GC-MS Analysis of Various Crude Extracts from the Leaves, Flowers, and Stems of *Datura metel* Linnaeus 1753 and the Potential Activity as Anesthetic Agents on Fish

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Abstract

Anesthesia made from the *Datura metel* (locally known as kecubung) has high potential as an alternative to synthetic fish anesthetics. This study aimed to identify the active compound in leaves, stems and flowers of *D. metel* and describe its potency as a fish anaesthetic agent. Samples of *D. metel* were collected from Takengon City, Aceh Tengah Regency, Indonesia. The phytochemical and fractionation tests were carried out at the Microbiology and Biotechnology Laboratory as well as the Chemistry and Biochemistry Laboratory, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indonesia. Maceration technique using n-hexan solvent for 24 hours at a ratio of 1:2 v/v. GC-MS analysis was carried out to identify the chemical compounds in the extracts. The results showed that, as many as eleven compounds in leaves, seven compounds in flowers, and two compounds in stems of *D. Metel* were potential as fish anesthetics agent. In leaves, several compounds that have the potential as anesthetics agent of fish include: seychellene; trans-caryophyllene; α-guaiene; 1h-3a,7-methanoazulene,2,3,6,7,8,8a-hexa; alloaromadendrene; δ-guaiene; (-)-caryophyllene oxide; epiglobulol; 2-pentadecanone,6,10,14-trimethyl-; phytol. In the flower, include: trans-caryophyllene; α-guaiene; seychellene; alloaromadendrene; (-)-caryophyllene oxide; phytol. While, in stems include: α-guaiene and seychellene. These substances have reportedly been utilized as anaesthetic agents for several fish, including *Colossoma macropomum*, *Rhamdia quelen*, and *Oreochromis niloticus*.

Keywords: α-guaiene, phytol, seychellene, anesthetics

1. Introduction

The use of synthetic anesthetics in fish has several risks, such as residues that have an impact on fish, consumers and the environment. Synthetic anesthetics commonly used in fish include 2-phenoxyethanol, benzocaine (etil paraaminobenzoate), carbon dioxide, eetomidate, and tricaine methanesulfonate (MA-222) (Purbosari *et al*., 2019). The risk of using synthetic anesthetics in fish causes hypoxemia, hypercapnia, hypoglycaemia, increased levels of lactic acid, erythrocyte swelling, elevated haematocrit and changes in blood electrolytes, hormones, cholesterol, urea and inter-renal ascorbic acid (Martins *et al*., 2019). Therefore, an inventory of natural anesthetics continues to be carried out to avoid residues. The advantages of natural anesthetics include low impact on the environment, no residue, cheaper, and effective at low concentrations compared to synthetic anesthetics (Purbosari *et al*., 2019). There are several herbal plants that have bioactive components as anesthetic agents that have been identified, where *Datura metel* (local name is kecubung) is one of them that has high potential as an anesthetic compound (Adebayo and Olufayo, 2017; Hariyanto *et al*., 2009; Palmi *et al*., 2019; Akbar *et al*., 2021; Saputra *et al*., 2021).

All parts of *D. metel* including roots, stems, leaves, flowers, fruits and seeds are reported to contain bioactive compounds including alkaloids, saponins, flavonoids, and phenols (Kuganathan and Ganeshalingam, 2011; Alabri, 2014). The concentration of alkaloids in the roots and seeds reached 0.4-0.9%, while in the leaves and flowers it reached 0.2-0.3%. High concentrations of alkaloids in *D. metel* can cause anticholinergic activity in the body, resulting in several cases such as hallucinations, delirium and convulsions. Based on BNN (National Anti-Narcotics Agency of Indonesia) (2014) data, *D. metel* is often used as a narcotic, where hallucinogenic compounds reach 3%.

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D. metel originated from Central America and southeastern Mexico which was then cultivated and spread widely because of the beauty of the flower and its medicinal benefits (Monira and Munan, 2012). *D. metel* has the same name for all races although it actually has chromosomal differences and produces different flower colors (Bergner, 1943). In the Gayo highlands (Aceh Tengah Regency, Indonesia), this plant is widely cultivated and used as an ornamental plant in the yard for the treatment of asthma. *D. metel* which grows in the Gayo highlands has stems that can grow tall and have reddish yellow flowers. *D. metel* has been used as a medicinal ingredient for centuries as an anti-bacterial, antiseptic, narcotic, and sedative (Ganesh *et al.*, 2015; Alam *et al.*, 2021). The people of Aceh use *D. metel* to mix hernia ingredients, treat toothache, rheumatism and fungal infections (Ristoja, 2012).

Studies on extraction solutions, chemical compositions of extracts from various parts of the plant genus *Datura* have been analyzed and their anesthetic activity tested on various types of fish such as *Cyprinus carpio* (Rahanandeh *et al.*, 2022), *Epinephelus* sp. (Saputra *et al.*, 2021), *Heterobranchus bidorsalis* (Adebayo and Olufayo, 2017), *Oreochromis niloticus* (Palmi *et al.*, 2019), and *Osphronemus gourami* (Mashuda *et al.*, 2020). In addition, the effectiveness of this plant anesthetic has also been tested on dogs (Bbalola *et al.*, 2014) and rabbits (Elsa *et al.*, 2001). Previous research by Kiruthika and Sornaraj (2011) revealed that there are four active compounds in *D. metel* flower extract including 1,4-cyclohexadiene, 1-methyl; acetic acid, trifluoro-, 2,2-dimethylpropylester; 4-trifluoroacetoxyoctane; cis-2-nitro-4-tbutylcyclohexanone. However, information regarding the content of active compounds (including stems, leaves and flowers) that have the potential as anesthetic in *D. metel* has not been reported completely. Therefore, identification is needed to obtain complete information to support the use of *D. metel* extract as a natural anesthetic that is safe and does not leave negative effects on fish, people, and the environment.

2. Material and methods

2.1. Site and Time

This research was carried out from September 2021 to January 2022. Sample preparation and extraction were carried out in the MIPA Laboratory of Almuslim University, Indonesia. The phytochemical and fractionation tests were carried out at the Microbiology and Biotechnology Laboratory as well as the Chemistry and Biochemistry Laboratory, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indonesia.

2.2. Sample Collection

Samples of *D. metel* were collected from Takengon City, Aceh Tengah Regency, Indonesia. The samples were then sorted between stems, leaves and flowers, then air-dried. The dried sample was then crushed with a waring blender to obtain the required sample size. The resulting yield was calculated based on weight percent extract/dry weight (w/w) and stored in a sample bottle at -4ºC (Silva *et al.*, 2012). The ingredients were then identified by comparing mass spectrum value based on the NIST (2005)

and Adams (2001) mass spectrum literature. Fractionation and isolation of essential oils were carried out according to the method of Benovit *et al.* (2015).

2.3. Extraction of Phytochemical Components

The leaves, stems and flowers that have been air-dried and crushed were then extracted by maceration technique using n-hexan solvent for 24 hours at a ratio of 1:2 v/v. The extract solution was filtered using Whatman paper no.1. A thick extract was generated by combining the maceration filtrates and vaporizing the solvent in a vacuum rotary evaporator at a temperature close to the solvent's boiling point (n-hexane 69°C).

2.4. GC-MS Analysis

GC-MS analysis was performed using a Shimadzu GC-MS-QP2010 Ultra equipped with a 30 m × 0.25 mm × 0.25 μm Rxi-1MS column (Restek), and the initial temperature of the 100ºC column was heated for 5 minutes, then the temperature was gradually increased up to 250ºC at a rate of 10ºC min^{-1}. The split injector and the GC-MS interface were each at a temperature of 250ºC. The detectors used were mass-selective and electron-impact mass ionization spectrometry programmed at 70 eV and a temperature of 250°C. The carrier gas used was helium with a flow rate of 2.0 mL min^{-1}, and an injection volume of 2 L. Data was recorded using GC-MS Solution Software (Shimadzu).

2.5. Chemical Identification

Chemical compounds in the extracts from the leaves, stems and flowers of *D. metel* were identified based on the retention time of GC on the column. Chromatograms were interpreted based on the NIST (2005) library consisting of two hundred thousand compounds. The name, molecular weight and percentage of the unknown compound were evaluated by Mass Spectrometry. The compounds were identified by comparing the results of Mass spectrometry measurements with data obtained from the literature and the NIST database.

3. Results

About 23-26 (a total of 52) compounds were identified in the hexane extract of *D. metel* from the leaves, flowers and stems (Table 1 and Figure 1). Based on the results of the GC-MS analysis and literature studies, the compounds that have the potential as anesthetics in *D. metel* are 11 compounds in the leaf, 7 compounds in the flower, and 2 compounds in the stem. Compounds that have the potential as anesthetics in *D. metel* are 11 compounds in the leaves, 7 compounds in the flowers, and 2 compounds in the stems. In the hexane extract of the *D. metel* leaves, compounds that have the potential to be anesthetics include seychellene; trans-caryophyllene; α-guaiene; 1h-3a,7-methanoazulene,2,3,6,7,8,8a-hexa; alloaromadendrene; δ-guaiene; (-)-caryophyllene oxide;epiglobulol; 2-pentadecanone,6,10,14-trimethyl-; phytol. In the hexane extract of the flowers of *D. metel*, compounds that have the potential to be anesthetics include trans-caryophyllene; α-guaiene; seychellene; alloaromadendrene; (-)-caryophyllene oxide; phytol. In the hexane extract of the stem of *D. metel*, compounds that have the potential to be anesthetics include α-guaiene dan seychellene.

Table 1. Compounds detected in leaves, flowers and stems of *Datura metel* with hexane solvent using GC-MS analysis

| No	Compounds	Leaves		Flowers		Stems	
		Retention Time (minute)	Area (%)	Retention Time (minute)	Area (%)	Retention Time (minute)	Area (%)
1	Guaia-1(10),11-diene	10.526	2.96	10.521	2.92	-	-
2	Seychellene	10.920	1.14	-	-	-	-
3	trans-Caryophyllene	10.997	1.39	10.995	0.65	-	-
4	α-Guaiene	11.236	9.41	11.236	6.90	11.196	3.13
5	Seychellene	11.397	11.20	11.392	6.84	11.356	3.82
6	1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexa	11.582	7.98	-	-	-	-
7	Alloaromadendrene	12.013	2.60	12.005	1.62	-	-
8	δ-Guaiene	12.094	5.88	12.100	4.73	-	-
9	Ledol	12.914	4.83	-	-	-	-
10	1H-Cycloprop[e]azulen-4-ol, decahydro-1,	13.000	0.57	12.902	1.09	-	-
11	(-)-Caryophyllene oxide	13.069	3.38	-	-	-	-
12	Kauran-18-al, 17-(acetyloxy)-, (4-beta.)- (C	13.444	0.65	-	-	-	-
13	Epiglobulol	13.959	0.94	-	-	-	-
14	6-ISOPROPENYL-4,8A-DIMETHYL-3,5,	14.438	1.20	-	-	-	-
15	2-Pentadecanone, 6,10,14-trimethyl-	15.998	3.01	-	-	15.990	2.89
16	NEOPHYTADIENE	16.045	0.96	16.001	0.96	16.035	1.51
17	Palmitic acid, Hexadecanoic acid, methyl e	16.825	1.51	-	-	-	-
18	Hexadecanoic acid, ethyl ester (CAS) Ethyl	17.550	2.25	-	-	-	-
19	1-Octadecanol (CAS) Stenol	18.498	2.29	-	-	-	-
20	Phytol	18.900	31.07	18.819	0.69	-	-
21	Ethyl linoleate	19.212	1.26	19.259	13.10	-	-
22	ETHYL LINOLEOLATE	19.259	1.17	-	-	-	-
23	1-Octadecanol (CAS) Stenol	20.445	2.36	-	-	-	-
24	α-Patchoulene	-	-	11.583	5.27	-	-
25	(-)-Caryophyllene oxide	-	-	13.060	1.84	-	-
26	Decanedioic acid, diethyl ester (CAS) Bisof	-	-	13.917	1.18	-	-
27	Tetradecanoic acid, ethyl ester (CAS) Ethyl	-	-	15.368	1.16	-	-
28	Pentadecanoic acid, ethyl ester	-	-	16.180	1.11	-	-
29	Hexadecanoic acid, methyl ester (CAS) Me	-	-	16.828	1.61	-	-
30	Ethyl 9-hexadecenoate	-	-	17.322	3.08	-	-
31	Hexadecanoic acid, ethyl ester (CAS) Ethyl	-	-	17.640	24.57	17.601	20.58
32	Octadecanoic acid, ethyl ester (CAS) Ethyl	-	-	18.553	1.34	18.290	0.70
33	ETHYL OCTADEC-9-ENOATE	-	-	19.315	5.03		
34	(E)-9-Octadecenoic acid ethyl ester	-	-	19.368	2.36	19.274	2.14
35	Octadecanoic acid, ethyl ester	-	-	19.586	9.07	19.547	5.01
36	Eicosanoic acid, ethyl ester	-	-	21.513	2.89	21.473	2.28
37	2-Decenal, (Z)-	-	-	-	-	8.340	2.48
38	2(3H)-Furanone, dihydro-5-pentyl- (CAS)	-	-	-	-	9.582	2.25
39	2-Undecenal, E-	-	-	-	-	9.825	0.70
40	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahy	-	-	-	-	10.505	1.08
41	Nonanoic acid, 9-oxo-, methyl ester	-	-	-	-	10.579	2.19
42	Nonanoic acid, 9-oxo-, ethyl ester (CAS)	-	-	-	-	11.563	6.08
43	9-Octadecenoic acid, methyl ester, (E)- (C	-	-	-	-	12.683	0.58
44	Nitrobenzene, 3,4,5-trimethoxy-	-	-	-	-	14.227	0.59
45	Tridecanoic acid, 12-methyl-, methyl ester	-	-	-	-	15.407	1.93
45	Pentadecanoic acid, methyl ester (CAS) Me	-	-	-	-	15.715	1.28
46	Heptadecanoic acid, ethyl ester (CAS) Ethy	-	-	-	-	16.178	4.23
47	Pentadecanoic acid, 14-methyl-, methyl est	-	-	-	-	16.880	22.27
46	Linoleic acid, 9,12-Octadecadienoic acid (Z	-	-	-	-	18.540	1.72
49	9-Octadecenoic acid, methyl ester, (E)-	-	-	-	-	18.624	2.42
50	Methyl stearate	-	-	-	-	18.899	5.57
51	9,12-Octadecadienoic acid (Z,Z)-	-	-	-	-	19.190	1.30
52	N-HENTRIACONTANOL-1	-	-	-	-	20.443	1.28
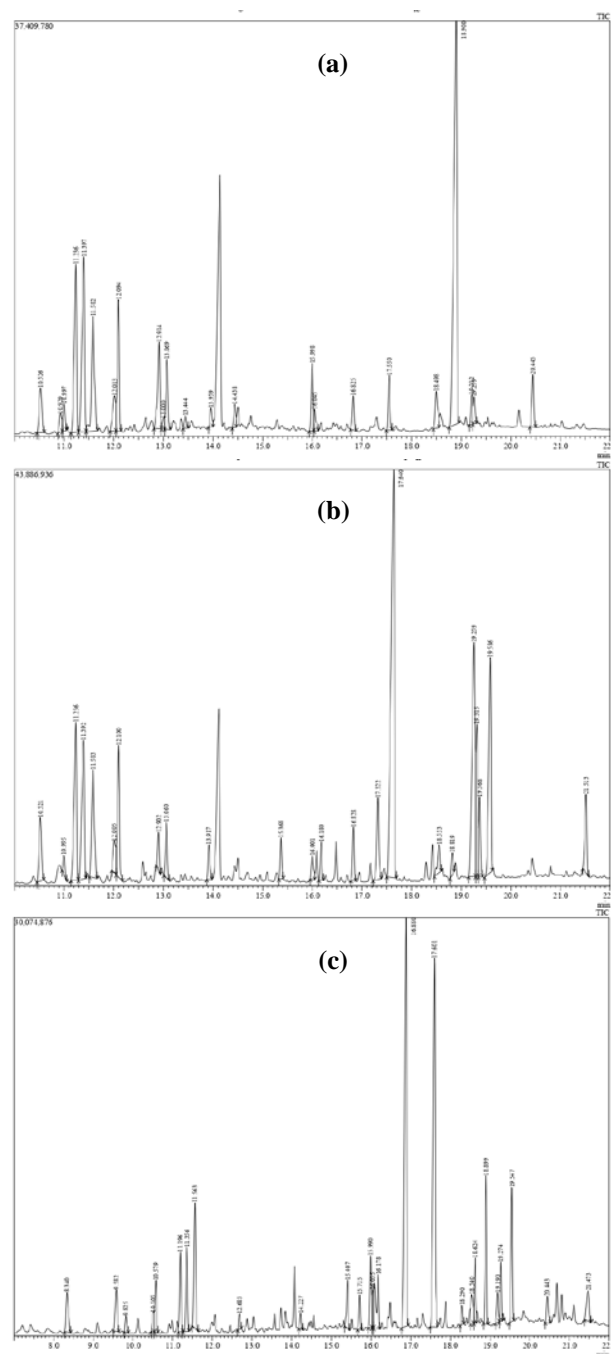
Figure 1. Chromatogram characteristics of hexane extract compounds from leaves (a), flowers (b), stems (c) of *Datura metel* plant.

4. Discussion

Compounds that have the potential to be anesthetics in the hexane extract of the leaves of *D. metel* reach 11 compounds, namely: seychellene (Swamy and Sinniah, 2015; Lu *et al.*, 2011); trans-caryophyllene (Pinho-da-Silva *et al.*, 2012); α-guaiene (Silva *et al.*, 2015; Jugran *et al.*, 2019); 1h-3a,7-methanoazulene,2,3,6,7,8,8a-hexa (Kartal *et al.*, 2002); alloaromadendrene (Benovit *et al.*, 2015; Almeida *et al.*, 2018); δ-guaiene (Silva *et al.*, 2015; Uritu *et al.*, 2018; Jugran *et al.*, 2019); (-)-caryophyllene oxide (Silva *et al.*, 2015; Almeida *et al.*, 2018; Benovit *et al.*, 2015; Sharif *et al.*, 2020); epiglobulol (Wang, 2018); 2-pentadecanone,6,10,14-trimethyl- (Sharif *et al.*, 2020);

and phytol (Sharif *et al.*, 2020). A total of eleven compounds were identified; Phytol is the dominant compound with a percentage of chromatogram area of 31.07% and retention time of 18.9 minutes, followed by seychellene and α-guaiene with a chromatogram area of 11.20% and 9.41%, with retention times of 11.397 and 11.236 minutes.

Phytol is a diterpene compound from the degradation of chlorophyll. This is because the extraction material is part of the leaves of the *D. metel* plant. Furthermore, phytol is a phytochemical herbal phytoconstituents compound, which is widely distributed in nature and was first obtained in the process of separating chlorophyll from alfalfa through a hydrolysis process (Taj *et al.*, 2021). These compounds are known to have biological activities including anti-microbial, anti-tumor, anti-cancer, anti-anxiety, anti-inflammatory, anti-diabetic and a number of other biological activities (Islam *et al.*, 2018). In vivo test using an animal model conducted by Santos *et al.* (2013) showed that Phytol compounds have good anti-pain activity when compared to morphine and indomethacin. Meanwhile, morphine is also known to have an anesthetic effect on both animals and humans (Dahan *et al.*, 2001; Jash and Gorai, 2015; Wang *et al.*, 2021).

Seychellene and α-guaiene are sequisterpene compounds identified in *Pogostemon cablin* oil (Swamy and Sinniah, 2015; Astuti *et al.*, 2022). The biological activities of *P. cablin* oil include anti-microbial, anti-inflammatory, anti-oxidant, anti-depressant, and cytotoxicity (Jain *et al.*, 2022). Furthermore, the results of research by Astuti *et al.* (2022) showed that *P. cablin* variant Tapak Tuan, Aceh Province, Indonesia with traditional distillation process contains anti-depressant compounds including patchouli alcohol (28.68 %), δ-guaiene (24.87 %), α-guaiene (16.89 %) and seychellene (8.32 %) based on the percentage of chromatogram area as a result of GC-MS analysis. Although, then, very few of these components were identified in the results of the further distillation of *P. cablin* oil using a rotary evaporator with a heating temperature of 125-160°C, which showed anti-depressant activity close to the anti-depressant activity of Kalxetin. Subsequent literature studies showed that the plant extract *P. cablin* has analgesic activity (Lu *et al.*, 2011; Junren *et al.*, 2021). However, there is no literature that states directly that both seychellene and α-guaiene compounds have anti-depressant and analgesic activity where both of these biological activities have a milder relationship to anesthetic activity.

The results of the GC-MS analysis of the hexane extract of the flowers of *D. metel* showed that the dominant compounds were fatty acids and fatty acid esters, respectively hexadecanoic acid, ethyl ester (CAS) ethyl (24.57 %), ethyl linoleic (13.10%) and octadecanoic acid, ethyl ester (9.07%) based on the percentage of chromatogram area. The three compounds were not identified to have anesthetic activity but have other biological activities such as anti-oxidants and anti-microbials (Durugbo *et al.*, 202), while compounds that have the potential to have anesthetic activity include: trans-caryophyllene (Pinho-da-Silva *et al.*, 2012), α-guaiene (Silva *et al.*, 2015; Jugran *et al.*, 2019), seychellene (Lu *et al.*, 2011; Swamy and Sinniah, 2015), alloaromadendrene (Benovit *et al.*, 2015; Almeida *et al.*,

2018), δ-guaiene (Silva *et al*., 2015; Uritu *et al*., 2018; Jugran *et al*., 2019), (-)-caryophyllene oxide (Silva *et al*., 2015; Almeida *et al*., 2018; Benovit *et al*., 2015; Sharif *et al*., 2020), and phytol (Sharif *et al*., 2020). In the seven compounds, the percentage of chromatogram area indicated by α-guaiene and seychellene was the highest, reaching 6.90% and 6.84%, respectively.

The hexane extract of the stem of *D. metel* showed compounds that have the potential to have anesthetic activity including α-guaiene (Silva *et al*., 2015; Jugran *et al*., 2019) and seychellene (Lu *et al*., 2011; Swamy and Sinniah, 2015) with the percentage of the chromatogram area is 3.13% and 3.82%. This percentage is smaller than that contained in flowers and leaves. The dominating compounds in the hexane extract are fatty acid ester compounds, namely pentadecanoic acid, 14-methyl-, methyl ester, and hexadecanoic acid, ethyl ester with a chromatogram area of 22.27% and 20.58%, respectively. Similar with the research conducted by Hossain *et al*. (2013) that the hexane extract from *D. metel* was dominated by fatty acid groups and their derivatives, but the presence of several types of sesquiterpene compounds in the hexane extract of leaves, flowers and stems of *D.*

metel identified was slightly different from previous studies. The molecular configurations read from the mass spectra of α-guaiene, seychellene and phytol compounds can be seen in Figure 2.

Various studies have been conducted to identify fish anaesthetic agents derived from natural substances. Inoue *et al*. (2003) revealed that clove oil was an effective anaesthetic for *Brycon cephalus* fish. It has also been reported that extracts from the plants *Lippia alba, Spilanthes acmella*, and *Nicotiana tabacum* have the potential to serve as anaesthetic agents for several species of fish (Cunha *et al*., 2011; Barbas *et al*., 2016; Agokei and Adebisi, 2010; Zulfahmi *et al*., 2019). Several secondary metabolic compounds found in datura metel, such as guaiene; seychellene; trans-caryophyllene; α-guaiene; 1h-3a,7-methanoazulene,2,3,6,7,8,8a-hexa; alloaromadendrene; δ-guaiene; (-)-caryophyllene oxide; epiglobulol; 2-pentadecanone,6,10,14-trimethyl-; phytol. Phytol was also observed in other plants (*Lippia alba* and *Colossoma macropomum*) that have been demonstrated to be effective as natural anesthetics for fish (Barbas *et al*., 2016; dos Santos Maia *et al*., 2019).
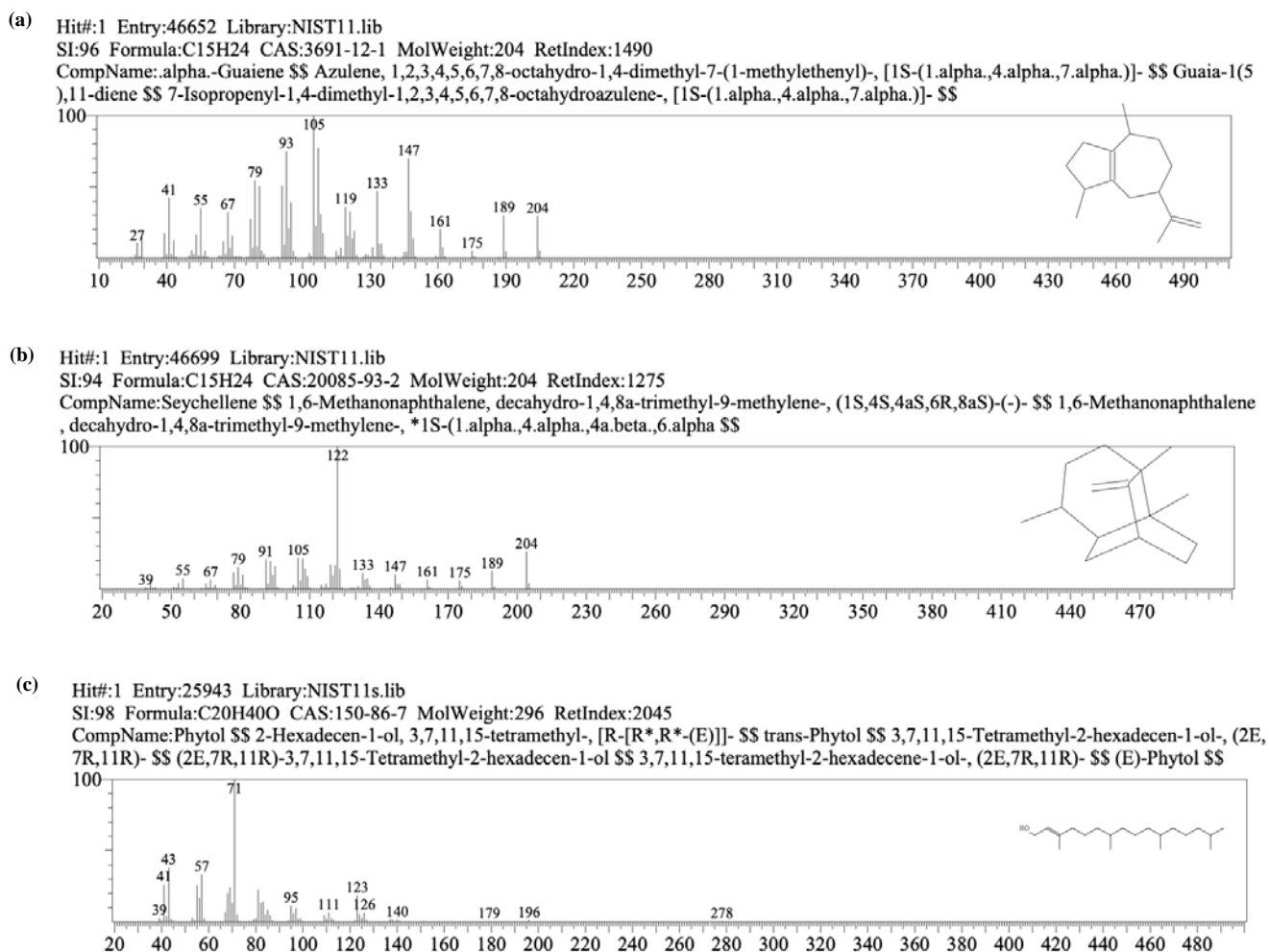
(a) Hit#:1 Entry:46652 Library:NIST11.lib
SI:96 Formula:C15H24 CAS:3691-12-1 MolWeight:204 RetIndex:1490
CompName:.alpha.-Guaiene $$ Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]- $$ Guaia-1(5),11-diene $$ 7-Isopropenyl-1,4-dimethyl-1,2,3,4,5,6,7,8-octahydroazulene-, [1S-(1.alpha.,4.alpha.,7.alpha.)]- $$

(b) Hit#:1 Entry:46699 Library:NIST11.lib
SI:94 Formula:C15H24 CAS:20085-93-2 MolWeight:204 RetIndex:1275
CompName:Seychellene $$ 1,6-Methanonaphthalene, decahydro-1,4,8a-trimethyl-9-methylene-, (1S,4S,4aS,6R,8aS)-(-)- $$ 1,6-Methanonaphthalene, decahydro-1,4,8a-trimethyl-9-methylene-, *1S-(1.alpha.,4.alpha.,4a.beta.,6.alpha $$

(c) Hit#:1 Entry:25943 Library:NIST11s.lib
SI:98 Formula:C20H40O CAS:150-86-7 MolWeight:296 RetIndex:2045
CompName:Phytol $$ 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- $$ trans-Phytol $$ 3,7,11,15-Tetramethyl-2-hexadecen-1-ol-, (2E,7R,11R)- $$ (2E,7R,11R)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol $$ 3,7,11,15-teramethyl-2-hexadecene-1-ol-, (2E,7R,11R)- $$ (E)-Phytol $$

Figure 2. Mass spectra of α-guaiene (a), Seychellene (b) and Phytol (c) according to the NIST library.

5. Conclusion

Compounds that have the potential as anesthetics in *D. metel* are 11 compounds in the leaves, 7 compounds in the flowers, and 2 compounds in the stems. In the hexane extract of the leaves of *D. metel*, compounds that have the potential to be anesthetics include seychellene; trans-caryophyllene; α-guaiene; 1h-3a,7-methanoazulene,2,3,6,7,8,8a-hexa; alloaromadendrene;δ-guaiene; (-)-caryophyllene oxide; epiglobulol; 2-pentadecanone,6,10,14-trimethyl-; phytol. In the hexane extract of the flowers of *D. metel*, compounds that have the potential to be anesthetics include trans-caryophyllene; α-guaiene; seychellene; alloaromadendrene; (-)-caryophyllene oxide; phytol. In the hexane extract of the stem of *D. metel*, compounds that have the potential to be anesthetics include α-guaiene dan seychellene. The leaves of *D. metel* have the best anesthetic potential because they have the most anesthetic components, so their use and testing are important to do in fish in future studies.

Conflict of interest

None.

Acknowledgments

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