test organism's in-vitro known as MIC & MBC.[16]

On account of this, our work turned to determine MIC and MBC of pure leaf extract of *Sargassum wightii* on chosen test pathogenic organisms of periodontium.

Materials and Methods

Naturally collected commercially available dried and finely powdered *sargassum wightii* leaf powder was obtained (ECOTIKA F&F ™ D-2 plot no. nu-108, Shaktinagar, Gandhidham, Kutch, Gujarat, India.) The product was free of extraneous matter.

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Microbial subtypes

The microbial variant used in this work were *Porphyromonas gingivalis*-33277, *F.nucleatum*-25586, *Tannerella forsythia*- 43037 and *Aggregatibacter actinomycetemcomitans*- 43718 American type culture collection, USA respectively.

Fig.1: Sargassum wightii leaf

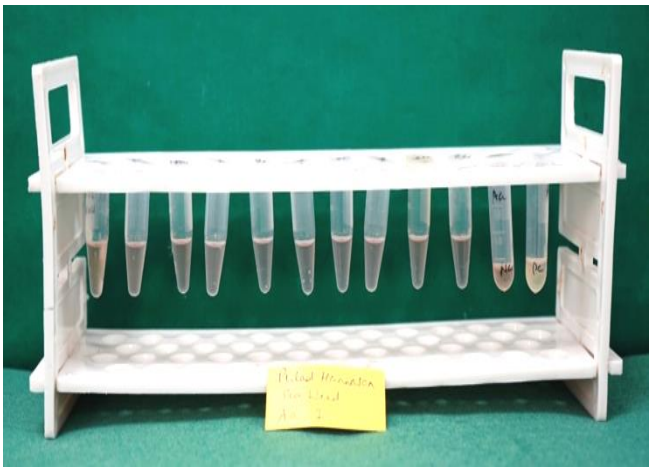
MIC parameters in contrast to selected microbes:

The assay was executed in 9 dilutions of the extract mixed with thioglycollate broth. First in a tube 0.02 ml of extract was infused to 0.38 ml thioglycollate broth. Then 0.2 ml of thioglycollate broth was dispersed in nine different tubes. In the 1st tube 0.02ml extract was mixed, this renders itself as 10^{-1} dilution. And from this 1st tube 0.02 ml transferred into 2nd tube that depicts itself as 10^{-2} dilution,

Similarly, this procedure is repeated ten times sequentially up to 10^{-9} dilution.

0.005 microbial stock variants are mixed into 2ml of thioglycollate broth to procure a suspension and 0.2 ml of this is mixed to nine sequential tubes followed by incubation for 2-3 days in anaerobic container at 98.6°F that revealed the growth of microbes through turbidity proving the bacterio-static efficacy of the extract i.e., MIC. (Fig. 2)

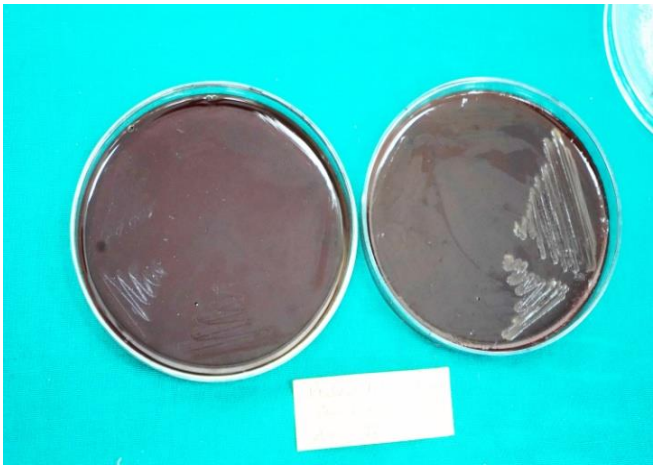
Fig. 2: Determination of Minimum inhibitory concentration



MBC parameters in contrast to selected microbes

Following the MIC assay, to assess the bactericidal values of the extract versus the chosen microbes, MBC assay was performed. Entire sequential dilution tubes then subjected to inoculation in the culture media i.e., placed in two culture dishes which was further cleaved into ten parts (five in each) consequently to the prepared sequential dilutions of the extract, which was then subjected to incubation for one day at 98.6°F within an anaerobic container to assess microbial colonization. The microbial growth count was performed using microscope following one hour of incubation. Observing the placed culture media, the concentration at which colonization of the microbes ceased, rendered the MBC values for the extract. (Fig. 3)

Fig. 3: Determination of minimum bactericidal concentration



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Ethical approval: PMNMDCH/1690/2020-21.

Results

The results showed that *P.gingivalis (Pg), F.nucleatum (Fn) T.forsythia (Tf)* and *Aggregatibacter actinomy-cetemcomitans (Aa)* showed prominent sensitivity to pure leaf extract with MIC parameters of 0.04mg/dl, 0.04mg/dl, 0.04mg/dl and 0.02mg/dl correspondingly (table 1), MBC parameters of 0.31mg/dl, 0.02 mg/dl, 0.04 mg/dl, 0.04 mg/dl correspondingly (table 2).

Discussion

The modality of therapeutic preference for chronic periodontitis is mechanical debridement. Numerous pharmaco-theraputic agents are used in conjunction to scaling and root planning. The t-half life of any antibiotic is noted to be finite and the adversity with abuse and resistance of over the counter antibiotics are two reasons to take interest in the topic of antimicrobial of natural derivatives like plant extracts.[17]

Being included as sea water subfamily with abundant bioactive molecules, *Sargassum Wightii* is largely found at tropical parts embracing a high commercial value which makes a prime species below the genus *Sargassum*.[18] Approximately half of our planet is covered by ocean waters consisting of diverse eco systems. Every organism in that lives in near association with each other in an unfriendly environment. The struggle for existence to occupy position, space in the system and sustain competitiveness for living demand to evolves the organisms to produce bioactive molecules having medicinal properties like antimicrobial, anti-inflammatory, anti-neoplastic, anti-viral and anti-fungal that can be benefited for humans along with their protection from preditors.[19]

Johnson marimuthu *et al.* in their examination of plant contents revealed the existence of secondary metabolites from direct concentrations of *Sargassum Wightii* such as saponins, steroids, alkaloids, phenols which could be utilized against microbes, virus, parasites, allergens, carcinomas, inflammation and platelet aggregation.[18]

Two possible antibacterial pathways were found according to a study done by Feng he et.al firstly, the death of cells takes place due to seep out of proteins and vital constituents when cell wall integrity is spoiled as polysaccharide's sticks on the plasma membrane and Secondly Karyorrhexis is initiated after its invasion into the plasma membrane targeting the vital DNA functions required for its survival making the bacteria incompetent to resist trauma.[20] More over anionic properties of sulfated polysaccharides and its association with nutrients of negative charge may cease the uptake of microbial nutriments, decreasing the pharmacokinetics when placed on culture plates tested in-vitro.[21]

Muthuraman and compeers (2014) chloroform extract showed significant anti-bacterial activity of *Sargassum wightii* in contrast to diverse microrganisms expressing its antimicrobial effect.[22] In another study, crude petroleum ether extract of *S.wightii* has the highest antibacterial efficacy contrary to *B.cerus* than other extracts, and MBC and MIC levels were 125 & 61.8 µg/ml respectively which was more when compared with current study results i.e. 3.12 & 0.4 µg/ml respectively.[23]

A study, by R. Vaikundamoorthy *et al.* (2018) reported antitumor potency of polysaccharide's extracted from *s.wightii* examining in contrast to mcf 7 & mda-mb-231 breast tumour cells utilizing calorimetric test to determine cell metabolites & their gradual reduction in development of two cells types, subjected at different doses.[14]

V. Raji *et al.* (2020) in their in-vitro study included absolution of fucoxanthin from *sargassum* species, expression of free radical's protective action, suppression of ACE-1 & its relation of fucoxanthin ion's related to active locations of angiotensin converting emzymes-1 in human by molecular docking examination to report *Sargassum wightii* could be utilized as a nutritive additive to control blood-pressure.[15] K T Magesh *et al.* showed enhanced antibacterial potency with increase in the concentration

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of extract offering assuring evidence that biologically effective secondary phyto-compounds could be considered for the therapeutics of diseases confined to oral cavity and its definitive antibacterial activity even with the crude extract.[24]

Though significant understanding exists concerning to *sargassum wightii* as anti-microbial agent in contrast to varied microbes but still inadequacy persists when it comes to prime periodontal pathogens.

Therefore, the present study is the foot mark work to determine the in-vitro antibacterial effect of *sargassum wightii* extract using microbiological assay over some periodontal-pathogens.

As a result, *Sargassum wightii* was good in terms of their sensitivity of pure leaf extract for all four chosen periodontal-pathogens. Hence; clinical in-vivo research should be planned & conducted to check the clinical efficacy of *s.wightii* which could be helpful for it enhances its scope of therapeutics.

Table 1: MIC of pure leaf extract of *Sargassum wightii*.

Microorganism	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
P.G	S	S	S	S	S	S	S	S	S	R
F.N	S	S	S	S	S	S	S	S	S	R
T.F	S	S	S	S	S	S	S	S	S	R
A.A	S	S	S	S	S	S	S	S	S	S

Table 2: MBC of ethanolic extract of *Sargassum wightii.*

Microorganisms	Repeats	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml	C
PG	1	NG	NG	NG	NG	NG	NG	18	210	225	50	300
	2	NG	NG	NG	NG	NG	NG	NG	180	200	210	300
FN	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	300
	2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	300
TF	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	180	250
	2	NG	NG	NG	NG	NG	NG	NG	NG	NG	150	200
AA	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	300	500
	2	NG	NG	NG	NG	NG	NG	NG	NG	NG	350	400

CONCLUSION

The current study sheds light on the antibacterial activity of the pure leaf extract of *sargassum wightii* against the chosen test periodontal-pathogens which was credited to its enhanced bacteriostatic and bactericidal properties against the chosen periodontal pathogens. Extensive studies and animal research are needed to confirm the in-vivo efficiency of the same in different combinations of pure *sargassum wightii* extract as herbal antibacterial agent for periodontal therapy.

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