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Article in International Journal of Bio-Science and Bio-Technology - February 2015

DOI: 10.14257/ijbsbt.2015.7.1.19

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The Uses of *Chlorella Vulgaris* as Antimicrobial Agent and as a Diet: the Presence of Bio-active Compounds which caters the Vitamins, Minerals in General

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Abstract

*The extracted and dried green micro algae by using solvents Acetone, Ethanol and Chloroform assay is tested to know its efficiency against four bacterial strains under the cultured conditions by adopting the standard agar disc diffusion technique. These agar plates are incubated at 37°C for 24 hour duration with four test bacterial strains in 10ml each of Acetone, Ethanol and Chloroform with extracted green micro algae. These extracts exhibit optimal effects on inhibitory efficiency. The observed highest inhibition zone is 13mm in *Chlorella Vulgaris* extracted with Ethanol against *klebsilla sp.*. The performed preliminary phytochemical analysis of this dried algal sample as per the Harborne method reveals that this assay withhold some of the valuable bioactive compounds. It is flavanoids, tannin, phenolic compounds, terpenes, cardiac glycosides, saponins and carbohydrates. The substantial evidence of the presence of these seven bioactive compounds revealed that *Chlorella vulgaris* is having major role as a useful precursor to obtain various bioactive compounds. This algal species exhibits its maximum inhibition property with *Klebsilla sp.* This enables to discover some of new derivatives with more investigation about its feasibility of finding new drugs by the application of procedures adopted in drug discovery and designing. The drugs derived from these algae species will find some specific application to curtail the growth of the bacteria which results the control of Vector infections without any side effects in a more specific manner.*

Keywords: *Chlorella vulgaris*, ethanol, acetone, chloroform, antimicrobial activity, phytochemical activity

1. Introduction

Algae are a rich resource for pharmaceuticals on the basis of presence of these biologically active primary and secondary metabolic compounds [1]. The secondary metabolites present in the Algae is in favor of organizing a numerous biological defense systems and they act against predation, herbivores and compete for a space [2].

The utility research studies based on Algal Bio Mass produced [12] was pharmaceutically important with respect to a first investigation reporting its antibiotic activities. The extensive research reports indicate that it has already been utilized as a traditional medicine, the highlights are of bacteria-static, bactericidal, antifungal, antiviral and antitumor activities. The abilities are due to the presence of fatty acids in these micro algae organisms, which develops antibacterial activity [4]. The anti bacterial activities are exhibited by the volatile extracts of *Spirulina platensis*, reports that it contains a "phytochemical compound" which is found in this plants are undergoing pharmacological interactions with the nutrients and dietary fibers which enables the humans in protecting them self against the diseases and

there are some interesting reports that their ability in slowing down the aging process. The studies which lead to the discovery of the medicinal plants with lot of medicinal values are referred as Phytochemical screening. It is wondered, without the understanding the basic knowledge about the cellular actions or metabolic reaction mechanisms, basic functionalities of these phytochemicals (Algae), and from the period of Hippocrates they are administrated as effective drugs. Few reports in the literature states that willow tree leaves were prescribed to abate fever and Salicin was prescribed for anti-inflammatory and pain relieving medicine. This has been extracted from the bark of white willow tree and it is used to synthesis staple and it is proven that it is the counter drug for aspirin [3]. These remarkable aspects encourage to investigate this phytochemical and its derivatives and validate its effectiveness as antibacterial character is present with *Chlorella vulgaris* a micro organism. As per the reports available in literature, the anti microbial substances are unsaturated lactones, cyanogenic glycosides, sulphur containing compounds, phenols, phenolic glycosides, saponins and phytoalexins [5]. The therapeutic use of algae has reported from 1950s and some of the systematic drug discoveries of algae and biologically active substances of antibiotics were reported. The positive and negative gram bacterial tests were carried out by the aqueous and solvent extracts of this algae [6] due to the presence of fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols [7] in this system it possesses antimicrobial character. Various results in-vitro antifungal activities of these extracts of green micro algae, diatoms and dinoflagellates have already been reported [8].

Very important major living and renewable resources Microalgae constitutes with carbon sequestration abilities contains more than sixty trace elements which includes minerals, iodine, bromine, proteins with bioactive substances. The Chemically Unique compounds with various biological activities of this fresh water algae micro organism have been isolated and few are under investigation, fewer are utilized to discover the drugs [9] In this efforts few bioactive, pharmacological substances have already been isolated [14]. The antimicrobial activities of these plant-based algae species have negligible side effects and have valuable therapeutic potential rather than the synthetic antimicrobials.

2. Materials and Methods

The sample microalgae species *Chlorella vulgaris* sp was collected from Erode and Coimbatore surface lake water bodies. The algal sample was cleaned and necrotic parts were removed. Then the sample was rinsed with sterile water to remove any associated debris. The pure culture of the sample was incubated in water medium. Pure cultures prior to the stationary phase of growth (10 days) were harvested and collected by centrifuging at 10,000 rpm for 3 min. The collected micro algal pellets were dried under shade and made into a coarse powder with mechanical grinder for further use. The algal dried powders (20gm) were successively extracted using serial Exhaustive Extraction Method with Chloroform, Acetone, Ethanol solvents. The dry powders of each extract were resuspended in the respective organic solvents at a concentration of 100 mg/ml for further phytochemical screening and antimicrobial activity.

Bacterial Strain Used

The following bacterial strains were used for this present study

- *Escherichia coli*.
- *Bacillus sp.*
- *Klebsiella sp.*

- *Pseudomonas sp.*

Preparation of Extracts

Crude method

The sample of 1 gm was transferred into clean screw cap bottle of 10 ml capacity. Solvent of 10ml is added separately. The extracts are separated and collected for storing at 4°C and utilised for the remaining analysis is to be carried out.

Determination of Antimicrobial Activity

The test organisms subject for determination of antimicrobial activity are swabbed on the air dried nutrient agar plates by using sterile cotton swabs. The sterilised discs are loaded with varying concentration of *Chlorella vulgaris*. The flame sterile disc loaded with 150 µl of sample extracts are placed with the surface of nutrient agar plates and swabbed bacterial cultures. The discs loaded with proper solvents as prescribed and controls are incorporated in order to maintain the culture at the incubation temperature of 37°C atleast for 24 hours. The antimicrobial activity is to be determined as per the normal procedure by measuring the zone of inhibition around the discs the diameter of the inhibition zone is measured exactly with the help of physical scales in millimeters.

Preliminary Phytochemical Screening

A systematic and complete study of crude drugs includes with complete investigation of both primary and secondary metabolites obtained from derived plant metabolisms. Various qualitative test as prescribed by standard procedure are carried out and various profiles are established for various extracts according to their chemical composition in nature^{11,13&15}. The extracts obtained are subjected to chemical tests as normal routine procedure in identification of various phytoconstituents which are followed as protocol as per the methods adopted by Harborne.

3. Results and Discussion

Antimicrobial Activity of *Chlorella Vulgaris*:

The antimicrobial activity of *Chlorella vulgaris* was carried out to determine inhibition against some of the common pathogen like *E.coli*, *Klebsilla sp.*, *Bacillus sp.* and *Pseudomonas sp.* which are pathogenic to humans and considered to cause water borne and vector diseases and is causing drastic impacts with huge impacts [10, 12].

The effect of Acetone, Ethanol, Chloroform extracted micro algae is tested for its inhibition efficiency in which, the Ethanol extracted sample showed the maximum inhibitory activity against *klebsilla sp.* (17mm). where as the *E.coli* (15mm), *Bacillus sp.* (13mm) and *Pseudomonas* (12mm), with exhibition of minimum inhibitory zone. The Acetone extract of the sample showed a maximum inhibitory activity against *Bacillus sp.* (15mm). Where the as *Pseudomonas* (13mm) and *klebsilla sp.* (12mm) and *E.coli* (08) show a minimum inhibitory zone. The chloroform extract of the sample showed the maximum inhibitory activity against *Bacillus sp.* (14mm). with *E.coli* (12mm), *Pseudomonas* (11mm) and *klebsilla sp.* (10mm), a minimum inhibitory zone. As per the available details of Tetracycline the maximum inhibitory the activity against *klebsilla sp.* (18mm) against *Bacillus sp.* (16mm), against *Pseudomonas* (15mm), and against *E.coli* (13mm) with minimum inhibitory zone (Table 1).

A very sensitive and an important phytochemical screening of the *Chlorella vulgaris* fraction are performed. It reveals the level of Flavonoids, Tannin, Phenolic compounds,

Terpenoids, Cardiac, glycosides, Saponins and Carbohydrates with positive results with the help of ethanol extract (Table 1).

Table 1. Antimicrobial Activity of *Chlorella Vulgaris* Extracted with Acetone, Ethanol and Chloroform. (Values are in millimeter)

S.No	Organisms	Zone of inhibition in Diameter		
		Acetone	Ethanol	Chloroform
1.	<i>E.coli</i>	8	15	12
2.	<i>Klebsilla sp.</i>	12	17	10
3.	<i>Bacillus sp.</i>	15	13	14
4.	<i>Pseudomonas</i>	13	12	11
Phytochemical screening of <i>Chlorella vulgaris</i>				
5.	Flavonoids	Positive		
6.	Tannin	Positive		
7.	Phenolic compounds	Positive		
8.	Terpenoids	Positive		
9.	Cardiac glycosides	Positive		
10.	Saponins	Positive		
11.	Carbohydrates	Positive		

4. Conclusions

As per the growth rate assessments and carbon sequestration capabilities and in testing its microbial inhibition character, it is concluded that this algae is having its ability of sequestrating carbon level in atmosphere and nitrate, phosphate, sulphate loads of waters, and it proves its worthiness in extracting bio-fuel.

The present research enables to know the ability of this micro-algae species to promote as an antimicrobial agent. This inhibition efficiency is mainly due to the presence of stable biologically active compounds. The bio active compounds presents in this system will have some specific chemical structures which enables to functions it as a beneficial substance and creates the effects of inhibition of harmful pathogens. The negligible side effect of this micro-algae species draws some of special interest to promote it in the form of a new medicinal food supplement. This micro algae will helps the living organisms to increase immunity even to humans, in order to confirm the results proper permissions are needed to carry out research trails with immuno modulated studies.

The reason behind it to be used as food supplement with anti microbial character is due to the presence of Flavonoids which are possessing vitamin like properties. The earlier research finding proposed that a short lived vitamin P is present in these type of compounds which contains flavonoids and the plants containing flavonoids are used by the folks to treat the disorders with respect to thyroid and other hormones deficiencies. Some of the recent investigation also identify that they possess antioxidant and antimicrobial, anti mutagenic, anti carcinogenic properties. The informations on tannins attributed to the wound-healing and anti-inflammatory properties, the Phenolic compounds are considered to be an essential part of

the human dietary methods since it contains antioxidant properties, Terpenes are found its use as an anti-spasmodic, carminative and stimulant, with an effective antiseptic property. It is useful in treating cold and fever, headache and body aches due to its ability to decrease inflammation. The anti-spasmodic (sedative) nature helps in relieving the pains in the bowel and its muscles. The therapeutic application of Cardiac glycosides finds its therapeutic application due to positive isotropic effects which enables it to treat heart-related pathologies. More recently, considerable *in vitro*, *in vivo* and epidemiological data support the roles for such drugs for the treatment of several diseases [6]. Saponins are having some strong antitumor effects and found beneficial in targeting and inhibiting the tumor angiogenesis by suppressing its inducer in the endothelial cells in blood vessels and preventing and adhering, invasion of metastasis of tumor cells. Carbohydrate are used in the field of skin tissue engineering, wound healing modulation and a food material. All these essential bio active compounds are present in the *Chlorella vulgaris* sample and it is to be refined and could be utilised as food supplement as well as medicine. This study enables to pave a way to discover a new drug and field trials are to be needed with proper licencing procedures.

Acknowledgements

The authors wish to express the gratitude for the support and financial help given by Department of Science and Technology, New Delhi for their support under Net Work Programme of Bio-Algae Carbon Sequestration research under the title “Carbon Sequestration and cultivating algae for green bio-fuel Production” and to PadmaBhusan, Dr. Arutselvar N. Mahalingam, Chairman Kumaraguru College of Technology.

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