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Seagrass Metabolomics: A New Insight towards Marine Based Drug Discovery

*Danaraj Jeyapragash, Ayyappan Saravanakumar
and Mariasingarayan Yosuva*

Abstract

Metabolomics is one of the new field of “Omics” approach and the youngest triad of system biology, which provides a broad prospective of how metabolic networks are controlled and indeed emerged as a complementary tool to functional genomics with well-established technologies for genomics, transcriptomics and proteomics. Though, metabolite profiling has been carried out for decades, owing to decisive mechanism of a molecule regulation, the importance of some metabolites in human regimen and their use as diagnostic markers is now being recognized. Plant metabolomics therefore aims to highlight the characterization of metabolite pool of a plant tissue in response to its environment. Seagrasses, a paraphyletic group of marine hydrophilous angiosperms which evolved three to four times from land plants back to the sea. Seagrasses share a number of analogous acquired metabolic adaptations owing to their convergent evolution, but their secondary metabolism varied among the four families that can be considered as true seagrasses. From a chemotaxonomic point of view, numerous specialized metabolites have often been studied in seagrasses. Hence, this chapter focus the metabolome of seagrasses in order to explore their bioactive properties and the recent advancements adopted in analytical technology platforms to study the non-targeted metabolomics of seagrasses using OMICS approach.

Keywords: seagrass, metabolomics, OMICS, non-targeted, drug discovery

1. Introduction

Over the past decades, metabolomics has emerged as a valuable tool for the comprehensive profiling and metabolic networks in the biological system. Pauling et al. [1] coined the term metabolomics which was first used in 1998 and even up to 2010 metabolomics was considered as an emerging one in the science field. Reports were documented on the complete genome ([2]; Yu et al., 2002), transcriptome [3] and proteome studies [4–6], but in recent years metabolome analyses using mass spectrometry (MS) - based platforms attracted attention. Even though, metabolite profiling have been carried out for decades, due to ultimate mechanism of a molecule regulation as constituents of metabolic pathways, the prominence of some metabolites in human regimen and their use as diagnostic markers is now being recognized [7].

Currently, metabolomics is a powerful tool for characterizing the metabolites and their metabolic pathways which provides a clear metabolic picture of biological samples. Metabolites are small molecules with diverse structures that are chemically transformed during the cellular metabolism [8]. The number of metabolites is expected to be significantly lowered than the number of genes, mRNAs and proteins which reduce the sample complexity. So far, the total number of metabolites in the plant kingdom is estimated to exist between 100000 to 200000, which make the task more challenging to detect more diverse group of metabolites [9]. Plant metabolomics therefore aims to highlight the characterization of metabolite pool of a plant tissue in response to its environment [10–13]. Since, metabolomics is a balanced approach that obtains inclusive information on the cell's, tissues or organisms metabolite content with low molecular weight, their configuration likely to be changed owing to diverse environmental conditions which reproduces different genetic background [14, 15].

Recent reports on the plant metabolome brought huge challenges to analytical technologies that have been used in current plant metabolomics programs. Some analytical approaches comprise metabolite profiling, metabolite target analysis and metabolite fingerprinting which can be employed according to focus of the research and research questions [16, 17]. Metabolite profiling does not certainly determine the absolute concentrations of metabolites; rather their comparative levels within a structurally related predefined group. Targeted metabolite analysis aims to determine the absolute concentration of metabolites using specialized extraction protocols with an adapted separation and detection methods [18]. Metabolite fingerprinting generally not used to detect individual metabolites, but rather it provides a fingerprint of all compounds which can be measured for sample comparison and discrimination analysis by non-specific rapid analysis of crude metabolite mixtures. However, single analytical technology is not enough to cover the whole metabolome owing to the metabolic diversity and their broad dynamic range in cellular abundance. Accordingly, different extraction techniques and combinations of analytical methods are often employed in order to acquire diverse group of metabolite coverage.

2. Mass spectrometry-based metabolomics analysis

Historically, metabolite concentrations were achieved either by spectrophotometric assays capable of detecting single metabolites or by simple chromatographic separation of mixtures with low complexity. However, over the past decade several methods with high accuracy and sensitivity have been established for the analysis of highly complex mixtures of compounds [19–21]. These methods include gas chromatography - mass spectrometry (GC-MS), liquid chromatography - mass spectrometry (LC-MS), fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and capillary electrophoresis - mass spectrometry (CE-MS). In addition, NMR coupled with chromatography have found great efficacy in addressing specific issues with respect to medical fields [22, 23] and conceivably more important to the unequivocal determination of metabolite structures [24]. However, NMR shows relatively low sensitivity and hence can be used for profiling the diverse group of metabolites from complex mixtures. The pros and cons of mass-spectrometric based metabolomics is given in **Table 1**.

Gas Chromatography - Mass Spectrometry assists the identification and robust quantification of few hundred metabolites in a single plant extracts, which results in inclusive coverage of the central pathways of primary metabolism [25]. GC-MS has a major advantage than other methods that it has long been used for profiling the

Metabolomics Technology	Advantages	Disadvantages
GC-MS	<ul style="list-style-type: none"> Well established chromatographic-mass spectrometric technology Less expensive Easy to perform Better stability and reproducibility Universal database for metabolite identification is available 	<ul style="list-style-type: none"> Time consuming Can only analyze volatile compounds upon derivatization Not able to identify the unknown compounds
LC-MS	<ul style="list-style-type: none"> Highly sensitive Method for sample pre-treatment is simple Wide coverage of metabolite could be identified Scanning range for different ions is usually less Different columns can be used depends on the polarity of compounds 	<ul style="list-style-type: none"> Less reproducibility Highly Expensive Difficult to identify highly polar and charged metabolites
FT-ICR-MS	<ul style="list-style-type: none"> High resolution mass spectrometric technique Extremely high mass accuracy High acquisition rates Highly flexible 	<ul style="list-style-type: none"> Involves series of steps Use large super conducting magnets Highly expensive than GC-MS and LC-MS

Table 1.
 Advantages and disadvantages of mass-spectrometric based metabolomics.

metabolites and therefore it has stable protocols for machine setup, their maintenance with chromatogram evaluation and interpretation. Though, single analytical system cannot cover the whole metabolome, GC-MS has a quite broad coverage of compounds classes including organic and amino acids, sugars, sugar alcohols, lipophilic compounds and phosphorylated intermediates [26]. During method validation, recovery experiments of all measurable compounds have been done and for unknown compounds, recombination experiments were executed to determine the recovery rates in which the extracts of two plant species are evaluated independently and also with mixtures [27, 28]. Liquid chromatography-based methods offer numerous advantages such as detection of broad range of metabolites, as they suffer from the lower reproducibility of retention time. In addition, they are more susceptible to ion suppression effects due to the predominant use of electrospray ionization, which renders the precise quantification more difficult [29–31].

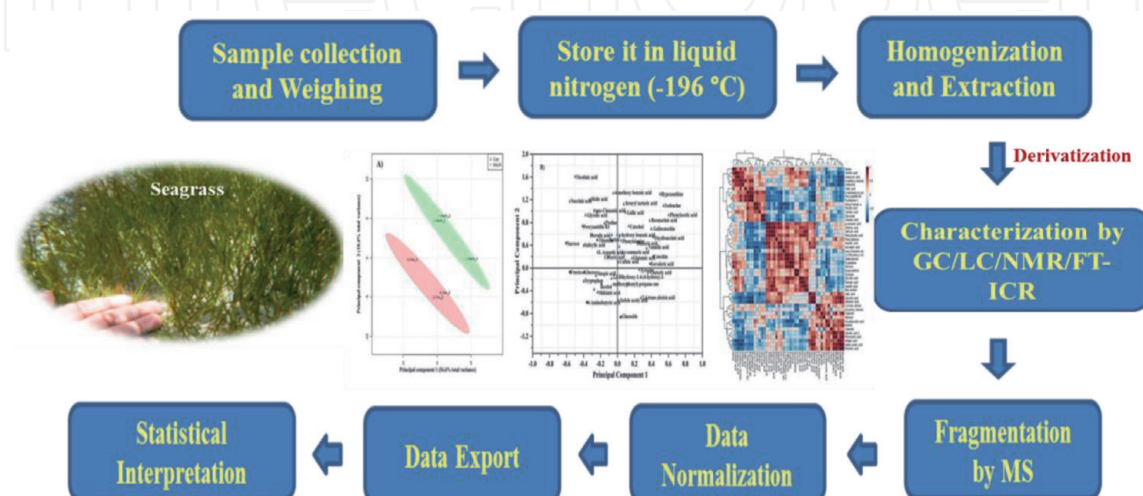


Figure 1.
 Schematic overview of MS based metabolomics.

FT-ICR-MS and CE-MS has been reported to be worth mentioning, where FT-ICR-MS has unsurpassed mass accuracy thereby allows the researcher to obtain an idea about the chemical composition of the specific compounds. In case of CE-MS, the low-abundance metabolites can be detected and affords good chromatographic separation [32, 33]. Of these techniques, GC-MS is mostly preferred for the separation of low molecular weight metabolites which can be either volatile or can be converted into volatile and thermally stable compounds via chemical derivatization prior to the analysis [34]. The experimental procedure for GC-MS based metabolomics analysis is represented in **Figure 1**.

3. Derivatization

Derivatization is a process by which a compound is chemically modified to produce a new compound that has properties which are more amenable to specific analytical procedure. Samples analyzed by gas chromatography requires derivatization in order to make them suitable for analysis. Derivatization procedure imparts volatility, decreases the adsorption in the injector, increases the stability of compounds; improve the resolution and detectability between coeluting compounds and overlapping which assist in structure determination [35]. A good derivatizing reagents and the procedure should produce the compound of interest with desired chemical modification and be efficient, reproducible and non-hazardous (www.piercenet.com). For GC, derivatization reaction can be done by three basic types: silylation, acylation and alkylation. Silylating reagents react with compounds containing active hydrogen and are most frequently used in GC. Acylating reagents react with compounds having high polar functional groups such as amino acids or carbohydrates. While alkylating reagents target the active hydrogen's on amines and acidic hydroxyl group [36].

4. Seagrasses

Seagrasses, a marine hydrophilus angiosperm live entirely in an estuarine or in the marine environment and nowhere else [37]. Seagrass ecosystem act as a breeding and nursery ground for numerous organisms and also help in promoting the commercial fisheries. It is considered to be one of the most productive ecosystems that retain the structural complexity and biodiversity shed light to some researchers to describe seagrass community as marine representation of the tropical rainforests [38]. Currently, seagrasses are assigned to four families Hydrocharitaceae, Cymodoceaceae, Posidoniaceae and Zosteraceae (den [39, 40]). According to angiosperm Group III System, all four families occurred exclusively to monocot order Alismatales [41]; while Les and Tippery [40] favored to treat the same clades as a subclass Alismatidae. The family hydrocharitaceae comprises of three genera namely *Enhalus*, *Halophila* and *Thalassia* and the Cymodoceaceae encompasses the highest variety of genera includes *Amphibolis*, *Halodule*, *Syringodium*, *Cymodocea* and *Thalassodendron*. Likewise, the Zosteraceae comprises of *Zostera*, *Heterozostera*, *Nanozostera*, *Phyllospadix* which are exclusive marine organisms and the family Posidniaceae are monogeneric [42–44]. Therefore, seagrasses can be considered as a unique ecological group occurring worldwide in different climatic zones and sharing their metabolic features with their terrestrial counterparts [37]. However, their metabolism must have undergone several adaptations to survive and colonize in shorelines and oceans worldwide [37]. The seagrass ecosystems and their functions were given in **Table 2**.

Seagrass and Seagrass beds	Properties
Common names	Eelgrass, turtle grass, tape grass, shoal grass, and spoon grass
Families: 4	<ul style="list-style-type: none"> • Hydrocharitaceae • Posidonaceae • Zosteraceae • Cymodoceaceae
Total Species: 72	In India: 14 species exist
Habitat	Found in salt and brackish water
Depth	1 meter - 58-meter depth
Seagrass Parts	
Leaves <ul style="list-style-type: none"> • Chloroplast • Lacunae • Cuticle • Viens 	Photosynthesis Help for buoyancy Exchange oxygen and carbondioxide in the water column Transport nutrients throughout the plants
Rhizome	Stabilize the seagrass beds under wave action
Roots	Absorb nutrients from the soil and transport to the plants
Growth and reproduction	Sexual reproduction and asexual clonal growth
Biodiversity <ul style="list-style-type: none"> • Small bodied 	Small thin leaves Small rhizome “Guerilla” strategy Short lived with fast turnover Low biomass Abundant flowering Many small seeds and seed bank
<ul style="list-style-type: none"> • Large bodied 	Large thick leaves Large rhizome “Phalanx” strategy Long-lived with slow turnover High biomass and holds space Patchy flowering Few large seeds and seeds germinate rapidly
Ecosystem benefits	Lungs of the sea Creation of Living Habitat Foundation of Coastal Food Webs Blue Carbon

Table 2.
Seagrass ecosystem and their benefits.

5. Seagrass metabolome

Seagrasses share a number of analogous acquired metabolic adaptations owing to their convergent evolution, but their secondary metabolism varies among the four families that can be considered as true seagrasses. During the period of ancient Tethys Sea, approximately 90 million years ago surrounded by Africa, Gondwanaland, and Asia, the terrestrial like species returned to the sea and thus explaining the “terrestrial-like” chemical profile of seagrass. From a chemotaxonomic viewpoint, numerous secondary metabolites have been often studied in seagrasses. The metabolome of seagrasses may differ with respect to geographical location, substrates and other physiological factors includes wide fluctuations in the salinity which are prone to synthesize novel metabolites with defined physiological, biochemical, defense and ecological roles [45, 46]. Preliminary suggestions confirmed

that seagrasses have pharmaceutically potent bioactive secondary metabolites [47], that are directed to prove to be a lead molecule for drug discovery [48]. The status of metabolomic study in seagrasses reported so far is tabulated in **Table 3**.

Seagrass	Methods used	Derivation method	Results	Potential application	Reference
<i>Zostera marina</i> <i>Zostera noltii</i>	GC/TOF	Trimethyl silylation	Adaptivemechanisms are involved through metabolic pathways to dampen the impacts of heat stress	Sucrose, fructose, and myo-inositol were identified to be the most responsive metabolites of the 29 analyzed organic metabolites.	Gu et al. [49]
<i>Cymodocea nodosa</i>	GC-QTOF-MS	Trimethyl silylation	Growth promoting metabolites (sucrose, fructose, myo-inositol, heptacosane, tetracosane, stigmasterol, catechin and alpha-tocopherol) were lower close to the zone, whereas metabolites involved with stress-response (alanine, serine, proline, putrescine, ornithine, 3,4-dihydroxybenzoic acid and cinnamic acid) were identified	Metabolomic fingerprinting of seagrass provides opportunities for early detection of environmental degradation in marine ecological studies	Kock et al. [50]
<i>Halodule pinifolia</i>	GC-MS	Trimethylsilyl etherification	GC-MS analysis revealed the presence of thirty-five compounds which include flavonoids, sugars, amino acids and plant hormones	Study has explored a newer marine source, <i>H. pinifolia</i> for RA, which is an emerging potential preclinical chemical entity	Jeyapragash et al. [51]
<i>Zostera marina</i>	GC-MS	Trimethyl silylation	Decreased carbohydrate decomposition products and tricarboxylic acid (TCA) cycle intermediate products, indicating that the energy supply of the eelgrass may be insufficient at high temperature	composition of the membrane system of eelgrass may change at high temperature and implying that high temperature may cause the membrane system to be unstable	Gao et al. [52]
<i>Halodule pinifolia</i>	GC-MS	Trimethyl silylation	98 metabolites in wild and 125 metabolites in SCC were identified. 77 primary and secondary metabolism pathways in wild, while 73 metabolism pathways in SCC were reported	Baseline information on <i>H. pinifolia</i> metabolism in the marine and artificial environments	Jeyapragash et al. [53, 54]

Seagrass	Methods used	Derivation method	Results	Potential application	Reference
<i>Zostera muelleri</i>	NMR	Trimethyl-silylation	Several potential bioindicators of low-light stress: a reduction of soluble sugars and their derivatives, glucose, fructose, sucrose and myo-inositol, N-methylnicotinamide, organic acids and various phenolic compounds were identified	Metabolomics measurements may be useful bio-indicators of low-light stress in seagrass	Griffith et al. [55]
<i>Halodule pinifolia</i>	GC-MS	Trimethyl-Silylation	Three thermo-protective metabolites such as trehalose (sugar), glycine betaine (amino acid) and methyl vinyl ketone (organic acid) were profiled from <i>H. pinifolia</i> (45°C) and is the first report on the occurrence of glycine betaine and methyl vinyl ketone from seagrasses and other aquatic species so far	Facilitate the further research on identifying gene to metabolite networks for an effective management of seagrass conservation by genetic manipulation	Jeyapragash et al. (2021)

Table 3.
 Status of metabolomics studies in seagrasses.

Primary metabolites from seagrasses reported to be similar, to that of any other terrestrial angiosperms [56]. Despite the higher phenol content, seagrasses found to be rich source of protein which alleviates the chronic problem of protein deficiency in developing countries like India [47]. In addition, seagrasses are a rich source of secondary metabolites such as simple phenolic compounds, phenylmethane and phenylethane derivatives, flavonoid and volatile derivatives with high commercial value [38]. Jeyapragash et al., reported that the plant growth regulators enhance the production of flavonoid production in the callus and cellular suspension cultured cells of seagrass *H. pinifolia* (**Figure 2**) Though, quantum of research has been published with respect to metabolome of seagrasses, there is still a dearth of knowledge as compared to the terrestrial plants. Seagrass offers remarkable opportunities to derive new commercially valuable phytochemicals when compared to algae [57, 58]. Therefore, the metabolite content from seagrasses constitutes another treasure of the ocean. Henceforth, the knowledge on metabolomics analysis from seagrass is decisive for understanding the complete metabolite picture and to explore their bioactive properties.

Seagrasses, the only higher plants solely living in the marine habitats and are ultimate importance for marine ecological systems close to the shorelines. Several studies dealt with the function of seagrasses as primary producers, shelter and food for fish, turtles and invertebrates as well as spawning areas for these organisms [59–61]. The reviews existing on seagrasses with different focus than the present one deal in more detail with other aspects of the ecological role of seagrasses,

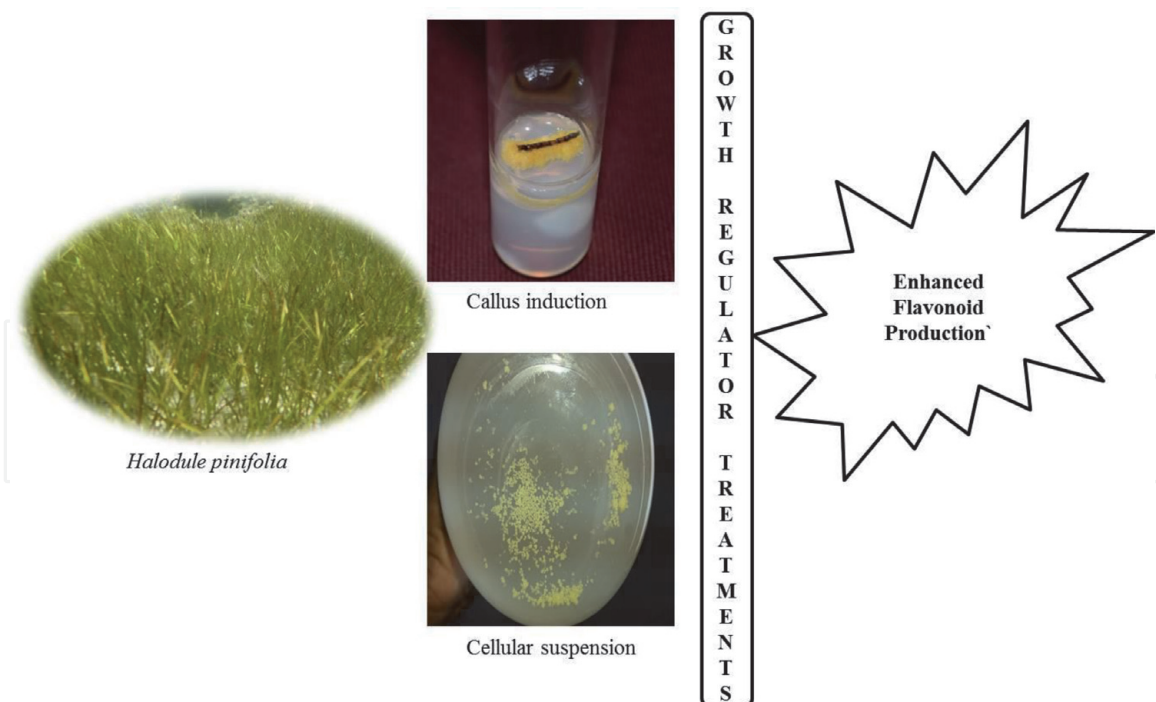


Figure 2. Growth regulators mediated flavonoid production in callus and cellular suspension of *H. pinifolia*.

particular the metabolite classes which are very few and primitive. Seagrasses reported to share the most features of primary and secondary metabolites with respect from the Alismatales order which live in land and freshwater habitats [62]. Kannan and Kannal [63] and Pradheeba et al. [56] reported that primary metabolites such as carbohydrate, protein and lipid content from seagrasses acts as a rich source of nutritional value and was evidenced by the obvious increase in the carbohydrate content of *E. acoroides*, *T. hemporichii* [63], *C. rotundata* [56] and lipid content of *C. nodosa* [64]. Higher protein content from seagrasses have also been reported in *E. acoroides* [63], *Ruppia cirrhosa* [64], *C. serrulata*, *S. isoetifolium*, *H. ovalis*, *H. pinifolia* [47], *C. rotundata* and *H. uninervis* [56] that are found to accumulate maximum concentration than the seaweeds [47].

Secondary metabolism occurs in seagrasses depends on the season and environmental conditions and was reported as a rich source of diverse natural products from simple to conjugated phenolic compounds such as phenolic acids, flavones, tannins and lignins [65, 66]. It was also reported that *P. oceanica* harbors compounds ranging from simple phenol derivatives, phenylmethane, phenylethane and phenylpropane derivatives [67]. Athiperumalsami et al. [47] reported the occurrence and absence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, saponins, quinones, tannins and secondary metabolites in the tropical seagrass *Cymodocea serrulata*, *Halodule pinifolia* and *Syringodium isoetifolium*. Heglmier and Zindron [62] reported 51 natural products in *P. oceanica* that includes phenols, flavones, phenylmethane, ethane derivatives. Though reports available on the seagrass derivatives, limited study dealt with the chemistry of secondary metabolites of seagrasses [62, 68]. The list of metabolites profiled and the metabolic pathways from wild seagrasses are tabulated in **Table 4** and **Figure 3**.

The presence of sulphated flavones was reported to be accumulated in *Halophila* and *Thalassia* species [69] and in *Z. marina* [70], but not found in *Syringodium* spp. and *P. oceanica*. Mc Millan [71] found the presence of either flavones or phenolic acid in 43 seagrass species while the sulphated flavones found specifically in five species namely, *Zostera*, *Phyllospadix*, *Enhallus*, *Thalassia* and *Halophila* species.

Compound Name	Molecular Formula	Molecular weight (g/ mol)	Exact Mass (g/mol)
D-Glucose	C ₆ H ₁₂ O ₆	180.156	180.063
Maltose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
D-Fructose	C ₆ H ₁₂ O ₆	180.156	180.063
Sucrose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
Inositol	C ₆ H ₁₂ O ₆	180.156	180.063
Methyl alpha-D-Glucopyranose	C ₇ H ₁₄ O ₆	194.183	194.079
D-Galactose	C ₆ H ₁₂ O ₆	180.156	180.063
Lactose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
L-Rhamnose	C ₆ H ₁₂ O ₅	164.157	164.068
D-Ribose	C ₅ H ₁₀ O ₅	150.13	150.053
Adenosine-2':3'- cyclic monophosphate	C ₁₀ H ₁₄ N ₅ O ₇ P	347.224	347.063
N-Acetyl-Î-D-glucosamine	C ₈ H ₁₅ NO ₆	221.209	221.09
Aspartyl-Leucine	C ₁₀ H ₁₈ N ₂ O ₅	246.263	246.122
Glycine	C ₂ H ₅ NO ₂	75.067	75.032
Threonine	C ₄ H ₉ NO ₃	119.12	119.058
Valine	C ₅ H ₁₁ NO ₂	117.148	117.079
Proline	C ₅ H ₉ NO ₂	115.132	115.063
Alanine	C ₃ H ₇ NO ₂	89.094	89.048
Thiamine	C ₁₂ H ₁₇ N ₄ OS ⁺	265.355	265.112
Methionine	C ₅ H ₁₁ NO ₂ S	149.208	149.051
Phenylalanine	C ₉ H ₁₁ NO ₂	165.192	165.079
Tyrosine	C ₉ H ₁₁ NO ₃	181.191	181.074
Methyl Pyroglutamate	C ₆ H ₉ NO ₃	143.142	143.058
Glutamic acid	C ₅ H ₉ NO ₄	147.13	147.053
Vanillic acid	C ₈ H ₈ O ₄	168.148	168.042
Oxalic acid	C ₂ H ₂ O ₄	90.034	89.995
gamma-Aminobutyric acid	C ₄ H ₉ NO ₂	103.121	103.121
Citrate	C ₆ H ₅ O ₇ ⁻³	189.099	189.004
Stearic acid	C ₁₈ H ₃₆ O ₂	284.484	284.272
Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	257.422	257.244
Potassium Gluconate	C ₆ H ₁₁ KO ₇	234.245	234.014
Nicotinic acid	C ₆ H ₅ NO ₂	123.111	123.032
Phosphoric acid	H ₃ PO ₄	97.994	97.977
Sodium Pyrophosphate	Na ₄ P ₂ O ₇	265.9	265.871
Acetamide	C ₂ H ₅ NO	59.068	59.037
Decanedioic acid	C ₁₂ H ₂₂ O ₄	230.304	230.152
Indoleacetic acid	C ₁₀ H ₉ NO ₂	175.187	175.063
1-Napthaleneacetic acid	C ₁₂ H ₁₀ O ₂	186.21	186.068

Compound Name	Molecular Formula	Molecular weight (g/ mol)	Exact Mass (g/mol)
4-Hydroxybenzaldehyde	C ₇ H ₆ O ₂	122.123	122.037
2,4-dihydroxybenzaldehyde	C ₇ H ₆ O ₃	138.122	138.032
3,4-Dihydroxybenzoic	C ₇ H ₆ O ₄	154.121	154.027

Table 4.
List of metabolites identified from wild seagrasses.

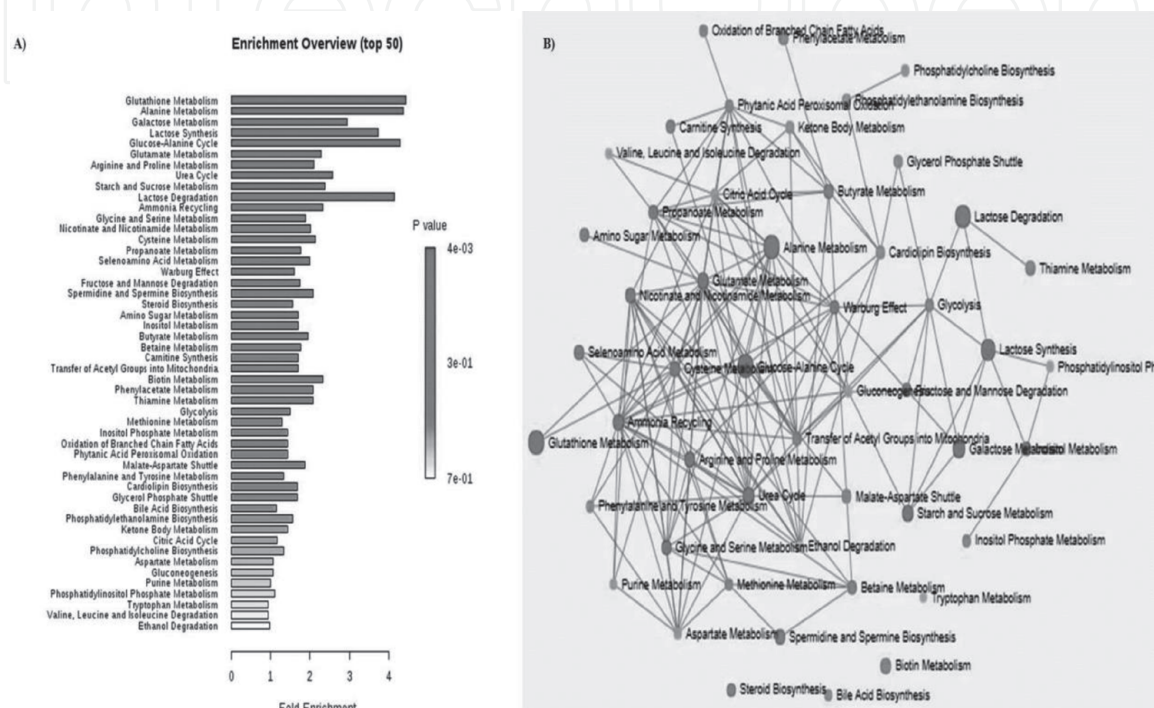


Figure 3.
Distribution of metabolic pathways of differential metabolites derived from wild seagrass (A) and their active networks (B) (adapted from [53, 54]).

The sulphated flavonoids have also been traced out in *Halophila ovalis* and *Thalassia testudinum* [72]. It was found that the flavonoid glycosides and acyl derivatives in *P. oceanica* and 15 flavonoid derivatives in *Halophila johnsonii* [73]. Similar to flavonoids and phenolic compounds, the sterol composition has also been reported from temperate seagrasses than the tropical seagrass. The presence of long chain fatty acids in *P. oceanica* [74] and α -hydroxy fatty acids in *Z. mulleri* [75]. The polar lipids and fatty acids from *Z. marina* and *Phyllospadix iwatensis* [76], phospholipids and glycolipids from *Z. marina* [77] and steroids, fatty acids from *Z. japonica* [78] confirms the prevalence of volatile compounds in seagrasses.

Jeyapragash et al. [53] investigated the systematic identification and characterization of metabolic changes in wild and SCC of *H. pinifolia* using GC-MS based metabolomics approach. It was found that the wild sample accumulated 98 metabolites, while SCC with 125 metabolites along with their relative abundance. The metabolites profiled from wild and SCC was used to map their biochemical pathways. Interestingly, the accumulated metabolites in wild were spanned with 77 primary and secondary metabolism pathways and 73 pathways in SCC. Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis confirmed insightful biochemical alterations occurred in wild and SCC such as glutathione metabolism, glutamate metabolism, phenylalanine and tyrosine metabolism in wild

with significant variations in citric acid metabolism, glycolysis, gluconeogenesis, oxidative phosphorylation related pathways of SCC respectively. Random forest analysis helped to group top 15 metabolites and their correct classification. The data obtained will provide baseline information on *H. pinifolia* metabolism in the marine and artificial environments. Furthermore, it helps to study the stress responsive mechanisms of seagrasses in the marine environment which would further aid in the dissection stress tolerance mechanism.

In addition, Jeyapragash et al. [79] reported the heat stress responsive metabolomics analysis of seagrass *H. pinifolia* in the marine environment. Since, ocean warming is a major global concern in the marine environment, the study focussed to understand the tolerance mechanism of seagrass *Halodule pinifolia* under temperature stress (24, 29, 37, and 45°C) using OMICS approach. Ecophysiological responses such as net photosynthesis ($\Delta F/F'm$) and dark respiration (F_v/F_m) were also studied. Results found that photosynthetic efficiency ($\Delta F/F'm$) significantly reduced due to heat stress, while the rate of dark respiration rate increased as compared to the control (24°C), respectively. Metabolomics study revealed that heat stress could cause huge metabolic alterations with respect to sugar, amino acids and organic acids. Interestingly, thermo-protective compounds such as trehalose (sugar), glycine betaine (amino acid) and methyl vinyl ketone (organic acid) were accumulated from *H. pinifolia* (45°C) which was the first report on the occurrence of glycine betaine and methyl vinyl ketone from seagrasses and other aquatic species so far. These findings would help the research groups to focus on the gene to metabolite networks mechanism for an effective management of seagrass conservation by genetic manipulation.

6. Seagrass cell suspension culture

Plants are considered as the factories of chemical compounds produced in order to carry out their biochemical pathways for survival and propagation [80]. All plants produce secondary metabolites which gained importance in pharmaceutical applications since ancient periods. The plant-based drug discovery gained importance with the development of anti-infectious and anti-cancer drugs which contributes to new bioactive molecules that are being isolated for the treatment of other diseases such as diabetes and obesity [81]. However, the important plant derived drugs are obtained commercially by the extraction from their respective plants. Currently, the natural plant habitats are vanishing due to environmental and geopolitical instabilities and so making it very difficult to procure important secondary metabolites and in the process many potential bioactive compounds have been left undiscovered. Plant cell culture is considered as a promising alternative approach for producing the bioactive compounds that are challenging to be obtained by chemical synthesis or plant extraction [82]. Plant cell culture studies have been carried out on the basis of the totipotent nature, in which the cell has the full set of genes necessary for secondary metabolisms [83]. The production of secondary metabolite via plant tissue culture have been commercialized since late 1950s, when atropine from the roots of *Atropa belladonna* was accumulated in roots and callus [84]. The list of metabolites profiled and their metabolic pathways from SCC of seagrasses were shown in **Figure 4** and tabulated in **Table 5**.

Improved plant cell culture techniques made possible to increase the target metabolite production under *in-vitro* conditions. However, agronomically focussed biotechnology is capable to make use of plant tissue culture; such methods are not usually available to marine biologist and notably, *in-vitro* culture of seagrasses has been far more problematic due to lack of the suitable culturing conditions [85].

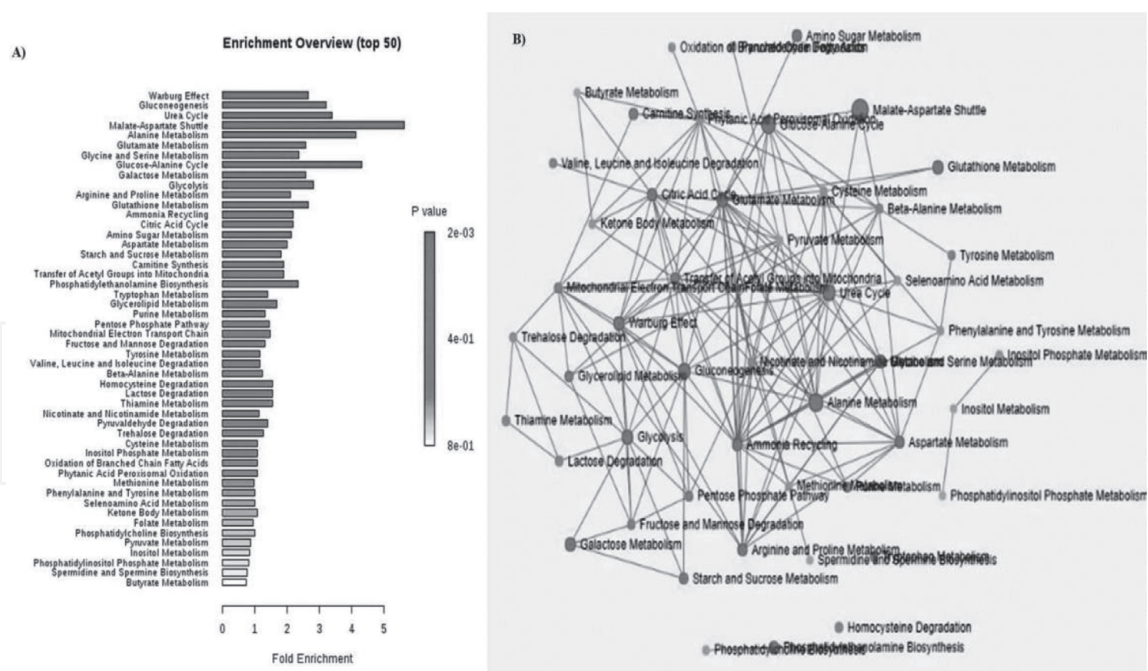


Figure 4. Distribution of metabolic pathways of differential metabolites derived from SCC of seagrass (A) and their active networks (B) (adapted from [53, 54]).

Available literature has brought that 14% of seagrasses are at an elevated risk of extinction under IUCN red list of threatened species [86]. Though numerous studies have been addressed the in-vitro culture of seagrass [38, 87–93], no report exist on the metabolomics analysis from suspension cultured cells of seagrass. *In-vitro* propagation techniques of different seagrass species for restoration and protocol for seagrass protoplast isolation are prevailing [94–98]. Among all these protocols, Carpeneto *et al.* [96] was successful in the cell wall regeneration of protoplast from *Posidonia oceanica* and *Cymodocea nodosa*. Recently, establishment of cell suspension culture has been achieved in *C. nodosa* [99], *Halodule pinifolia*, *C. rotundata* and *C. serrulata* [38]. Jeyapragash *et al.*, [53, 54] reported that the metabolites synthesized from seagrass *H. pinifolia* in the marine and artificial environment will be highly similar with a total of 98 metabolites in wild and 125 metabolites in SCC respectively and however the cellular suspension accumulated the higher content. The study suggested that the use of seagrass cellular suspension for metabolomics engineering will provide a new facet for novel metabolite identification and characterization. Nevertheless, there lay famine knowledge in the metabolite accumulation pattern and their different biotechnological and pharmaceutical applications. Enhancement of metabolite biosynthesis can also be achieved via the precursors or elicitor treatments of plant cells (**Figure 5**). Precursors are the compound which act as intermediate in or at the beginning of biosynthetic route, treatments using the same stands a good chance of increasing the yield of the desired product. Exogenous supply of biosynthetic precursors to the culture medium induces the high yield of targeted products (Whitmer *et al.*, 1998; [100]).

Plant also synthesizes the secondary metabolites to protect themselves in response to various environmental stresses. It might be physical, chemical or a biological factor which induces the higher secondary metabolism known as elicitors. The use of elicitors in cell suspension cultures has been developed to enhance the yield of secondary metabolites, wherein elicitation of target compounds can be induced by the addition of trace number of elicitors [101]. Biotic and abiotic

Query	Match	HMDB	PubChem
Rosmarinic acid	C ₁₈ H ₁₆ O ₈	360.318	360.085
Caffeic acid	C ₉ H ₈ O ₄	180.159	180.042
p-Coumaric acid	C ₉ H ₈ O ₃	164.16	164.047
Protocatacheuic acid	C ₇ H ₆ O ₄	154.121	154.027
p-Anisic acid	C ₈ H ₈ O ₃	152.149	152.047
Vanillic acid	C ₈ H ₈ O ₄	168.148	168.042
Naringenin	C ₁₅ H ₁₂ O ₅	272.256	272.068
4-hydroxybenzoic acid	C ₇ H ₆ O ₃	138.122	138.032
Fructose-6-phosphate	C ₆ H ₁₃ O ₉ P	260.135	260.03
Glucose-6-phosphate	C ₆ H ₁₃ O ₉ P	260.135	260.03
Glucose	C ₆ H ₁₂ O ₆	180.156	180.063
Phosphoenol pyruvic acid	C ₃ H ₅ O ₆ P	168.041	167.982
Pyruvic acid	C ₃ H ₄ O ₃	88.062	88.016
Citric acid	C ₆ H ₅ O ₇ ⁻³	189.099	189.004
Fumaric acid	C ₄ H ₄ O ₄	116.072	116.011
3-PGA	C ₃ H ₇ O ₇ P	186.056	185.993
Ketoglutaric acid	C ₅ H ₆ O ₅	146.098	146.022
Malic acid	C ₄ H ₆ O ₅	134.087	134.022
Succinic acid	C ₄ H ₆ O ₄	118.088	118.027
Mannose	C ₆ H ₁₂ O ₆	180.156	180.063
Oxaloacetic acid	C ₄ H ₄ O ₅	132.071	132.071
Sucrose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
D-Fructose	C ₆ H ₁₂ O ₆	180.156	180.063
Raffinose	C ₁₈ H ₃₂ O ₁₆	504.438	504.169
Trehalose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
Turanose	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
Mannitol	C ₆ H ₁₄ O ₆	182.172	182.079
Inositol	C ₁₂ H ₂₂ O ₁₁	342.297	342.116
Xylitol	C ₅ H ₁₂ O ₅	152.146	152.146
Alanine	C ₃ H ₇ NO ₂	89.094	89.048
Asparagine	C ₄ H ₈ N ₂ O ₃	132.119	132.053
Aspartic acid	C ₄ H ₇ NO ₄	133.103	133.038
Glutamic acid	C ₅ H ₉ NO ₄	147.13	147.053
Glycine	C ₂ H ₅ NO ₂	75.067	75.032
Proline	C ₅ H ₉ NO ₂	115.132	115.063
Serine	C ₃ H ₇ NO ₃	105.093	105.043
Threonine	C ₄ H ₉ NO ₃	119.12	119.058
Valine	C ₅ H ₁₁ NO ₂	117.148	117.079
2,4-dihydroxybenzoic acid	C ₇ H ₆ O ₃	138.122	138.032
2-hydroxybutyric acid	C ₄ H ₈ O ₃	104.105	104.047

Query	Match	HMDB	PubChem
Gamma-aminobutyric acid	C ₄ H ₉ NO ₂	103.121	103.121
Dimethylamine	(CH ₃) ₂ NH	45.085	45.058
Ethanolamine	C ₂ H ₇ NO	61.084	61.053
Thiamine	C ₁₂ H ₁₇ N ₄ OS ⁺	265.355	265.112
Nicotinic acid	C ₆ H ₅ NO ₂	123.111	123.032
Pyridoxine	C ₈ H ₁₁ NO ₃	169.18	169.074
Phenylalanine	C ₉ H ₁₁ NO ₂	165.192	165.079
Tryrosine	C ₉ H ₁₁ NO ₃	181.191	181.074
Shikimic acid	C ₇ H ₁₀ O ₅	174.152	174.053
Acotinic acid	C ₆ H ₆ O ₆	174.108	174.016
Xylonic acid	C ₅ H ₁₀ O ₆	166.129	166.048
Ascorbic acid	C ₆ H ₈ O ₆	176.124	176.032
Guanine-2'3'-cyclic monophosphate	C ₁₀ H ₁₂ N ₅ O ₇ P	345.208	345.047
Pantothenate	C ₉ H ₁₆ NO ₅	218.229	218.103
Sphingosine	C ₁₈ H ₃₇ NO ₂	299.499	299.28
N-acetylglucosamine	C ₈ H ₁₅ NO ₆	221.209	221.09
Aspartyl leucine	C ₁₀ H ₁₈ N ₂ O ₅	246.263	246.122
2-hydroxy glutaric acid	C ₅ H ₈ O ₅	148.114	148.037
Glyceric acid	C ₃ H ₆ O ₄	106.077	106.027
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.311	354.095
Rhamnose	C ₆ H ₁₂ O ₅	164.157	164.068
Guanosine monophosphate	C ₁₀ H ₁₅ N ₅ O ₁₁ P ₂	443.202	443.024
Ribose	C ₅ H ₁₀ O ₅	150.13	150.053
Adenosine-2'3'-cyclic Monophosphate	C ₁₀ H ₁₄ N ₅ O ₇ P	347.224	347.063
Dihydroquercetic acid	C ₁₅ H ₁₂ O ₇	304.254	304.058
Adenosine-2-Monophosphate	C ₁₀ H ₁₄ N ₅ O ₇ P	347.224	347.063
p-hydroxybenzoic acid	C ₇ H ₆ O ₃	138.122	138.032
Quinic acid	C ⁷ H ¹² O ⁶	192.167	192.063
Tryptophan	C ¹¹ H ¹² N ² O ²	204.229	204.09
Pyroglutamic acid	C ₅ H ₇ NO ₃	129.115	129.043
Salicylic acid	C ₇ H ₆ O ₃	138.122	138.032
Methionine	C ₅ H ₁₁ NO ₂ S	149.208	149.051
Lactic acid	C ₃ H ₆ O ₃	90.078	90.032
Isovaleric acid	C ⁵ H ¹⁰ O ²	102.133	102.068
2-oxyglutaric acid	C ₅ H ₆ O ₅	146.098	146.022
2-hydroxyisobutyric acid	C ₄ H ₈ O ₃	104.105	104.047
1-methylnicotinic acid	C ₇ H ₈ NO ₂ ⁺	138.146	138.056
Hypoxanthine	C ₅ H ₄ N ₄ O	136.114	136.039

Query	Match	HMDB	PubChem
Indole Acetic acid	C ₁₀ H ₉ NO ₂	175.187	175.063
Naphthelene acetic acid	C ₁₂ H ₁₀ O ₂	186.21	186.068

Table 5.
 List of metabolites identified from suspension cultured cells seagrasses.

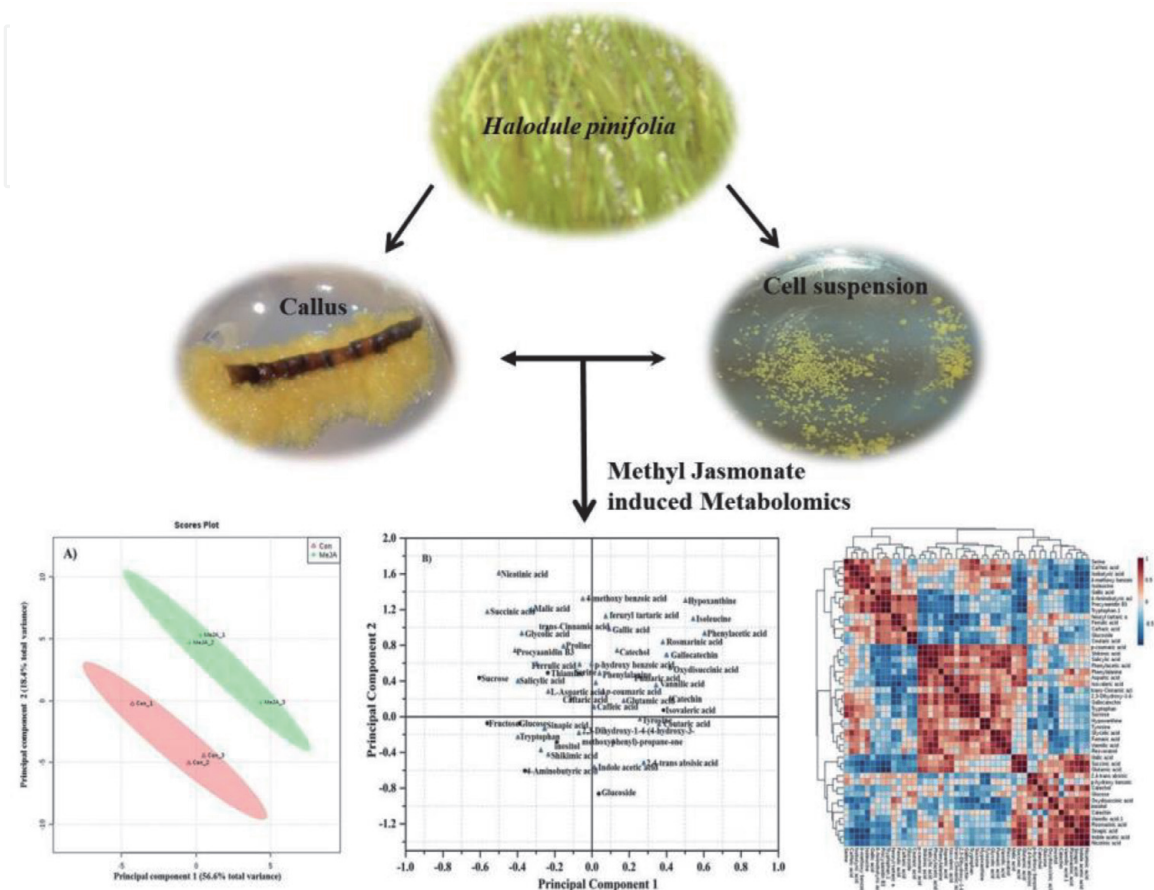


Figure 5.
 Elicitor induced comparative metabolomics of wild and cellular suspension of seagrass.

elicitors are available which depends on the target compounds that need to be synthesized.

7. Seagrasses—a source for marine based drug discovery

Ravn *et al.* [102] reported that phenolic acids such as *p*-coumaric acid, caffeic acid, ferulic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, gentisic acid found predominant in *Halophila ovalis*, *Thalassia hemprichii*, *Halodule* sp., *Cymodocea* spp., *Enhalus acoroides*, *Syringodium isoetifolium* and other seagrass species. In addition, the pharmaceutically potent rosmarinic acid has been isolated from *Z. marina* [102] *Z. noltii* [103], *H. pinifolia* [51] and also from the detritus of *Z. noltii* and *Z. marina* [57]. Along with rosmarinic acid, caffeic acid and chlorogenic acid found to be present in some trace amounts, since caffeic acid acts as a precursor for rosmarinic acid in the shikimic acid biosynthesis. It was also been reported that the predominance of caffeic acid was accumulated in the leaves of *P. oceanica* [104] and *Thalassodendron ciliatum* [105]. Jeyapragash *et al.* [51], profiled 45 metabolites

from *Halodule pinifolia*, which includes caffeic acid, coumaric acid, chlorogenic acid and rosmarinic acid which found predominant than other compounds. Recently, biofilm associated, multidrug resistant *Pseudomonas aeruginosa* infection remain a challenging problem in the clinical field since the conventional antibiotic therapy are largely inefficient and new approaches are needed. Inactivating the QS virulence mechanism with anti-infective agent is an attractive approach to prevent bacterial infections without resistance development [54]. Seagrass *Halodule pinifolia* (Miki) Hartog has been shown to exhibit potential antimicrobial activities against *P. aeruginosa* PAO1. Preliminary screening on antibiofilm activity showed that the methanolic extract of *H. pinifolia* exhibited potential inhibition of biofilm formation (96%) as compared to the control respectively. Eight bioactive compounds such as 4-hydroxybenzoic acid, rosmarinic acid, 4-methoxybenzoic acid, *p*-coumaric acid, protocatechuic acid, caffeic acid, naringenin, vanillic acid, were profiled. Of these compounds, 4-methoxybenzoic acid (4-MBA) showed maximum bacterial growth inhibition that act as a lead molecule with minimum inhibitory concentration (MIC). Furthermore, 4-MBA at MIC concentration reduced the virulence factors and down regulated the level of QS mediated virulence transcripts. The study suggests that seagrasses may act as a newer source for the marine based drug discovery and may act as anti-infective agent against biofilm-mediated harmful pathogens.

8. Conclusion

To summarize, experiments in seagrass metabolomics to date helped us to validate a vast array of metabolites and their alterations in response to various stress mechanisms. This approach has previously enabled to recognize a large number of metabolites whose accumulation is affected upon the exposure of organisms under stress conditions. Nevertheless, despite the many advancements that have been achieved in this field, much work is still needed to identify the seagrass metabolites and their novel metabolic pathways connected to stress response and their tolerance mechanism and to interpret the extensive organization and interaction among gene to metabolite networks. This chapter provides knowledge on the systematic identification and metabolic characterization of seagrass metabolites using metabolomics approach. The bioactive potential of compounds derived from seagrasses paves a way to lead as potential inhibitors of many harmful pathogens in the pharmaceutical sectors and therefore, seagrass explored as newer marine source for the development of plant-based drugs. Further, in-vitro cultures of seagrass afford an alternate model for the up-regulation of enhanced bioactive compound synthesis. Moreover, various stress related metabolomics approach of wild seagrasses should be studied in order to derive diverse group of bioactive metabolites as much as possible, so as to fill the knowledge gap of seagrass metabolites and step forward towards the commercialization of bioactive natural products from seagrasses.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Abbreviations

GC-MS	Gas Chromatography-Mass Spectrometry
LC-MS	Liquid Chromatography Mass Spectrometry
FT-ICR-MS	Fourier Transform- Ion Cyclotron Resonance-Mass Spectrometry
SCC	suspension cultured cells
NMR	Nuclear Magnetic Resonance
4-MBA	4-methoxy benzoic acid
MIC	minimum inhibitory concentration
KEGG	Kyoto Encyclopaedia of Genes and Genomes
IUCN	International Union for conservation of Nature
CE-MS	Capillary Electrophoresis-Mass Spectrometry

Author details


Danaraj Jeyapragash^{1*}, Ayyappan Saravanakumar² and Mariasingarayan Yosuva²

¹ Department of Biotechnology, Karpagam Academy of Higher Education (Deemed to be University), Eachanari, Coimbatore, Tamil Nadu, India

² Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India

*Address all correspondence to: pragashdb@gmail.com

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References

- [1] Pauling L, Robinson AB, Teranishi R, Cary P. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proceedings of the National Academy of Sciences of the United States of America. 1971;68:2374-2376. DOI: 10.1073/pnas.68.10.2374.
- [2] Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature. 2000;408:796-815. DOI: 10.1038/35048692
- [3] Fowler S, Thomashow MF. Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell. 2002;14(8):1675-1690. DOI: 10.1105/tpc.003483.
- [4] Froehlich JE, Wilkerson CG, Ray WK, McAndrew RS, Osteryoung KW, Gage DA, Phinney BS. Proteomic study of the *Arabidopsis thaliana* chloroplast envelope membrane utilizing alternatives to traditional two-dimensional electrophoresis. Journal of Proteome Research. 2003;2:413-425. DOI: 10.1021/pr034025j.
- [5] Hajduch M, Ganapathy A, Stein JW, Thelen JJ. A systematic proteomic study of seed filling in soybean. Establishment of high-resolution two-dimensional reference maps, expression profiles, and an interactive proteome database. Plant Physiology. 2005;137:1397-1419. DOI: 10.1104/pp.104.056614.
- [6] Santoni VJ, Vingh J, Pflieger D, Sommerer N, Maurel C. A proteomic study reveals novel insights into the diversity of aquaporin forms expressed in the plasma membrane of plant roots. Biochemical Journal. 2003;373:289-296. DOI: 10.1042/BJ20030159.
- [7] Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: From diagnostics to systems biology. Nature Reviews Molecular Cell Biology. 2004;5:763-769. DOI: 10.1038/nrm1451.
- [8] Jorge TF, Mata AT, António C. Mass spectrometry as a quantitative tool in plant metabolomics. Philosophical transactions. Philosophical Transactions of the Royal Society A. 2016;374:20150370. DOI: 10.1098/rsta.2015.0370.
- [9] Oksman-Caldentey KM, Inze D. Plant cell factories in the post-genomic era: New ways to produce designer secondary metabolites. Trends in Plant Science. 2004;9:433-440. DOI: 10.1016/j.tplants.2004.07.006
- [10] Fiehn O, Kopka J, Dörmann P, Altmann T, Trethewey RN, Willmitzer L. Metabolite profiling for plant functional genomics. Nature Biotechnology. 2000; 18(11):1157-1161.
- [11] Hall RO, Bernhardt ES, Likens GE. Relating nutrient uptake with transient storage in forested mountain streams. Limnology and Oceanography. 2002;47:255-265. DOI: 10.4319/lo.2002.47.1.0255.
- [12] Seger C, Sturm S. Analytical aspects of plant metabolite profiling platforms: Current standings and future aims. Journal of Proteome Research. 2007;6:480-497. DOI: 10.1021/pr0604716.
- [13] Sumner LW, Lei Z, Nikolau BJ, Saito K. Modern plant metabolomics: Advanced natural product gene discoveries, improved technologies, and future prospects. Natural Product Reports. 2015;32:212-229. DOI: 10.1039/c4np00072b.
- [14] Nicholson JK, Lindon JC, Holmes E. Metabonomics: Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data.

- Xenobiotica.1999;29:1181-1189. DOI: 10.1080/004982599238047.
- [15] Olivier DK, Van Wyk BE, Van Heerden FR. The chemotaxonomic and medicinal significance of phenolic acids in *Arctopus* and *Alepidea* (Apiaceae subfamily Saniculoideae). *Biochemical Systematics and Ecology*. 2008;36:724-729. DOI: 10.1016/j.bse.2008.07.003.
- [16] Fiehn O. Combining genomics, metabolome analysis, and biochemical modeling to understand metabolic networks. *Comparative and Functional Genomics*. 2001;2(3): 155-168. DOI: 10.1002/cfg.82.
- [17] Fiehn O. Metabolomics-the link between genotypes and phenotypes. *Plant Molecular Biology*. 2002;48(1-2): 155-171. DOI:10.1023/A:1013713905833.
- [18] Hill CB, Roessner U. Metabolic profiling of plants by GC-MS. *The Handbook of Plant Metabolomics* (eds. W. Weckwerth and G. Kahl), Wiley; 2013.1-23pp. DOI: 10.1002/9783527669882.ch1.
- [19] Hirai MY, Saito K. Post-genomics approaches for the elucidation of plant adaptive mechanisms to Sulphur deficiency. *Journal of Experimental Botany*. 2004;55(404):1871- 1879. DOI: 10.1093/jxb/erh184.
- [20] Roessner U, Willmitzer L, Fernie AR. High-resolution metabolic phenotyping of genetically and environmentally diverse potato tuber systems. Identification of phenocopies. *Plant Physiology.*, 2001;127:749-764. DOI: 10.1104/pp.010316
- [21] Soga Y, Ueno Y, Naraoka H, Ohashi Y, Tomita M, Nishioka T. Simultaneous determination of anionic intermediates for *Bacillus subtilis* metabolic pathways by capillary electrophoresis electrospray ionization mass spectrometry. *Analytical Chemistry*. 2002;74:2233-2239. DOI: 10.1021/ac020064n.
- [22] Lindon JC, Holmes E, Nicholson JK. Pattern recognition methods and applications in biomedical magnetic resonance. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2001;39:1-40. DOI: 10.1016/S0079-6565(00)00036-4.
- [23] Wasim M, Hassan MS, Brereton RG. Evaluation of chemometric methods for determining the number and position of components in high-performance liquid chromatography detected by diode array detector and on flow H-1 nuclear magnetic resonance spectroscopy. *Analyst*.2003;128:1082-1090. DOI: 10.1039/B304313D.
- [24] Meiler J, Will M. Genius: A genetic algorithm for automated structure elucidation from C-13 NMR spectra. *Journal of the American Chemical Society*.2002;124:1868-1870. DOI: 10.1021/ja0109388.
- [25] Halket JM, Zaikin VG. Derivatization in mass spectrometry – 1 Silylation. *European Journal of Mass Spectrometry*.2003; 9:1-21. DOI: 10.1255/ejms.527.
- [26] Sumner LW, Mendes P, Dixon RA. Dixon, plant metabolomics: Large-scale phytochemistry in the functional genomics era. *Phytochemistry*. 2003;62: 817-836. DOI: 10.1016/s0031-9422(02)00708-2.
- [27] Roessner U, Wagner C, Kopka J, Trethewey RN, Willmitzer L. Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *The Plant Journal*.2000;23:131-142. DOI: 10.1046/j.1365-313x.2000.00774.x.
- [28] Roessner U, Willmitzer L, Fernie AR. Metabolic profiling and biochemical phenotyping of plant systems. *Plant Cell Reports*.2002;21:189-196. DOI: 10.1007/s00299-002-0510-8.
- [29] Aharoni A, de Vos CH, Verhoeven HA, Maliepaard CA, Kruppa G, Bino RJ,

- Goodenowe DB. Non-targeted metabolome analysis by use of Fourier transform ion cyclotron mass spectrometry. *OMICS A Journal of Integrative Biology*. 2002;6(3):217-234. DOI: 10.1089/15362310260256882
- [30] Plumb RS, Stumpf CL, Granger JH, Castro-Perez J, Haselden JN, Dear GJ. Use of liquid chromatography/time-of-flight mass spectrometry and multivariate statistical analysis shows promise for the detection of drug metabolites in biological fluids. *Rapid Communications in Mass Spectrometry*. 2003;17:2632-2638. DOI: 10.1002/rcm.1250.
- [31] Swart PJ, Bronner GM, Bruins AP, Ensing K, Tepper PG, De Zeeuw RA. HPLC-UV atmospheric-pressure ionization mass-spectrometric determination of the dopamine-D2 agonist N-0923 and its major metabolites after oxidative-metabolism by rat-liver, monkey liver, and human liver Microsomes. *Toxicology Methods*. 1993;3:279-290. DOI: 10.3109/15376519309068445.
- [32] Taylor J, King RD, Altmann T, Fiehn O. Application of metabolomics to plant genotype discrimination using statistics and machine learning. *Bioinformatics*. 2002;18:241- 248. DOI: 10.1093/bioinformatics/18.suppl_2.s241.
- [33] Unger M, Dreyer M, Specker S, Laug S, Pelzing M, Neusüss C, Holzgrab U, Bringmann G. Analytical characterisation of crude extracts from an African *Ancistrocladus* species using high-performance liquid chromatography and capillary electrophoresis coupled to ion trap mass spectrometry. *Phytochemical Analysis*. 2004;15:21-26. DOI: 10.1002/pca.737.
- [34] Hall J. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*. 2002;53(366):1-11. DOI: 10.1093/jexbot/53.366.1.
- [35] Knapp DR. *Handbook of Analytical Derivatization Reactions*. Wiley, One Wiley Drive, Somerset, NJ 08873;1979. 741 pp. DOI: 10.1002/jps.2600690549.
- [36] Grob R, Barry E. *Modern Practice of Gas Chromatography*. Wiley Inter science; 2004. 817 - 818 pp.
- [37] Papenbrock J. Highlights in Seagrasses Phylogeny, Physiology, and Metabolism: What Makes them Special? *ISRN Botany*. 2012. 15 p. DOI: 10.5402/2012/103892.
- [38] Subhashini P, Dilipan E, Thangaradjou T, Papenbrock J. Bioactive natural products from marine angiosperms: Abundance and functions. *Natural Products and Bioprospecting*. 2013;3:129-136. DOI: 10.1007/s13659-013-0043-6.
- [39] Hartog CD, Kuo J. Taxonomy and biogeography of seagrasses. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology, and Conservation*, Springer, Berlin; 2006. 1 - 23pp.
- [40] Les DH, Tippery NP. In time and with water the systematics of alismatid monocotyledons. In: Wilkin, P., Mayo, S.J. (Eds.), *Early Events in Monocot Evolution*. Cambridge University Press, Cambridge. 2013. 118 - 164 pp.
- [41] Bremer B, Bremer K, Chase MW, Fay MF, Reveal JL, Soltis DE, Soltis PS, Stevens PF, Anderberg AA, Moore, MJ, Olmstead RG, Rudall PJ, Sytsma KJ, Tank DC, Wurdack K, Xiang JQY, Zmarzty S. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*. 2009;161:105-121. DOI: 10.1111/j.1095-8339.2009.00996.x.
- [42] Browne CM, Milne R, Griffiths C, Bolton JJ, Anderson RJ. Epiphytic seaweeds and invertebrates associated with south African populations of the

rocky shore seagrass *Thalassodendron leptocaulis* - a hidden wealth of biodiversity. African Journal of Marine Science. 2013;35:523-531. DOI: 10.2989/1814232X.2013.864332.

[43] Duarte MC, Bandeira S, Romeiras MM. Systematics, and ecology of a new species of seagrass (*Thalassodendron*, Cymodoceaceae) from southeast African coasts. Novon A Journal for Botanical Nomenclature. 2012;22:16-24. DOI: 10.3417/2010079

[44] Mabberley DJ. Mabberley Plant-Book, 3rd edition. Cambridge University Press, Cambridge; 2008.

[45] Puglisi MP, Engel S, Jensen PR, Fenical W. Antimicrobial activities of extracts from indo-Pacific marine plants against marine pathogens and saprophytes. Marine Biology. 2007;150: 531-540. DOI: 10.1007/s00227-006-0376-3.

[46] Ross C, Puglisi MP, Paul VJ. Antifungal defenses of seagrasses from the Indian River lagoon. Florida. Aquatic Botany. 2008;88:134-141. DOI: 10.1016/j.aquabot.2007.09.003.

[47] Athiperumalsami T, Kumar V, Jesudass LL, Survey and phytochemical analysis of seagrasses in the Gulf of Mannar, southeast coast of India. Botanica Marina. 2008;51: 269-277. DOI: 10.1515/BOT.2008.038.

[48] Kumar CS, Sarada DVL, Gideon TP, Rengasamy R. Antibacterial activity of three south Indian seagrasses, *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capensis*. World Journal of Microbiology and Biotechnology. 2008; 24(9):1989-1992. DOI: 10.1007/s11274-008-9695-5.

[49] Gu J, Weber K, Klemp E, Winters G, Franssen SU, Wienpahl I, Huylmans AK, Zecher K, Reusch TB, Bornberg-Bauer E, Weber AP. Identifying core features of adaptive metabolic

mechanisms for chronic heat stress attenuation contributing to systems robustness. Integrative Biology. 2012;4 (5):480-493.doi: 10.1039/c2i b00109h.

[50] Kock WD, Hasler-Sheetal H, Holmer M, Tsapakis M, Apostolaki ET. Metabolomics and traditional indicators unveil stress of a seagrass (*Cymodocea nodosa*) meadow at intermediate distance from a fish farm. Ecological Indicators. 2012; 109:105765. DOI: 10.1016/j.ecolind. 2019.105765

[51] Jeyapragash D, Saravanakumar A, Begum S, Kumar S, Thangaradjou T. GC-MS based metabolomics analysis and occurrence of rosmarinic acid in the tropical seagrass *Halodule pinifolia*. International Journal of Pharmaceutical research. 2018;10(4):277-285. DOI: 10.31838/ijpr/2018.10.04.023.

[52] Gao Y, Jiang Z, Du M, Fang J, Jiang W, Fang J. Photosynthetic and metabolic responses of eelgrass *Zostera marina* L. to short-term high-temperature exposure. Journal of Oceanology and Limnology. 2018;1-11. DOI: 10.1007/s00343-019-7319-6.

[53] Jeyapragash D, Yosuva M, Karuppiah V. Seagrass *Halodule pinifolia* active constituent 4-methoxybenzoic acid (4-MBA) inhibits quorum sensing mediated biofilm and virulence production of *Pseudomonas aeruginosa*. Microbial Pathogenesis (Elsevier). 2020a;147:104392. DOI: 10.1016/j.micpath.2020.104392.

[54] Jeyapragash D, Yosuva M, Saravanakumar A. Comparative metabolomics analysis of wild and suspension cultured cells of seagrass *Halodule pinifolia*. Aquatic Botany (Elsevier). 2020b;167:103278. DOI: 10.1016/j.aquabot.2020.103278.

[55] Griffiths LL, Melvin SD, Connolly RM, Pearson RM, Brown CJ. Metabolomic indicators for low-light stress in seagrass. Ecological Indicators.

- 2020; 114:106316. DOI:10.1016/j.ecolind. 2020.106316
- [56] Pradheeba M, Dilipan E, Nobi EP, Thangaradjou T, Sivakumar K. Evaluation of seagrasses for their nutritional value. *Indian Journal of Geo-Marine Science*. 2011;40(1):105-111.
- [57] AchamLale S, Rezzonico B, Grignon-Dubois M. Evaluation of *Zostera detritus* as a potential new source of zosteric acid. *Journal of Applied Phycology*. 2009;21:347- 352. DOI: 10.1007/s10811-008-9375-8
- [58] Nuissier G, Diaba F, Dubois MG. Bioactive agents from beach waste: *Syringodium flotsam* evaluation as a new source of L-Chico-inositol. *Innovative Food Science and Emerging Technologies*.2008;9:396-400. DOI: 10.1016/j.ifset.2007.12.002.
- [59] Gillanders B.M.. Seagrasses, fish, and fisheries. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology, and Conservation*. Springer, Berlin; 2006. 503-536 pp.
- [60] Gobert S. Cambridge ML, Velimirov B, Pergent G, Lepoint G, Bouquegneau JM, Dauby P, Pergent-Martini C, Walker DI. *Biology of Posidonia*. In: Larkum, A. W. D., Orth, R. J., Duarte, C. M. (Eds.), *Seagrasses: Biology, Ecology, and Conservation*. Springer, Berlin; 2006. 387-408 pp. DOI: 10.1007/1-4020-2983-7_17.
- [61] Ruiz JM, Boudouresque CF, Enriquez S. Mediterranean seagrasses. *Botanica Marina*. 2009;52:369-381. DOI: 10.1515/BOT.2009.058.
- [62] Heglmeier A, Zidorn C. Secondary metabolites of *Posidonia oceanica* (Posidoniaceae). *Biochemical Systematics and Ecology*. 2010;38:964-970. DOI: 10.1016/j.bse.2010.07.001
- [63] Kannan R, Kannan L. Biochemical and caloric composition of seaweeds and seagrass of the Palk Bay, south east coast of India. In: VN Raja Rao, Anand Bishen Singh N, Mahendra Pal Singh Dehradun editors. *Algological Research in India: Festschrift*. In: , editors; 2002 471 p.
- [64] El-Hady HHA, Daboor SM, Ghoniemy AE. Nutritive and antimicrobial profiles of some seagrass from Bardawil Lake, Egypt, *Egyptian Journal of Aquatic Research*. 2007. 33 (3):103-110.
- [65] Arnold TM, Targett NM. Marine tannins: The importance of a mechanistic framework for predictive ecological roles. *Journal of Chemical Ecology*. 2002;28:1919-1934. DOI: 10.1023/a:1020737609151.
- [66] Vergeer LHT, Aarts TL, De Groot JD. The “wasting disease” and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquatic Botany*.1995;52:35-44. DOI: 10.1016/0304-3770(95)00480-N.
- [67] Haznedaroglu MZ, Zeybek U. HPLC determination of chicoric acid in leaves of *Posidonia oceanica*. *Pharmaceutical Biology*. 2007;45:745-748. DOI: 10.1080/13880200701585717.
- [68] Larkum AWD, Drew EA Ralph PJ. Photosynthesis in seagrasses. In: Larkum, A.W.D., Orth, R.J., Duarte, C. M. (Eds.), *Seagrasses: Biology, Ecology, and Conservation*. Springer, Berlin; 2006.159-192 pp.
- [69] Harborne JB, Williams CA. Occurrence of sulphated flavones and caffeic acid esters in members of Fluviales. *Biochemical Systematics and Ecology*. 1976;4:37-41. DOI: 10.1016/0305-1978(76)90007-7.
- [70] Barron D, Varin L, Ibrahim RK, Harborne JB, Williams CA. Sulphated flavonoids, an update. *Phytochemistry*.

- 1998; 27: 2375-2395. DOI: 10.1016/0031-9422(88)87003-1,
- [71] McMillan C. Flowering under controlled conditions by *cymodocea serrulata*, *halophila stipulacea*, *syringodium isoetifolium*, *zostera capensis* and *Thalassia hemprichii* from Kenya. Aquatic Botany.1980;8:323-336. DOI: 10.1016/0304-3770(80)90062-5.
- [72] Rowley DC, Hansen MST, Rhodes D, Sotriffer CA, Ni H, McCammon JA., Bushman FD, Fenical W. Thalassiolins A–C: new marine-derived inhibitors of HIV cDNA integrase. Bioorganic and Medicinal Chemistry. 2002;10(11):3619-3625. DOI:10.1016/s0968-0896(02)00241-9.
- [73] Meng Y, Krzysiak AJ, Durako MJ, Kunzelman JI, Wright JLC. Flavones and flavone glycosides from *Halophila johnsonii*. Phytochemistry.2008;69: 2603-2608. DOI:10.1016/j.phytochem.2008.07.007.
- [74] Viso AC, Pesando D, Bernard P, Marty JC. Lipid components of the Mediterranean seagrass *Posidonia oceanica*. Phytochemistry. 1993;34:381-387. DOI: 10.1016/0031-9422(93)80012-H.
- [75] Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F. Microalgal biomarkers: A review of recent research developments. Organic Geochemistry. 1999;29:1163. DOI: 10.1016/S0146-6380(98)00062-X.
- [76] Vaskovsky VE, Khotimchenko SV, Xia B, Healthy L, Hefang L. Polar lipids and fatty acids of some marine macrophytes from the yellow sea. Phytochemistry. 1996;42(5):1347- 1356. DOI: 10.1016/0031-9422(96)00117-3.
- [77] Goncharova SN, Sanina NM, Kostetsky EY. Role of lipids in molecular thermo adaptation mechanisms of seagrass *Zostera marina*. Biochemical Society Transactions. 2000; 28(6):887-890. DOI: 10.1042/0300-5127:0280887.
- [78] Hu H, Wang G, Liu J, Cao H, Zheng X. Studies on phenolic compounds from *Polygonum aviculare*. Zhongguo Zhongyao Za zhi. 2006;31(9):740-742.
- [79] Jeyapragash D, Saravanakumar A, Yosuva M, Packiyavathy IASV, Jeba Sweetly D. Chlorophyll fluorescence, dark respiration and metabolomic analysis of *Halodule pinifolia* reveal potential heat responsive metabolites and biochemical pathways under ocean warming. Marine Environmental Research. 2021;164:105248. DOI: 10.1016/j.marenvres.2020.105248.
- [80] Zhi-lin Y, Chun-chao D, Lian-quiring C. Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system. African Journal of Biotechnology. 2007;6(11):1266-1271.
- [81] Saklani A, Kutty SK. Plant-derived compounds in clinical trials. Drug Discovery Today. 2008;13(3–4):161-171. DOI: 10.1016/j.drudis.2007.10.010.
- [82] Kloewe ME, Gaurav V, Roberts SC. Pharmaceutically active natural products synthesis and supply vial plant cell culture technology. Molecular Pharmaceutics. 2008;5:243-256. DOI: 10.1021/mp7001494.
- [83] Verpoorte R, van der Heijden R, ten Hoopen HJG Memelink J. Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. Biotechnology Letters.1999;21:467-479. DOI: 10.1023/A:1005502632053.
- [84] West-jr FR, Mika ES. Synthesis of atropine by isolated roots and root-callus cultures of *Belladonna*. Botanical Gazette., 1957:119:50-54. DOI: 10.1086/335961.
- [85] Bird KT, Jewett-Smith J. Development of a medium and culture

- system for in vitro propagation of the seagrass *Halophila engelmannii*. Canadian Journal of Botany. 1994;72:1503-1510. DOI: 10.1139/b94-185.
- [86] Short FT, Polidoro B, Livingstone SR, Carpenter KE, Bandeira S, Bujang JS, Calumpang HP, Carruthers TJB, Coles RG, Dennison WC, Erftemeijer PLA, Fortes MD, Freeman AS, Jagtap TG, Kamal AHM, Kendrick GA, Kenworthy WJ, Nafie YAL, Zieman, JC. Extinction risk assessment of the world's seagrass species. Biological Conservation. 2011;144(7):1961-1971. DOI: 10.1016/j.biocon.2011.04.010.
- [87] Balestri E, Piazzini L, Cinelli F. In vitro germination and seedling development of *Posidonia oceanica*. Aquatic Botany. 1998;60:83-93. DOI: 10.1016/S0304-3770(97)00017-X.
- [88] Bird KT, Cody BR, Jewett-smith J, Kane M.E. Salinity effects on *Ruppia maritima* L. cultured in vitro. Botanica Marina.1993;36:23-28. DOI: 10.1515/botm.1993.36.1.23.
- [89] Bird KT, Brown MS, Henderson TT, Hara CEO, Robbie JM. Culture studies of *Ruppia maritima* L. bicarbonate and sucrose-based media. Journal of Experimental Marine Biology and Ecology. 1996;199:153-164. DOI: 10.1016/0022-0981(95)00195-6.
- [90] Koch EW, Durako MJ. In vitro studies of the submerged angiosperm *Ruppia maritima*: Auxin and cytokinin effects on plant growth and development. Marine Biology. 1991; 110(1):1-6. DOI: 10.1007/BF01313085.
- [91] Loques F, Caye G, Meinesz A. Axenic culture of selected tissue of *Posidonia oceanica*. Aquatic Botany. 1990;37(2):171-188. DOI: 10.1016/0304-3770(90)90090-8.
- [92] Thangaradjou T, Kannan L. Survival and growth of transplants of laboratory raised axenic seedlings of *Enhalus acoroides* (L.f.) Royle and field-collected plants of *Syringodium isoetifolium* (Aschers.) Dandy, *Thalassia hemprichii* (Ehrenb.) Aschers. And *Halodule pinifolia* (Miki) den Hartog. Journal of Coastal Conservation. 2008;12:135-143. DOI: 10.1007/s11852-008-0036-5.
- [93] Wilson JG, Bennett IJ. Nutrient requirements of in vitro cultured *Halophila ovalis* and *Posidonia coriacea*: Nitrogen source. Plant Cell, Tissue and Organ Culture.2008;92: 155-163. DOI: 10.1007/s11240-007-9318-0.
- [94] Balestri E, Cinelli F. Isolation of protoplast from the seagrass *Posidonia oceanica* (L.) Delile. Aquatic Botany.1992;43:301-304. DOI: 10.1016/0304-3770(92)90074-S.
- [95] Balestri E, Cinelli F. Isolation and cell wall regeneration of protoplast from *Posidonia oceanica* and *Cymodocea nodosa*. Aquatic Botany. 2001;70:237-242. DOI: 10.1016/S0304-3770(01)00157-7.
- [96] Carpaneto A, Naso A, Paganetto A, Cornara L, Pesce ER, Gambale F. Properties of ion channels in the protoplast of the Mediterranean seagrass *Posidonia oceanica*. Plant, Cell and Environment. 2004;27:279-292. DOI: 10.1111/j.1365-3040.2003.01139.x.
- [97] Garril A, Tyerman SD, Findlay GF. Ion channels in the plasma membrane of protoplasts from the halophytic angiosperm *Zostera muelleri*. The Journal of Membrane Biology. 1994;42:381-393. DOI: 10.1007/BF00233443.
- [98] Mazzella L, Mauzerall D, Lyman H, Alberte RS. Protoplast isolation and photosynthetic characteristics of *Zostera marina* L. (eel grass). Botanica Marina. 1981;24(5):285- 290. DOI: 10.1515/botm.1981.24.5.285.
- [99] Zarranz Elso M, Gonzalez-Henriquez N, Garcia-Jimenez P, Robaina RR. Restoration of *Cymodocea*

nodosa seagrass meadows through seed propagation: Germination in vitro, seedling culture and field transplants. *Botanica Marina*.2010;53:173-181. DOI: 10.1515/BOT.2010.019

[100] Silvestrini A, Gabriella P, Bruno B, BarbaraM, Robertvan H, Robert V. Effects of alkaloid precursor feeding on a *Camptotheca acuminata* cell line. *Plant Physiology and Biochemistry*. 2002.40 (9):749-753.DOI:10.1016/S0981-9428 (02)01436-5.

[101] Radman R, Teresa S, Christopher B, Tajalli K. Elicitation of plants and microbial cell systems. *Biotechnology and Applied Biochemistry*. 2003; 37(1): 91-102. DOI: 10.1042/ba20020118.

[102] Ravn H, Pedersen MF, Borum J, Andary C, Anthoni U, Christophersen C, Nielsen PH. Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of eelgrass (*Zostera marina* L.). *Ophelia*., 1994;40:51-61. DOI: 10.1080/00785326.1994.10429550.

[103] Males Z, Plazibat M. Investigation of chemical composition of *Zostera noltii*. *Hornem. Farmaceutski Glasnik*. 2000;56(4):109-118.

[104] Dumay O, Costa J, Desjobert JM, Pergent G. Variations in the concentration of phenolic compounds in the seagrass *Posidonia oceanica* under conditions of competition. *Phytochemistry*. 2004;65:3211-3220. DOI: 10.1016/j.phytochem.2004.09.003.

[105] Hamdy AHA, Mettwally WSA, El Fotouh MA, Rodriguez B, El-Dewany AI, El-Toumy S AA, Hussein MAA. Bioactive phenolic compounds from the Egyptian Red Sea seagrass *Thalassodendron ciliatum*. *Zeitschrift für Naturforschung C*. 2012;67:291- 296. DOI: 10.1515/znc-2012-5-608.