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ASSESSMENT OF BIO OIL PRODUCTION FROM MIXED ALGAL CULTURES FROM GODAVARI REGION AGAINST MICROFLORA RESPONSIBLE FOR GI TRACK INFECTIONS.

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ABSTRACT

The intricate groups of microorganisms that colonize the human gastrointestinal tract assume a vital part in human health. The advance of culture-autonomous sub-atomic systems has given new bits of data within the creation and diverse qualities of the enteric microbiota. People have complex microbial groups accepted to add to wellbeing support and, when in lopsidedness, to the improvement of maladies. Deciding the microbic sythesis in patients and sound controls might therefore offer novel therapeutic targets. In this paper, microbial pathogens were isolated from patients GI track and the antimicrobial activity of the Mixed Algal cultures isolated from Tammileru lake and Godavari river (A1, A2, A3, A4, & A5) microalgae was tested on the isolated pathogens. Totally, 20 bacterial and 6 fungal strains were isolated, of which *Eschericia coli* (34.1), *Enterococci* spp (20%), *Lactobacilli* spp (22%), *Staphylococcus* spp. (30.2%) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (12.8%) were the major pathogens. Only three *Staphylococcus aureus* (*S. aureus*) strains and *Pseudomonas* strains showed resistance to methicillin. The mixed algal extract showed interesting antimicrobial properties, which mostly inhibited the growth of isolated *S. aureus, P. aeruginosa, Escherichia coli* and *Klebsiella* spp. These results suggest that the two algae have potential as resources for the development of antimicrobial agents.

KEYWORDS: Carbene compounds, methylated caffeine, XRD, malarial parasite.

INTRODUCTION

Gastrointestinal microbiota is the intricate group of microorganisms that live in the stomach related tracts of people and different creatures, including insects. The gut metagenome is that the total of the appreciable variety of genomes of gut microbiota. The gut is one specialty that human microbiota can inhabit (Saxena and Sharma, 2016). The GI tract of a well evolved creature has vertebrate has 5 major areas: throat, stomach, tiny GI tract, cecum and enormous gut. Contingent on the animal species, any of these areas might be additionally compartmentalized or separated into subareas. The 3 basic minor departures from the overall subject may be recognized, i.e. ruminant, cecal and "straight tube." The initial two varieties are found in animals only or dominatingly herbivorous. Both include adjustments of the tract in which coarse, stringy sustenance is deferred in travel and presented to microbial corruption from which the animal infers nutritional advantage (Savage, 1977). Around the world particularly in the developing countries the GI track infections lead morbidity and mortality worldwide, particularly in developing countries. The system of vertebrates incorporates advanced settlements of cells and particles that interface to administer security from difficulties by infective microorganisms (microscopic organisms, infections, parasites). The intestinal micro biota applies both destructive and valuable impacts on human health and subsequently is the biggest wellspring of microbial stimulations). For all intents and functions all surfaces of the organic structure conferred to the world square measure generally occupied by miniaturized scale living beings. The digestive tract constitutes a particularly rich and assorted microbial territory. Around 800–1000 distinctive bacterial species and 7000 unique strains occupy the gastrointestinal tract. The treatment of anti-infection will influence the target infectious agent moreover as commensal occupants of the human host. The degree of the effect on non-target microbial populaces relies on upon the specific anti-microbial utilized, its method of activity and the level of resistance in the group (Varsha and Bunger, 2014. Once in a while an unevenness in the commensal gut microbiota because of anti-microbial organization can bring about intestinal

issues, for example, anti-toxin related loose bowels (AAD) (Jernberg et al., 2010). The Micro organisms that cause the Gastro Intestinal diseases like *Salmonella, Shigella, Yersinia* (Dekker and Frank, 2015) *Campylobacter, E coli*. *Aspergillus species* (e.g. *fumigatus, flavus* and *niger*), *Candida albicans; Candida tropicalis* causes the GI track Infections (Laura et al., 2014).

2. Experimental Section

Algal cultures
A1-Spyrogyra
A2-Nostoc
A3-Clorella
A4-Scencedesmus
A5-Botryococcus braunii

Study Participants

Twenty two healthy volunteers (8 male and 14 female) of individuals who worked at the clinic between the ages of 23 and 54 were recruited. Participants were excluded if they were under the age of 18, had used antibiotics or probiotics within the last two weeks, or were currently undergoing chemotherapy. All subjects signed and Informed Consent forms prior to the study.

Pathogenic Micro-Organisms Collection

For 2 months (from April 2014 to June 2014), a total of 100 aliquots were generated. Fecal specimens and fecal swabs were sent to the Microbiology Laboratory for further analysis. All consecutive, non-duplicates strains were studied.

Bacterial Strains and Growth Conditions

Fecal swabs and diluted aliquots were plated in blood agar and in appropriate selective culture medium (Mc Conkey agar for Gram-negative bacteria, Sabouraud agar for fungi). The plates were incubated at 37 °C for 18–24 h. The incubation of Sabouraud plates was prolonged up to a week. All isolates were identified to the species level by routine methods viz., disc diffusion test and MIC etc.

Biofilm Assay

A qualitative assessment of biofilm formation was done as described by Christensen et al. The TSBglu (10 mL) was inoculated with a loop ful of microorganism from overnight culture plates and incubated for 24 h at 37 ºC. The tubes were decanted and washed with PBS (pH 7.2) and dried. Dried tubes were stained with 0.1% crystal violet. Excess stain was washed with deionized water. Tubes were then dried in inverted position for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Experiments were performed in triplicate and repeated for three times.

Bacterial Susceptibility (MIC Determination)

Minimum Inhibitory Concentration (MIC) of mixed algal extract was determined using the broth microdilution method [CLSI, 2012a and 2012b]. Briefly, algal extract has been tested on cultures of bacteria (5×10^5 CFU per mL) in 96-well microtiter plates in Mueller-Hinton broth. After 18–24 h of incubation at 37 °C, the concentration of the extract from marine algae which prevented a visible bacterial growth was identified as the MIC. All tests were performed in triplicate and were executed three times.

RESULTS

Bacterial Isolates

A total of 100 samples were collected from study participants (8 males, 14% females) with GI tract infections. The age of patients ranged from 23 to 54 years; 86 samples were positive for microorganisms, whereas 14 were negative.

A total of 110 microorganisms were found, including Gram-negative bacteria, Gram-positive bacteria and fungi. Table 1 summarizes the list and distribution of pathogens isolated from the positive samples.

E.coli had the highest number of isolates (39), followed by *Enterococci spp* (24), *Streptococci spp* (21), *Clostridium spp* (17) *S. aureus* (5) and *P.aeruginosa* (4). Among Fungi, 52.2% were *Candida* spp., while the remaining 47.8% were *A. niger, Trichoderma* and *Fusarium spp*.

2.2. Biofilm Assay

Four out of Five isolates of *S. aureus* and 27 out of 39 *E.coli* were biofilm producers (Table 2). One out of four strains of *P. aeruginosa* were shown to be able to induce significant biofilm production (producers), while only one strain was a non biofilm producer.

E. coli strains were similarly distributed among producers, slight producers, and non-producers, while most of *Clostridium* spp, *Enterococci* spp and *Streptococci* spp. were biofilm producers and a few (< 10%) were non biofilm producers.

Table 1: Microorganisms isolated from positive column swabs.

	Strain Name	No of isolated strains	%
Bacteria	*Eschericia coli*	39	42.9
	Enterococcus spp	24	26.4
	Staphylococcus aureus	05	5.5
	Pseudomonas aeruginosa	04	4.4
	Clostridium spp	17	18.7
	Streptococci spp	21	23.1
Fungi	*Trichoderma spp*	06	26.1
	Aspergillus spp	03	13.0
	Candida spp	12	52.2
	Fusarium spp	02	8.7

Table 2: Production of biofilm by bacteria isolated from ear swabs. In brackets: number of strains. No. = number of strains and relative percentage (%). * = Congo Red Agar method; ** = Christensen method.

Strain	Producers		Slight Producers		Non Producers	
	No.	%	No.	%	No.	%
S. aureus	4	80			1	20
P. aeruginosa	1	25	2	50	1	25
E. coli	27	69	5	13	7	18
Enterococci spp	13	54.1	8	33.3	3	12.5
Clostridium spp	11	64.7	5	29.4	1	5.8
Streptococci spp	14	66.7	1	4.8	6	28.6

2.3. Antibacterial Activity of Mixed Algal Extract

Table 3 shows the MIC values obtained after treatment of main pathogenic agents of GI infections (*S. aureus*, *P. aeruginosa*, *E. coli*, *etc.*). MIC values obtained after treatment of *P. aeruginosa* and *S. aureus* strains with Mixed algal extract were included between 1.1×10^8 and 3.6×10^8 cells/mL and between 1.2×10^8 and 1.7×10^9 cells/mL, respectively. MIC values for *Streptococci spp* and *Enterococci spp* exposed to Mixed algal extract were included between 5.1×10^{10} and 9.2×10^{10} cells/mL and between 2.7×10^9 and 7.1×10^9 cells/mL, respectively.

Table 3: Antimicrobial activity of extracts from algae on selected pathogens. In brackets: number of strains. * Eight strains of *E. coli*; six strains of *Klebsiella* spp.; SD = Standard Deviation.

Pathogen	MIC cells/mL Mean ± S.D.		
	MIC Range	MIC 50	MIC 90
S. aureus	1.2×10^8 to 1.7×10^9	2.4×10^8	1.7×10^9
Streptococci spp	5.1×10^{10} to 9.2×10^{10}	5.1×10^{10}	5.1×10^{10}
Enterococci spp	2.7×10^9 to 7.1×10^9	2.7×10^9	2.7×10^9
P.aeruginosa	1.1×10^8 to 3.6×10^8	1.1×10^8	3.6×10^8

MIC 50 and MIC 90 values obtained after treatment of bacteria with mixed algal extract were shown in the above table.

4. DISCUSSION

Knowledge of the gut microbial genes/pathways and cellular mechanisms of action of polyphenols on the human gut microbial ecosystem will allow us to better assess the fate of polyphenols and, ultimately, enhance/ improve our understanding of the impact of polyphenols on host health. In this study, *S. aureus* and *P. aeruginosa* were the main agents of GI tract infections. It can be supposed that the infection could be facilitated by cold and damp climate as well as by stagnation of water into the oral and nasal cavities due to bathing and swimming.

Through over two months sample collection from participants, a satisfactory susceptibility of isolated strains to antibiotics was studied (data not shown). Therefore, notwithstanding the anatomical site is not favorable to drug diffusion, these results show that in normal conditions antibiotics can eradicate the pathogens.

To date, bacterial infections in the clinical practice are relevant to the biofilm formation by bacteria, and more than 60% of infections seem to be occurred with the presence of biofilms [Stewart and Costerton, 2001]. Recent studies showed that ear, nose and throat (ENT) diseases are biofilm related [Vlastaraskos et al., 2007]. In our study, 80% of *S. aureus* and 25% of *P. aeruginosa* isolated from GI infected specimens were biofilm producers.

In this study, the evaluation of the antimicrobial activity of mixed algal extract against the etiological agents of GI tract infections has been attempted. Results show that the mixed algal extract showed antimicrobial activity,

notably against *P. aeruginosa, S. aureus, Enterococci spp and Streptococci spp.*

Algal extracts are known to contain polyphenols, notably gentisic acid, (+) catechin and (−) epicatechin [Lopez et al., 2015]. Considering that the antimicrobial activity of polyphenols is already known [Coppo and Marchese, 2014], the activity of the extract can be supposed to be due to these compounds.

The interest of scientists for antimicrobials from microalgae started in early 1940s with the pioneering work of Pratt [1942] who studied the antibacterial activity of chlorellin produced by *Chlorella*.

Subsequently, Duff *et al.* [1966] found remarkable antimicrobial properties in some Bacillariophyceae, Chrysophyceae and Cryptophyceae. Lustigman [1988] stated that *Dunaliella* produces a broad spectrum of antibiotic substances depending on the site of collection. Notably, the antibiotic activity was observed in cultures isolated from high-polluted waters, presumptively as a result of a mechanism of survival in a highly competitive environment [Lustigman, 1988].

The crude extract from Mixed algal culture was shown to inhibit the growth of *S. aureus*, and a number of antibiotic substances was found in these algae [Chang et al., 1993].

Our results show that extract from Mixed algal culture can yield compounds provided with antimicrobial activity useful for the treatment of otolaryngological diseases due to bacterial agents.

Therefore, taking into account the present therapeutic difficulties due to the occurrence of resistant strains, the identification of the active molecule(s) can be a therapeutic perspective of high interest.

Further studies will be needed to recognize the pharmacophores responsible for the antimicrobial activity and to elucidate the mechanisms of action of these algae extracts.

Thus, the biomass derived from algae is considered a source of valuable chemical constituents that can have application in human and animal nutrition, in agriculture and in pharmaceutical and cosmetics industries. Therefore, the development of biotechnological tools to ensure the supply of algal biomass of sufficient quality and quantity is desirable to meet the growing demand [Stengel and Connan, 2015].

REFERENCES

1. Saxena, R.; Sharma, V.K (2016). "A Metagenomic Insight Into the Human Microbiome: Its Implications in Health and Disease". In D. Kumar; S. Antonarakis. *Medical and Health Genomics*. Elsevier Science. p. 117.
2. Savage DC (1977) Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol, 31: 107–133.
3. Varsha A. Singh, Ruhi Bunger. "Probiotics and Gut Health". JIMSA, January-March 2014; 27: 1.
4. Cecilia Jernberg, Sonja Lo¨ fmark, Charlotta Edlund and Janet K. Jansson. "Long-term impacts of antibiotic exposure on the human intestinal microbiota". Microbiology, 2010; 156: 3216–3223.
5. John Dekker and Karen Frank. "Salmonella, Shigella and Yersinia". Clin Lab Med., 2015 June; 35(2): 225–246.
6. Laura W. Lamps, Keith K.T. Lai, Dr. Danny A. Milner Jr. "Fungal Infections of the Gastrointestinal Tract in the Immunocompromised Host-An Update". Adv Anat Pathol., 2014 July; 21(4): 217–227.
7. Stengel, D.B.; Connan, S. Marine algae: A source of biomass for biotechnological applications. *Method Mol. Biol.*, 2015; *1308*, doi: 10.1007/978-1-4939-2684-8-1.
8. Chang, T.; Ohta, S.; Ikegami, N.; Miyata, H.; Kashimoto, T.; Kondo, M. Antibiotic substances produced by a marine green alga, *Dunaliella primolecta*. *Bioresour. Technol.*, 1993; *44:* 149–153.
9. Lustigman, B. Comparison of antibiotic production from four ecotypes of the marine alga, *Dunaliella*. *Bull. Environ. Contam. Toxicol.*, 1988; *40:* 18–22.
10. Duff, D.C.B.; Bruce, D.L.; Antia, N.J. The antibacterial activity of planktonic algae. *Can. J. Microbiol.* 1966; *12:* 877–884.
11. Pratt, R.H. Studies on *Chlorella vulgaris* V: Some properties of the growth inhibitor formed by Chlorella cells. *Am. J. Bot.*, 1942; *29:* 142–148.
12. Coppo, E.; Marchese, A. Antibacterial activity of polyphenols. *Curr. Pharm. Biotechnol.*, 2014; *15:* 380–390.
13. Lòpez, A.; Rico, M.; Santana-Casiano, J.M.; Gonzàlez-Dàvila, M. Phenolic profile of *Dunaliella tertiolecta* growing under high levels of copper and iron. *Environ. Sci. Pullut. Res. Int.*, 2015; *22:* 14820–14828.
14. Vlastarakos, P.V.; Nikolopoulos, T.P.; Maragoudakis, P.; Tzagaroulakis, A.; Ferekidis, E. Biofilmsin ear, nose, and throat infections: How important are they? *Laryngoscope*, 2007; *117:* 668–673.
15. Stewart, P.S.; Costerton, J.W. Antibiotic resistance of bacteria in biofilms. *Lancet*, 2001; *358:* 135–138.
16. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. Document M02-A11*; Clinical and Laboratory Standards Institute (CLSI): Wayne, PA, USA, 2012.
17. Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition*. *Document M07-A9*; Clinical and Laboratory Standards Institute (CLSI): Wayne, PA, USA, 2012.