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Phytochemistry and anti-microbial activity of *Chara*

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Abstract

This study was carried out to investigate the phytochemical and biological screening of algae *Chara* [Green algae]. The *Chara* material is collected from the pond of Chitradurga fort. Phytochemical screening was assured by chemical analysis. The methanol extract revealed the presence of D-Norandrostane-16-carboxylate, 17B-hydroxyandrost-4-en-3-one. The extract of *Chara* was tested for the presence of primary and secondary metabolites and antibacterial activity.

Keywords: *Chara*, GCMS, TLC, NMR phytochemical, antibacterial, Gram negative bacteria, pathogenic, bacteria.

Introduction

Approximately 7310 species of algae present in fresh water. Among these most of them are blue green algae and Green algae. Nature has provided many things for human kind over years including for the tool and therapeutic intervention [Nakanishi]. To refer herbs, herbal cosmetics dietary supplements or alternative medicine.

Algae are autotrophs, a group of chlorophyll containing thalloid plant, synthesize their food material. Algae, fungi and lichens are grouped together under the division of thallophyta.

Chara is a genus of green algae belongs to Characeae. It is multicellular and superficially resemble land plants because of stem like and leaf like structures. They are found in fresh water particularly in lime stone areas throughout the northern temperature zone, where they grow submerged or attached to the muddy bottom. They prefer less oxygenated and hard water and are not found in where mosquito larvae are present. They are covered with calcium carbonate crystals.

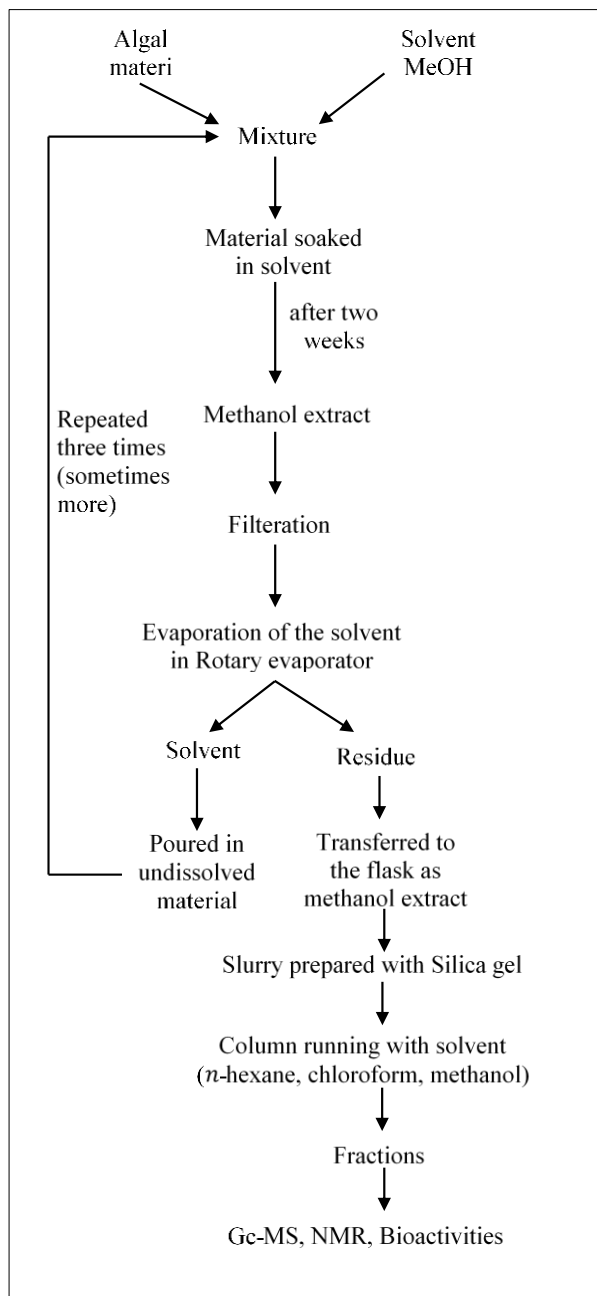
Materials and methods**Collection and extraction of algae**

Chara was collected from the pond of Chitradurga fort located in Karnataka, India. The sample is cleaned and shade dried. The completely dried algae was used for various phytochemical analysis like test for carbohydrates, proteins, aminoacids, alkaloids, steroids, lipids, flavonoids, and oils.

Bacterial activity

Wells were placed in the medium with the help of sterile metallic borers with their centers at least 24mm apart. Bacterial inoculation about 2 to 8 hours old containing approximately 10^6 - 10^8 colony forming units (cfu)/ml were spread on the surface of nutrient agar with the help of sterile cotton swabs. Which were rotated firmly against the upper inside well of the express fluid. The entire agar surface of the plate was streaked three times with the swab while turning the plate at 60° C between each streaking. Antibacterial drug served as negative and positive control respectively, were incubated immediately at 37°C for 14 to 19 hours or more activity was determined by measuring the diameter of inhibition zones showing complete inhibition (in mm) and later growth inhibition was calculated with reference to a positive control by referring Ghazala and Shameel *et al.* (2005) [16].

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Extraction of methanol extract

Extraction and identification of steroids and fatty acids

The dried algal material was soaked in methanol for 2 weeks at room temperature. The mixture was filtered to remove an undissolved portion and the filtrate was evaporated in a rotary evaporator under reduced pressure. This was repeated three times and eluted in a dark coloured thick and syrupy residue.

The residue was dissolved in a small quantity of methanol and poured on silica gel (about 10g) to prepare slurry. Its mesh size 60-120 unit-2 was selected before that the sample was allowed for Thin layer chromatographic techniques (TLC) for its good separation according to the different solvent system [Chloroform(10): Chloroform: methanol (9:1) N-hexane: Chloroform (10:1). From this chloroform is used for the extraction column was taken and 60 to 120mesh was selected and was pre-sterilized in an oven at 80°C for 24 hours. Similarly, all the glass wares used were also pre heated and used.

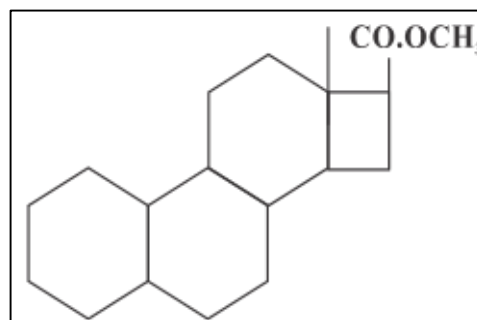
A medium sized column was packed with fresh silica gel –G using chloroform as a solvent which was allowed to run for one day. After packing the column, the slurry was loaded and

a layer of silica gel was placed above it which prevent the disturbance of slurry. The eluted fraction was collected in a separate conical flask according to their solvent system. Totally three different fractions were obtained. After drying this fraction and TLC banding pattern a single fraction was selected for further analysis like Infrared radiation(IR) Gas chromatography mass spectroscopy (GC-MS) and Nuclear magnetic resonance(NMR).Based on these analytical data and referring the Ghazala and Shameel *et al.* (2005) [16], the fatty acid D-norandrostane-16-carboxylic and a steroid -17- β -hydroxyandrost-4-en-3-one is predicted.

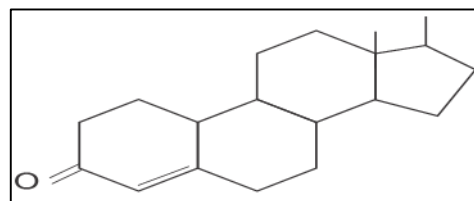
Qualitative determination of phytochemicals of Chara

Phytochemicals	Inference
Carbohydrates	+
Polysaccharides	-
Protein	-
Alkaloid	+
Steroids	+
Flavonoids	-
Lipids	+
Saponin	+
Tannin	+
Oils	+

GCMS/NMR Analysis



D-Norandrostane-16-Carboxylate

17- β -hydroxyandrost-4en-3-one.

Result and discussion

Phytochemical analysis

The preliminary investigation of phytochemical of crude extract of *Chara* revealed the presence of following primary and secondary metabolite.

Tannin: Tannin possess general antimicrobial and antioxidant (Rievere *et al.*, 2009) The present study confirms the presence of tannin in methanol extract of *Chara*, can be used as antimicrobial agents Tannin have been found to have antiviral, antimicrobial, Antiparasitic effect anti inflamantory, a ntulcer and antioxidant property for possible therapeutic application (Kolodziej and Kiderlen, (2005 and lavanya rayapu, fazina makkar 2017) [20] and tannin containing remedies are in use as antihelminthic and antioxidant properties shows Vinoth kumar, R Murugesan S and Bhuvaneshwari, S. 2015).

Flavonoids

These are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro* flavonoids The major groups of phenolic compound reported for their antimicrobial and antiviral activity (Lavanya Rayapu, Fazina Makkar, Kajal Chakraborty, Lokanatha Valluru 2017) [20] and (Azhagu Raj. R, Mala K and A. prakasam 2015) [4] that works reveals that flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have found to be antimicrobial substances against many pathogens.

Saponin: Saponin are specific physical, chemical and biological activities, they make useful as medicine it is used in hyper cholesterolemia, hyperglycemia antioxidant, anticancer, anti-inflammatory and weight loss etc.

The plant extract were also revealed the presence of saponin which are known to produce of saponin inhibitory effect on inflammation and saponin have the property of precipating and coagulating RBC etc. The present study confirms the presence of saponin in methanol extract of *Chara*. (Ranjani Devi M, Premraj Loganathan, Arputharaj P and JMV Kalaiarasai 2018)

Steroids: The present study confirms the presence of steroid in the extract of *Chara*. They have been reported to have antibacterial properties (Raquel 2007) and they are very important compounds especially due to their relationships with compounds such as sex hormones (Okwu 2001). It suggests that the extract of *Chara* can be used as antimicrobial agents.

Alkaloids: The present study confirms the alkaloid and mainly primary and secondary metabolite. They are mainly helpful to insecticide property and toxicity property and cardiovascular problems solved. (Dr Mazen A EL Sakke (2010)

Fatty acid: The present study confirms the fatty acids they are mainly helpful to the anti inflamantory, antiviral, antiplatelet, cardiotionic, wound healing (Sohail Ahamad, Sabirahmad 2008

B) Antimicrobial activity analysis

The purified extract was further analysed for its antimicrobial activity against *Xanthomonas compestris*, *pseudomonas syringue* and *Agrobacterium tumefaciens*. Using well diffusion method. The following tables displays the inhibition zone obtained by *Chara* extract and standard antibiotic Ciproflaxin.

Xanthomonas campestris [MTCC-3386]

Organism	Inhibition zone
Ciprofloxacin	18
<i>Chara</i> extract	10
DMSO	10

Pseudomonas syringae [MTCC-1604]

Ciprofloxacin	15
<i>Chara</i> extract	12
DMSO	10

Agrobacterium tumefaciens [MTCC-431]

Ciproflaxin	20
<i>Chara</i> extract	10
DMSO	10

The rate of antibacterial activities of the *Chara* extract were calculated by considering the length zone of inhibition. The inhibition zone was measured in millimetre (mm).

The *Chara* extract showed maximum inhibition zone in *Pseudomonas syringae* (12mm) where as in *xanthomonas campestris* and *Agrobacterium tumefaciens* the inhibition zone was 10mm

Antibacterial activity

The purified extract was further analysed for its antimicrobial activity against *Xanthomonas campestris*, *Pseudomonas syringue*, and *Agrobacterium tumefaciens*. Well diffusion method revealed that the *Chara* extract showed maximum inhibition zone in *Pseudomonas syringue* than *Agrobacterium tumefaciens* and, *Xanthomonas compestris*. Literature reveal that activity of green algae has verified on various bacterial strains. In the present work *Chlorosarcinopsis* sp, acholrophycean unicellular alga when extracted with different solvents, exhibited various range of activity against all bacterial strains tested. Antibacterial against both Gram negative and Gram positive bacteria compared to the benzene and methanol extract and its contains many alkaloids. (Dhanalakshmi M Angayarkanni, J 2013) [14] (Table-1,2and 3) Green algae members showed higher antimicrobial activity than red algae (Periasamy mansuya, Pandurangan aruna Sekaran Sridhar, Jebamalmal suresh kumar 2010)

The antimicrobial activity in Gram negative bacteria *Pseudomonas* species shows maximum inhibition in marine algae and fresh water algae (Nishanthi Rajendran, Karpanai selvan and *et al.* 2014)

Methanol have higher antibacterial activity than that of extracts obtained organic solvents (Fables *et al.* 1995)

Recent studies shows that algae have more antimicrobial activity (Vinay Kuma, Prem Saran Tirumalai, Abha Singh, Ak Bhatnagar, JN Shrivastava)

Conclusion

During this investigation, *Chara* extract were tested for the presence of primary and secondary metabolite. The tests showed the presence of carbohydrates, proteins, alkaloids, steroids, flavonoids and fatty acids, like D-norandrostanae-16-carboxylic acid and steroid like 17B-hydroxyrost-4-en-3-one in *Chara* and showed the presence of antimicrobial activity. According to our observation, it was observed that the highest percentage of antimicrobial activity in *Pseudomonas syringae* than *Xanthomonas compestris* and *Agrobacterium tumefaciens*. This whole research has been carried out to know the medicinal properties and importance.

References

1. Anthoni U, Nielsen PH, Smith-Hansen, Wium-Andersen S, C Christssophersen. Charannin, a quaternary ammonium ion antibiotic from the green alga *Chara globularis*. J org. chem. 1987; 52:694-695.
2. Anthoni VC, Christophorsen J, Omedsen S, Wium Anderson, Jacobsen. Biologically active sulphur compounds from the green algae *Chara* phytochemistry, 1980.
3. Arnott HJ, FG Es Pautard. Calcification in plants. In biological classification: cellular and molecular aspects

- Appleton century Craft Educational Division, New York. 1970, 375-446.
4. Azhagu raj, R Mala, A Prakasam. Phytochemical analysis of marine macro alga *Caulerpa racemosa* global biosciences, 2015, 3055-3067.
 5. Azza M, Abd El-Aty, Amal A Mohamed, Farag A Samahan. *In vitro* antioxidant and antibacterial activities of two fresh water *Cyanobacteria* species, *oscillatoria* agardhii and *Anabena* sphaerica national research center cairo Egypt 2014, 069-075.
 6. Abu-Ghannam N, Gupta S. An assessment of the antioxidant and anti-microbial activity of six species of edible Irish seaweeds International food research journal. 2010; 17:205-220.
 7. Benjannet J, Chaari A backhouf. Structure antibacterial activity relationship of secondary metabolite from *Ajugapseudonia* Rb leaves. Nat prod Res. 2006; 20:299-304.
 8. Balusabarathnum, George Seghalkiran, Joseph selvin, Chippushakir, Premnath. Bioactivity of the red algae *Aspargopsis* taxi formis collected from the south western coast of India Brazilian Journal Of Oceanography. 2010; 58(2)93-100,200
 9. Barreto MJ, JM Meyar. Isolation and antimicrobial activity of a lanosol derivative from *Osmundaria serrate* and a visual exploration of its biofilm covering South African journal of botany. 2006; 72:521-528.
 10. Balakrishnanap Jjenifer, ES Sakkilingam. Algal documentation and phytochemical studies of red algae *Gracilaria* corticata of Manapad coast, Tamilnadu Journal of pharmacoscopy and phyto chemistry. 2013; 2(4):193-197.
 11. Chu W, A Radhakrishana. Research on bioactive molecules. Achievements and the way forward ICJSME. 2008; 2:521-524.
 12. Dalia F, Abd Elemegeed, Doaa A Ghareeb, Muhammad EL-Saadani. Phytochemical constituents and bio screening activities of green algae International journal of agricultural policy and research. 2014; 2(11):73-378.
 13. Dipankar Chaudhari, Nikhil baban Ghate, shampa Deb, Souravpanja. Assessment of the phytochemical constituents and antioxi activity of a bloom forming micro algae *Euglena tuba* Biological research 2014; 47:24.
 14. Dhanalakshmi M, Angayarkanni J. Phytochemical and antimicrobial activity of *Chlorosarcinopsis* species. International journal of scientific technology research ISSN, 2013, 2277-8616.
 15. Einokow I, Stefanow K, Alxeandrova M, Dimitrove konaklieva S, S Papov. Lipid composition of some Burgarian cladophora species. Bot Mur. 1996; 39:79-82.
 16. Ghazala B, M Shameel. Phytochemistry and bioactivity of some fresh water green algae from Pakistan. Pharma Bid. 2005; 43:358-369.
 17. Godwin TW. Sterols: In Stewart, W D P. ed. algal physiology and biochemistry London. Blackwell, 1974, 266-280.
 18. Jacobsen N, LEK Pederson. Synthesis and insectidal properties of derivative of propane-1, 3-dithiol analogy of the insecticidal derivatives of dithiolane and trithiane from the alga *Chara globularis* Theillier. Pestic sci. 1983; 14:90-97.
 19. Justella Wilfred, Johnson marimuthu @Antonisamy and Solomon jeeva. Antimicrobial activity of certain freshwater micro algae from Thamirabarani River, 2001.
 20. Lavanya Rayapu, Fazina Makkar, Kajal Chakraborty, Lokanatha Valluru. Phyto chemical evaluation and antimicrobial activity of *gracilaria opuntia*: An important anti diabetic red marine macroalgae. International Journal of current pharmaceutical research ISSN, 2017, 0975-7066.
 21. Mohammed Deyab, Taha Elkatony, Fatma ward Qualitative, and quantitative. Analysis of phytochemical studies on Brown Seaweed, *Dictyota dichotoma* IJEDR. 2016 4(2):2321-9939.
 22. Ranjani Devi M, Premraj Loganathan, Arputharaj P, Kalaiarasi. Pharmacognostical and phytochemical analysis of *Sargassum cinereum* (Turner) C. Agardh. Journal of pharmacognosy and phytochemistry 2018; 7(1):2233-2238.
 23. Vinoth Kumar R, Murugesan S, Bhuvanewari S. Phytochemical analysis of red alga *Champia parvula* collected from Mandapam coast of Tamil Nadu, India. International journal of advances in pharmaceuticals ISSN. 2015, 2320-4923.
 24. Vinay kumar, Prem Saran Tirumalai, Abha Singh, AK. Bhatanagar JN Shrivatsava. Natural Compounds from Algae and spirulina platensis and its Antimicrobial activity. Indo global journal of pharmaceutical sciences 2013; (3):212-223.