Chapter

Seagrass Metabolomics: A New Insight towards Marine Based Drug Discovery

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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 100,000 to 200,000, 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 bought 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
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].

| Metabolomics Technology | Advantages                                                                 | Disadvantages                                                                 |
|------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| GC–MS                  | • Well established chromatographic-mass spectrometric technology             | • Time consuming                                                             |
|                        | • Less expensive                                                            | • Can only analyze volatile compounds upon derivatization                    |
|                        | • Easy to perform                                                           | • Not able to identify the unknown compounds                                 |
|                        | • Better stability and reproducibility                                       |                                                                               |
|                        | • Universal database for metabolite identification is available              |                                                                               |
| LC–MS                  | • Highly sensitive                                                          | • Less reproducibility                                                       |
|                        | • Method for sample pre-treatment is simple                                 | • Highly expensive                                                           |
|                        | • Wide coverage of metabolite could be identified                           | • Difficult to identify highly polar and charged metabolites                  |
|                        | • Scanning range for different ions is usually less                        |                                                                               |
|                        | • Different columns can be used depends on the polarity of compounds        |                                                                               |
| FT-ICR-MS              | • High resolution mass spectrometric technique                             | • Involves series of steps                                                   |
|                        | • Extremely high mass accuracy                                              | • Use large super conducting magnets                                         |
|                        | • High acquisition rates                                                    | • Highly expensive than GC–MS and LC–MS                                      |
|                        | • Highly flexible                                                           |                                                                               |

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

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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 alkylation 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 biodiverity 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.
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, Gondwana-land, 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 |
|-----------------------|-------------------|---------|-----------------------|-----------|
| Zostera marina, Zostera noltii | GC/TOF Trimethyl silylation | Adaptive mechanisms 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] |
| Cymodocea nodosa | 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] |
| Halodule pinifolia | 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, H. pinifolia for RA, which is an emerging potential preclinical chemical entity | Jeyapragash et al. [51] |
| Zostera marina | 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] |
| Halodule pinifolia | 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 H. pinifolia metabolism in the marine and artificial environments | Jeyapragash et al. [53, 54] |
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,
particulary 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 form 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 accu-
mulate manximum 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 com-
pounds ranging from simple phenol derivatives, phenylmethane, phenyethane and phenypropane derivatives [67]. Athiperumalsami et al. [47] reported the occurrence and absence of alkaloids, antroquinones, 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 availed 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 segrass species while the sulphated flavones found specifically in five species namely, *Zostera, Phyllospadix, Enhallus, Thallasia* and *Halophila* species.

![Figure 2.](image)

*Figure 2.* Growth regulators mediated flavonoid production in callus and cellular suspension of *H. pinifolia.*
| Compound Name                      | Molecular Formula | Molecular weight (g/mol) | Exact Mass (g/mol) |
|-----------------------------------|-------------------|--------------------------|-------------------|
| D-Glucose                         | C₆H₁₂O₆           | 180.156                  | 180.063           |
| Maltose                           | C₁₂H₂₂O₁₁         | 342.297                  | 342.116           |
| D-Fructose                        | C₆H₁₂O₆           | 180.156                  | 180.063           |
| Sucrose                           | C₁₂H₂₂O₁₁         | 342.297                  | 342.116           |
| Inositol                          | C₆H₁₂O₆           | 180.156                  | 180.063           |
| Methyl alpha-D-Glucopyranose      | C₇H₁₄O₆           | 194.183                  | 194.079           |
| D-Galactose                       | C₆H₁₂O₆           | 180.156                  | 180.063           |
| Lactose                           | C₁₂H₂₂O₁₁         | 342.297                  | 342.116           |
| L-Rhamnose                        | C₆H₁₄O₅           | 164.157                  | 164.068           |
| D-Ribose                          | C₅H₁₀O₅           | 150.13                   | 150.053           |
| Adenosine-2’-3’- cyclic monophosphate | C₁₀H₁₄N₅O₇P     | 347.224                  | 347.063           |
| N-Acetyl-Î-D-glucosamine          | C₈H₁₅NO₆          | 221.209                  | 221.09            |
| Aspartyl-Leucine                  | C₁₆H₁₈N₂O₅        | 246.263                  | 246.122           |
| Glycine                           | C₂H₅NO₂           | 75.067                   | 75.032            |
| Threonine                         | C₄H₉NO₃           | 119.12                   | 119.058           |
| Valine                            | C₂H₅NO₂           | 117.148                  | 117.079           |
| Proline                           | C₂H₅NO₂           | 115.132                  | 115.063           |
| Alanine                           | C₂H₅NO₂           | 89.094                   | 89.048            |
| Thiamine                          | C₁₂H₁₇N₄OS⁺       | 265.355                  | 265.112           |
| Methionine                        | C₂H₁₀NO₂S         | 149.208                  | 149.051           |
| Phenylalanine                     | C₉H₁₀NO₂          | 165.192                  | 165.079           |
| Tyrosine                          | C₉H₁₁NO₃          | 181.191                  | 181.074           |
| Methyl Pyroglutamate              | C₉H₁₁NO₃          | 143.142                  | 143.058           |
| Glutamic acid                     | C₅H₁₀NO₄          | 147.13                   | 147.053           |
| Vanillic acid                     | C₈H₉O₄            | 168.148                  | 168.042           |
| Oxalic acid                       | C₂H₂O₄            | 90.034                   | 89.995            |
| gamma-Aminobutyric acid           | C₂H₅NO₂           | 103.121                  | 103.121           |
| Citrate                           | C₈H₁₀O₇⁻³         | 189.099                  | 189.004           |
| Stearic acid                      | C₁₈H₃₆O₂          | 284.484                  | 284.272           |
| Hexadecanoic acid                 | C₁₆H₃₂O₂          | 257.422                  | 257.244           |
| Potassium Gluconate               | C₆H₁₁KO₂          | 234.245                  | 234.014           |
| Nicotinic acid                    | C₆H₈NO₂           | 123.111                  | 123.032           |
| Phosphoric acid                   | H₃PO₄             | 97.994                   | 97.977            |
| Sodium Pyrophosphate              | Na₉P₂O₇           | 265.9                    | 265.871           |
| Acetamide                         | C₂H₅NO            | 59.068                   | 59.037            |
| Decanedioic acid                  | C₁₂H₂₂O₄          | 230.304                  | 230.152           |
| Indoleacetic acid                 | C₁₀H₁₀NO₂         | 175.187                  | 175.063           |
| 1-Napthaleneacetic acid           | C₁₂H₁₀O₂          | 186.21                   | 186.068           |
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

| Compound Name                  | Molecular Formula | Molecular weight (g/mol) | Exact Mass (g/mol) |
|-------------------------------|-------------------|--------------------------|--------------------|
| 4-Hydroxybenzaldehyde         | C7H6O2            | 122.123                  | 122.037            |
| 2,4-dihydroxybenzaldehyde     | C7H6O3            | 138.122                  | 138.032            |
| 3,4-Dihydroxybenzoic          | C7H6O4            | 154.121                  | 154.027            |

Table 4. List of metabolites identified from wild seagrasses.
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 (ΔF/F’m) and dark respiration (Fv/Fm) were also studied. Results found that photosynthetic efficiency (ΔF/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 sincelate 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].
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

Figure 4.
Distribution of metabolic pathways of differential metabolites derived from SCC of seagrass (A) and their active networks (B) (adapted from [53, 54]).
| 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   |
| Protocatechuic acid           | C_{9}H_{8}O_{4}  | 154.121 | 154.027   |
| p-Anisic acid                 | C_{9}H_{8}O_{3}  | 152.149 | 152.047   |
| Vanillic acid                 | C_{9}H_{8}O_{4}  | 168.148 | 168.042   |
| Naringenin                    | C_{15}H_{12}O_{5} | 272.256 | 272.068   |
| 4-hydroxybenzoic acid         | C_{9}H_{8}O_{3}  | 138.122 | 138.032   |
| Fructose-6-phosphate          | C_{6}H_{12}O_{4}P | 260.135 | 260.03    |
| Glucose-6-phosphate           | C_{6}H_{12}O_{4}P | 260.135 | 260.03    |
| Glucose                       | C_{6}H_{12}O_{6} | 180.156 | 180.063   |
| Phosphoenol pyruvic acid      | C_{6}H_{12}O_{5}P | 168.041 | 167.982   |
| Pyruvic acid                  | C_{3}H_{4}O_{3}  | 88.062  | 88.016    |
| Citric acid                   | C_{6}H_{8}O_{7}^{-3} | 189.099 | 189.004   |
| Fumaric acid                  | C_{4}H_{8}O_{4}  | 116.072 | 116.011   |
| 3-PGA                         | C_{3}H_{7}O_{8}P  | 186.056 | 185.993   |
| Ketoglutaric acid             | C_{4}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   |
| Oxalacetic acid               | C_{6}H_{4}O_{6}  | 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_{24}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_{3}H_{7}NO_{3} | 132.119 | 132.053   |
| Aspartic acid                 | C_{4}H_{7}NO_{4} | 133.103 | 133.038   |
| Glutamic acid                 | C_{4}H_{7}NO_{4} | 147.13  | 147.053   |
| Glycine                       | C_{2}H_{5}NO_{2} | 75.067  | 75.032    |
| Proline                       | C_{2}H_{5}NO_{2} | 115.132 | 115.063   |
| Serine                        | C_{2}H_{5}NO_{2} | 105.093 | 105.043   |
| Threonine                     | C_{2}H_{5}NO_{3} | 119.12  | 119.058   |
| Valine                        | C_{5}H_{11}NO_{2} | 117.148 | 117.079   |
| 2,4-dihydroxybenzoic acid     | C_{9}H_{8}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             | C₄H₉NO₂                | 103.121    | 103.121    |
| Dimethylamine                       | (CH₃)₂NH               | 45.085     | 45.058     |
| Ethanolamine                        | C₂H₇NO                 | 61.084     | 61.053     |
| Thiamine                            | C₄H₂N₄O⁺               | 265.355    | 265.112    |
| Nicotinic acid                      | C₆H₅NO₂                | 123.111    | 123.032    |
| Pyridoxine                          | C₄H₇NO₃                | 169.18     | 169.074    |
| Phenylalanine                       | C₉H₁₁NO₂                | 165.192    | 165.079    |
| Tryrosine                           | C₉H₁₁NO₃                | 181.191    | 181.074    |
| Shikimic acid                       | C₇H₁₀O₅                | 174.152    | 174.053    |
| Acotinic acid                       | C₆H₆O₆                 | 174.108    | 174.016    |
| Xylonic acid                        | C₆H₉O₆                 | 166.129    | 166.048    |
| Ascorbic acid                       | C₆H₇O₆                 | 176.124    | 176.032    |
| Guanine-2’3’-cyclic monophosphate   | C₁₀H₁₃N₂O₅P             | 345.208    | 345.047    |
| Pantothenate                        | C₁₈H₃₇NO₂               | 218.229    | 218.103    |
| Sphingosine                         | C₁₈H₃₇NO₂               | 299.499    | 299.28     |
| N-acetylglucosamine                 | C₁₈H₃₇NO₂               | 221.209    | 221.09     |
| Aspartyl leucine                    | C₁₀H₁₄N₂O₆              | 246.263    | 246.122    |
| 2-hydroxy glutaric acid             | C₅H₈O₅                 | 148.114    | 148.037    |
| Glyceric acid                       | C₃H₆O₄                 | 106.077    | 106.027    |
| Chlorogenic acid                    | C₁₆H₁₅O₉                | 354.311    | 354.095    |
| Rhamnose                            | C₆H₁₂O₅                 | 164.157    | 164.068    |
| Guanosine monophosphate             | C₁₀H₁₅N₅O₇P₂             | 443.202    | 443.024    |
| Ribose                              | C₅H₁₀O₅                 | 150.13     | 150.053    |
| Adenosine-2’3’-cyclic Monophosphate | C₁₀H₁₄N₅O₇P             | 347.224    | 347.063    |
| Dihydroquercetic acid               | C₁₅H₁₂O₇                | 304.254    | 304.058    |
| Adenosine-2- Monophosphate          | C₁₀H₁₄N₅O₇P             | 347.224    | 347.063    |
| p-hydroxybenzoic acid               | C₆H₆O₃                 | 138.122    | 138.032    |
| Quinic acid                         | C₇H₁₂O₆⁺                | 192.167    | 192.063    |
| Tryptophan                          | C₁₁H₁₄N₂O₂⁺              | 204.229    | 204.09     |
| Pyroglutamic acid                   | C₆H₇NO₃                | 129.115    | 129.043    |
| Salicylic acid                      | C₇H₆O₃                 | 138.122    | 138.032    |
| Methionine                          | C₅H₁₇NO₃S               | 149.208    | 149.051    |
| Lactic acid                         | C₇H₁₄O₃                 | 90.078     | 90.032     |
| Isovaleric acid                     | C₇H₁₄O₂                 | 102.133    | 102.068    |
| 2-oxyglutaric acid                  | C₅H₈O₅                 | 146.098    | 146.022    |
| 2-hydroxyisobutyric acid            | C₅H₉O₅                 | 104.105    | 104.047    |
| 1-methyl nicotinic acid             | C₆H₅NO₂⁺                | 138.146    | 138.056    |
| Hypoxanthine                        | C₅H₄N₄O                 | 136.114    | 136.039    |
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

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