Mass Spectrometry in Bioprospecting of Plant Natural Products in the Amazon Rainforest

L. Soto

Abstract

The Amazon rainforest is a megadiverse region of cultural complexity. Ethnic groups have been using an extensive plant repository for several applications. Bioprospecting the potential bioactive molecules requires a set of analytical techniques capable of elucidating the chemical composition of complex samples. Chromatography coupled with mass spectrometry is one of the most accurate and robust strategies available for identifying and quantifying natural products. This brief review outlines the most common mass spectrometry techniques and applications in plant bioprospecting in the Amazon region during the last ten years.

Source: [3]

Keywords: bioprospecting, natural products, mass spectrometry, LC-MS, GC-MS, Amazon.

Content

1. Introduction

The Amazon rainforest covers around 5 500 000 km^2 across nine South American countries, mainly Brazil. The tropical rainforest is rich in biome, constituting the world's largest collection of flora and fauna. This biome houses millions of insect species, thousands of plants, birds, and mammals. Some approximations indicate that the Amazon host around 16 000 tree species,[1] and that 0.25 km^2 of Ecuadorian rainforest contains more than 1100 species of trees.[2]

The Amazon is also culturally complex. It includes thousands of indigenous territories and more than 30 million people and 320 different ethnic groups.[3] These communities have their own traditional norms and knowledge that shapes the social organization and their interaction with the environment. Over generations, they transferred traditional medicine that employs natural resources to treat various health conditions.

The Amazon region has a proven application of natural products and ethnobotanical surveys play a considerable role in bioprospecting new bioactive compounds. An ethnobotanical study for collecting data on medicinal plants in the Valley of Juruena, Brazil, found 332 plant species of 90 families reported by interviewed people. Those plants were used for various gastrointestinal and respiratory ailments[4]. In 2015, another ethno-directed study with the quilombola communities in Oriximina, Brazil, examined the activity of 11 plants with in vitro cultures parasites for malaria. The results showed the antiplasmodial potential of *Aspidosperma rigidum* and *Bertholletia excelsa* [5].

Chromatography, mass spectrometry (MS), and nuclear magnetic resonance (NMR), are the most common analytical tools for exploring natural products. Mass spectrometry is widely used in diverse fields to identify and quantify the chemical composition of complex samples. Mass spectrometry is a rapid, sensitive, and accurate method that measures the mass-to-charge ratio of analytes. Fragmentation of molecules with tandem mass spectrometry can provide more relevant information on the chemical structure. Along with mass spectrometry, chromatographic separation can enhance selectivity. The retention time of the compounds gives an additional identity indication.

This review aims to explore the current mass spectrometry techniques applied to the bioprospection of natural products from plants in the Amazon region.

2. Methodology

In this non-systematic review, most of the articles were obtained from the ScienceDirect database with the query "((bioprospecting OR bioprospection) AND (amazon) AND (mass AND spectrometry OR ms OR hrms))". The publication period was 2012-2022. Additionally, searches from Scopus, Google Scholar, Web of Science, and specialized journals were included. The articles are mainly in English. Other languages, such as Portuguese and Spanish, could be considered to explore other databases in a further systematic study.

3. MS techniques

The most common analytical technique was gas chromatography (GC) coupled to single quadrupole mass analyzer, followed by other liquid chromatography (LC) coupled to Orbitrap, time-of-flight, and triple quadrupole analyzers. Nuclear magnetic resonance (NMR) was also used in some studies for identity confirmation.

The predominance of GC-MS single quadrupole over other techniques could be explained by the scope of applications and its popularity. The scope of many studies was on investigating small and volatile molecules, while the heavier and thermo-labile ones were not considering for which other techniques are usually necessary. The GC-MS single quadrupole instrument is a standard instrument in many analytical laboratories and used in routine analysis.

Besides, it is worth mentioning that the findings are limited to the articles in this review and the way in how the articles were collected, mainly via the search query described in the methodology.

GC-MS

Gas chromatography-mass spectrometry (GC-MS) has been extensively used in plant bioprospecting of essential oils and volatile compounds. GC-MS was the most common technique found in this review, and it could be due to the specific application scope to volatile analytes and that it is commonly available. For the analysis, samples are usually taken from aerial parts, for instance, leaves and small stems. Some of the analytes were small molecules such as essential oils, terpenoids, and alkaloids. For certain plant species, these compounds and the chemical composition were reported for the first time.

GC-MS is a well-studied and standardized technique. In all GC-MS studies, the electron impact ionization was 70 eV. The most common mass analyzer was the single quadrupole, and the mass range was approximately between 35 and 500 m/z. The mass spectra are compared with spectra libraries and specialized books. For qualitative analysis and relative quantification, most studies used the linear retention index with Van Den Dool and Kratz equation, series of n-alkenes C8-C38, along with NIST and FFNSC libraries [6] [7] [8] [9]. While for quantitative analysis, one study prepared an external calibration. [10] Herein, some details about the applications of this technique are presented.

GC-MS single quadruple has been of particular interest to study essential oils. Samples from *Diospyros guianensis, Carapa guianensis and Aspidosperma nitidum* showed larvicidal activity [11]. In order to determine the bioactive compounds, GC-MS analysis has been helpful in the identification of some constituents, for example, fatty acids, limonoids, and plumbagin. A possible synergic effect of some of these compounds was also suggested. For some compounds, the structure elucidation additionally involved NMR and LC-MS.

The seasonal variation of the chemical composition of some essential oils has been studied. The *Mesosphaerum suaveolens (L.) Kuntze,* an endemic plant from the Brazilian Amazon, has a different composition of essential oils during the rainy, intermediate, and dry seasons, as shown in *Figure 1*. [6] The dry season showed a greater linalool presence than other seasons. Contrary to this finding, Pimentel et al. [10] found a greater concentration of linalool in the wet season for another plant, *Aniba rosaeodora Ducke.* This suggests that essential oils are not directly influenced by environmental factors but intrinsic ones. Pimentel et al. [10] also found greater effectiveness against phytopathogens for those essential oils that were extracted during the wet season. The study also suggested linalool as the main active compound. In a similar study, the larvicidal activity of essential oils from *E. uniflora, L. camara, O. basilicum, P. neochilus* against *A. aegypti* showed greater effectiveness in the dry season [7].

Geographical and environmental factors could also influence the chemical profile of the plants. Plant samples that were collected at different locations differed in their essential oils composition [8]. Seasons can also modify the overall extraction yield and composition. Lower volatile compounds can be explained by a higher sun radiation exposure[6]. An examination of chemical profile against these and other factors could help to determine the best conditions for material collection depending on the compounds of interest.

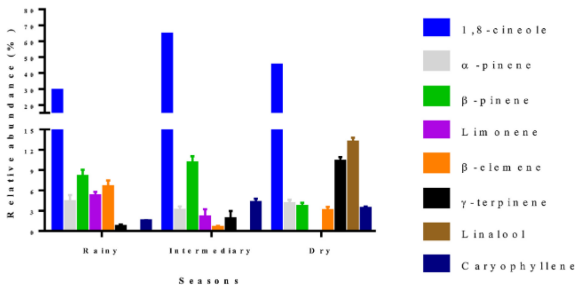

Figure 1 Essential oil composition variation over the seasons for *Mesosphaerum suaveolens*. Figure from Luz et al. [6]

The shell of *Carapa guianensis Aubl.*, a plant that could be a potential eco-friendly larvicide, showed a possible synergic effect of fatty acids and limonoids. A mixture of essential oil extracts has been used to study the combined effect on bioactivity [11].

Potential natural products against helminth infections have also been reported in the context of growing resistance to common drugs. For example, a study of anthelmintic activity of the leaves and fine stems of *Hyptis dilatata* and *Mesosphaerum suaveolens* showed that the oils, containing terpenes, can have a synergistic action as nematocidal [8]. The effect of monoterpenes has been attributed to their interference with receptors or enzymes [8]. However, the exact mechanism of terpenes in biological systems is not yet well understood.

Some mosses have been traditionally used as medicinal plants [12]. GC-MS has helped to examine their chemical composition. For the study of *Neckeropsis undulata (Hedw.) Reichardt*, distillation-extraction, GC, and GC-MS single quadrupole were applied[9]. Some compounds were 1-octen-3-ol, α-muurolol, naphthalene, and n-hexanal. The relative quantitation of each compound was calculated by peak normalization in GC coupled to a flame ionization detector (FID) [9]. In a similar study for *Calymperes palisotii Schwaegr*, 13 compounds were identified and relatively quantified by GC/MS single quadrupole. 3-methyl-2-pentanone, phenylacetaldehyde, and E-nerolidol were the major constituents. E-nerolidol and furfural were first reported for mosses[13]. Epizonarene, α-selinene, β-selinene have been also identified as constituents of mosses oil extracts [14].

The smokes of oleoresin have been attributed positive effects for headaches. The smoke from *Burseraceae pitch (breu)* consisted mainly of terpenoids that were transferred to the smoke by volatilization. GC-MS single quadrupole identified at least 32 compounds[15].

New compounds are usually reported for the first time in some studies, for example, new compounds in the family *Verbenaceae* [16], and secondary metabolites for *E. amazonica* and *M. dubia* [17]. At the same time, some authors suggest that the presence of specific molecules could be attributed to other organisms [13].

LC-MS Orbitrap

Unlike GC-MS, mainly limited to studying small, thermostable, and volatile compounds, liquid chromatography-mass spectrometry (LC-MS) opened a wide range of discovery possibilities. Non-volatile chemical compositions have not been explored yet for some medicinal plants as these molecules may require specific sample preparations, derivatization or cannot be determined by GC-MS. For this analysis, some of the samples were leaves, stems, bark, and seeds. The most common ion source interphase between LC and the mass spectrometer was electrospray ionization (ESI).

Some studies used the Orbitrap mass analyzer. An extensive analysis of the potential antimicrobial activity of 59 plant species from 33 families was carried out. 12 plants showed antibacterial activity. The study also isolated and identified three secondary metabolites for the first time. The analytical methods were chromatography, NMR, and high-resolution mass spectrometry (HRMS). An MS analysis was done with ESI in positive and negative mode with Orbitrap in the mass range of 100 to 850 Da. Alkaloid and triterpenic compounds were identified [17]. In another study, a hybrid mass analyzer linear trap quadrupole (LTQ) - Orbitrap was used to identify flavonoids in *Lippia origanoides* (Verbenaceae), a plant known for its medicinal and food applications. The ESI source was in positive and negative mode. In this case, the resolution was 30000, and the mass ranged from 140 to 1500 m/z [16]. For fragmentation, a data dependent scan was set with a collision energy of 35 eV and precursor width of 2.0 m/z [16].

Ampelozizyphus amazonicus Ducke is a plant with medicinal attributes used in the Amazon region. In a study, an extract was analyzed by HPLC-HRMS. In this case, the ESI source was in negative mode. The Orbitrap allowed a resolution of 60000 for the full mass and 7500 for the data-dependent acquisition. The collision energy for the collision-induced dissociation (CID) was 35 eV, and the width for the precursor ion was 2.0 m/z. For identification, the fragmentation patterns were relevant to propose several saponin chemical structures [18].

LC-MS TOF

A few studies included time-of-flight (TOF) analyzers. Spilanthol content and bactericide/antibiofilm activity have been determined for the *Acmella oleracea*, a plant with anti-cariogenic properties. HPLC-MS/MS with a hybrid Q-TOF (quadrupole-TOF) mass analyzer was used to quantify the concentration of spilanthol in the leaves. The ESI operated in positive mode and the range was from 50 to 1000 m/z [19]. In another study of *Carapa guianensis*, a plant with potential eco-friendly larvicidal activity, HPLC-MS was used with ESI and Q-TOF to analyze the seed shells [11].

MALDI TOF

The TOF analyzer is well suited to the pulse nature of laser desorption of matrix-assisted laser desorption/ionization (MALDI) and could represent an opportunity to explore large, unstable, and polymeric biomolecules by direct infusion. Research papers using MALDI-TOF are even less common. MALDI – TOF in positive mode has been used to determine the molecular weight of a phenolic compound (761.2 Da) with antioxidant activity from epicarp samples of *Garcinia brasiliensis*, a plant with attributed anti-inflammatory, antioxidant and antitumor properties [20].

4. Alternative approaches

In-vivo SPME and LC-MS QqQ

An in-vivo solid phase microextraction (SPME) sampling technique has been documented. Twenty Amazonian plant species were sampled in vivo using SPME probes. Probes are inserted under the plant's bark, left for a contact time, then transferred to plastic tubes under vacuum. Samples are extracted from the probes with ethanol solution, evaporated, and reconstituted before the analysis. With this approach, the damage to the plant is minimized, the sampling is faster than the standard preparation procedures, and it could be suitable for sampling covering large remote areas. For the LC-MS analysis, this study used ESI along a triple quadrupole (QqQ) mass analyzer for the range 150 - 600 m/z. [21]

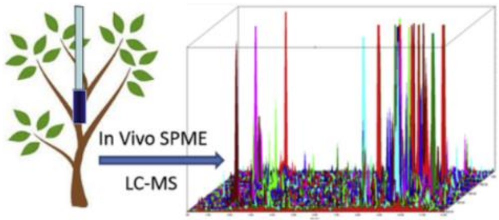

Figure 2 Graphical abstract for in-vivo sampling with SPME probe. Image from Musteata et al.[21]

Off-line counter-current chromatography

Counter-current chromatography (CCC) has been reported to improve the identification of constituents in complex samples as a purification step prior to mass spectrometry analysis. In a study of the phenolic profile of *Lippia origanoides*, counter-current chromatography was used as a preparatory step for fractionation of a sample extract. This approach increased the number of identified compounds[16]. CCC has also been used to fractionate *Garcinia brasiliensis* epicarp extract samples [20].

5. Data processing

MS spectra is compared to chemo-taxonomic data available in literature and databases. Some studies used the software Xcalibur, and a common mass spectra library was NIST. Open-source processing software, e.g. MZMine, or collaborative mass spectra annotation, e.g. Global Natural Products Social Molecular Networking (GNPS), seem to be not common in these studies. Multivariate analysis and a machine learning algorithm has been applied. Partial least squares - discriminant analysis (PLS-DA) and random forest analysis helped to differentiate between two types of oleoresins and find a potential chemical marker based on mass spectrometry data[22].

6. Conclusion and future perspectives

Mass spectrometry experiments offer a variety of possibilities for bioprospecting. This brief review outlined some of the most common mass spectrometry techniques and their applications in bioprospecting plants in the Amazon region. It includes an overview of GC-MS, LC-MS, Orbitrap, TOF, and MALDI, and describes the typical samples, analytes, technique settings, and data processing. This review is limited to the revised articles here and the way in which the articles were collected, mainly via the search query. GC-MS single quadrupole was found to be the most common technique. This could be explained because of the intended application to identify or quantify small molecules e.g., essential oils, and the greater popularity of this instrument. The main challenge remains in expanding the analytical capabilities offered by MS to analyze a broader spectrum of natural products. In this regard, high-resolution mass spectrometry, ambient mass spectrometry, mass spectrometry imaging and other ionization modes could lead to a better understanding of the complex chemical configuration of plants in the Amazon region and their potential applications.

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