



GC-MS analysis for the assessment of the phytochemical constituents of the filamentous green algae *Pithophora roettleri* (Roth) Wittrock

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Abstract

Pithophora roettleri (Roth) Wittrock is a freshwater macroalgae which is believed to possess high medicinal property. The present work was designed to investigate the phytochemical constituents in ethanolic extract of *Pithophora roettleri* (Roth) Wittrock by Gas chromatography - Mass spectrometry analysis. The phytochemicals elucidated from the algae revealed the presence of various interesting compounds and their mass spectra were interrelated with the National Institute of the Standards and Technology (NIST) and WILEY7 library. A total of 20 compounds were identified in ethanolic extract which comprises terpene, hydrocarbons, esters and others that possess various biological activities.

Keywords: freshwater algae, organic extract, GC-MS, phytochemical, antimicrobial activity

Introduction

Algae were possibly the first species capable of photosynthesis, and the only photosynthesizers for billions of years before plants appeared on earth. Algae yield an estimated 30 to 50 percent of the global net oxygen. This oxygen is essential for respiration to humans and other terrestrial creatures [1]. They are usually inhabitants of aquatic biotopes either freshwater or marine ecosystem [2]. To cope with different environmental conditions, algae synthesize and accumulate a wide range of bioactive compounds [3]. Researchers discovered that all kinds (green, red and brown) of algae contain a remarkable amount of components such as amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes and cyclic polysulphides valuable for human health and responsible for antibacterial, antialgal, antifungal, antioxidant and antiviral activities [4]. Members of the family Pithophoraceae are naturally tropical and occur widespread from tropical to subtropical regions commonly in freshwater environments [5, 6]. *Pithophora* is common in tropical and temperate regions throughout the world. It is tolerant of temperatures > 30°C and low light irradiances, an advantage for mat-forming alga in shallow littoral areas of lakes. Nitrate has been identified as the major factor limiting growth in such environments. Recent studies show that algae are promising sources to provide new biochemically active compounds [7]. Hence, the present work was designed to investigate the phytochemical constituents by Gas chromatography-Mass spectrometry analysis of ethanolic extract of the algae *Pithophora roettleri* (Roth) Wittrock.

Methodology

Collection of algal material

The fresh sample of *Pithophora roettleri* (Roth) Wittrock was collected from Freshwater lake (Krishna lake), Pollachi, Coimbatore, Tamil Nadu, India and it was authenticated in Botanical Survey of India, Southern Regional Center, Coimbatore, Tamil Nadu, India. The sample was thoroughly washed with lakewater to remove epiphytes followed by tap

water to remove other extraneous materials, it was then shade dried and powdered coarsely [8].

Extraction of chemical constituents

Twenty grams of the dried powdered algal sample was successively extracted by Soxhlet apparatus, according to the method [9] adopted by using solvent with analytical reagent (AR) quality. Solvent extraction was implemented with 25g of algal biomass powder with extraction solvent 250 ml of ethanol on a Soxhlet system [10]. The extraction process was performed until the solution was clear, followed by solvent evaporation by transferring it to a hot air oven, where they were dried at 40°C and the crude extract was stored at 4°C [11]. Portions of the extracts were used for phytochemical analysis carried out by gas chromatography-mass spectrometry (GC-MS) method.

Gas chromatography and mass spectrometry

GC-MS analysis was performed at Yaazh Xenomics, Coimbatore, Tamil nadu, India. The ethanolic extract of the algal sample was injected (1µl) in SHIMADZU GCMS-QP-2010plus model, which is equipped with Rtx-5MS column of 30.0mm length. 0.32mm in diameter and 0.50µm of thickness. The column temperature and injector temperature was fixed at 50°C and 270°C respectively. Helium (5.5 lb/in2) was used as a carrier gas. The sample injector with split ratio was 25 throughout the experiment periods. In the chromatography part, the initial temperature 0-150(0min hold) which is then rose to 150-200(10min hold) followed by 200-270(10min hold).

The compounds were separated by GC-MS, then they were eluted from the column and allowed to pass into the detector which was capable of creating an electronic signal, which was processed by the computer for generating chromatogram. Then the compounds which entered into the electron ionization (mass spectroscopy) detector were bombarded with a stream of electrons causing them to break into fragments. These fragments were actually charged ions with certain mass. The m/z (mass/charge) ratio obtained was

calibrated from the graph, called the mass spectrum, and is the fingerprint of the molecule. The interpretation of the mass spectrum obtained was identified using WILEY7 and NIST05 (National Institute of Standards and Technology) mass spectral databases, where, the unknown component was identified by comparing the known component already stored in the WILEY7 and NIST05 (National Institute of Standards and Technology) library.

Results

In the present study, *Pithophora roettleri* (Roth) Wittrock was successively extracted using Soxhlet apparatus and the chemical constituents of the extracts were analyzed by GC-MS. The analyzed algal sample's chromatogram confirmed the presence of various interesting compounds at different

retention times as illustrated in (Figure 1). These compounds were identified through mass spectrometry using WILEY7 and NIST05 (National Institute of Standards and Technology) library attached with GC.

The resulted 20 compounds elucidated in the ethanolic extract of *Pithophora roettleri* (Roth) Wittrock were presented in Table 1 along with their retention time, peak area (%), molecular formula, molecular weight and molecular structure. Based on the peak area (%), the following compounds Pentadecane, Benzaldehyde, 2-nitro-, 1-Octadecyne, Oxirane, tetradecyl-, 9-Octadecenoic acid (Z)-, Phytol, 2-Tridecyne, dl-2-Ethylhexyl chloroformate, 1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) were found to be in higher percentage and considered to be most prevailing.

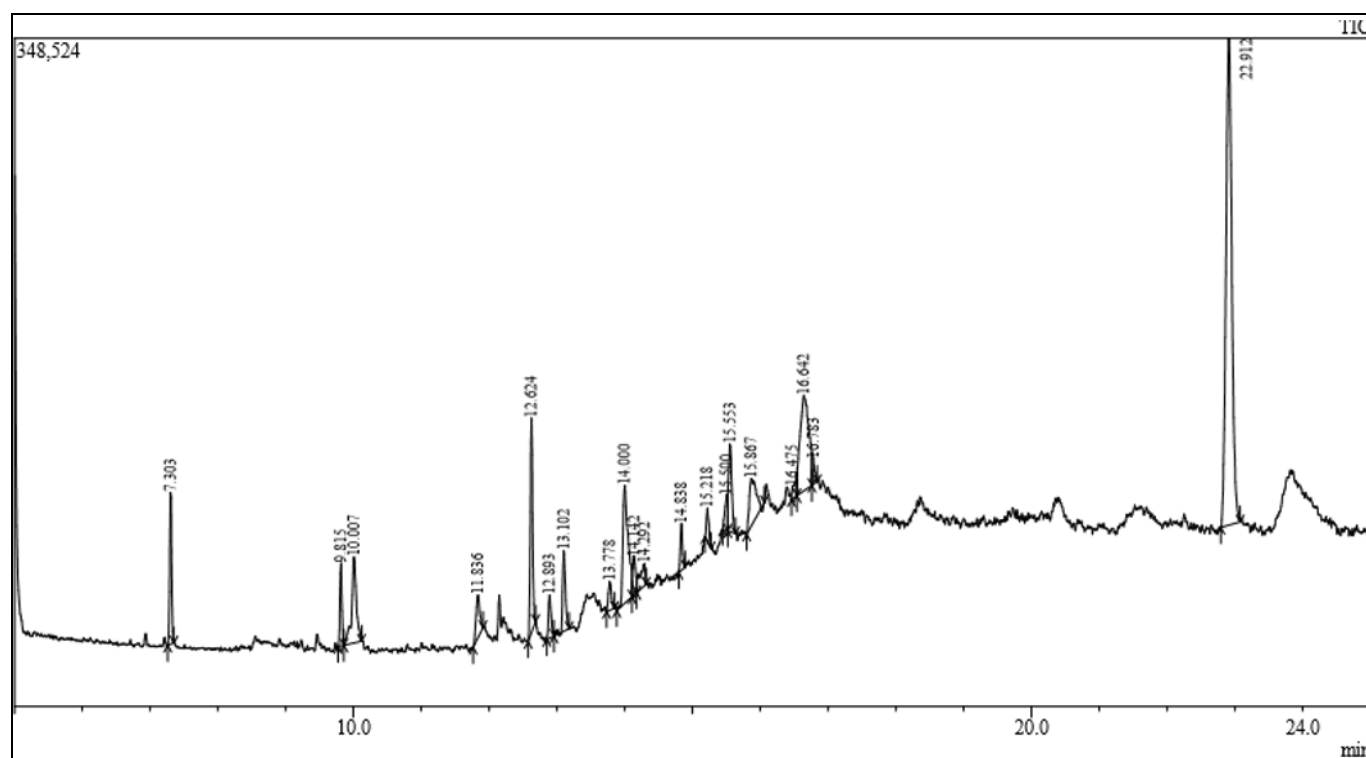
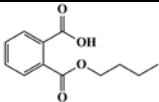
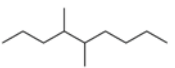
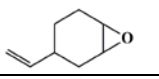

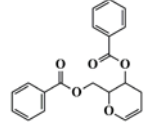
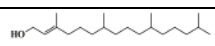
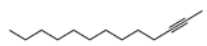
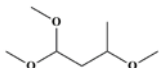
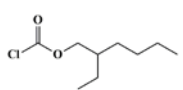
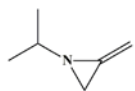
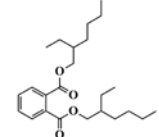


Fig 1: GC-MS chromatogram of ethanol extract of *Pithophora roettleri* (Roth) Wittrock

Table 1. Phytoconstituents of *Pithophora roettleri* (Roth) Wittrock by GCMS Study.

S.No	RT	Peak Area %	Name of compound	Molecular Formula	Molecular Structure	Molecular weight
1	7.30	4.00	Pentadecane	C ₁₅ H ₃₂		212.41
2	9.81	2.24	Tetradecane	C ₁₄ H ₃₀		198.39
3	10.07	5.48	Benzaldehyde, 2-nitro-	C ₇ H ₅ NO ₃		151.12
4	11.83	2.20	10-bromo-7-hydroxy-11-iodolaurene	C ₁₂ H ₂₂ BrIO		389.11
5	12.62	6.45	1-Octadecyne	C ₁₈ H ₃₄		250.46
6	12.89	1.54	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	C ₁₀ H ₂₀ O		156.27
7	13.10	3.12	Oxirane, tetradecyl-	C ₁₆ H ₃₂ O		240.42
8	13.77	1.16	1-Hexene, 5,5-dimethyl-	C ₈ H ₁₆		112.21
9	14.00	8.25	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂		282.46

10	14.14	1.48	1,2-Benzenedicarboxylic acid, monobutyl ester	C ₁₂ H ₁₄ O ₄		222.24
11	14.29	1.63	Nonane, 4,5-dimethyl-	C ₁₁ H ₂₄		156.31
12	14.83	1.44	1,2-EPOXY-4-VINYLCYCLOHEXANE	C ₈ H ₁₂ O		124.18
13	15.21	1.03	Phosphonic acid, dioctadecyl ester	C ₃₆ H ₇₅ O ₃ P		586.95
14	15.50	1.30	3-Hydroxy-2,3(4H)-dihydropyran-2-methanol, dibenzoate	C ₂₀ H ₁₈ O ₅		338.35
15	15.55	3.73	Phytol	C ₂₀ H ₄₀ O		296.53
16	15.86	4.10	2-Tridecyne	C ₁₃ H ₂₄		180.33
17	16.47	0.94	Butane, 1,1,3-trimethoxy-	C ₇ H ₁₆ O ₃		148.20
18	16.64	11.05	dl-2-Ethylhexyl chloroformate	C ₉ H ₁₇ ClO ₂		192.68
19	16.64	0.97	Aziridine, 2-methylene-1-(1-methylethyl)-	C ₆ H ₁₁ N		97.16
20	22.91	37.89	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄		390.56

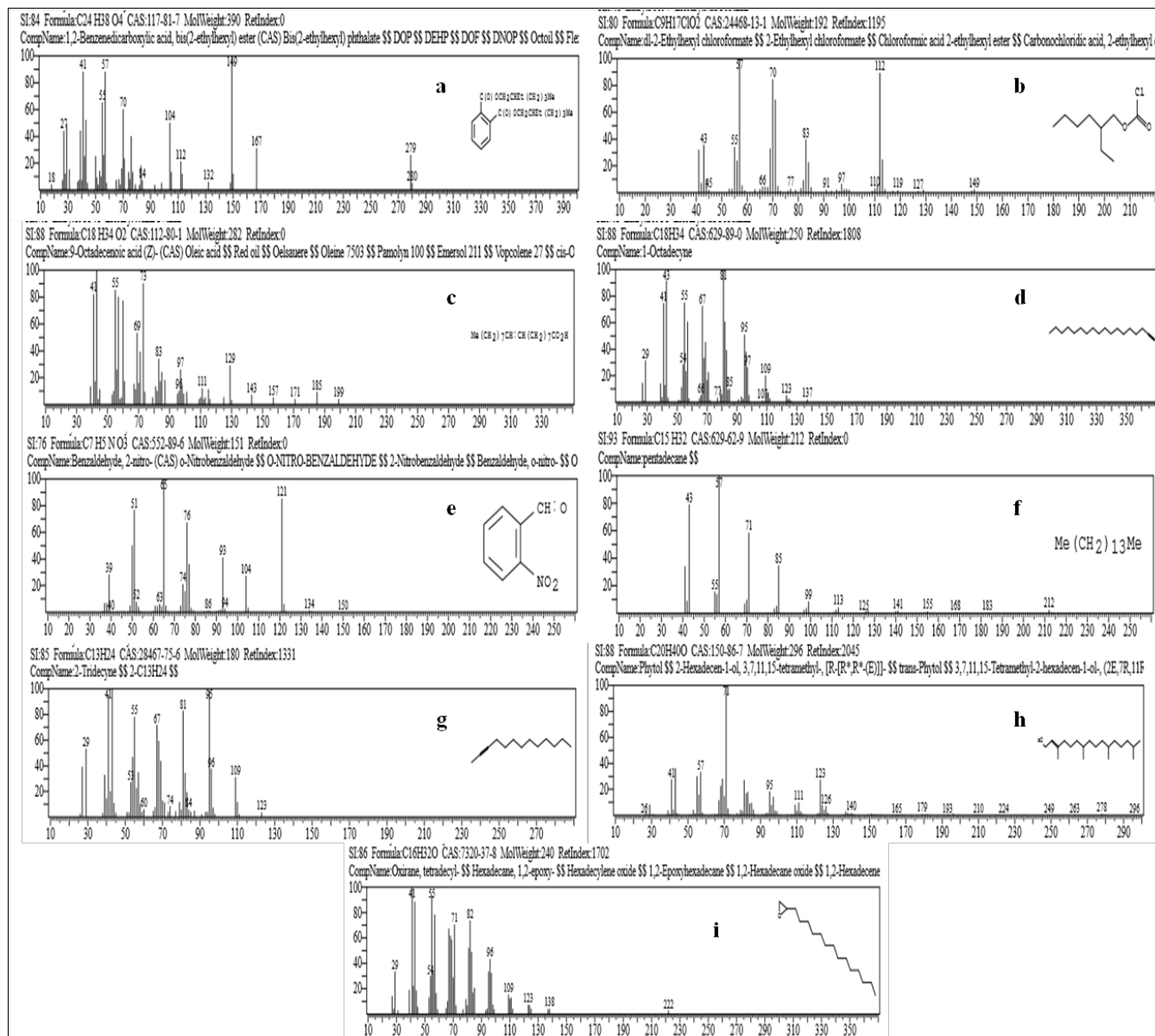


Fig 2: Mass spectra of a) 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, b) dl-2-Ethylhexyl chloroformate, c) 9-Octadecenoic acid (Z)-, d) 1-Octadecyne, e) Benzaldehyde, 2-nitro-, f) Pentadecane, g) 2-Tridecyne, h) Phytol, i) Oxirane, tetradecyl

Table 2: Important major compounds with their pharmacological activity of *Pithophora roettleri* (Roth) Wittrock

S.No	Name of compound	Peak area %	Nature of the compound	Activity
1	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	37.89	Ester	Neurodegenerative disorders, anti-cancer activity ¹²
2	dl-2-Ethylhexyl chloroformate	11.05	Ester	Pharmaceutical, agrochemical, and personal care applications ¹³
3	9-Octadecenoic acid (Z)-	8.25	Linoleic acid ester	Anti-inflammatory, antiandrogenic, cancer preventive, dermatitogenic, hypocholesterolemic, 5- α reductase inhibitor, anemiagenic, insectifuge ¹⁴
4	1-Octadecyne	6.45	Alkyne	No activity reported.
5	Benzaldehyde, 2-nitro-	5.48	Organic aromatic compound	Dyes and colorants ¹⁵
6	Pentadecane	4.00	Hydrocarbon	Antimicrobial and antioxidant ^{16,17}
7	2-Tridecyne	4.10	Alkyne compound	No activity reported.
8	Phytol	3.73	Diterpene	Antimicrobial, anticancer, cancer preventive, diuretic, anti-inflammatory ^{18,19,20}
9	Oxirane, tetradecyl-	3.12	Epoxide	antifungal and antibacterial activities ²¹

The prevailing compounds were previously reported with numerous important bio-activities (Table 2) and their mass spectra were depicted in Figure 2. 1, 2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (peak area: 37.89%) (Figure 2(a)) revealed positive anti-cancer activity of this compound

on PC3, MCF, HCT-116, A549, and MIAPACA cell lines and proved to be a strong immunomodulatory B-cell stimulant. Hence, 1, 2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester was proposed as a potential biomarker. dl-2-Ethylhexyl chloroformate (peak area: 11.05%) (Figure

2(b)) is an ester, which is a chemical building block used in the synthesis of specialty chemicals for pharmaceutical, agrochemical and personal care applications. Used as raw material in the formation of peroxydicarbonates and used as polymer initiators for plastics. 9-Octadecenoic acid (Z)- (peak area: 8.25%) (Figure 2(c)), an unsaturated fatty acid that is most widely distributed and abundant fatty acid in nature. It is used commercially in the preparation of oleates and lotions, and as a pharmaceutical solvent. Benzaldehyde, 2-nitro- (peak area: 5.48%) (Figure 2(e)) is used as a starting material in synthetic chemistry and used as an intermediate during the synthesis of various Indigo dye. Further, it serves as a useful photo removable protecting group. Phytol (peak area: 3.73%) (Figure 2(h)) is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1, antioxidant and a preventive agent against epoxide-induced breast cancer carcinogenesis. It is an important component of all plants as it is known to possess antimicrobial, anticancer, antidiuretic activity. (Figure 2(f) and 2(i)) Pentadecane and Oxirane, tetradecyl- is a hydrocarbon and epoxide respectively possesses antimicrobial and antioxidant properties.

Conclusion

The gas chromatograms of ethanolic algal extract of *Pithophora roettleri* (Roth) Wittrock confirmed the presence of various interesting compounds at different retention time. Twenty compounds were detected in the ethanolic algal extract and among them nine compounds were found to be most prevailing in which, 1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester with 37.89% of peak area was found to be present in higher concentration than others such as Pentadecane, Benzaldehyde, 2-nitro-, 1-Octadecyne, Oxirane, tetradecyl-, 9-Octadecenoic acid (Z)-, Phytol, 2-Tridecyl-, dl-2-Ethylhexyl chloroformate, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl). Hence, the algae *Pithophora roettleri* (Roth) Wittrock have been found to possess significant phytoconstituents that might be attributed to the medicinal characteristics, agrochemical, chemical synthesis and personal care applications. Thus, isolation and characterization of individual phytochemical constituents may proceed to discover novel drugs for their pharmacological and other applications.

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