NUTRACEUTICALS FROM FRESHWATER MICROALGAE

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ABSTRACT

Microalgae are excellent reservoirs of adiverse range of nutraceuticals.The presence of secondary metabolites, pigments, vitamins makes microalgae unique reservoir of nutraceuticals. Many strains of microalgae are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial and antifungal;in addition, to it, they are also rich in adiverse range of antioxidants, vitamins and pigments. There is a drastic increase in cases of antibiotic resistance and exploring the antimicrobial efficacy of microalgae can aid in resolving the problem. In this study we have investigated the activity of Microalgae *Anabaena variabilis*, *Synechococcus elongates* and*Spirulinaplatensis*against the human pathogenic bacteria namely *Enterococcus sp, Esherichiacoli, Pseudomonas sp, Klebsiella sp*.Experimental results show that microalgal extracts are capable of inhibiting the growth of pathogenic bacterial strains. Methanol (60%) extract of *Anabaena variabilis*gave 30mm zone of inhibition against *E.coli*and methanol extract of *Synechococcus elongates* gave the highest antimicrobial activity against *Enterococcus* (32mm inhibition zone) and *Pseudomonas sp.* (26mm inhibition zone). *S.platensis* shows highest antimicrobial activity against *E.coli*with Methanol (100%). As per the formation of zones, ethanol (100%) extract, butanol, water, acetone and methanol (100%) extract gave a high visibility of zone of inhibition.Exploring the nutraceutical efficacy of microalgae can efficiently address theplethora of drug related issues and, in addition, serve as asource of nutrients.

Keywords: Nutraceutical, Microalgae, Antimicrobial activity,antibiotic resistance.

INTRODUCTION

Microalgae are unique reservoirs of nutraceuticals and produce a potential source of biologically active secondary metabolites, which are compounds that are essential for cell metabolism[9]. The nutraceutical efficacy of the microalgal strains is by virtue of the diverse metabolites, antioxidants enzymes[1,2], pigments and vitamins, present in these extraordinary photosynthetic microbes.Many of these compounds possess antibiotic and pharmacological effects such as toxicity for eukaryotic organismsantibacterial[3], antifungal[11], antiviral[17]. The high portion of the antimicrobial producing strains may be associated with an ecological role, playing a defensive action to maintain their niche, or enabling the invasion of strain into an established microbial community.Allelopathyis defined as the release of chemicals or toxins by plants or microorganisms, thataffect their potential competitors for resources[18, 12]. It is a ubiquitous phenomenon in theaquaticecosystem, and there are diverse organisms involved like angiosperms, macrophytes, and macroalgae. According to Molisch[14], allelopathy covers biochemical interactions, both stimulatory and inhibitory, among different primaryproducers or between primary producers and microorganisms. Freshwater microalgae are rich in several bioactive secondary metabolites with diverse chemical structure, which may achieve high concentrations in the aquatic medium when microalgal blooms. Some of the compounds released by microalgae have phytoplankton or aquatic plants.

Extracellular components from cultures of “dominant” microalgae have been reported to be isolated indicating the clear effect of extracellular components in the succession of microalgal populations[9, 21]. Subsequent studies have continued to support the role that these allelochemicals in controlling annual variability in phytoplankton communities.Microalgae are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications. Many compounds from microalgae are useful for thewelfare of humanity. Microalgae have been identified as a rich source of biologically active compounds with antiviral, antiplasmodial, antibacterial,antifungal and many more activities[20].Marine microalgae can serve as anexcellent source of large scale production of vitamins like Vitamin E and Vitamin B complex. The pigments like carotenoid and phycobiliprotein extracted from microalgae find wide commercial applications as feed additives, as colouring agents, as drugs and in thecosmetic industry.

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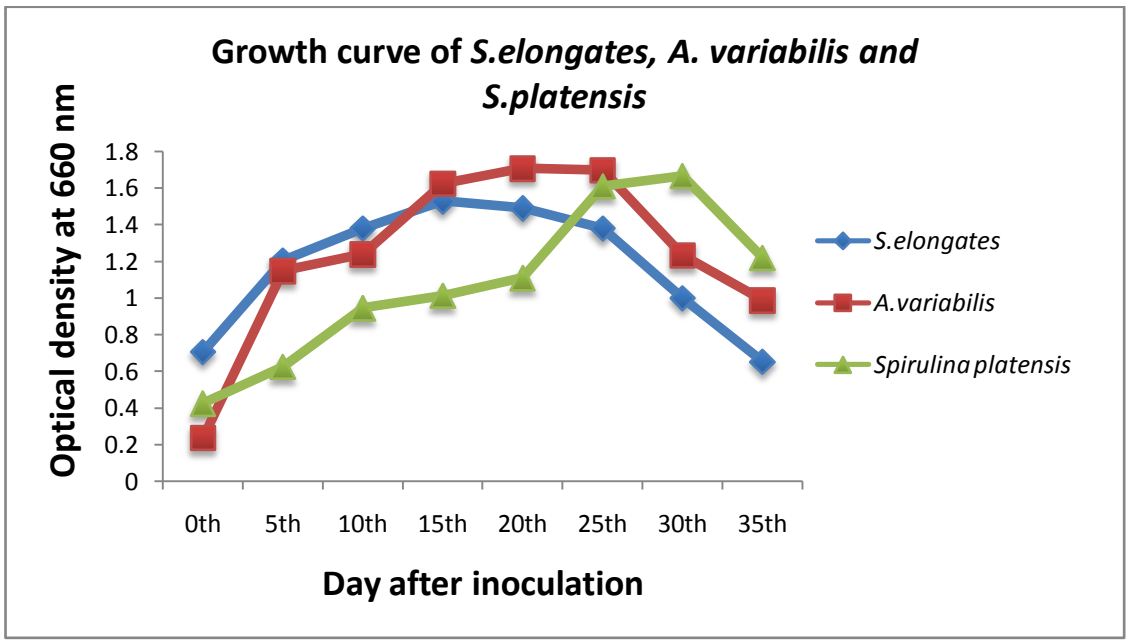

Graph 1- Growth curve of Microalgae

Table 1: Antibacterial activity of *Anabaena variabilis*

Extracts	E.coli	Pseudomonas sp	Enterococcus sp	Klebsiellasp
Methanol (60%)	-	22±1.8mm	19±0.8mm	32±2.6mm
Methanol (80%)	32±2.8mm	-	-	21±1.9mm
Ethanol	33±2.4mm	18±1.2mm	29±1.9mm	-
Butanol	26±1.9mm	22±2.1mm	30±2.7mm	25±1.8mm
Control	34±2.4mm	31±1.8mm	33±2.8mm	30±2.6mm

The aim of thestudy reported here is to investigate the nutraceutical potential of three freshwater microalgae as anantibacterial agent against the bacteria of clinical significance.

MATERIALS & METHODS

MicroalgalCultures

Microalgal strains were isolated from anearby pond and identified as *A. variabilis, S. elongates* and*S.platensis*as pertaxonomicclassification. The purified cultures were grown in BG11 medium and kept at optimum conditions.

Bacterial Cultures

Four pathogenic strains namely *E.coli, Enterococcus sp, Pseudomonas sp,* and *Klebsiella*sp,were isolated and cultivated. These isolated strains were cultivated on nutrient agar media and were incubated at 37^0 C for 24 hours.

Growth Estimation of Microalgal Cultures

Cultures were harvested on16^{th}day, and optical density was observed in every 5^{th} day at O.D. (660nm) of the incubation period.

Estimation of Phenolic content

Estimation of phenolic content was done by FolinCiocalteu reagent[13]. Dilute extract of microalgae was mixed with FolinCiocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3(4ml,1M). The mixtures were allowed to stand for 15 minutes, and the total phenols were determined by colorimetry at 765nm.

Estimation of Polysaccharides

The polysaccharide content was determined using Phenol-sulfuric –acid method[4]. The concentration of polysaccharides was estimated by harvesting cells and extracting with water (0.5 ml).The supernatant was discarded,and 4M NaOH was added. Initially, it was washed with 100% ethanol and later 90% ethanol was added to the supernatant followed bycentrifugation. Later on,4M NaOHwas added to the precipitate and kept for 1 hour.10% TCA was added to the supernatant and kept overnight.

Table 2: Antibacterial activity of *Synechococcuselongates*

Extracts	E.coli	Enterococcus sp.	Pseudomonas sp.	Klebsiella sp.
Ethanol	15±0.21mm	25±1.5mm	11±0.7mm	10±0.6mm
Methanol (60%)	23±0.9mm	20±1.8mm	8±0.7mm	-
Methanol (80%)	26±1.2mm	21±1.9mm	-	-
Methanol (100%)	30±2.1mm	29±2.1mm	-	20±1.1mm
Water	18±1.3mm	17±0.8mm	-	-
Acetone	22±1.3mm	-	23±1.9mm	27±1.2mm
Acetic acid	28±1.9mm	16±0.9mm	-	22±1.9mm
Butanol	30±2.5mm	24±1.8mm	23±1.9mm	-
Met:Acetone (1:1)	9±0.8mm	-	8±0.4mm	6±0.4mm
But:methanol (1:1)	12±0.9mm	10±0.8mm	7±0.4mm	-
Ethanol:Acetic acid (1:1)	14±0.6mm	9±0.5mm	-	-
Control	32±2.5mm	30±1.8mm	28±1.5mm	29±1.9mm

Table 3: Antibacterial activity of *Spirulinaplatensis*

Extracts	E.coli	Pseudomonas sp	Enterococcus sp	Klebsiellasp
Methanol (60%)	25±1.8mm	-	32±2.8mm	19±0.8mm
Methanol (80%)	28±2.1mm	26±1.8mm	27±1.2mm	-
Ethanol	-	20±1.2mm	-	25±1.8mm
Butanol	29±1.8mm	30±2.1mm	26±2.1mm	-
Control	33±2.4mm	34±1.9mm	35±2.8mm	30±2.5mm

-=No inhibitory effect; width 1 to 3mm = weak activity; width 8 to 15mm = moderate activity; width > 15mm = strong activity.

Table 4: Phenol and Polysaccharide content in Cyanobacterial extracts

Microalgae	Phenol	Polysaccharide
Anabaena variabilis	25.12± 0.28 mg/g	80± 1.9 µg
Spirulinaplatensis	33.4± 0.83 mg/g	150± 2.5 µg
Synechococcuselongates	25.35± 1.2 mg/g	120± 3.8 µg

Estimation of Antibacterial activity

The antibacterial activity of microalgal extracts was evaluated by agar plate diffusion test and disc method.

AGAR PLATE METHOD (cup plate method)

In this technique wells are made in the agar plates by using a screw cork of approximately 10mm diameter.The wells were then filled with the microalgal extracts using a micropipette.The Petri plates were then covered, sealed and incubated at a temperature of 26^{0}C for a period of 48-72 hours.The plates were then analyzed for their antimicrobial activity on the basis of formation of azone of inhibition.

DISC METHOD

In this technique, small discs were cut out from WATTMAN FILTER PAPER having a diameter (8mm).These discs were sterilized and were soaked in microalgal extracts for around twenty minutes and were allowed to dry.These discs were then placed in the Petri plates and were incubated at a room temperature of 26^{0}C for 48-72 hours in anincubator.

RESULTS & DISCUSSION

Many microalgae produce compounds with potent biological activities. A number of highly potent microalgal natural products have been uncovered as potential lead compounds for further drug development. In this research, microalgae were collected and cultured in BG11 medium. Spectrophotometrical analysis of the microalgal strains at 660nm by culturing them in BG-11 media depicted the growth. It was observed that *Spirulinaplatensis* showed maximum growth after the25th day. Whereas *A.variabilis* attained its maximum growth after the20th day and in

S.elongates it was after the15th day, indicating the fast growth of *S. elongates*(Fig. 1).

Based on their growth characteristics, three species were selected for the production of antimicrobial agents against pathogenic organisms like *E.coli, Pseudomonas sp., Enterococcus sp., Klebsiella sp.*Experimental results show that microalgal extracts are capable of inhibiting the growth of pathogenic bacterial strains. Methanol (60%) extract of *Anabaena variabilis*gave 30mm zone of inhibition against *E.coli*(Table 1),and methanol extract of *Synechococcus elongates* gave the highest antimicrobial activity against *Enterococcus* (32mm inhibition zone) and *Pseudomonas sp.* (26mm inhibition zone)as shown in Table 2. *S.platensis* shows highest antimicrobial

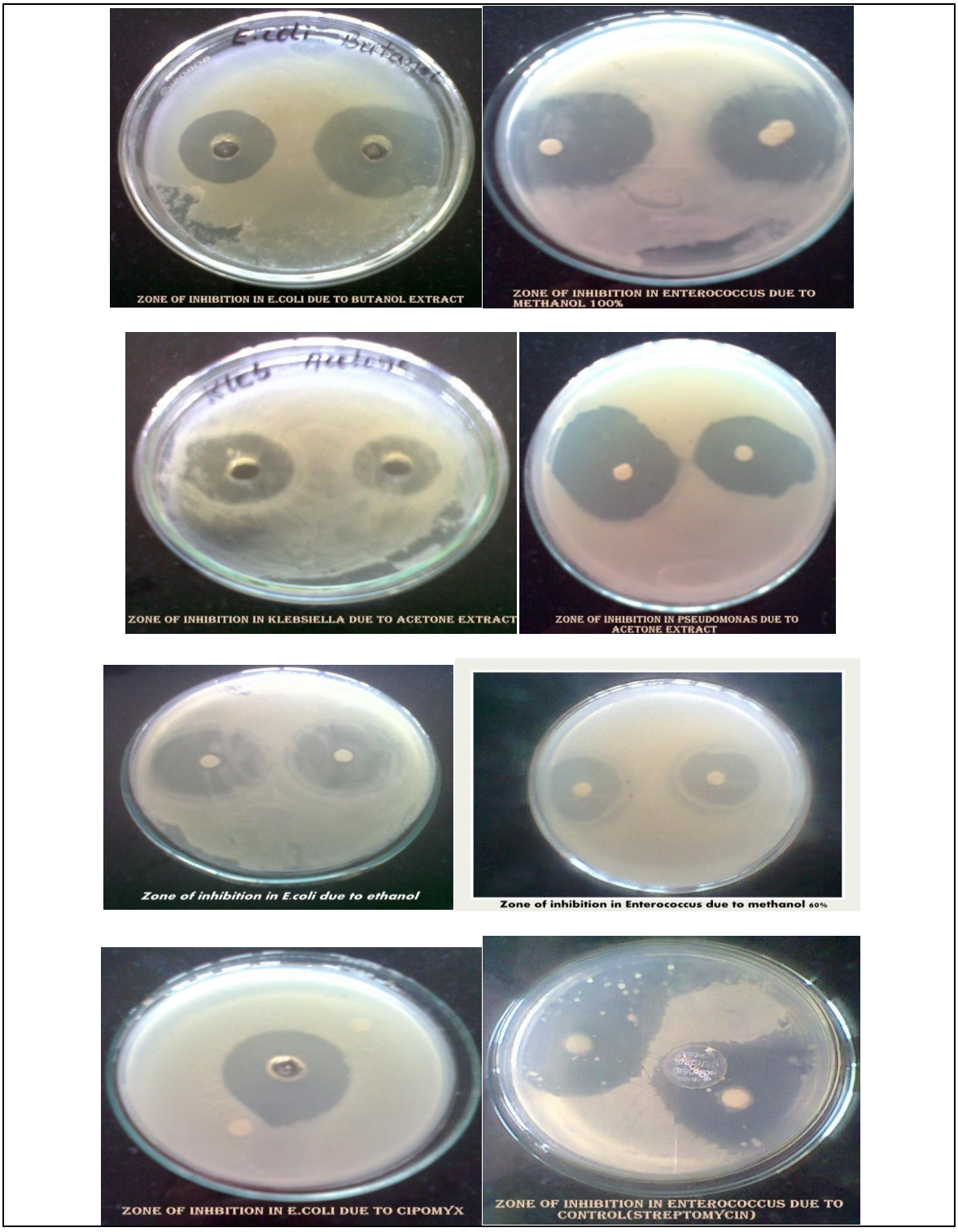

Figure 1: Antibacterial activity of Microalgal Extracts.

activity (Table 3) against *E.coli*with Methanol (100%). As per the formation of zones, ethanol (100%) extract, butanol, water, acetone and methanol (100%) extract gave a high visibility of zone of inhibition as compared to remaining ones (Figure 1).

As microalgae are very resistant to extreme environmental conditions, they are assuming increasing importance in frontier areas of biotechnology. Microalgae produce potent toxins, but they also produce useful bioactive compounds, including substances with antitumor, antiviral, anticancer, antibiotic and antifungal activity, UV protectants and specific inhibitors of enzymes[20]. There are reports on antimicrobial effects from microalgal aqueous and organic solvent extractand have been visualized in bioassays using selected microorganisms. In the investigations, microalgae have demonstrated the antimicrobial effects of many strains like*Nostoc, Anabaena, Synechococcus, Oscillatoria*against a variety of microbes.Kim[10]showed that 50% of the microalgae genera existed in heterocytous filament form. A total of 100 such samples were taken against 10 different strains of fungi and proved to have fungicidal activity. The unusual metabolites produced by the microalgae have been visualized as either or as products of pharmaceutical importance.Vardi[21]reported the allelopathic relationship between microcystin-producing *Microcystis sp.* and the dinoflagellate, *Peridinium gatunense* in the mesotrophic Sea of Galilee. The compounds responsible for the allelopathic response can be attributed to microcystins.The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes.

Phenol and Polysaccharide concentration was observed maximum in*S.platensis* as compared to *A. variabilis* and *S.elongates*. The phenol and polysaccharide content in *S.platensis* was 33.4 mg/g and 150µg respectively whereasin *S.elongates* it was 25.35 mg/g and 120µg (Table 4). It was very low in *A.variabilis* that is 25.12 mg/g and 80µg. Microalgal production of extracellular polymers, mainly EPS is well documented (De Phillips *et al.,* 1998). Exopolysaccharides (EPS) have been reported to play a significant role in providing protection to the cell and also act as antimicrobial agents.

Blue greenmicro algae (Microalgae) can act as a source of novel, biologically active compounds such as phycobilins, phenols, (antioxidants) terpenoids, steroids and polysaccharides. The occurrence of phenolic compounds in microalgae is less documented than that in higher plants. Algal phenolic compounds were reported to be potential antioxidants to combat free radicals, which are harmful to our body and food systems. Several epidemiological studies revealed that phenolic compounds present in thediet are helpful in treating coronary heart disease and osteoporosis stroke and other degenerative diseases. Furthermore, phenols have been reported to exhibit pharmacological properties such as ananticarcinogenic, antiviral, antimicrobial, anti-inflammatory or anti-tumor.

De Phillips[6] has reported the production of extracellular polymers, mainly EPS in microalgae. Polysaccharides play a diverse role in nature and get modified under stress condition[16]. Caiola[5]have reported the important role of Exopolysaccharides (EPS) by actings as protecting layer in the cell boundary. They also play a role in soil aggregation because they possess gluing properties[15]. There have been reports on the presence of functional groups on EPS, contributing to the binding of heavy metals[8, 19].

Microalgae are also used in aquaculture, wastewater treatment, food, fertilizers, and production of secondary metabolites including exopolysaccharides, vitamins, toxins, enzymes and pharmaceuticals. Future research should focus on isolating new microalgalstrains capable of yielding high-value products and methods to enhance the productivity of these products through genetic engineering.Optimization of incubation conditions and fermenter designs in order to increase productivity are essential for the large-scale industrial production of themicroalgal products.

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