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RESEARCH ARTICLE

CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY OF *ULVA LACTUCA* LINN

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Abstract:
The present study was aimed to reveal the chemical constituents' present in the medicinally important seaweed *Ulva lactuca* Linn using GC-MS analysis. Ten grams of powdered sample was extracted with 60 mL ethanol overnight (cold extraction) and filtered through ash less filter paper and the filtrate was concentrated to 1 mL by bubbling nitrogen into the solution. 2 µL of the ethanolic extract of *Ulva lactuca* was employed for GC-MS analysis. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes. The compound biological activity prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA and PASS. The GC-MS analysis provided different peaks determining the presence of seventeen different phytochemical compounds viz., Phytol (10.51%), Hexadecanoic acid, ethyl ester (20.24%), (E)-9-Octadecenoic acid ethyl ester(9.59%) which was present in this plant considered to have diarrhea, anemia, anti-inflammatory, hepatitis, and anticancer activity. The presence of various bioactive compounds confirms and supplemented the traditional application of *Ulva lactuca* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Keywords: GC-MS; PASS; Phytochemistry; Bio-potentials

Introduction

Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries[1]. So the use and search for drugs and dietary supplement derived form plants have increased in recent years[2]. Seaweeds which are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc., have been found to have several biological properties. Seaweeds have been used in the treatment of various infectious diseases. Many substances obtained from seaweeds have been used for decades in medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties[3-5].

Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters[6]. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards[7,8].

GC-MS is a simple, rapid and accurate method for analyzing plant material [9]. GC-MS fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time. The GC-MS method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products[10]. The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceal sustainable studies. GC-MS finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug[11].

Ulva lactuca Linn, green seaweed that is widespread in the Hare island coastal region of the Gulf of Mannar on southeast coast of India. (Order*: Ulvales*; Family: *Ulvaceae*), is mainly used for food, animal feed, and agriculture. The majority of seaweeds from the Gulf of Mannar have not been examined for their bioactive substances [12]. To fufill the lacuna, the present study was aimed to reveal the chemical constituents present in the *Ulva lactuca* Linn using GC-MS analysis.

Materials and methods

Collection and preparation of plant material

The fresh green seaweed *Ulva lactuca* Linn. were collected from the Hare island coastal region of the Gulf of Mannar on southeast coast of India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Plant sample extraction

The powdered plant material was analyzed using the Clarus 500 GC-MS (Perkin Elmer). Ten grams of powdered sample was extracted with 30 mL ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g) and the extract was concentrated to 1 mL by bubbling nitrogen into the solution. 2 µL of the *Ulva lactuca* ethanolic extract was employed for GC-MS analysis (Merlin et al., 2009).

Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The 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. The compound biological activity prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA and PASS prediction. The biological activity spectrum of PASS is designed according to the algorithm specified below.

$$u_j = a_i \, \mathrm{ArcSin}\{r_i(2p_{ij}-1)\},\ u_{0j} = a_i \, \mathrm{ArcSin}\{r_i(2p_j-1)\}$$
$$S_j = \mathrm{Sin}\,(u_j/m),\ S_{0j} = \mathrm{Sin}(u_{0j}/m)$$
$$P_{rj} = (1 + (s_j - s_{0j})/\,(1 - s_j s_{0j}))/2$$

For the compound under prediction structural descriptors are generated. For each activity the following values are calculated.

Results and Discussion

In this present study the GC-MS analysis leads to the prediction of chemical constituents present in the ethanolic extract of *U. lactuca*. 17 compounds were found in the ethanolic extracts of *U. lactuca*. The active principles with their rentention time (RT). Molecular formula, Molecular weight (MW), and peak area value (%) are presented in Table 1 and Figure 1. The identified compounds bioactivities were predicted using Dr. Duke's Phytochemical & Ethnobotanical Databases and PASS prediction. The results revealed the presence of 17 different phytocompounds viz., 7-Hexadecene, (Z) (1.50%), 8-Heptadecene (3.78%), Tetradecanoic acid, ethyl ester (CAS) (2.07%), Phytol 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]- (CAS) (10.51%), Doconexent (2.24), Hexadecanoic acid (CAS) (3.18%), Hexadecanoic acid, ethyl ester (20.24%), Ethyl 6,9,12,15-octadecatetraenoate (3.35%), (E)-9-Octadecenoic acid ethyl ester (9.59%), Octadecanoic acid, ethyl ester (1.66%), Hexadecanoic acid, 2,3-dihydroxypropyl ester, (ñ) (1.77%), 2-(2-mercaptopro-2-nyl-aminoimino)-pentane (2.29%), Hexadecanoic acid, 1,1-dimethyl-1,2-ethanediyl ester (CAS) (1.17%), Heneicosane (CAS) (1.83%), Heptacosane (CAS) (2.02%), Nonacosane (CAS) (3.83%) and Dodecane, 5,8-diethyl (2.07%). The GC-MS spectrum confirmed the presence of 17 major components with the retention time 18.69, 20.60, 23.31, 25.27, 26.72, 27.08, 27.69, 31.04, 31.63, 33.28, 35.40, 37.07, 38.08, 40.53 and 45.60 respectively. The results of the present study also supplement the usage of the studied plant which possesses several known and unknown bioactive compounds with bio-activity namely, pulmonary edema, irritation, tetany, diarrhea, muscle weakness, pulmonary edema, anemia, respiratory failure, drowsiness,

depatotoxic, hematotoxic, hepatitis, antiulcerative, sleep disturbance, anti-inflammatory, hyperthermic, hypercholesterolemic, antineoplastic.

Marine macroalgae, or seaweeds as they are more commonly known, are one of nature's most biologically active resources, as they possess a wealth of bioactive compounds. Compounds isolated from marine macroalgae have demonstrated various biological activities[14-20]. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

GC-MS finger printing profile is useful as a phytochemical marker to dinstinguish the medicinalylly important seaweeds from its adulterants. In addition to this, the results of the GC-MS profile can be used as pharmacognostical msrker for the identification of the medicinally important seaweed. The result of the present study supported and supplemented the previous observations on the medicianlly important plants[21-25].

Similar to our observation, Sahaya Sathish et al.[26] also anlsysed bioactive constituents present in leaf extracts of *Vitex altissima* L. They also identified 21 phytochemical compounds and predicted biological activities using Dr. Duke's Phytochemical and Ethnobotanical Databases. John De Britto et al. [27] predict the biological activity profile of known seven secondary metabolites viz., taxol, vinblastine, vincristine, topotecan, irinotecan, etoposide and teniposide using PASS.

In the present study predicted biological activities using Dr. Duke's Phytochemical and Ethnobotanical Databases and PASS for GC-MS predicted unknown compounds. We observed boundless activity for the major constituents in the ethanolic extracts of *U. lactuca*. The presence of various bioactive compounds confirms the application of *U. lactuca* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Conclusion

The result of the present investigation revealed the chemical constituents present in the ethanolic extracts of *Ulva lactuca.* The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders.

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Table 1: Chemical constitents of *U. lactuca*

Name of the compound	RT	Peak area %	Mol. formula	Mol. Wt.	Biological activity
7-Hexadecene,(Z)	18.69	1.50	$C_{16}H_{32}$	224	Thioredoxin inhibitor, Pulmonary edema, Irritation, Tetany, Diarrhea.
8-Heptadecene	20.60	3.78	$C_{17}H_{34}$	238	Pulmonary edema, Irritation, Tetany, Diarrhea, Anemia, Respiratory failure.
Tetradecanoic acid, ethyl ester (CAS)	23.31	2.07	$C_{16}H_{32}O_2$	256	Muscle weakness, Pulmonary edema, Anemia, Respiratory failure, Drowsiness, Diarrhea.
Phytol 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]	25.27	10.51	$C_{20}H_{40}O$	296	Hepatotoxic, Anemia, Hematotoxic, Hepatitis, Antiulcerative, Tetany, Sleep disturbance, Irritation.

Doconexent	26.72	2.24	$C_{22}H_{32}O_2$	328	Antiinflammatory, Hyperthermic, Anemia, Hypercholesterolemic, Hematotoxic, Pulmonary edema,Antineoplastic.
Hexadecanoic acid (CAS)	27.08	3.18	$C_{16}H_{32}O_2$	256	Muscle weakness, Tetany, Anemia, Diarrhea, Pulmonary edema, Respiratory failure.
Hexadecanoic acid, ethyl ester	27.69	20.24	$C_{18}H_{36}O_2$	284	Muscle weakness, Pulmonary edema, Anemia, Respiratory failure, Drowsiness, Diarrhea.
Ethyl 6,9,12,15-octadecatetraenoate	31.04	3.35	$C_{20}H_{32}O_2$	304	Muscle weakness, Pulmonary edema, Anemia, Respiratory failure, Drowsiness, Diarrhea.
(E)-9-Octadecenoic acid ethyl ester	31.63	9.59	$C_{20}H_{38}O_2$	310	Muscle weakness, Pulmonary edema, Anemia, Respiratory failure, Diarrhea, Sleep disturbance, Tetany.
Octadecanoic acid, ethyl ester	32.09	1.66	$C_{20}H_{40}O_2$	312	Muscle weakness, Pulmonary edema, Anemia, Respiratory failure, Diarrhea, Tetany.
Hexadecanoic acid, 2,3-dihydroxypropyl ester, (ñ)	33.28	1.77	$C_{19}H_{38}O_4$	330	Muscle weakness, Drowsiness, Sleep disturbance, Anemia, Hyperthermic, Hepatitis.
2-(2-mercaptopro-2-nyl-aminoimino)-pentane	35.40	2.29	$C_8H_{18}N_2S$	174	Anemia, sideroblastic, Anemia, Hyperthermic.
Hexadecanoic acid, 1,1-dimethyl-1,2-ethanediyl ester	37.07	1.17	$C_{36}H_{70}O_4$	566	Antiinflammatory, Pulmonary edema, Muscle weakness.
Heneicosane (CAS)	38.08	1.83	$C_{21}H_{44}$	296	Tetany, Anemia, Hypercholesterolemic,
Heptacosane (CAS)	40.53	2.02	$C_{27}H_{56}$	380	Tetany, Anemia.
Nonacosane (CAS)	42.85	3.83	$C_{29}H_{60}$	408	Tetany, Anemia, Pulmonary edema.
Dodecane, 5,8-diethyl	45.60	2.07	$C_{16}H_{34}$	226	Tetany, Pulmonary edema, Muscle weakness,

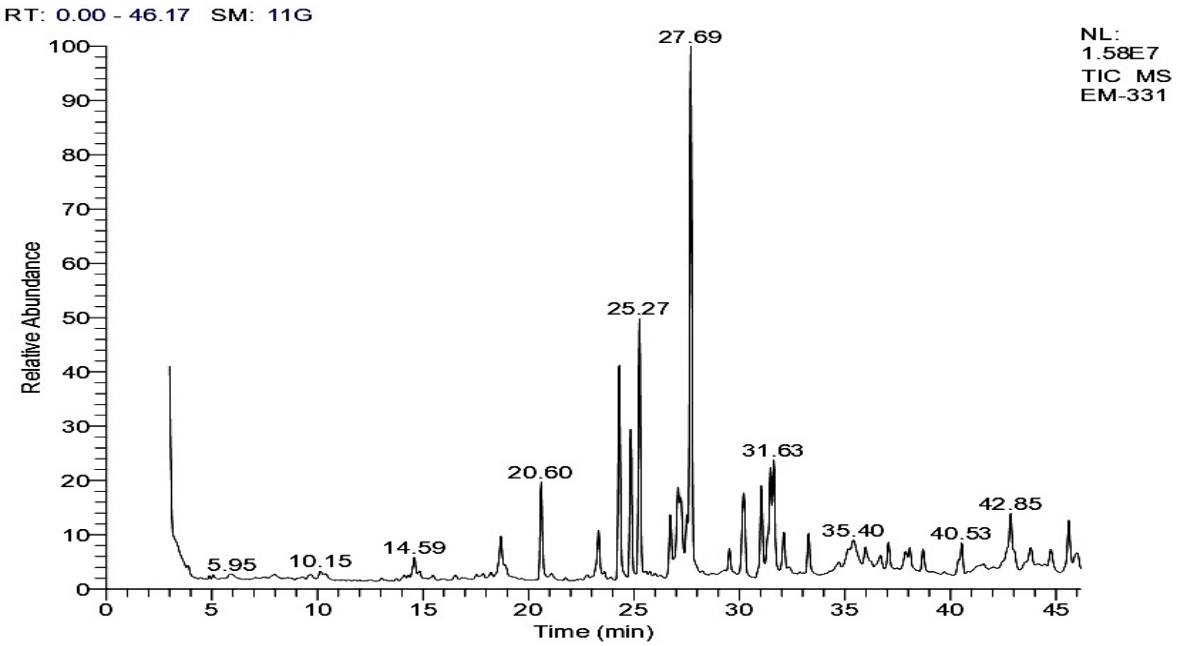

Fig. 1: GC-MS chromatogram of *U. lactuca* ethanolic extract

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