International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 264-269
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF FRESHWATER ALGAE *RHIZOCLONIUM HIEROGLYPHICUM*

B. Vijaya Rani[1*] and B. Vasantha Kumari[2]

[1]Research Scholar (Reg. No. 8077), Department of Botany, S.T. Hindu College, Nagercoil-629002, Tamil Nadu, India, affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, India.
[2]Principal and Head of the Department of Botany, Sree Ayyappa College for Women, Chunkankadai-629003, Tamil Nadu, India, affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, India.

***Corresponding Author Email: vijayaranirvg@gmail.com**

ABSTRACT

Algae are very important component of aquatic ecosystem, known for producing several biologically active compounds. In the present study, filamentous green algae, Rhizoclonium hieroglyphicum was collected from Marthandeswarar temple pond of Kanniyakumari district in SouthIndia. The Benzene, Methanol, Chloroform, petroleum ether and hexane extracts of Rhizoclonium hieroglyphicum were subjected to phytochemical screening. The result clearly revealed that it contains alkaloids, flavonoids, steroids, terpenoids, phenols, saponins, glycosides, oil and resin, and free from anthroquinone, tannin and phlobatannins. GC-MS analysis showed that 11 different compounds of varied nature were present in the benzene extract.

KEY WORDS

Rhizoclonium hieroglyphicum, secondary metabolites, extracts, phytochemical screening, GC-MS analysis.

INTRODUCTION

Ecologically, algae are the most widespread of the photosynthetic plants, constituting the bulk of carbon assimilation through microscopic cells (Ramaraj *et al*., 2014). The green algae are the most diverse group of algae with more than 7000 species growing in a variety of habitats (Mongillo,2000). The green algae include unicellular and colonial flagellates as well as various coccoid, filamentous forms, and macroscopic multicellular weeds. Algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antiviral, antioxidant, cytotoxic agents (Schaeffer and Krylov, 2000; Del Val *et al.,* 2001 Zbakh *et al.,* 2014) and the bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlobotannins, diterpenoids, sterols, quinones, lipids and glycerols (Kumar *et al*., 2012, Tang *et al*., 2012 and Kumbhar *et al*., 2014).

Rhizoclonium species were described by some taxonomists, such as: Kützing (1843), Blair (1983), Posada & Crandall (1998) etc. The filamentous algae *Rhizoclonium hieroglyphicum* is a common inhabits of freshwater locations. *R. hieroglyphicum* grow attached to other substrates by rhizoids. It produces little or no mucilaginous secretion and the salts tend to crystallize on the filaments of older specimens, gives it a rougher, grittier feel than other filamentous algae. It is also more readily colonized by epiphytic diatoms and other algae and provides a protected for aging environment for the smaller pond creatures such as protozoa, worms, small crustaceans and insect larvae (Fabrowska *et al.,* 2015 and Pochon. *et al.,* 2015; Zeinab A El-Swaify, 2017).

Phytochemical analysis of algae can help the manufacturers for identification and selection of raw materials for drug production. Despite all the efforts made to date, phytochemical screening of *R. hieroglyphicum* have not been extensively studied yet. In this context an attempt was taken to identify the phytochemical constituents of *R.hieroglyphicum* by phytochemical screening. The benzene extract of *R. hieroglyphicum* was also analysed by GC-MS to identify different constituents present in it.

MATERIALS AND METHODS

Algae Collection

The filamentous algae *Rhizoclonium heiroglyphicum* (Ag) Kuetz was collected from the Marthandeswarar temple pond at Eraniel, in Kanniyakumari District, Tamil Nadu, India in August 2016. The collected samples were identified using the manuals of Prescott (1978) and Krishnamoorthy (2000).

Preparation of Algal Extract

The collected fresh algal mats were thoroughly washed with distilled water to remove dirts, epiphytes and debris and the filaments were aseptically grown in containers with liquid Bold's Basal Medium (BBM) (Bischoff and Bold, 1963).The culture conditions were maintained at temperature 25^{o}C, light intensity; 25µmole/m2/sec and pH was 8.0 under (12h: 12h) light: dark period (Mubeen *et al.,* 2011). Aseptically grown filaments were dried for one week under shade and cut into small pieces and grind well, powdered with the help of a mixer grinder and stored in airtight bottles in refrigerator. About 20gm of powdered material was uniformly packed in filter paper and extracted with benzene, methanol, chloroform, petroleum ether and hexane in Soxhlet apparatus. The process of extraction was continued for 24 hours or till the solvent in the extractor became colourless. After that the extract was taken in a beaker kept on hot plate and heated at 30 – 40^{0} C till the solvent got evaporated. Dried extract was kept in refrigerator at 40 ^{0}C for future use.

Phytochemical Screening of *Rhizoclonium heiroglyphicum*

Preliminary phytochemical analysis was carried out for all the extracts of *Rhizoclonium heiroglyphicum* as per standard methods described by Brain and Turner 1975 and Evans 1996.

1. Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) Mayer's test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Mayer's reagent

Mercuric chloride (1.358g) is dissolved in 60ml of water and potassium iodide (5g) is dissolved in 10ml of water. The two solutions are mixed and made up to 100ml with water.

b) Wagner's test

Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Wagner's reagent

Iodine (1.2g) and potassium iodide (2g) is dissolved in 5ml of water and made up to 100ml with distilled water.

2. Detection of Flavonoids

a) Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) H_2SO_4 test

Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

3. Detection of Steroids

Liebermann- Burchard test

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

4. Detection of Terpenoids

Salkowski's test

About 0.2g of the extract of the whole sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish-brown coloration of the inner face indicated the presence of terpenoids.

5. Detection of Anthroquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and

heated. Formation of pink colour indicated the presence of anthraquinones.

6. Detection of Phenols

a) Ferric chloride test

Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicated the presence of phenol.

b) Lead acetate test

Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of phenol.

7. Detection of Saponins

Froth test

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) showed the presence of saponins.

8. Detection of Tannins

Ferric chloride test

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered, and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicated the presence of tannins.

9.Test for Glycosides: 10 ml of 50% H_2SO_4 was added to the 1 ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10 ml of Fehling's solution (5 ml of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

10.Test for phlobatannins: 10 ml of extract was boiled with 1% HCl in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins

11. Detection of Oils and Resins

Spot test

Test solution was applied on filter paper. It developed a transparent appearance on the filter paper. It indicated the presence of oils and resins.

GC-MS ANALYSIS

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°c. The source temperature was maintained at 180°c. GCMS was analysed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass OCPTVS-Demo SPL software.

RESULTS AND DISCUSSION

Microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to be produced by chemical synthesis (Kaushik and Chauhan, 2008). Most of those compounds are accumulated in the microalgal biomass; others are excreted during growth into the environment (Jaki *et al.*,2001). Inhibitory activities against growth of microorganisms and development of animal and plant cells are common indicators for screening antibacterial, antifungal, antiviral, cytotoxic and antitumor substances (Febles *et al.*, 1995).

The qualitative phytochemical analysis of benzene, methanol, chloroform, petroleum ether and hexane extracts of *Rhizoclonium heiroglyphicum* revealed that benzene extract had better activity than other extracts (Table 1). Oil and resin were present in all the extracts except chloroform. Flavonoids and phenols were present in benzene and hexane extracts while glycosides were present in chloroform and hexane extracts. Balch JF and Balch PA (2000) reported that most commonly known phytochemicals such as phenols, flavonoids and tannins with antioxidant property counteract the body's reactions to allergens, viruses and carcinogens and glycosides can be used in the treatment of congestive heart failure and cardiac arrhythmia.

Alkaloids and saponin were seen only in the benzene extract, likewise steroids were present only in chloroform extract. Terpenoids were present in benzene and petroleum ether. Anthroquinones, tannin

and phlobatannins were absent in all five extracts of *Rhizoclonium heiroglyphicum.* From the study, it was observed that the algae possess medicinally important phytochemicals such as flavonoids, alkaloids, terpenoids, phenols, saponin, glycosides and steroids. The results of GC-MS study indicated the presence of 11 different compounds (Fig 1). The compounds exhibited a wide range in their nature. Largest peak area (40.37%) was observed for the compound Toluene while the smallest peak (1.25) compound 7a,9c-(Iminoethano) phenanthrol [4, Benzene, 1-phenyl-4-(2-cyano-2-p..., 5H-Naphtho[2,3-c]carbazole, 5-me... also observed as depicted in Table 2.

The obtained results in this study suggested that the identified phytochemical compounds may be the bioactive constituents and this *Rhizoclonium hieroglyphicum* is proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit.

Table 1. Priliminary phytochemical screening of *Rhizoclonium heiroglyphicum*

Sl No	Phytochemicals	Observations	*Rhizoclonium*				
			Be	Me	Ch	Pe	He
1	**Alkaloids** Mayer's test Wagner's test	Cream colour Reddish brown solution/precipitate	+	-	-	-	-
2	**Flavonoids** Lead acetate test H_2So_4 test	Yellow orange Reddish brown/ Orange colour precipitate	+	-	-	-	+
3	**Steroids** Liebermann-Burchard test	Violet to blue or Green colour formation	-	-	+	-	-
4	**Terpenoids** Salkowski test	Reddish brown precipitate	+	-	-	+	-
5	**Anthroquinone** Borntrager's test	Pink colour	-	-	-	-	-
6	**Phenols** Ferric chloride test Lead acetate test	Deep blue to Black colour formation white precipitate	+	-	-	-	+
7	**Saponin**	Stable persistant	+	-	-	-	-
8	**Tanin**	Brownish green/ Blue black	-	-	-	-	-
9	**Phlobatannins**	Red precipitate	-	-	-	-	-
10	**Glycosides**	Yellow colour	-	-	+	-	+
11	**Oil and Resin**	Filter paper test	+	+	-	+	+

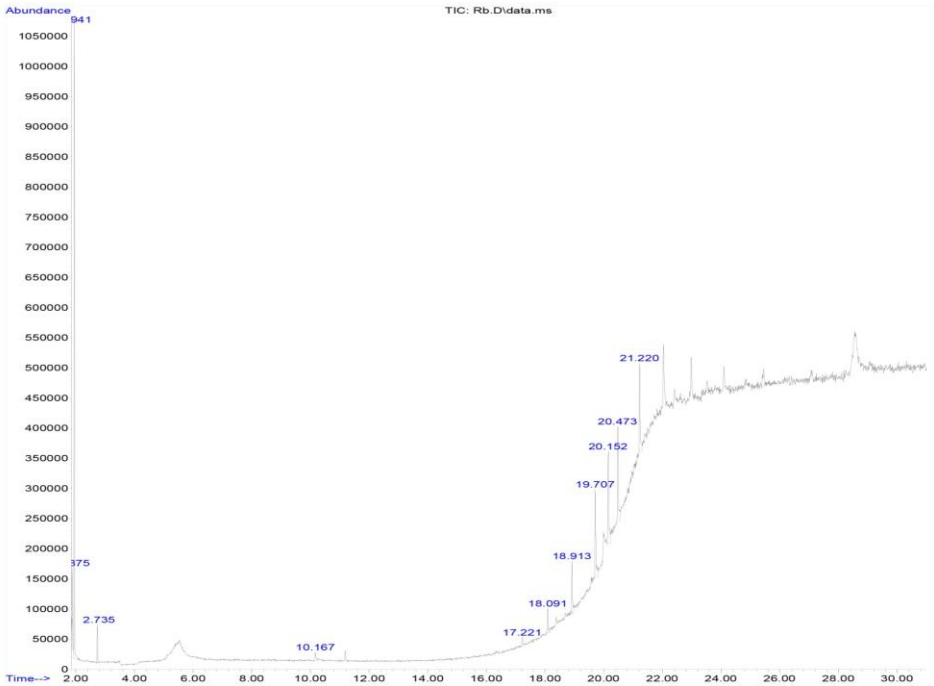

Figure 1. GCMS activity in benzene extract of *Rhizoclonium heiroglyphicum.*

Table 2. GC-MS activity in benzene extract of *Rhizoclonium hieroglyphicum*

Peak Number	Retention Time	Name of the Compounds MassHunter\Library	Ref\#CAS\# Qual	Peak Area %
1	1.875	2,4-Hexadiene, 2-methyl- 1,3-Pentadiene, 2,3-dimethyl- 1,4-Hexadiene, 5-methyl-	2902 028823-41-8 76 2918 001113-56-0 76 2892 000763-88-2 68	4.87
2	1.941	Toluene Toluene Toluene	2455 000108-88-3 91 2449 000108-88-3 91 2454 000108-88-3 90	40.37
3	2.735	Benzene, 1,3-dimethyl- Benzene, 1,3-dimethyl- o-Xylene	5102 000108-38-3 95 5100 000108-38-3 94 5076 000095-47-6 94	2.43
4	10.167	Phenol, 2,4-bis(1,1-dimethylethyl)- Phenol, 3,5-bis(1,1-dimethylethyl)- Phenol, 2,4-bis(1,1-dimethylethyl)-	66115 000096-76-4 46 66113 001138-52-9 41 66109 000096-76-4 41	1.38
5	17.221	7a,9c-(Iminoethano) phenanthro[4,... Benzene, 1-phenyl-4-(2-cyano-2-p... 5H-Naphtho[2,3-c] carbazole, 5-me...	128432 024695-70-3 20 128506 027869-56-3 18 128505 100025-44-3 18	1.25
6	18.091	Tris(tert-butyldimethylsilyloxy) ... Arsenous acid, tris (trimethylsil... 2-Ethylacridine	230548 1000366-57-5 22 178799 055429-29-3 22 66996 055751-83-2 18	2.74
7	18.913	9H-Fluorene-4-carboxylic acid, 9... 1,2,4-Benzenetricarboxylic acid, ... 1,2-Benzenediol, 3,5-bis (1,1-dim...	166892 1000304-78-2 30 214443 033975-29-0 30 79220 001020-31-1 22	5.12
8	19.707	Heneicosane, 3-methyl- Octadecane, 1-iodo- Tetratriacontane, 17-hexadecyl-	153226 006418-47-9 43 202263 000629-93-6 42 242899 055256-07-0 38	9.81
9	20.152	Bis(2-ethylhexyl) phthalate Phthalic acid, monoamide, N-ethy... Phenol, 2-methyl-4-(1,1,3,3-tetr...	207665 000117-81-7 46 165110 1000322-88-9 41 77582 002219-84-3 38	10.91
10	20.473	(+-)-cis-3,4-Dimethyl-2-phenylte... Benzo[h]quinoline, 2,4-dimethyl- Tris(tert-butyldimethylsilyloxy) ...	66930 092772-63-9 32 67018 000605-67-4 30 230548 1000366-57-5 27	10.89
11	21.220	Benzo[h]quinoline, 2,4-dimethyl- 1,1,1,3,5,5,5-Heptamethyltrisilo... Cyclotrisiloxane, hexamethyl-	67018 000605-67-4 35 79666 001873-88-7 35 79619 000541-05-9 35	10.22

CONCLUSION

Algae are also used as raw materials for many industrial productions, which are consumed as food in many Asian countries. In the present study, the phytochemical screening of the algae *Rhizoclonium hieroglyphicum* was done in benzene, methanol, chloroform, petroleum ether and hexane extracts. The analysis revealed that metabolites with higher medicinal activities such as flavonoids, alkaloids and steroids were present. Anthraquinones, tannin and phlobatannins were absent in all five extracts. GC-MS analysis results showed that 11 different compounds of varied nature were present in the extract. Thus, the algae *Rhizoclonium hieroglyphicum* can be a significant source of important compounds which can be used in formulation of drugs by the pharmaceutical industries.

ACKNOWLEDGEMENT

The author (B.Vijaya Rani) expresses sincere thanks to the Principal of Sree Ayyappa College for Women, Chunkankadai and the Head of the Department of

Botany, S.T. Hindu College, Nagercoil, for providing research facilities to carry out the present investigation.

REFERENCES

Ramraj.R., Tsai. D.D.W., and Chen.P.H. 2014. Freshwater microalgae niche of air carbondioxide mitigation. Ecol. Eng., 6847-6852.

Mongillo J, Zierdtarshaw L. Encyclopedia of Environmental Science. Greenwood, United States. 2000;504.

Schaeffer DJ, Krylov VS. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol Environ Saf. 2000; 45(3):208-27.

Del Val AG, Platas G, Basilio A, Gorrochategui J, Suai I, Vincente F, *et al*. Screenina of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain), International journal of Microbiology. 2001; 4:35-40.

Zbakh H, Chiheb I, Motilva V, Riadi H. Antibacterial, Cytotoxic and Antioxidant Potentials of *Cladophoraprolifera*(Roth) Kutzing Collected from the Mediterranean Coast of Morocco. American Journal of Phytomedicine and Clinical Therapeutics. 2014; 2(10):1187-1199.

Kumar N, Barotm JI, Kumar RN. Phytochemical analysis and antifungal activity of selected seaweeds from Okha Coast, Gujarat,India life Sciences Leaflets, 2012, 57-70.

Kumbhar RD, Rode SP, Sabale AB. Phycochemical Screening of Seaweeds from Sindhudurg District of Maharashtra Int J Pharm. Sci. Rev. Res. 2014; 29(1):77-81.

Tang YV, Phang SM, Chu WL, Ho A, Teo SH, Lee HB. Cyclic tetrapyrrolic photosensitizers from *Cladophorapatentiramea* (Cladophoraceae, Chlorophyta) and *Turbinariaconoides* (Sargassaceae, Phaeophyta) for photodynamic therapy. J ApplPhycol, 2012; 24(4):783-90.

Fabrowska J, Łeska B, Grzegorz S. Freshwater *Cladophoraglomerata*as a new potential cosmetic raw material. Chemik. 2015; 69:8:491-497.

Pochon X,Atalah J, Susanna AW, Grant AHAshleigh W, Christian B. *Cladophoraruchingeri* (C. Agardh) Kützing, 1845 (Cladophorales, Chlorophyta): a new biofouling pest of green-lipped mussel *Pernacanaliculus* (Gmelin, 1791) farms in New Zealand Aquatic Invasions. 2015; 10(2):123-133.

Received:06.08.18, Accepted: 09.09.18, Published:01.10.2018

Zeinab A El-Swaify2017 Phytochemical study on *Cladophora*species from the Nil River Edges, Egypt International Journal of Chemical Science Online ISSN: 2523-2843, Print ISSN: 2523-6075 www.chemicaljournals.com Volume 1; Issue 2; November 2017; Page No. 13-22

Prescott, G.W. 1978. How to know freshwater algae. 3rd Edn, Wes. C. Brown company Publication, Iowa, USA. pp. 1-280.

Krishnamoorthy, V., 2000. Algae of India and neighbouring countries - I. Chlorophycota, Oxford and IBH Publishers, New Delhi. pp. 210.

Bischoff, H.W., and Bold, H.C. 1963. Phycological studies. IV. Some soil algae from enchanted rock and related algal species. Univ. Texas Publicat., 6318: 1-95.

Mubeen, I., W. Hussain, Haq, I.U., and Malik, K.A. 2011. Study of native algal species for growth potential and lipid yield. Cur. Res. I. Bio. Sci., 3(3):240-245.

Brain KR, Turner TD. Wright - Scientechnica. 1st Ed. Bristol: 1975. Practical evaluation of phytopharmaceuticals; p. 144

Evans WC. Trease and Evans Pharmacognosy. 14th Ed. London: WB Saunders Ltd; 1996. pp. 119–159.

Kaushik, P. and A. Chauhan, 2008. *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis*. Indian J. Microbiol., 48: 348-352.

Jaki, B., O. Zerbe, J. Hilemann and O. Sticher, 2001. Two novel cyclic peptides with antifungal activities from the cyanobacterium *Tolypothrixbyssoidea* (EAWAG 95). J. Nat. Prod.,64:154-158.

Febles, C.I., A. Arias, M.C. Gil-Rodriguez, A. Hardisson and A.S. Lopez, 1995. *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife. Anuario Inst. EstudiosCanarios, 34: 181-192.

Balch JF, Balch PA: Prescription for Nutritional Healing: A A Practical A-to-Z Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs & Food Supplements. 5th edition. New York: Penguin Putnam Inc; 2000:267–270

Kützing, F.T. (1843): Phycologia Generalis. – 458 pp., FA Brockhaus, Leipzig.

Blair, S.M. (1983): Taxonomic treatment of the *Chaetomorpha* and *Rhizoclonium* species (Cladophorales; Chlorophyta) in New England. – Rhodora. 85: 197–209.

Posada, D. & Crandall, K.A. (1998): Modeltest: testing the model of DNA substitution. – Bioinformatics 14: 817–818.

***Corresponding Author:**
B. Vijaya Rani*
Email: vijayaranirvg@gmail.com