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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.

Studied plants were identified and authenticated as mentioned in [2]. The leaves of *P. asirensis* and *P. amboinicus* (Fig.1), were collected in 2012 from Yafae district, Yemen. Only plants judged to be mature and disease-free were harvested in the early morning hours. 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.

(A) *P. asirensis*

(B) *P. amboinicus*

Figure 1 Medicinal plants studied in this work

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	
- Column	Agilent 2.1mmx50mm Zorbax SB-C18 1.8µm beads.
- Column temp.	35 ºC.
- Mobile phase	A= 0.1% formic acid in water; B=0.1% formic acid in acetonitrile.

- Gradient	2%B at 0 min; 2%B at 1min; ramp to 50%B at 35 min; ramp to 95%B at 40min; ramp back to 2%B at 42 min; hold at 2%B until 60 min. Stop time=60 min (no post-time).
- Flow rate	250 µL/min.
- Autosampler temp.	held at 6 ºC.
- Injection volume	1 µL.
MS Conditions	
- Source	Positive ESI.
- Internal standard supplied to ESI source	at 20µL/min via isocratic pump and ionized by secondary ESI needle.
- Drying gas flow	10L/min.
- Nebulizer gas	30psi.
- Drying gas temp.	350 ^{0}C.
- V capillary	3500V.
- Scan (in positive ion mode)	m/z 100-3200.
- Fragmentor	60V $(M+H)^{+}$ identification and 130V for fragmentation.
- Resolution	10,000 transients/scan with a cycle time of 0.89 cycles/sec.
- Reference masses	m/z 121.050873 and 922.009798.

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. Whereas, 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.

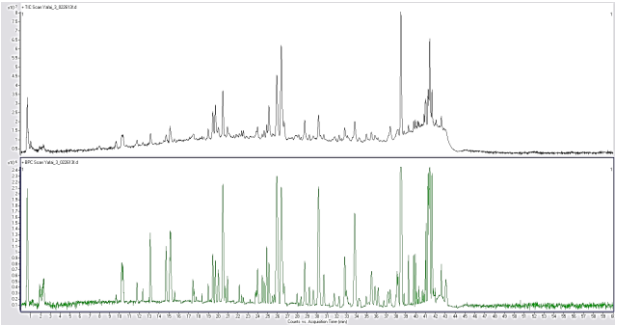


Figure 2 Positive TIC & positive BPC showing disperse *P. asirensis* plant compounds peaks

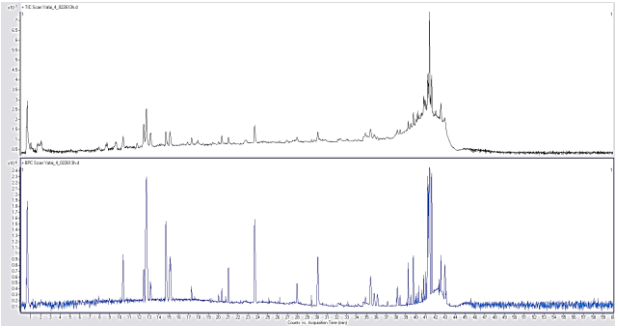


Figure 3 Positive TIC & positive BPC showing disperse *P. amboinicus* plant compounds peaks

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; Acetylcaranine 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 41.470min. 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.

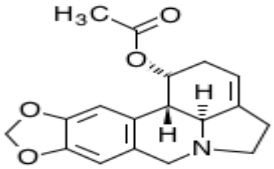
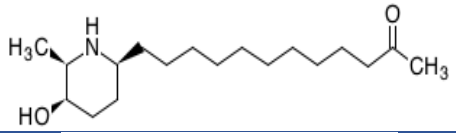
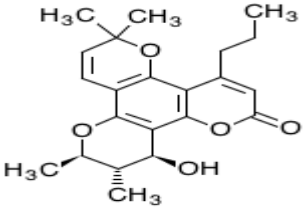
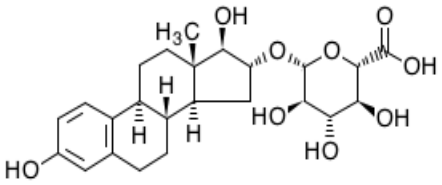
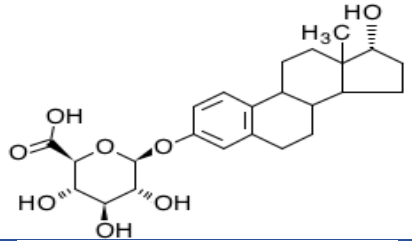
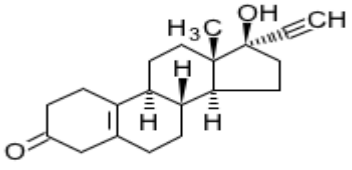
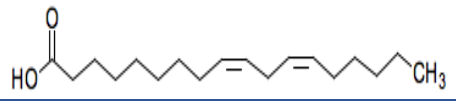
On the other hand, the methanolic extract of *P. amboinicus* plant showed twelve bioactive compounds using the same analysis technique. Four alkaloids (*i.e.* cassine, (S)–coclaurine, lentiginosine, and bellendine) were obtained in the retention time ranged 12.924-38.423

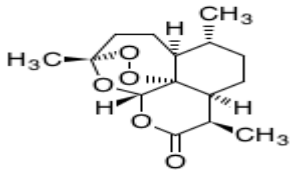
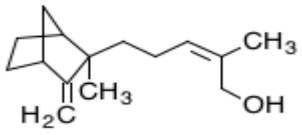
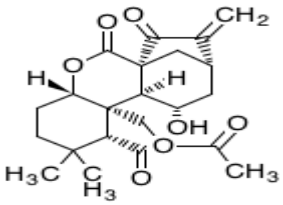
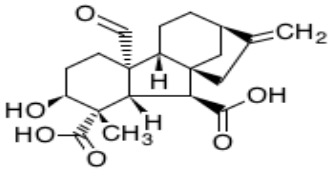
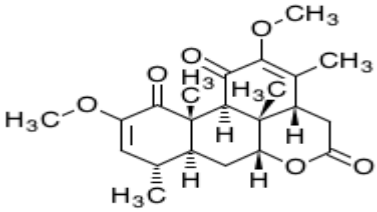
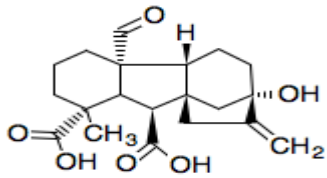
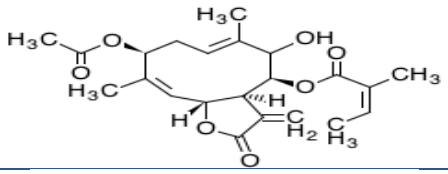
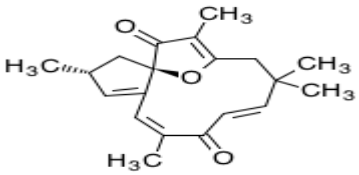
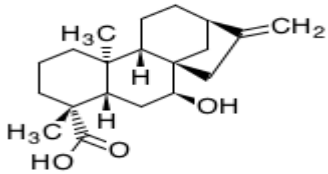
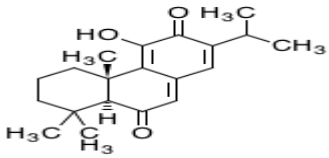
min. The three lipids found in this plant were peaked within the range 32.712-41.135 min. Caprylic acid, 8-Amino-7-oxononanoate, and glyceryl monostearate as fatty acids were obtained at 22.811-42.003 min and had exact masses 144.1152, 187.1208, and 358.30875 g/mol correspondingly. Two types of monoterpenoids were detected that were thymol (26.974 min; 150.1045 g/mol), and boschnialactone (39.783 min; 154.0994 g/mol).

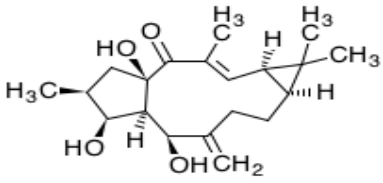
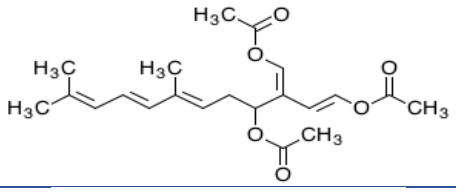
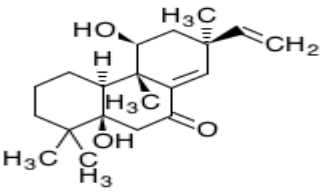
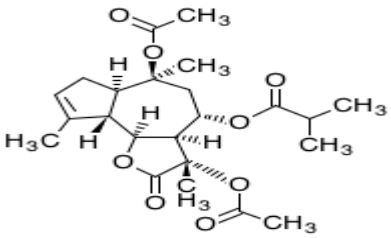
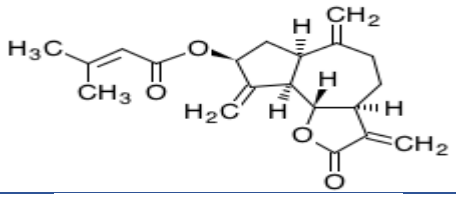
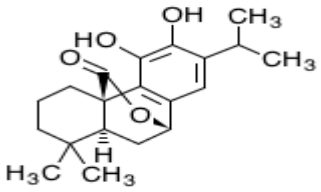
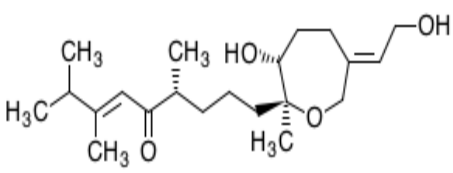
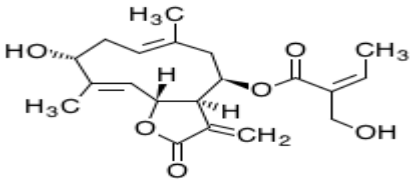
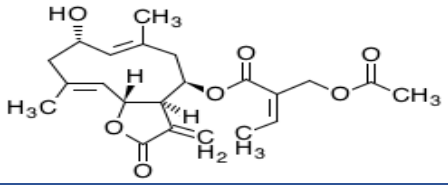
4. Conclusion

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.

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	Structure
1	16.824	**Alkaloid** (Isoquinoline alkaloids)	Acetylcaranine; Belamarine	313.1314	$C_{18}H_{19}NO_4$	
2	38.464	**Alkaloid** (Piperidine alkaloids)	Cassine	297.2668	$C_{18}H_{35}NO_2$	
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	$C_{18}H_{32}O_2$	

8	14.563	**Terpenoid** (Sesquiterpenoid)	Qing Hau Sau; Artemisinin	282.1467	$C_{15}H_{22}O_5$	
9	17.263	**Terpenoid** (Sesquiterpenoid)	beta-Santalol	220.1827	$C_{15}H_{24}O$	
10	18.992	**Terpenoid** (Diterpenoid)	Isodonal	404.1835	$C_{22}H_{28}O_7$	
11	19.621	**Terpenoid** (Diterpenoid)	Gibberellin A36	362.1729	$C_{20}H_{26}O_6$	
12	19.692	**Terpenoid** (Triterpenoid)	Quassin; Nigakilactone D	388.1886	$C_{22}H_{28}O_6$	
13	20.018	**Terpenoid** (Diterpenoid)	Gibberellin A19; Gibberellin 19	362.1729	$C_{20}H_{26}O_6$	
14	21.033	**Terpenoid** (Sesquiterpenoid)	Eupatocunin	404.1835	$C_{22}H_{28}O_7$	
15	21.824	**Terpenoid** (Diterpenoid)	Jatrophone	312.1725	$C_{20}H_{24}O_3$	
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	318.2195	$C_{20}H_{30}O_3$	
17	23.865	**Terpenoid** (Diterpenoid; Abietane)	Taxodione	314.1882	$C_{20}H_{26}O_3$	

18	23.977	**Terpenoid** (Diterpenoid)	Lathyrol	334.2144	$C_{20}H_{30}O_4$	
19	24.647	**Terpenoid** (Sesquiterpenoid)	Rhipocephalin	376.1886	$C_{21}H_{28}O_6$	
20	26.067	**Terpenoid** (Diterpenoid)	Ineketone	318.2195	$C_{20}H_{30}O_3$	
21	26.314	**Terpenoid** (Sesquiterpenoid)	Polhovolide	436.2097	$C_{23}H_{32}O_8$	
22	26.673	**Terpenoid** (Sesquiterpenoid)	Vernoflexin	328.1675	$C_{20}H_{24}O_4$	
23	28.157	**Terpenoid** (Diterpenoid)	Carnosol	330.1831	$C_{20}H_{26}O_4$	
24	33.865	**Terpenoid** (Diterpenoid)	Montanol	352.2614	$C_{21}H_{36}O_4$	
25	34.291	**Terpenoid** (Sesquiterpenoid)	Deacetyleupaserrin	362.1729	$C_{20}H_{26}O_6$	
26	37.492	**Terpenoid** (Sesquiterpenoid)	Eupaserrin	404.1835	$C_{22}H_{28}O_7$	

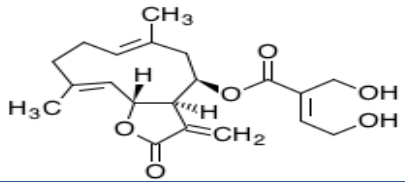
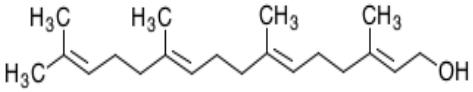
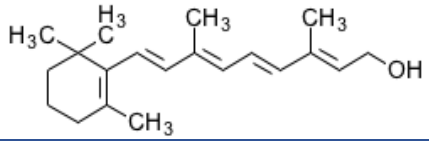
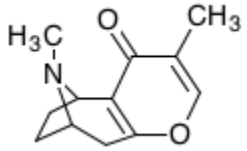
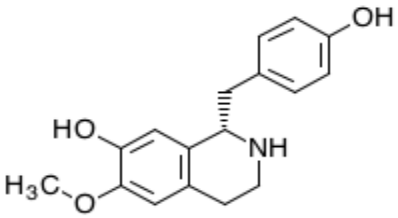
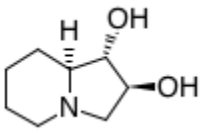
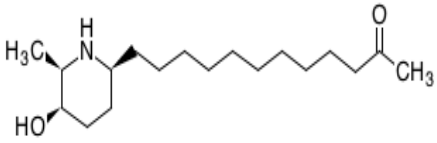
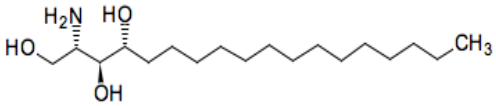
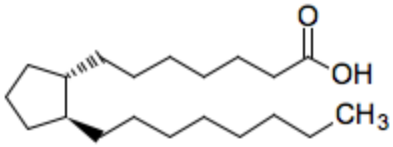
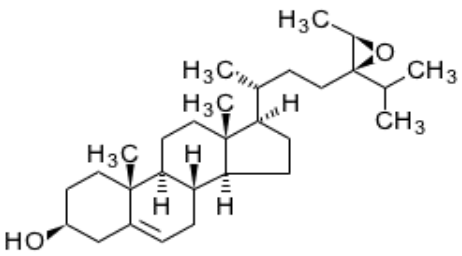
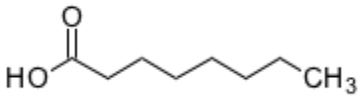
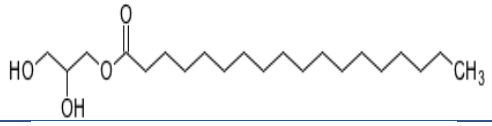
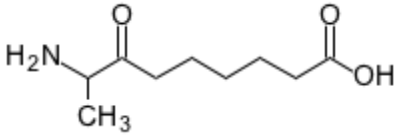
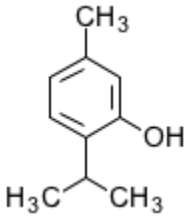
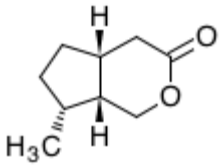
27	38.118	**Terpenoid** (Sesquiterpenoid)	Eupatoriopicrin	362.1729	$C_{20}H_{26}O_6$	
28	41.470	**Terpenoid** (Diterpenoid)	Geranylgeraniol; 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15- tetramethyl	290.2619	$C_{20}H_{34}O$	
29	40.983	**Vitamin** (Fat-soluble vitamin)	Retinol; all-trans-Retinol; Vitamin A; Vitamin A1	286.2297	$C_{20}H_{30}O$	

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	Structure
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	$C_8H_{15}NO_2$	
4	38.423	**Alkaloid** (Piperidine alkaloid)	Cassine	297.2668	$C_{18}H_{35}NO_2$	
5	32.712	**Lipid** (Sphingolipid)	Phytosphingosine; 4-D-Hydroxysphinganine	317.2930	$C_{18}H_{39}NO_3$	
6	40.413	**Lipid** (Eicosanoid)	Prostanoic acid	310.2872	$C_{20}H_{38}O_2$	
7	41.135	**Lipid** (Sterol)	24R,24'R)-Fucosterol epoxide	428.3654	$C_{29}H_{48}O_2$	

8	22.811	**Fatty acid** (Saturated)	Octanoic acid; Caprylic acid;	144.1152	$C_8H_{16}O_2$	
9	42.003	**Fatty acid** (Saturated)	Glyceryl monostearate	358.30875	$C_{21}H_{42}O_4$	
10	39.784	**Fatty acid** (Fatty acyl)	8-Amino-7-oxononanoate; 8-Amino-7-oxononanoic acid	187.1208	$C_9H_{17}NO_3$	
11	26.974	**Terpenoid** (Monoterpenoid)	Thymol	150.1045	$C_{10}H_{14}O$	
12	39.783	**Terpenoid** (Monoterpenoid)	Boschnialactone	154.0994	$C_9H_{14}O_2$	

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مقالة بحثية

تحليل بعض المكوّنات النشطة حيويّاً في نباتين طبيّين يمنيّين باستخدام HPLC-ESI-MS

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المُلخّص

تمتلك النّباتات العديد من المكوّنات النّشطة حيويّاً والّتي تلعب دوراً هامّاً في الطّبّ الشّعبي، بسبب امتلاكها فوائد صحّيّة في علاج العديد من الأمراض خصوصاً المكوّنات الأيضية الثّانويّة للنبات. مع هذا، لاتزال المعلومات التّفصيليّة حول هذه المركّبات محدودة. يهدف العمل الحالي إلى التّحقّق من وجود عدد من المركّبات النّشطة حيويّاً في نباتين يمنيّين هما نباتي العضرب والشعوس باستخدام الطور العكوس لكروماتوجرافيا السّائل عالي الأداء المرتبط بتأيّن الرّذاذ المكهرب- طيف الكتلة في وضعيّة التأيّن الموجب. وتعطي هذه الطّريقة التّجريبيّة المتّبعة توصيفاً للعديد من المكوّنات مثل القلويدات، الأحماض الدّهنيّة، السّيترويدات، والتّيربينودات. تسلّط النّتائج المتحصّل عليها الضّوء على أهمّيّة النّباتات المدروسة كمصدر طبيعي واعد للحصول على المركبّات النّشطة حيويّاً.

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

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