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GC-MS Characterization, Isolation and Antimicrobial Activities of Isolated Compounds from Actinomycetes Isolates from *Porphyra indica* Seaweed

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Abstract

Objective: Aim of the study is isolation of bioactive compounds from actinomycetes isolates from seaweed *Porphyra indica.*

Methods: GC-MS analysis of actinomycetes isolates from seaweed *Porphyra indica* was carried out and found six major compounds. Isolated compounds 1-4 were characterized by IR, ^{1}HMR, C^{13} NMR AND MASS. *In-vitro* anti bacterial study of isolated compounds was carried out by agar diffusion method against three microorganism *Bacilluscereus, Staphylococcus aureus, Escherichia Coli.*

Results: Four compounds were isolated namely Compound-1 (7H-Furo (3, 2-G) (1) Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)); Compound-2 (heptacosyl octadec-9-enoate); Compound-3-(4, 4, 14-Trimethyl-9, 19-cyclo-9, 10-secocholesta-1(10), 9(11)-diene) and Compound-4 (16-((Octyloxy) carbonyl)-15-hydroxyhexadec-8-enoic acid).

Conclusion: Compound-3 and 4 showed potent antibacterial activity when compared to the standard.

Key words: *Porphyra indica,In-vitro* anti bacterial, GC-MS analysis

INTRODUCTION

India has one of the oldest, richest and diverse traditions associated with the use of medicinal plants. During the course of history the cure of disease and the use of medicinal plants have been much influenced by religious practices and it is being felt that there has always a magic in plants an “unknown spirit” mysterious and omnipotent powerful resource. Natural meds have been utilized for millennia to further develop wellbeing and prosperity of human progress. Indeed, even in regions where present day prescriptions are accessible the interest of natural medications and the use has been expanding quickly lately. In this setting it is important to take on recovery system, which assists with guaranteeing that natural medications have worthy quality, wellbeing and viability. It has been assessed that in created nations like United States, plant drugs comprise as much as 25% of the all out drugs, while in quick agricultural nations like China and India, the commitment is just about as much as 80%. Consequently, the monetary significance of restorative plants is considerably more to nations like India than to rest of the world. These nations give two third of the plants utilized in present day arrangement of medication and the medical care arrangement of provincial populace rely upon native frameworks of medication. Natural products are the useful starting material for the preparations of synthetic drugs. It has been estimated that 56% of lead compounds for the medicines in the British National Formulary are natural products. Nature has persistently given humanity a wide and basically different armory of pharmacologically dynamic mixtures that keep on being used as exceptionally viable medications to battle a variety of dangerous illnesses or as lead structures for the advancement of novel artificially inferred drugs that reflect their model from nature. Marine ecosystem represents almost 95% of biosphere and is particularly promising because of the rightly adapted species which found on this harsh environment. The marine environment is rich source of both biological and chemical diversity. This unique diversity provides a rich source of chemical compounds with potentials for industrial development such as pharmaceuticals, cosmetics, nutritional supplements, enzymes, polymers, biofuels, bioremediators, antifoulants, fine chemicals and agrochemicals. Thousands of such compounds have been identified from the marine resources. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, bryozoans, sea weeds and marine organisms. The marine environment also represents a largely unexplored source for isolation of new microbes (bacteria, fungi, actinomycetes, microalgae-cyanobacteria and diatoms) that are potent producers of bioactive secondary metabolites. Marine organisms have a shorter history of utilization in the treatment and prevention of human disease such as anticancer, antibacterial, antiplasmodial, anti-inflammatory and antiviral agents [1-5].

MATERIALS AND METHODS

Isolation of actinomycetes isolates

Porphyra indica seaweeds were collected from coastal area of India. Fresh seaweeds were rinsed using sterile sea water to remove epiphytes, salt, sand, microorganisms and other suspended materials associated with seaweeds. Then seaweeds were cut and added to 5 mL of sterile seawater. 0.1 ml of diluted sample was placed on Strach Nitrate Agar medium by pour plate technique and incubated at 30 °C for 7 - 10 days. On the basis of

morphological features, colonies were randomly picked up and purified by using streak plate's method.For longer storage, it was grown on nutrient broth for seven days and glycerol was added to make the final concentration of 15% and stored at -20 °C [5].

GC-MS analysis of actinomycetes isolates from seaweed *Porphyra indica*

GC-MS analysis of actinomycetes isolates from seaweed *Porphyra indica* was carried, chromatogram was recorded and interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), WILEY 8 and FAME having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained [6, 7].

Isolation and purification of compounds

Bulk fermentation was performed on 10 liters scale of the different actinomycetesisolates from *Porphyra indica*. The production of bioactive compounds in the fermentation broth began at day 3 and reached maximum at day 7. The fermentation broth was separated into filtrate (10 liters) filtered through Whatman No. 1 filter paper under vacuum. The filtrate was extracted with acetone and ethyl acetate respectively. Different chromatographic techniques were used on the metabolites obtained from the isolates to deliver compounds. Obtained compounds were characterizedby IR, 1H-NMR, 13C-NMR and MS spectra and compared with literature data to identify the isolated compounds [8].

Antibacterial activity of isolated compounds

In-vitro anti bacterial study of isolated compounds was carried out by agar diffusion method against three microorganism *Bacilluscereus, Staphylococcus aureus, Escherichia Coli* [9, 10].

RESULTS AND DISCUSSION

GC-MS analysis of actinomycetes isolates from seaweed *Porphyra indica*

The phytocomponents of **actinomycetes isolates from seaweed *Porphyra indica*** were studiedand the literature study reveals that six of the phytochemical constituents were found to be major (Fig-1).

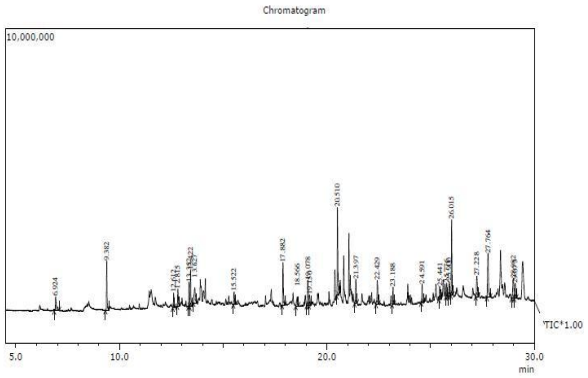

Fig-1 GC-MS analysis of actinomycetes isolates from seaweed *Porphyra indica*

Isolated compounds and its characterization

Compound-1 (7H-Furo (3, 2-G) (1) Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s))

IR (KBr) cm^{-1} spectrum it exhibits a broad band at 3279 cm^{-1} for the hydroxyl group, band at 1631.18 cm^{-1} for C=O group, band at 1568 cm^{-1} and 1178 cm^{-1} for C-O-C group. H^1 NMR ($CDCl_3$) spectrum it exhibits peak at δ 1.595 (6H, s) for two methyl groups, peak at δ 2.043 (1H, s) for OH group, peak at δ 4.244 (2H, t) for CH group present in furan nucleus, peak at δ 6.9, 7.2, 9.0 for present in the Benzopyran nucleus. In its C^{13} NMR it exhibits peak at δ 26.955 for methyl groups, peak at δ 79.558 for tertiary alcohol, peak at δ 160.269 for C-O group in the furan/benzopyran nucleus, peak at δ 97.43, 100.75, 113.96, 117.7, 124.397, 136.40, and 140.79 for carbon present in the furan/benzopyran nucleus. The compound with molecular weight 246.09 exhibit m/z peak at 247 for $[M-H]_+$ ion in its mass spectrum.

Compound-2 (heptacosyl octadec-9-enoate)

IR spectrum of Compound-2 showed signals at 2916.24 (C-H s), 1721(C=O), 1646(C=C) and 1319(C-O). The ^1H NMR spectrum of Compound-2 showed peaks at δ 0.91 (6H, t,) for a methyl group, a broad singlet at δ 1.25, 1.62 (6H, s), showed the presence of long chain methylene groups. The doublet of doublets at δ 4.10 and 4.20 each integrating for one proton each were due to protons under oxygen residue. The multiplet at δ 5.15 for two protons suggests the presence of two unsaturated protons in the compound. The protons which resonated at 2.20 and 2.10 suggest the presence of methylene groups adjacent to unsaturated double bond and carbonyl group respectively. The ^{13}C- NMR spectrum of CN-2 exhibited peaks at δ 12.82 for methyl groups, at 60.99 for the methylene carbon attached to the oxygen function, signals at δ 22.44, 24.23, 27.22, and the bunch of signals centered at δ 29.43, indicates the presence of long chain methylene groups. The signals at δ 31.93, 33.82 and 33.93 were due to the alpha carbons to the unsaturated carbon atoms which resonated at δ 129.70 for two carbon atoms. The signal at δ 172.18 is attributed to the carbonyl group.

Compound-3 (4, 4, 14-Trimethyl-9, 19-cyclo-9, 10-secocholesta-1(10), 9(11)-diene)

IR spectrum: KBr (λmax cm−1) showed signals at 2872.0(C-H), 1678.07 (C=C stretch), 1490.97 (-CH_3) and 1402.24(CH_2 bend). In the ^1H- NMR spectrum, it exhibits signals at 5.29(t, 1-H, =CH), 1.96(m, 2-H, CH_2), 1.92(t, 5-H, CH), 1.41(m, 6-H, CH_2), 1.6(m, 7-H, 2.48(s, 19-H, CH_2), 1.19(d, 12-H, CH_2), 1.16 (s, 18-H, CH_3), 5.29(t, 11-H, =CH), 1.83 (m, 25-H, CH), 1.32(s, 29-H, CH_3), 1.4(s, 30-H, CH_3). The ^{13}CNMR spectra shows peak at C-1 at 121.7, C-10 at 140.30, C-11 at 129.7 and C-28, 29 at 25.14. The molecular ion peak of compound-3 was found to be 410.05.

Compound -4 (16-((Octyloxy) carbonyl)-15-hydroxyhexadec-8-enoic acid)

IR spectrum of Compound-3 showed functional group signals at 3392.45 (OH), 2878.01(C-H stretch),

1742.56(C=O), 1612.58(C=C) and 1250.31(C-O). The ^{1}H- NMR spectrum of Compound-4 exhibits a triplet at δ 0.88 due to a methyl group, a broad singlet at δ 1.22 and at δ 1.62 indicating the presence of long chain methylene groups, δ 1.81 due to methylene groups adjacent to unsaturated system, triplet at δ 2.32 due to methylene groups attached to carbonyl carbon. Further the pair of multiplet signals at δ 4.22 is due to a methylene group attached to the oxygen functional group, and signal at δ 5.12 for the proton of hydroxyl group. The multiplet signal at δ 5.40 is due to two unsaturated protons. It was supported by ^{13}C-NMR signals 172.46 (C=O), 128.99 (C=C), 78.24 (CH-OH) and 61.91 (CH_2-OH). The mass spectrum of Compound-4 exhibited a pseudo molecular ion at m/z 426 for $[M+H+Na]^+$ ion.

In-vitro anti bacterial study of isolated compounds (1-4) against *Bacilluscereus*

Table-1 and fig-2 shows the antibacterial activity of isolated compounds 1-4. Compound 3 and 4 showed potent activity when compared to standard (Ampicillin).

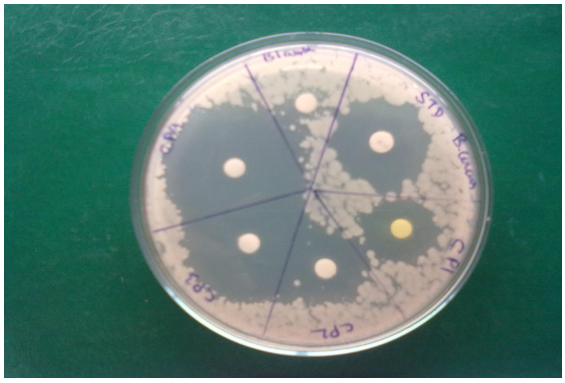

Fig-2 Anti bacterial study of isolated compounds (1-4) against *Bacilluscereus*

Table-1 Zone of inhibition of isolatedcompounds (1-4) against *Bacilluscereus*

S.No	Name of the compounds	Zone of inhibition
1	Standard (Ampicillin)	27mm
2	Isolated Compound 1	20mm
3	Isolated Compound 2	21mm
4	Isolated Compound 3	26mm
5	Isolated Compound 4	32mm

In-vitro anti bacterial study of isolated compounds (1-4) against *Staphylococcus aureus*

Table-2 and fig-3 shows the antibacterial activity of isolated compounds 1-4. Compound- 3 and 4 showed potent activity when compared to standard (Ampicillin).

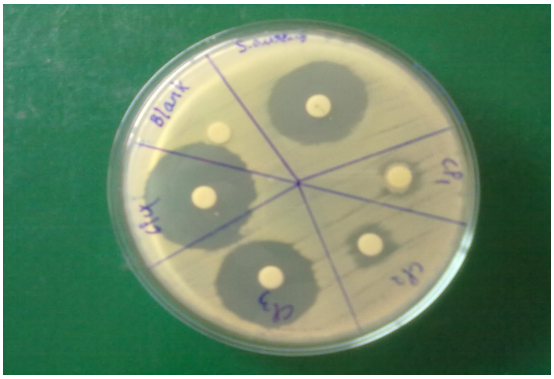

Fig-3Anti bacterial study of isolated compounds (1-4) against *Staphylococcus aureus*

Table-2 Zone of inhibition of isolated compounds (1-4) against *Staphylococcus aureus*

S.No	Name of the compounds	Zone of inhibition
1	Standard (Ampicillin)	27mm
2	Isolated Compound 1	12mm
3	Isolated Compound 2	14mm
4	Isolated Compound 3	29mm
5	Isolated Compound 4	32mm

In-vitro anti bacterial study of isolated compounds (1-4) against *Escherichia Coli*

Table-3 and fig-4 shows the antibacterial activity of isolated compounds 1-4. Compound-4 showed potent activity when compared to standard (Ampicillin).

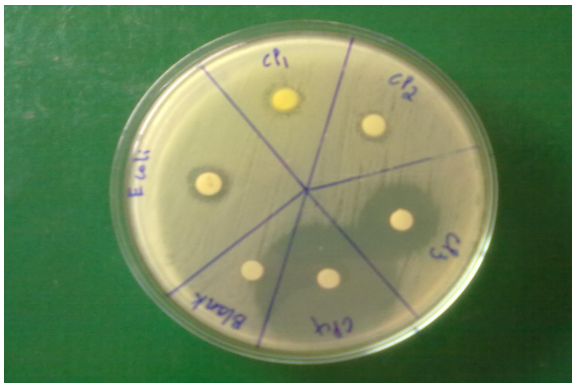

Fig-4 Anti bacterial study of isolated compounds (1-4) against *Escherichia Coli*

Table-3 Zone of inhibition of isolated Compounds (1-4) against *Escherichia Coli*

S.No.	Name of the compounds	Zone of inhibition
1	Standard (Ampicillin)	17mm
2	Isolated Compound 1	9mm
3	Isolated Compound 2	8mm
4	Isolated Compound 3	21mm
5	Isolated Compound 4	35mm

CONCLUSION

In conclusion four compound were isolated from actinomycetes isolates from seaweed *Porphyra indica.* Compound-3 and 4 showed potent antibacterial activity against three microorganism *Bacilluscereus, Staphylococcus aureus, Escherichia Coli.*

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Authors Contributions -All the authors have contributed equally.

Conflicts Of Interests -Declared none

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