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Phytochemical analysis and bioactive compound separation of sea weed, *Hypnea valentiae*

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Abstract- Marine algae have been the source of active compounds. They are used for various applications. Their use in traditional medicine has been reported since time immemorial. In the present study, one such seaweed, *Hypnea valentiae* was investigated. The ethanol, acetone, methanol and water extracts of *Hypnea valentiae* was subjected to phytochemical analysis to know the secondary metabolites present in the extracts. The methonal extracts were also subjected to GC-MS analysis and identified 16 compounds. The antimicrobial activity also use studied in of all extract of seed weed, *Hypnea valentiae.*

Keywords:- Seaweed, extract, TLC, GC-MS, Antimicrobial activity.

1. INTRODUCTION

Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae potential. About 6000 species of seaweeds have been identified and are grouped into green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae. These natural products, are known as secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and related active metabolites, and have been extensively used in the drug and pharmaceutical industry (Eluvakkal *et al.,* 2010).

Recently, researches have proved that compounds originating from marine algae exhibit various biological activities (Kim and Wijesekara, 2010).

Phytochemical are naturally present and are biologically significant and plays an essential role in decencies themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. The secretion of these compounds varies among the seaweeds. Some produce more and same produce in minimal quantity. There is an evidence form laboratory study that phytochemical in marine algae may reduce the risk of cancer, possibly due to dietary fibers, polyphone antioxidants and anti inflammatory effects. The phytochemical research approach is considered effective in discovering bioactive profile of the marine algae of therapeutic importance. Therefore, there is a new trend to isolate novel bioactive compounds and constituents from edible seaweeds (Li *et al.,* 2011). Preliminary phytochemical screening is a part of chemical evaluation. The two main method used are qualitative and quantitative tests are used to quantify or determine the amount of active constituents present. Now-a-days these phytochemical become more popular due to their countless medicinal uses. Phytochemical play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemical do not have any side effects. Since the phytochemical cure diseases without causing any harm to human beings these can also be considered as "man- friendly medicines" Sahirabanu *et al.* (2015). The present studied mainly to the phytochemical analysis and bioactive compound separation from seaweed, *Hypnea valentiae.*

2. MATERIALS AND METHODS

Sample preparation

5g of sample was cut in to small pieces and were grinded well with 5ml of suitable solvent using mortal and pestle. Then the sample was filtered using whatman filter paper. After filtration the sample was store at -20^{0}C.

PHYTOCHEMICAL ANALYSIS

Carbohydrate

2ml sample was mixed with 2ml of benedict reagent and boiled. Observed the formation of reddish brown precipitate.

Redusing sugar

The sample was shaken with distilled water and filtered using filter paper. The filtrate was boiled with drops of fehling's solution A and B for minutes. Observed the formation of orange-red precipitate.

Amino acid

3ml of sample was boiled using water both for 10minutes. Observed the appearances of purple or bluish color.

Proteins

Few drops of 4% NaOH and 1% of $CuSo_4$ solution were taken in 3ml of sample. Observed the appearance of violet or pink color.

Chloride

3ml of sample was prepared in HNO_3 and few drops of 10%$AgNO_3$ solution were added. Observed the formation of white precipitate $AgCl_3$ is observed.

Tannin

2-3ml of sample was added in 5% $feCl_3$ solution and observed the formation of deep blue-block color reactions.

Alkaloid

2ml of sample, 2ml conc.Hcl and added few drops of Mayer's reagent. Observed the formation of green or white color.

Flavonoid

5ml of ammonia solution,1ml of sample and few drop of conc H_2SO_4 were taken. Observed disappearance of yellow colour.

Phlobatannin

Sample was boiled with 1% Hcl. Observed the formation of red precipitate.

Steroid

0.5ml sample, 2ml chloroform and 1ml of H_2SO_4 added. Observed the appearance of red color.

Terpenoid

2ml of sample, 2 ml of chloroform and 2 ml conc. H_2SO_4 were added and observed chloroform layer formed red fluorescence.

Phenolic compound

5ml sample was diluted with distilled water and added a few drop of neutral 5% ferric chloride solution . Observed appearance of green color.

Saponin

0.5ml sample was shaken with 2ml of distilled water they produced foam continues for 10 minutes.

Glycoside

2ml sample was hydrolyzed with 0.5ml of Hcl solution and neutralized with 0.5 ml NaOH solution. Then a few drops of solution A and B were added. Observed the formation of precipitate.

Anthraquinone

0.5ml of sample was boiled with 10% Hcl using water both for 10 minutes. Then it was filtered and allowed to cool. Then 10%ammonia, equal volume of chloroform was added into few drops of filtrate. Then it observed the formation of rose-pink color.

SAPARATION OF BIOACTIVE COMPOUND

Thin Layer Chromatography

Pour the solvent chloroform and methanol (1:1) into the developing container, to a depth of just less than 0.5 cm. The TLC sheets were cuts horizontally (Merck) and the convenient size 5 cm x 10 cm using a pencil marked a line across the sheets. The sample spotted on the pencil marked TLC sheet. After spotting the TLC plate, allow to run with solvent mixer. The spots were visualized by exposure to iodine.

$$R_f \text{ value} = \frac{\text{Distance travelled from the component spot}}{\text{Distance travelled from the solvent front}}$$

ANALYSIS OF BIOACTIVE COMPOUND USING GAS CHROMATOGRAPHY MOSS SPECTROMETRY (GCMS)

The separated compound was analyzed the GCMS in CSIR institution Kerala.

INVITRO STUDY

Antimicrobial activity

Antibacterial activity was carried out by Naz *et al.*(2007). The commercially available 5.6g Muller Hinton agar (HiMedia) was dissolved into 500ml of distilled water. The mixture was warmed on water bath can till agar dissolved. Then the agar was sterilized in an autoclave at 15 lbs pressure, 121^0C for 15 mins. The sterilized medium was allowed to cool at room temperature and poured into sterilized petri dishes under laminar air flow chamber, allowing them to solidify on a plane table. After solidification 0.1% inoculums suspension were swabbed uniformly and wells were bored into the medium using a sterile 6 mm diameter cork borer. Then 150µl of samples were loaded on the well separately using micropipettes and the plates were kept for incubation at 37^0C for 24 hrs. At the end of the incubation, inhibition zones formed around the wells were measured in mm with transparent Hi-Antibiotic Zone scale. The study was performed in triplicate and mean values were recorded.

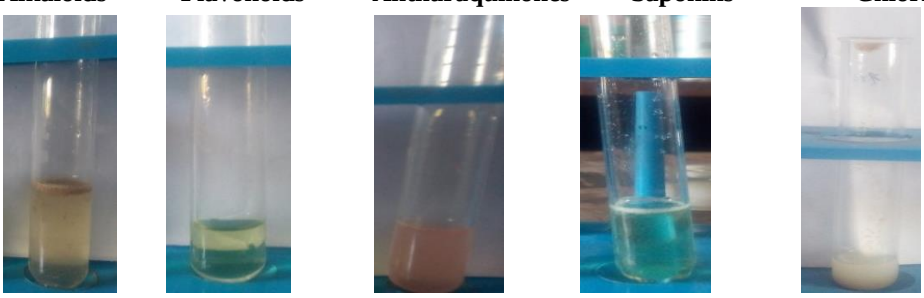
3. RESULT AND DISCUSSION

Seaweeds are primitive non-flowering plants without roots, stems and leaves. They contain different vita-mins, minerals, trace elements, proteins and bioactive substances (Harmesh *et al.*, 2014). Many polysaccharides are recovered from seaweeds, with the most important of them beingagar, alginic acid, laminarine, fucoidin, galactans, carrageenan, xylan and mannans (Sari *et al.*, 2013). In the present study, preliminary phytochemical screening of fifteen different chemical compounds (alkaloids terpenoids, steroids, tannin, saponins, flavonoids, phlobatannins, glycosides, anthraquninones, chloride, carbohydrate, redusing sugar, amino acid, protein, phenolic compound) were tested in four different extracts. Saponins did not show any positive result for their presence in any of the five extracts tested. alkaloid,

saponins, flavonoids, chloride, anthraquninones, showed the maximum presence in five different extracts. Alkaloids compound only present in acetone ethanol and methanol. Alkaloids compound showed the positive result presence in acetone extract. Among the four different extracts, methanol extract showed the presence of maximum number (4) of compounds. Next to that, Acetone extracts showed three compounds. ethanol and water extracts showed eight compounds each and ethanol extracts showed only four compounds.

Kahkonen *et al.* (1999) stated that flavonoids are probably the most important natural phenol due to their broad spectrum of chemical and biological activities, including anti oxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants of a wide range of reactive oxygen species and inhibitors of lipid peroxidation and as potential therapeutic agents against a wide variety of diseases. It has been reported that the presence of phyto-constituents such as flavonoids, tannins and polyphenols prevent a number of diseases through their free radical scavenging activity (Duan *et al.,* 2006), and these phenolic compounds, which include phenol, tannin and flavonoids, have been found in appreciable amounts in the three seaweeds.

Alkaloids **Flavonoids** **Antharaquinones** **Saponins** **Chloride**



Phytochemical analysis results

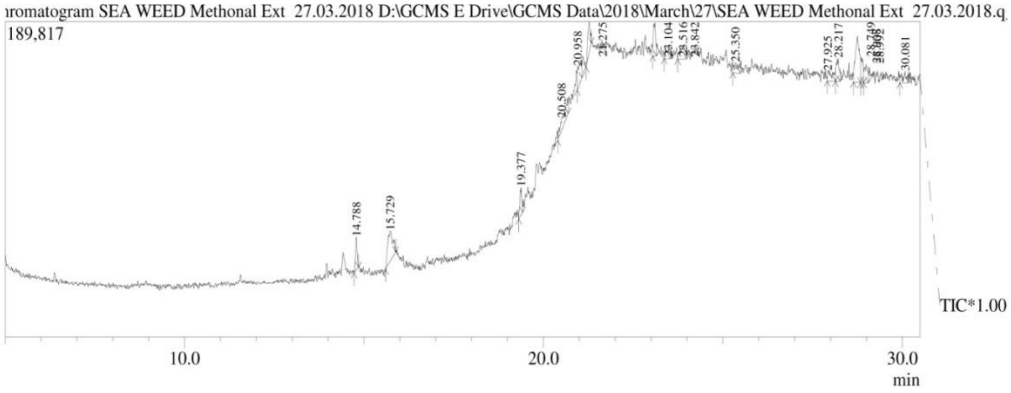
Many bioactive molecules have been isolated and purified by using paper thin-layer and column chromatographic methods. Column chromatography and thin-layer chromatography (TLC) are still mostly used due to their convenience, economy, and availability in various stationary phases (Ammar *et al.,* 2017). Silica, alumina, cellulose, and polyamide exhibit the most value for separating the phytochemicals. Plant materials include high amounts of complex phytochemicals, which make a good separation difficult (Ammar *et al.,* 2017). Therefore, increasing polarity using multiple mobile phases is useful for highly valued separations. Thin-layer chromatography has always been used to analyze the fractions of compounds by column chromatography. Silica gel column chromatography and thin-layer chromatography (TLC) have been used for separation of bioactive molecules with some analytical tools (Zhang *et al.,* 2001). In the present study the bioactive compound separation from seaweed, *Hypnea valentiae* using thin layer chromatography. The results revealed that the methanol extract (non polar) of *H.valentiae* have approximately one compounds. The compound was separated fluorescent by nature. The Rf value is0.92.

The main phyto constituent n-Hexadecanoic acid- Tetradecanoic acid (RT 18.205, 57.89%) Oleic acid-9-Octadecenoic acid-6-Octadecenoic acid (RT 19.846, 7.08%), Hexadecanoic acid-ethyl ester-Ethyl tridecanoate (RT 18.496, 5.16%) and Octadecanoic acid (RT 20.050, 2.77%) was present in the crude extract of *H.musciformis*. These compounds may be involved in biological activity (Balamurugan *et al.,* 2013). Antibacterial activities of the algal extracts were reportedly due to the presence of lauric, palmitic (hexadecanoic acid), linolenic, linoleic, oleic, stearic (octadecanoic acid) and myristic acids (tetradecanoic acid) (Agoramoorthy *et al.,* 2007).

TLC Result

Sivakumar *et al.* (2014) reported that crude ethyl acetate extract of *Ulva faciata* contains bis (2-ethylhexyl) phthalate and 1,2-benzenedicarboxylic acid- butyl as main chemical constituents active against bacteria. The methanolic extract of *H. valentiae contains* six major components including palmitic acid, methylester, trichloromethyloxirane, linolenic acid, ethylester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 11-octadecenoic acid, methylester and 12,15-octadecadienoic acid, methylester. High percentages of palmitic acid, n-heptacosane, 2-methylhexadecan-1-ol, methoxy acetic acid, 2-tridecylester and myristic acid. Most of the identified components have been reported to possess

antimicrobial activity that could be responsible for the antifungal potential reported in the present study (Shobier *et al.*, 2016). In this present study GC-MS analysis of bioactive compound from methanol extract of sea weed, *H. valentiae.* The active principles with their retention time (28.74), molecular formula, molecular weight (390) and Peak area (15.20) were tabulated in Table.1. Only sixteen major compounds were identified in the above extract. The prevailing compounds are of this (alcoholic compound, plasticizer compound, aromatic ether compound, flavonoid, amino compound, steroid, phenolic halogen, alkaloid , fluro benzoic acid ester , acidic compound, aromatic halogen and silica). The identified compounds have the property of antifungal, antioxidant and antimicrobial activity.



GCMS Analysis

Table: 5GCMS Analyses gas chromatography mass spectrometry analysis of bioactive compound separated seaweed, *Hypnea valentiae*

No	RT	Name of the Compound	Formula	M.W	Peak Area %	Compound Nature	**Activity
1	14.788	Oxiranemethanol, 3-(1-Pentadecynyl)-, (2R-trans)-	C18H32O2	280	4.75	Alcoholic compound	Antimicrobial
2	15.729	Diethyl Phthalate	C12H14O4	222	12.69	Plasticizer compound	Antimicrobial Anti-fouling
3	19.377	1,3-Benzodioxole, 5-[[2-(2-Butoxyethoxy)Ethoxy]methyl] -6-propyl-	C19H30O5	338	4.57	Aromatic ether compound	No activity reported
4	20.508	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl-	C10H18O3	186	7.92	Flavonoid fraction	Antimicrobial Anti-inflammatory
5	20.958	2-Propen-1-amine, N-2-Propenyl-	C6H11N	97	6.29	Amino compound	Antimicrobial Anti-inflammatory
6	21.275	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha)-	C28H46O	398	6.83	Steroid	Antimicrobial Anti-inflammatory Antiasthma Diuretic Hepatoprotective Antioxidant
7	23.104	Cyclohexanol, 4-bromo-2,2-dichloro-6,6-dimethyl-,trans	C8H13BrCl2O	274	6.39	Phenolic halogen compound	Antimicrobial Anti-inflammatory Antioxidant
8	23.516	Norpseudoephedrin-Propionyl-Artefakt.	C12H15NO	189	3.41	Alkaloid	Antimicrobial Anti-inflammatory Antioxidant
9	23.842	Pentafluorobenzoic acid,	C15H15F5O2	322	5.40	Fluro	Antimicrobial

		oct-3-en-2-yl ester				benzoic acid ester	Preservative
10	25.350	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	C12H38O5Si6	430	3.77	Silica compound	No activity reported
11	27.925	5-(2-Methylsulfanyl-Pyrimidin-4-yl)–Thiophene-2-Sulfonic acid (2-Cyano-ethyl)-Thiophen-2-ylmethyl ammine-	C17H16N4O2S4	436	4.38	Alkaloid compound	Antimicrobial Anti-inflammatory Antioxidant
12	28.217	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-Hexasiloxane.	C12H38O5Si6	430	5.13	Silica compound	No activity reported
13	28.749	1,2-Benzenedicarboxylic acid.	C24H38O4	390	15.20	Plasticizer compound	Antimicrobial Anti-fouling
14	28.908	Propanoic acid, 3,3'-[oxybis(2,1-Ethanediylthio)]Bis	C10H18O5S2	282	4.83	Acidic compound	Antimicrobial
15	28.992	1-Di(tert-butyl)silyloxy-2-phenylethane	1-Di(tert-butyl)silyloxy-2-phenylethane	264	4.73	Aromatic ether compound	No activity reported
16	30.081	1-((1-Methoxypropan-2-yl)oxy)propan-2-yl 2,3,4,5,6-pentafluorobenzoate.	C14H15F5O4	342	3.72	Aromatic halogen compound	Antimicrobial

Many pharmacological studies on algae have reported that the chemical compounds produced by marine algae have different biological activities such as anti-inflammatory, anticancer, anti-HIV, anti mutagenic and scavenging free radicals (Bechelli *et al.,* 2011). Antimicrobial activity depends on both algal species and the solvents used for their extraction (Radhika *et al.,* 2012). The antimicrobial activity of algae extracts is generally assayed using various organic solvents, such as acetone, ether, chloroform, methanol (Codeire *et al* ,2006). An organic solvent always provides a higher efficiency in extracting com-pounds for antimicrobial activity (Tuney *et al,* 2006). In this present study,antibacterial activity of different extract of seaweed, *H.valentiae* against some pathogenic bacteria's against some pathogenic bacteria *Escherichia coli, Bacillus, Streptococcous, Entrobacter and Pseudomonas*. No inhibition zone as formed on all pathogenic bacteria's.

In vitro screening of organic solvent extracts from several marine macro algae, including *Pterocladia capillacea* (Gmelin), showed specific activity in inhibiting the growth of five virulent strains of bacteria pathogenic to fish species such as *Vibrio anguillarum*, *V. tandara*, *P. fluorescens* and *Aeromonas hydrophila* (Wetky, *et al.,* 2008).

4. CONCLUSSION

The present study find out the maximum amount of flavniodspresent in this seaweed, *Hypneavalentiae*. So the present study highly recommended that the seaweed*Hypneavalentiae* can be use a drug of cancer and heart diseases.

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