RESEARCH ARTICLE

HPLC-ESI-MS ANALYSIS OF SOME BIOACTIVE SUBSTANCES IN TWO YEMENI MEDICINAL PLANTS

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Abstract

Plants have abundant bioactive components and play an important role in folk medicine, owing to their health benefits in the treatment of many diseases, partially due to the secondary metabolite compositions. Nonetheless, detailed information on these substances is still limited. The recent work was aimed at investigating the bioactive substances of two Yemeni medicinal plants (i.e. Plectranthus asirensis and Plectranthus amboinicus) using reversed-phase high-performance liquid chromatography-electrospray ionization-mass spectrometry in a positive ionization mode. The proposed method provided a tentative identification of several constituents such as alkaloids, fatty acids, steroids, and terpenoids. The obtained results highlight the importance of studied plants as a promising natural source of bioactive compounds.

Keywords: Medicinal plants, P. asirensis, P. amboinicus, Bioactive components, HPLC-ESI-MS.

1. Introduction

According to several researches, plants are the huge storage of natural foods, raw materials for food and drug industries that can be used as enriched diet, food flavors and colors, fragrances, anti-oxidants, anti-microbial…etc [1-7]. Medicinal plants are extensively used in diseases remedies due to their contents of bioactive compounds within the secondary metabolism of the plant and play a vital role in the treatment of many diseases. [1, 2, 5]

Secondary metabolites such as phenolic compounds, alkaloids, flavonoids, terpenoids, tannins, saponins, cardiac glycosides, essential oils…etc. are important in plant defense against herbivory and adaption to environmental stress [8-10]. They are structurally and chemically diverse groups of compounds and have a wide range of applications in the field of medicine, agriculture, veterinary and numerous other areas. Phenolic compounds are a kind of secondary metabolite found commonly in plants and are known to possess different biological effects. They have been classified into several categories: simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans, and lignins [11]. Flavonoids are ubiquitous plant secondary metabolites. They comprise major subgroups like anthocyanins, flavonols, flavones, flavanones, catechins and tannins [12]. Some of these compounds are present in plant tissue as red, blue, and purple pigments which help the plant in reproduction by recruiting pollinators and seed dispersers [13]. Flavonoids exhibit a wide range of pharmacological effects including antioxidant, anticancer, cardiovascular, and anti-inflammatory activity, anti-allergic effects, etc. [7, 14-16]. Alkaloids are a highly diverse group of low molecular-weight, nitrogen-containing organic compounds derived mostly from amino acids or the transamination process. Plants produce approximately 12,000 different alkaloids, which can be classified according to their carbon skeletal structures [17]. Alkaloids show broad pharmacological uses such as anti-oxidant and anti-bacterial activity [18, 19]. Tannins, the high molecular polymeric phenolics produced by secondary plant metabolism have a range of pharmacological properties such as anti-oxidant, antibacterial, anticancer activity [20-22] etc. and ecological functions such as important constituents in nutrient cycling, provide defense against herbivore and pathogen and plant growth regulating activities [23, 24]. Glycosides are characterized by a sugar portion attached by a specific bond to non-sugar portions; it may be phenol, alcohol or sulfur compounds. Cardiac glycosides have been reported to have anti-arrhythmic activity [25] and anti-proliferative activity [26]. Plants rich in glycosides are reported for medicinal properties including antibacterial activity [27, 28]. Several lipids such as glycerides and phospholipids associated with beneficial proteins or fatty acids like short and medium and...
polyunsaturated fatty acids bring about biological and health promoting activities. [29-31]

Plant saponins are a group of naturally occurring secondary metabolites in which glycosyl residues are attached to a triterpenoid (triterpene or steroidal) aglycon [32]. In plants, saponins are mostly found in angiosperms [33, 34] and they have a large number of biologically and pharmacologically active compounds use in anti-oxidant, anti-inflammatory and anti-cancer activities. [35, 36]

Coumarins have been reported as bioactive components used as antioxidants and inhibitors of a wide variety of microbes. [11, 37, 38]

Vitamins as vital nutrients cannot be synthesized by human body and have biological effects on health. [39]

The separation, identification and quantification of components in medicinal plant extracts continuously have been a challenging duty. Liquid chromatography linked to mass spectrometer is now available at low-cost benchtop instruments and since last decades the importance of the LC-MS technique in analytical, medicinal, industrial, environmental, and agricultural fields has steadily increased. Today, this technique has brought numerous improvements as well as new and interesting applications, which indicate the LC-MS analysis of these complex matrices at less than 1g, even easier, better and more cost-effective. [40-42]

Several ionization methods such as electron ionization, chemical ionization, etc. could not be able to overcome the propensity of the analyte fragmentation. Whereas the development of electrospray ionization-mass spectrometry (ESI-MS) became very valuable in the formation of gas-phase ions from large biologically important macromolecules and analysis, structural characterization as well as identification based on the basis of molecular mass. [43]

As far as we know, some medicinal plants such as Plectranthus asirensis and Plectranthus amboinicus have rarely mentioned in literatures refer to their chemical components or the analysis processes [2, 44-49] and we believe in this aspect it is the time to study their natural components.

The recent work focused on using HPLC- positive ion ESI-MS technique to find out some bioactive components in a methanolic extract of P. asirensis and P. amboinicus plants which are set under the same family (i.e. Lamiaceae).

2. Experimental Section

2.1 Chemicals and Reagents

All chemicals and reagents in the present work have been of analytical grade and they were used as received.

The plant materials were sorted and cleaned and the samples were air-dried and stored in a dark place at room temperature. The dried leaves were then ground into powder, sieved and packaged into clean polyethylene containers until use.

2.2 Sample Preparation

10 mg of each powdered plant (0.210-0.350 mm in size) were extracted with 500 µL of methanol. Then, 5µL of this extract was injected onto the instrument for positive ion reverse-phase LC-MS.

2.3 HPLC-MS Analysis

The work undertaken in this research was performed on an Agilent 1200 HPLC system consisting of a binary pump, autosampler, thermostatted column compartment, and the mass spectrometry is an Agilent G1969A LC/MSD TOF (facility of Biotechnology Center University of Wisconsin, Madison, USA). Other details are mentioned in Table 1 below:

Table 1: LC-Ms Method details.

| HPLC Conditions                   | Details                                   |
|-----------------------------------|-------------------------------------------|
| - Column                          | Agilent 2.1mmx50mm Zorbax SB-C18 1.8µm beads. |
| - Column temp.                    | 35 °C.                                    |
| - Mobile phase                    | A= 0.1% formic acid in water; B=0.1% formic acid in acetonitrile. |
### MS Conditions

- **Source**: Positive ESI
- **Internal standard supplied to ESI source**: at 20 µL/min via isocratic pump and ionized by secondary ESI needle.
- **Flow rate**: 250 µL/min.
- **Autosampler temp.**: held at 6 ºC.
- **Injection volume**: 1 µL.
- **Gradient**

  | Ramp Time | %B |
  |------------|----|
  | 0 min | 2% |
  | 1 min | 2% |
  | 35 min | 50% |
  | 40 min | 95% |
  | 60 min | 2% |
- **Flow rate**: 250 µL/min.
- **Autosampler temp.**: held at 6 ºC.
- **Injection volume**: 1 µL.

| Gradient | Flow rate | Autosampler temp. | Injection volume | Source | Internal standard supplied to ESI source | Flow rate | Autosampler temp. | Injection volume | MS Conditions |
|----------|-----------|--------------------|------------------|--------|----------------------------------------|-----------|--------------------|------------------|----------------|
| 2% B at 0 min; 2% B at 1 min; ramp to 50% B at 35 min; ramp to 95% B at 40 min; hold back to 2% B at 42 min; hold at 2% B until 60 min. Stop time=60 min (no post-time). | 250 µL/min. | held at 6 ºC. | 1 µL. | Positive ESI | at 20 µL/min via isocratic pump and ionized by secondary ESI needle. | 250 µL/min. | held at 6 ºC. | 1 µL. | Positive ESI |

### 3. Results and Discussion

Previously, researchers dedicated their efforts to study phytochemistry, traditional uses, side effects, and future perspectives of *P. amboinicus*; investigate of the influence of different solvents to recover higher phytochemicals from a local *P. amboinicus* and GC-MS analysis of bioactive nonvolatile compounds; identify of essential oil compositions of *P. asirensis* analyzed by various gas chromatography techniques (GC–MS, GC–FID) using two different stationary phase columns (polar and nonpolar) and HPLC-PDA profiling of phenolic constituents; and isolate, identify and quantity of the major compounds using high resolution UPLC-MS analysis, [44–49]. The recent work however was performed using HPLC-MS operated in positive ion mode for two analyzed plants (Figs. 2 and 3), the number of charged species normally observed in an electrospray spectrum is reflected in the number of basic sites on a molecule that can be protonated at low pH.

The positive total ion chromatograms (+TIC) in Figures 2 and 3 represent several peaks in the 0.5 to the 44-minute range and the impurities were largely obscured in the chromatographic baseline. The positive overlay base peak chromatogram (+BPC) feature was used to further improve the detection of impurities. Because +BPC looks less noisy and more strongly correlated with a given molecules’ chromatographic profile, it is a way to visualize a small portion of a much larger data set.

The +BPC is constructed from the base peak abundance of each scan in the analysis, where the base peak in a spectrum is the ion with the maximum abundance. Creating the +BPC of the background-subtracted data for the plants’ compounds analysis showed that there were more impurities previously hidden in the chromatographic baseline.

As the coupling of HPLC with MS is possible through ESI ionization source [46, 50], analysis of a methanolic extract of *P. asirensis* and *P. amboinicus* plants by this technique detected numerous bioactive compounds some of them are arranged in Tables 2 and 3.

Twenty-nine bioactive compounds have been approved in *P. asirensis* as follows; Acetylcarnine and cassine alkaloids were detected at retention time (RT) 16.824 and 38.464 min respectively. Calanolide-A as a coumarin derivative had been found at 28.769 min with an exact mass of 370.1789 g/mol. A one unsaturated fatty acid (*i.e.* linoleic acid) had been peaked at 41.021 min while three lipids appeared between 18.992 and 23.977 min. The most bioactive compounds that found in *P. asirensis* were terpenoids as mono-, di-, tri-, and sesqui-terpenoids and all twenty-one investigated terpenoids set among 14.563 to 41.470 min. Retinol (Vit. A) a one well-known fat-soluble vitamin had been detected at 40.983 min with exact mass equals 286.2297 g/mol.

On the other hand, the methanolic extract of *P. amboinicus* plant showed twelve bioactive compounds using the same analysis technique. Four alkaloids (*i.e.* cassine, (S)-coclaurine, lentiginosine, and bellendine) were obtained in the retention time ranged 12.924-38.423
min. The three lipids found in this plant were peaked within the range 32.712-41.135 min. Caprylic acid, 8-Amino-7-oxononanoate, and glyceryl monostearate as fatty acids were obtained at 22.811-42.003 min and had exact masses 144.1152, 187.1208, and 358.3075 g/mol correspondingly. Two types of monoterpenoids were detected that were thymol (26.974 min; 150.1045 g/mol), and boschnialactone (39.783 min; 154.0994 g/mol).

### Table 2: Some important compounds identified from the methanolic extract of *P. asirensis* by LC-MS

| NO. | RT (min) | Bioactive Compounds | Name of the Compound | Exact Mass | Molecular Formula |
|-----|----------|---------------------|----------------------|------------|-------------------|
| 1   | 16.824   | Alkaloid (Isoquinoline alkaloids) | Acetylcarnarine; Belamarine | 313.1314 | C_{18}H_{19}N_{4} |
| 2   | 38.464   | Alkaloid (Piperidine alkaloids) | Cassine | 297.2668 | C_{18}H_{20}N_{2} |
| 3   | 28.759   | Coumarin | Calanolide A | 370.1789 | C_{22}H_{26}O_{5} |
| 4   | 18.992   | Lipid (Steroid) | 16-Glucuronide-estriol; 16alpha,17beta-Estriol 16-(beta-D-glucuronide) | 464.2046 | C_{24}H_{32}O_{9} |
| 5   | 22.548   | Lipid (Steroid) | Estradiol-17alpha 3-D-glucuronoside | 448.2097 | C_{24}H_{32}O_{8} |
| 6   | 23.977   | Lipid (Steroid) | Norethynodrel | 298.1933 | C_{20}H_{26}O_{2} |
| 7   | 41.021   | Lipid/Fatty acid (Unsaturated fatty acid) | Linoleic acid; (9Z,12Z)-Octadecadienoic acid; Linoleate | 280.2402 | C_{18}H_{32}O_{2} |

### 4. Conclusion

In this study an extensive fingerprinting and metabolite profiling of the components in the methanolic extract obtained from two medicinal plants leaves had been carried out using the HPLC-positive ion ESI-MS method. In comparison with the previous studies, it has been found several bioactive compounds in the selected Yemeni folk medicinal plants that make them a natural source use to cure diseases and increase immunity.
| Page | Retention Time | Type | Compound | Formula | Molecular Weight |
|------|----------------|------|----------|---------|-----------------|
| 8    | 14.563         | Terpenoid (Sesquiterpenoid) | Qing Hau Sau; Artemisinin | C₁₅H₂₂O₅ | 282.1467 |
| 9    | 17.263         | Terpenoid (Sesquiterpenoid) | beta-Santalol | C₁₅H₂₅O | 220.1827 |
| 10   | 18.992         | Terpenoid (Diterpenoid) | Isodonol | C₁₇H₂₅O₇ | 404.1835 |
| 11   | 19.621         | Terpenoid (Diterpenoid) | Gibberellin A36 | C₃₅H₅₂O₈ | 362.1729 |
| 12   | 19.692         | Terpenoid (Triterpenoid) | Quassin; Nigakilactone D | C₂₃H₃₈O₆ | 388.1886 |
| 13   | 20.018         | Terpenoid (Diterpenoid) | Gibberellin A19; Gibberellin 19 | C₃₅H₅₂O₈ | 362.1729 |
| 14   | 21.033         | Terpenoid (Sesquiterpenoid) | Eupatocunin | C₁₅H₂₂O₇ | 404.1835 |
| 15   | 21.824         | Terpenoid (Diterpenoid) | Jatrophone | C₃₅H₅₂O₈ | 312.1725 |
| 16   | 22.131         | Terpenoid (Diterpenoid) | ent-7alpha-Hydroxykaur-16-en-19-oic acid; (+)-Kaur-16-en-7beta-ol-19-oic acid; ent-7alpha-Hydroxykaur-16-en-19-oate | C₃₅H₅₂O₈ | 318.2195 |
| 17   | 23.865         | Terpenoid (Diterpenoid; Abietane) | Taxodione | C₃₅H₅₂O₈ | 314.1882 |
| No. | Retention Time | Compound Type       | Compound Name | Molecular Formula |
|-----|----------------|---------------------|---------------|-------------------|
| 18  | 23.977         | Terpenoid (Diterpenoid) | Lathyrol     | \( C_{35}H_{50}O_4 \) |
| 19  | 24.647         | Terpenoid (Sesquiterpenoid) | Rhipocephalin | \( C_{23}H_{36}O_6 \) |
| 20  | 26.067         | Terpenoid (Diterpenoid) | Ineketone     | \( C_{35}H_{55}O_4 \) |
| 21  | 26.314         | Terpenoid (Sesquiterpenoid) | Polhovolide   | \( C_{35}H_{55}O_6 \) |
| 22  | 26.673         | Terpenoid (Sesquiterpenoid) | Vernoflexin   | \( C_{35}H_{55}O_7 \) |
| 23  | 28.157         | Terpenoid (Diterpenoid) | Carnosol      | \( C_{35}H_{55}O_4 \) |
| 24  | 33.865         | Terpenoid (Diterpenoid) | Montanol      | \( C_{35}H_{55}O_4 \) |
| 25  | 34.291         | Terpenoid (Sesquiterpenoid) | Deacetylpaserrin | \( C_{35}H_{55}O_4 \) |
| 26  | 37.492         | Terpenoid (Sesquiterpenoid) | Eupaserrin    | \( C_{35}H_{55}O_7 \) |
### Table 3: Some important compounds identified from the methanolic extract of *P. amboinicus* by LC-MS

| NO. | RT (min) | Bioactive Compounds | Name of the Compound | Exact Mass   | Molecular Formula |
|-----|----------|---------------------|----------------------|--------------|-------------------|
| 1   | 12.924   | Alkaloid (Tropane alkaloid) | Bellendine           | 205.1103     | C_{12}H_{15}NO_{2} |
| 2   | 28.338   | Alkaloid (Isoquinoline alkaloid) | (S)-Coclaurine; (S)-1,2,3,4-Tetrahydro-1-[(4-hydroxyphenyl)methyl]-6-methoxy-7-isoquinolinol | 285.1365     | C_{17}H_{19}NO_{3} |
| 3   | 35.163   | Alkaloid (Indolizidine alkaloid) | Lentiginosine        | 157.1103     | C_{8}H_{15}NO_{2} |
| 4   | 38.423   | Alkaloid (Piperidine alkaloid) | Cassine              | 297.2668     | C_{18}H_{20}NO_{2} |
| 5   | 32.712   | Lipid (Sphingolipid) | Phytosphingosine; 4-D-Hydroxyphytosphinganine | 317.2930     | C_{19}H_{29}NO_{3} |
| 6   | 40.413   | Lipid (Eicosanoid) | Prostanoic acid      | 310.2872     | C_{20}H_{32}O_{2} |
| 7   | 41.135   | Lipid (Sterol) | 24R,24'R-Fucosterol epoxide | 428.3654     | C_{29}H_{42}O_{2} |
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تحليل بعض المكوّنات النشطة حيويًا في نباتين طبيّين يمنيين باستخدام HPLC-ESI-MS

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المُلخص

تمتلك النّباتات العديد من المكوّنات النشطة النّشطة حيويًا والتي تلعب دورًا هامًا في الطّب الشعبي، بسبب امتلاكها فوائد صحّية في علاج العديد من الأمراض. خصوصاً المكوّنات الأيضية الثانوية للنباتات، مع هذا، لا تزال المعلومات التفصيليّة حول هذه المركّبات محدودة. يهدف العمل الحالي إلى التحقّق من وجود عدد من المركّبات النشطة حيويًا في نباتين يمّيين، هما نبات العضرب والشعوس، باستخدام الطور العكوس لكروماتوجرافيا السائل عالي الأداء المرتبط بتأيّن الرّذاذ المكهرب-طيف الكتلة في وضعية التأيّن الموجب. وتعطي هذه الطريقة التّجريبيّة المبتدأً توصيفًا للعديد من المكوّنات مثل القلويدات، الأحماض الدّهنيّة ليبوسائد، السّيترويدات، واللّيبرينويّات. تسلّط النّتائج المتحصّل عليها الضّوء على أهمّيّة النّباتات المدروسة كمصدر طبيعي واعد للحصول على المركّبات النّشطة حيويًاً.

الكلمات الرئيسية: نباتات طبّيّة، العضرب، الشعوس، مكوّنات نشطة حيويًا، HPLC-ESI-MS.

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