

Bioactive compounds evaluation
of the mudskippers
(*Periophthalmus chrysospilos*,
Boleophthalmus boddarti,
Boleophthalmus dussumieri and
Periophthalmodon schlosseri) in
the estuarine area of Musi River,
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Type

Research paper

Keywords

mudskippers, musu river, bioactive compounds

Abstract

This research was conducted to assess the various bioactive compounds in the meat of some mudskippers found in the Sungsang 2 Village, Banyuasin regency, South Sumatra. The research had been synthesized using n-hexane from February to May 2018 at Agricultural Technology Production Laboratory, Sriwijaya University. Subsequently, the mudskippers (Periophthalmus chrysospilos, Boleophthalmus boddarti, and Periophthalmodon schlosseri) extract was synthesized and further analysed by using GC-MS analysis. The study of bioactive compounds in mudskippers was carried out by evaporating in vacuum evaporation at 30°C. The results showed that Periophthalmus chrysospilos has five classes of bioactive compounds such as steroids, terpenoids, carotenoids, cannabinoids, and alkaloids and Boleophthalmus boddarti has five classes of bioactive compounds such as steroids, carotenoids, terpenoids, bufadienolides, and carotatoxins. Furthermore, Boleophthalmus dussumieri has four classes of bioactive compounds such as steroids, carotenoids, terpenoids, and carotatoxins and Periophthalmodon schlosseri has three classes of bioactive compounds such as steroids, carotenoids and terpenoids. This research discusses the potential benefit of all bioactive compounds in mudskippers for alleviating and treating people with enuresis in children through the literature review of the most cited bioactive compounds.

1 **Bioactive compounds evaluation of the mudskippers (*Periophthalmus***
2 ***chrysopilos*, *Boleophthalmus boddarti*, *Boleophthalmus dussumieri* and**
3 ***Periophthalmodon schlosseri*) in the estuarine area of Musi River, South**
4 **Sumatera, Indonesia**

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11 some mudskippers founding in the Sungsang 2 Village, Banyuasin regency, South Sumatra. The
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13 Technology Production Laboratory, Sriwijaya University. Subsequently, the mudskippers
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18 terpenoids, carotenoids, cannabinoids, and alkaloids and *Boleophthalmus boddarti* has five classes
19 of bioactive compounds such as steroids, carotenoids, terpenoids, fufadienolides, and
20 carotatoxins. Furthermore, *Boleophthalmus dussumieri* has four classes of bioactive compounds
21 such as steroids, carotenoids, terpenoids, and carotatoxins and *Periophthalmodon schlosseri* has
22 three classes of bioactive compounds such as steroids, carotenoids and terpenoids. This research
23 discuss the potential benefit of all bioactive compounds in mudskippers for alleviate and treating
24 people with enuresis in child through the literature review of the most cited bioactive compounds.

25 **Keywords:** Mudskippers, Musi river, Bioactive compounds

26 **1. Introduction**

27 Mudskipper is a genus from the family of Gobiidae and subfamily Oxudercinae that is
28 habitually known as ikan gelodok at Musi river, South Sumatera (Shukla et al., 2014; Polgar et al.,
29 2017; Lauriano et al., 2018; Ridho et al., 2019). Mudskippers are commonly found in brackish
30 river water, and beaches with certain salinity concentration, and mangrove areas of the Indo-
31 Pacific region (Tytler and Vaughan, 1983). Mudskippers are amphibious fish that are able to
32 tolerate in both freshwater and marine on muddy and sandy bottom habitat and spawn in estuarine
33 areas (Hu et al., 2016; Hong et al., 2017). Mudskippers are also capable to spend extended periods

out of water (Martin et al., 2013; Konstantinidis et al., 2016; Martinez et al., 2018). They appear during low tide, forage on tropical mudflats, seek mate and preserve territories for some portion of their daily cycle (Dabruzzi et al., 2019). According to the classification of amphibious gobies, 40 species mudskippers in the world have been currently identified which are divided into four genera of Oxudercinae, namely *Boleophthalmus*, *Periophthalmus*, *Periophthalmodon* and *Scartelaos* (Clayton 1993; Graham 1997; Graham and Wegner 2010; Ishimatsu and Gonzales 2011; Takeda et al., 2012; Murdy 2011a, You et al., 2018).

Juveniles of this Gobiidae are herbivores that eat more diatoms and filamentous algae, while the major food of adult Gobiidae are crustaceans (95.5%), polichaetas (86.4%) and land insects (85%) (Bob-Manuel, 2011). The burrows of these habitats are categorically suitable for the mudskippers for feeding grounds, spawning areas and nursery grounds (Masuda et al., 1984; Bob-Manuel, 2011; Satapoomin and Poovachiranon, 1997; Redjeki, 2013). Mudskippers are commonly consumed and employed specifically traditional medicine in many countries such as China, Malaysia and Japan (Feulner 2013; Gadhavi et al., 2017; Ikram et al., 2010; looi et al., 2016 and Yang, X. X., 2017). Medicinal mudskippers were often prepared from the natural products which contain of different bioactive compounds (Mahadevan et al., 2019; Wu et al., 2009). On the other hand, many fisherman and general public around Musi river ignore the existence and benefits of mudskippers.

Corticosteroids, one of bioactive compound in mudskippers, are steroid hormone class for overcoming inflammatory (Panda and Mabalirajan, 2018; Heughten et al., 2018). These hormones is one contributing factor to alleviate enuresis (bedwetting) in child. Enuresis is most common for a child or young person's behaviour that is denoted by repeated voiding of urine into the bed or clothing (Apos et al., 2017 and shafi et al., 2019). the study area represents bioactive compound like corticosteroid compounds in mudskippers that can reduce enuresis problem in children (Jain et al., 2016). Based on these things, the authors intend to evaluate the bioactive compounds in the four species mudskippers including *Periophthalmus chrysophilos*, *Boleophthalmus boddarti*, *Boleophthalmus dussumieri* and *Periophthalmodon schlosseri*.

2. Materials and Methods

2.1 Fishes and Experimental Design

Mudskippers were obtained from estuarine area around the Sungsang 2 village, Banyuasin regency, South Sumatera. Mudskippers, apparently healthy, were randomly caught using fishing equipment and transported to the Agricultural Technology Production Laboratory, Sriwijaya University for cleaning and removing the bones from the fish. After the fillet process, the mudskippers were divided into four groups with *Periophthalmus chrysophilos* (8 grams), *Boleophthalmus boddarti* (100 grams), *Boleophthalmus dussumieri* (13.8 grams) and *Periophthalmodon schlosseri* (100 grams) which were each homogenized.

Crushed fish flesh dissolved in n-hexane solvent with a sample ratio of 1: 4 and macerated for 24 hours. The next step is filtering with calico cloth and filter paper and evaporating with vacuum evaporator at at 30°C with a speed of 200 rpm. Each extract sample was reconstituted using n-hexane solvent, then each 1 µL was taken to be injected and analyzed in GC-MS alternately.

2.2 Analysis Technique

The data in this research were expressed in the form of chromatograms that contain graphs

and were equipped with a list of detected chemical compounds, along with the structure of compounds, retention times, areas, and probability of compound types. Data analysis was performed by identifying the chemical compounds detected to find out the natural name (nature of compound name), synonym, and the class of compounds of these chemical compounds. Identification is done by comparing the compound name detected with a data base on the literature in the form of books and journals. We included studies reported as full-text articles, those published as abstracts only and unpublished data.

2.3 Gas Chromatography and Mass Spectroscopy (GC-MS) Analysis

Qualitative analyses of mudskipper extract were expressed as a GCMS-QP 2010 Plus (Shimadzu, Japan) system equipped under computer control at 70 eV (Mohammed and Imad, 2013). The 1 μ l of the methanol extract was entered into the GC-MS with a micro injector and followed by scanning for 45 min. The temperature was maintained in the oven at 100°C and Helium gas was utilized as a carrier as well as an eluent. The helium flow was arranged to 1 ml per min and electron energy was fired by a mass detector about 70 eV. The compound of elite 5 was used as separation column (100% dimethyl poly siloxane) (Hameed et al., 2016; Kareem et al., 2015). The identity of the compounds in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries.

3. Results and Discussions

3.1 GC-MS Results of *Periophthalmus chrysopilos* extract

Gas chromatography and mass spectroscopy analysis of bioactive compounds was carried out in *Periophthalmus chrysopilos* extract that was presented in Fig. 1. It can be seen that the vertical lines in the chromatogram pattern point out the short periods of time when the ion beam is deflected-off the plate (and thus off the beam monitor) which is necessary when changing the position of the photographic plate, a process that takes about 2-3 seconds with our present arrangement. This pen deflection affords a useful signal of the exact quantities of the chromatogram being noted with the mass spectrometer and facilitates in the reconnection of the spectra on the plate with the chromatogram.

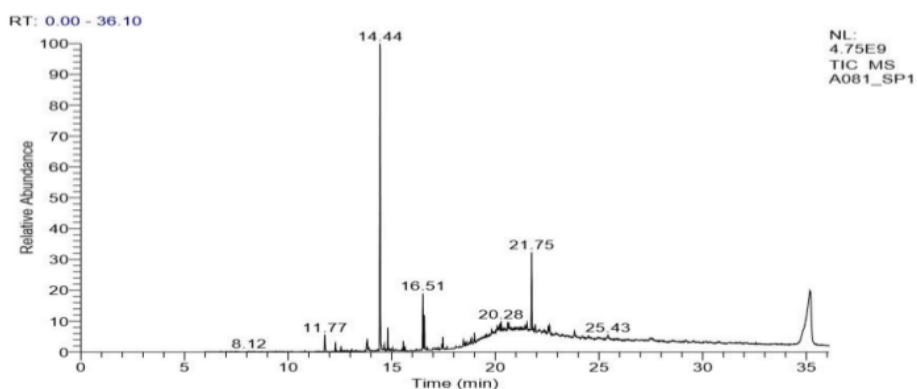


Figure 1. Chromatogram result of peak chemical compounds in *Periophthalmus chrysopilos*. (Source: LHP, 2018)

The GC-MS chromatogram showed that the presence of five major peaks of bioactive compounds was detected and the components corresponding to the peaks were determined as follows. The highest peak in chromatogram pattern could be seen that the extract of *Periophthalmus chrysospilos* has retention time 14.44 minutes. Based on the GC-MS signal, the sample has many bioactive compounds which was shown by some signals detected. It shows that GC-MS analysis successfully proves *Periophthalmus chrysospilos* extract for treating people with inflammatory disease especially enuresis.

3.2 Bioactive compounds in the *Periophthalmus chrysospilos* extract

The results of *Periophthalmus chrysospilos* extract revealed the amounts of bioactive compounds such as terpenoids, alkaloids, steroids, carotenoids and cannabinoids. Terpenoid compounds were detected as many as 4 types of compounds with a total of 4 compounds. Carotenoid compounds were detected as many as 1 type of compound with a total of 4 compounds. Cannabinoid compounds were detected as many as 1 type of compound with a total of 2 compounds; and alkaloid compounds detected as many as 1 type with a total of 1 compound. Cannabinoid bioactive compounds were also detected in this sample with a total of 1 compound. Cannabinoid compounds include secondary metabolites which are usually found in cannabis plants. According to Maggyvin and Sinuraya (2017), cannabinoids are classified by source and origin. Phytocannabinoids are derivative compounds from cannabis and other plants, including delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). The details of *Boleophthalmus boddarti* extract elucidated in following Table 1.

Table 1. The amount of bioactive compounds in the *Periophthalmus chrysospilos* extract.

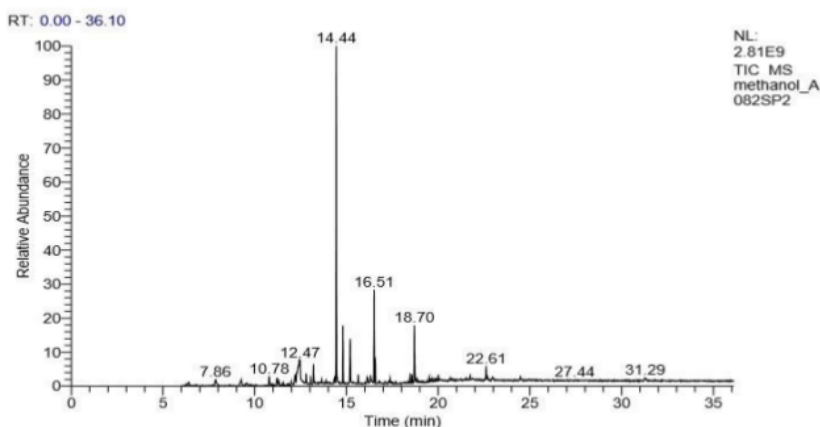
No.	Compound Names	Compound Classes	Total Numbers
1.	<i>D-Limonene</i>	Terpenoids	1
2.	<i>Limonene</i>	Terpenoids	1
3.	<i>Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15, 16-dimethoxy-</i>	Alkaloids (<i>Aspidospermidin</i>)	1
4.	<i>Choltesan-3-ol, 2-methylene-</i>	Steroids	6
5.	<i>Azulene, 1,4-dimethyl-7-(1-methylethyl)-</i>	Terpenoids	1
6.	<i>17.alfa.,21á-28, 30-Bisnorhopane</i>	Steroids	1
7.	<i>4-Androsten-6á-ol-3,17-dione</i>	Steroids	3
8.	<i>3,9-Epoxypregn-16-ene-14,20-diol, 7,11,18-triacetoxy-3-methoxy-</i>	Steroids	1
9.	<i>Cholestan-26-oic acid, 3,7,12-trihydroxy-</i>	Steroids	1
10.	<i>Rhodopin</i>	Carotenoids	1
11.	<i>Cannabinol, trifluoroacetate</i>	Cannabinoids	4
12.	<i>Cholestan-3,5-diol, 5-acetate</i>	Steroids	2
13.	<i>Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime</i>	Steroids	1
14.	<i>9,19-Cyclolanostan-3-ol, 24,24-epoxymethanoacetate</i>	Terpenoids	1
15.	<i>8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3 á-methoxy-4,4-dimethyl-</i>	Steroids	11

152	16.	Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-	Steroids	10
153	17.	2-Phenanthrenecarboxylic acid, 1-(1,3-dithian-	Steroids	1
154		2-ylmethyl)-7-hydroxy-2,4b-dimethyl-		
155		1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-		
156		.methyl ester		

157 The highest bioactive compounds in *Periophthalmus chrysospilos* extract were steroid
 158 compounds which were detected as many as 10 type⁸ of compounds with a total of 24 compounds.
 159 The total number of compounds detected are named Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-
 160 , where the total amount of compounds is 10 compounds. According to Moss (1989) androstane is
 161 an anabolic steroid group that belongs to the basic carbon cycle, which is unsaturated and is an
 162 alkyl substitute in the 17th carbon chain.

163 3.3 GC-MS Results of *Boleophthalmus boddarti* extract

164 The result of GC-MS analysis of *Boleophthalmus boddarti* extract describes as the
 165 chromatogram form for all chemical compounds that was read by the detector (Fig.2). The vertical
 166 lines were found in this chromatogram pattern. Those indicate more than one bioactive compounds
 167 in *Boleophthalmus boddarti* extract. The presence of five major peaks of bioactive compounds was
 168 detected and the most compound are from steroids. The highest peak in chromatogram pattern
 169 could be seen that *Boleophthalmus boddarti* extract has retention time 14.44. Based on the GC-MS
 170 signal, the sample has many bioactive compounds which was shown by some signals detected. It
 171 shows that GC-MS analysis successfully proves *Boleophthalmus boddarti* extract for treating
 172 people with inflammatory disease especially enuresis.



173 **Figure 2.** Chromatogram result of peak chemical compounds in *Boleophthalmus boddarti*.
 174 (Source: LHP, 2018)

175 3.4 Bioactive compounds in the *Boleophthalmus boddarti* extract

176 The results of *Boleophthalmus boddarti* extract revealed the amounts of bioactive
 177 compounds such as steroids, carotenoids, terpenoids, bufadienolide, carotatoxin, cholesterol, and
 178 corticosteroid shown in Table 2. Compounds of steroid groups were detected as many as 24 types

179 of compounds with a total of 44 compounds. Specific steroid compounds namely corticosteroids
180 are detected with 2 types of compounds and a total of 3 compounds. The details of *Boleophthalmus*
181 *boddarti* extract elucidated in following **Table 2**.

182 **Table 2.** The amount of bioactive compounds in the *Boleophthalmus boddarti* extract

183 No.	184 Compound Names	185 Compound Classes	186 Total 187 Numbers
188 1.	189 <i>Curan-17-oic acid, 19,20-dihydroxy-, methyl</i> 190 <i>ester</i>	191 Steroids	192 2
193 2.	194 <i>Cholestan-3-ol, 2-methylene-</i>	195 Steroids	196 12
197 3.	198 <i>Citronellol epoxide</i>	199 Terpenoids	200 1
201 4.	202 <i>Falcarinol</i>	203 Carotatoxin (secondary 204 cancer anti metabolites)	205 1
206 5.	207 <i>Cholestan-3-one, cyclic 1,2-ethanediyl aetal</i>	208 Steroids	209 1
210 6.	211 <i>..psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-</i> 212 <i>9,1'-dimethoxy</i>	213 Carotenoids	214 8
215 7.	216 <i>Androsten-4-en-11-ol-3,17-dione, 9-</i> 217 <i>thiocyanato-</i>	218 Steroids	219 2
220 8.	221 <i>Androsten-5-en-17-one-3,11-</i> 222 <i>bis[(trimethylsilyl)oxy]-, O-</i> 223 <i>(phenylmethyl)oxime</i>	224 Steroids	225 1
226 9.	227 <i>Prosta-5,13-dien-1-oic acid, 9,11,15-</i> 228 <i>tris[(trimethylsilyl)oxy]-, trimethylsilyl ester</i>	229 Steroids	230 6
231 10.	232 <i>Rhodoxanthin</i>	233 Carotenoids	234 2
235 11.	236 <i>Rhodopin</i>	237 Carotenoids	238 2
239 12.	240 <i>5á-Ergost-24-en-26-oic acid, 5,6á-epoxy-4á,</i> 241 <i>18,22-trihydroxy-3-methoxy-1-ox o-, ë-</i> 242 <i>lactone, diacetate,</i>	243 Steroids	244 1
245 13.	246 <i>Ethyl iso-allocholate</i>	247 Steroids	248 1
249 14.	250 <i>Gamabufotalin</i>	251 Bufadienolide (steroid 252 aglycones in frogs)	253 1
254 15.	255 <i>Betamethasone acetate</i>	256 Corticosteroid	257 2
258 16.	259 <i>Cholestan-26-oic acid, 3,7,12-trihydroxy-</i>	260 Steroids	261 2
262 17.	263 <i>..psi.,psi.-Carotene, 3,4-didehydro-</i> 264 <i>1,2,7',8'-tetrahydro-1- methoxy-2-ox o</i>	265 Carotenoids	266 1
267 18.	268 <i>..psi.,psi.-Carotene, 3,3',4,4'-tetrahydro-</i> 269 <i>2',2'-dihydro-1-hydroxy-1'methoxy</i>	270 Carotenoids	271 1
272 19.	273 <i>Prosta-5,13-dien-1-oic acid, 9,11,15-</i> 274 <i>tris[(trimethylsilyl)oxy]-, trimethylsilyl ester</i>	275 Steroids	276 2
277 20.	278 <i>17á-Acetoxy-1',1'14-carboethoxy-1á,2á-</i> 279 <i>dihydrocy cloprop[1,2]-5á-androst-1-en-3-</i> 280 <i>one</i>	281 Steroids	282 2
283 21.	284 <i>Cholesterol</i>	285 Cholesterol	286 1
287 22.	288 <i>26-Nor-5-cholesten-3á-ol-25-one</i>	289 Steroids	290 1
291 23.	292 <i>17-(1,5-Dimethylhexyl)-10,13-dimethyl-</i> 293 <i>2,3,4,7,8,9,10,11,12,13,14,15,16,17-</i>	294 Steroids	295 1

224		<i>tetradecahydro-</i>		
225		⁹ <i>1Hcyclopenta[a]phenanthren-3-ol</i>		
226	24.	<i>Androst-4-en-9-thiocyanomethyl-11-ol-3,17-</i>	Steroids	1
227		<i>dione</i>		
228	25.	¹⁰ <i>Androst-5-en-3-one, 19-acetoxy-4,4-</i>	Steroids	1
229		<i>dimethyl-,oxime</i>		
230	26.	¹⁰ <i>3-Isopropyl-6a,7,10b-trimethyl-8-(2-oxo-2-</i>	Corticosteroid	1
231		<i>phenylethyl)dodecahydrobenzo[f]chromene-</i>		
232		<i>7-carboxylic acid, methyl ester</i>		
233	27.	<i>Androst-8-en-3-ol, 4,4,14a-trimethyl-17-(2-</i>	Steroids	1
234		<i>bromo-1-methylethyl)</i>		
235	28.	<i>1',1'-Dicarboethoxy-1a,2a-dihydro-3'H-</i>	Steroids	1
236		⁸ <i>cloprop[1,2]cholesta-1,4,6-trien-3-one</i>		
237	29.	<i>Prosta-5,13-dien-1-oic acid, 9,11,15,19-</i>	Steroids	1
238		<i>tetrakis(trimethylsilyl)oxy]-, methyl ester</i>		

239 Corticosteroids are drugs that contain steroid hormones that are useful to increase the
 240 steroid hormones in the body that are needed, and relieve inflammation and help the body's
 241 excessive working system including asthma, allergy and multiple sclerosis (Cidlowski, 2013;
 242 Panda and Mabalirajan, 2018; Rozaliyani, 2011). According to the analysis of Prasad *et al* (2018),
 243 corticosteroids also reduce the mortality risk from tuberculosis effect at least in the short term. A
 244 quite unique compound was detected from this *Boleophthalmus boddarti* extract, the compound
 245 named Gamabufotalin which is a bioactive compound of the bufadienolide types. According to
 246 Dmitrieva *et al.* (2000), Bufadienolides are biosynthetic steroid that is independent of the side
 247 chain of cholesterol division. Bufadienolides (BDs) also include corticic steroid groups, which are
 248 usually obtained from amphibian poison extracts, these compounds are often used as traditional
 249 medicine in Asia.

250 3.5 GC-MS Results of *Boleophthalmus dussumieri* extract

251 The result of GC-MS analysis of *Boleophthalmus dussumieri* extract describes as the
 252 chromatogram form for all chemical compounds that was read by the detector (**Fig.3**). The vertical
 253 lines were found in this chromatogram pattern. Those indicate more than one bioactive compounds
 254 in *Boleophthalmus dussumieri* extract. The presence of four major peaks of bioactive compounds
 255 was detected by GC-MS like steroids, terpenoids, carotenoids and carotatoxins. The highest peak
 256 in chromatogram pattern could be seen that *Boleophthalmus dussumieri* extract has retention time
 257 22.61. Based on the GC-MS signal, the sample has significant bioactive compounds which was
 258 shown in the **Table 3**.

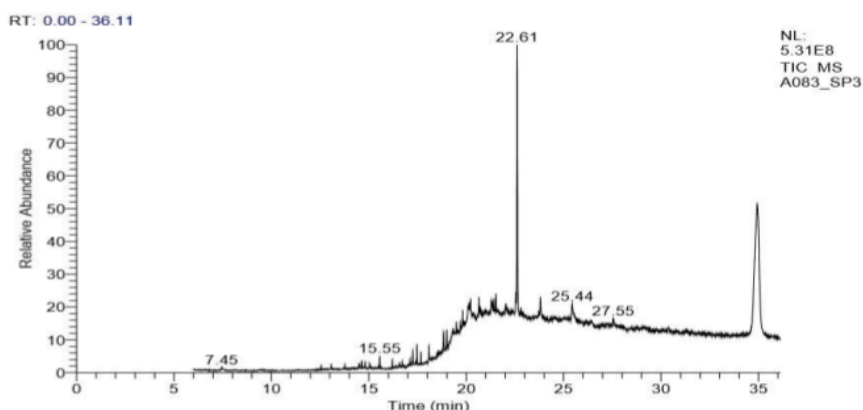


Figure 3. Chromatogram result of peak chemical compounds in *Boleophthalmus dussumieri*.
 (Source: LHP, 2018)

3.6 Bioactive compounds in the *Boleophthalmus dussumieri* extract

Bioactive compounds detected from *Boleophthalmus dussumieri* extract include steroids, terpenoids, carotenoids and carotatoxins. The compounds of steroid classes are as many as 11 types of compounds, with a total of 26 compounds. The group of carotenoid compounds was detected as many as 6 types of compounds, with a total of 13 compounds. The group of terpenoid compounds was detected by 2 types of compounds, with a total of 2 compounds. Carotatoxin compounds were also detected in this *Boleophthalmus dussumieri* extract, with a total of 1 compound. The details of *Boleophthalmus dussumieri* extract elucidated in following **Table 3**.

Table 3. The amount of bioactive compounds in the *Boleophthalmus boddarti* extract

No.	Compound Names	Compound Classes	Total Numbers
1.	<i>Limonene</i>	Terpenoids	1
2.	<i>D-Limonene</i>	Terpenoids	1
3.	<i>Corynan-17-ol, 18,19-didehydro-10-methoxy-,acetate (ester)</i>	Steroids	2
4.	<i>Rhodopin</i>	Carotenoids	5
5.	<i>Ç-Sitosterol</i>	Steroids	1
6.	<i>Spirost-8-en-11-one, 3-hydroxy</i>	Steroids	13
7.	<i>Cholest-5-en-3-ol, 24-propylidene-</i>	Steroids	2
8.	<i>Stigmasta-5,24(28)-dien-3-ol</i>	Steroids	1
9.	<i>..psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy</i>	Carotenoids	4
10.	<i>5á-Ergost-24-en-26-oic acid, 5,6á-epoxy-4á, 18,22-trihydroxy-3-methoxy-1-ox o-, ë-lactone, diacetate,</i>	Steroids	1
11.	<i>..psi.,psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-ox o</i>	Carotenoids	1

288	12.	¹⁰ 5-Chloro-6beta-nitro-5alpha-cholestan-3-one	Steroids	1
289				
290	13.	² Prosta-5,13-dien-1-oic acid, 9,11,15-tris(trimethylsilyloxy)-, trimethylsilyl ester	Steroids	1
291				
292	14.	Cholest-4-ene, 3á-(methoxymethoxy)-	Steroids	1
293	15.	Rhodoxanthin	Carotenoids	1
294	16.	4'-Apo-á,psi.-carotenoic acid	Carotenoids	1
295	17.	⁸ Astaxanthin	Carotenoids	1
296	18.	Androst-7-ene-6,17-dione, 2,3,14-trihydroxy	Steroids	2
297	19.	Prosta-5-en-1-oic acid, 9,11,15-tris(trimethylsilyloxy)-, methylsilyl ester	Steroids	1
298				
299	20.	Falcarinol	Carotatoxin	1

300 The name of steroid compounds with the highest total number of compounds is Spirost-8-
 301 en-11-one, 3-hydroxy. According to Rajendran et al. (2017), ¹²rost-8-en-11-one, 3-hydroxy
 302 including steroid compounds which have anti-cancer bioactivity. Spirost-8-en-11-one, 3-hydroxy
 303 has the chemical formula C₂₇H₄₀O₄. The group of carotenoid compounds detected with the
 304 highest amount is Rhodopin, where the total amount of these compounds is 5 compounds.
 305 According to Komori et al. (1998), Rhodopin is a carotenoid compound which is usually produced
 306 by Rhodospirillum rubrum bacteria which has the chemical formula C₄₀H₅₈O.

307 Bioactive carotatoxin alkaloids are also detected from *Boleophthalmus dussumieri extract*.
 308 The name of the carotatoxin group is falcarinol and the total of compound is only 1 compound.
 309 According to Zaini et al. (2012), falcarinol (FaOH) is one of the compounds found in carrots, this
 310 compound has shown bioactive action in several types of cell cultures. Falcarinol has been shown
 311 to be cytotoxic to the culture of acute lymphoblastic leukemia cells.

312 3.7 GC-MS Results of *Periophthalmodon schlosseri extract*

313 The result of GC-MS analysis of *Periophthalmodon schlosseri extract* describes as the
 314 chromatogram form for all chemical compounds that was read by the detector (**Fig. 4**). The vertical
 315 lines were found in this chromatogram pattern. Those indicate more than one bioactive compounds
 316 in *Boleophthalmus boddarti extract*. The presence of five major peaks of bioactive compounds was
 317 detected and the most compound are from steroids. The highest peak in chromatogram pattern
 318 could be seen that *Boleophthalmus boddarti extract* has retention time 14.44. Based on the GC-MS
 319 signal, the sample has many bioactive compounds which was shown by some signals detected. It
 320 shows that GC-MS analysis successfully proves *Boleophthalmus boddarti extract* for treating
 321 people with inflammatory disease especially enuresis.

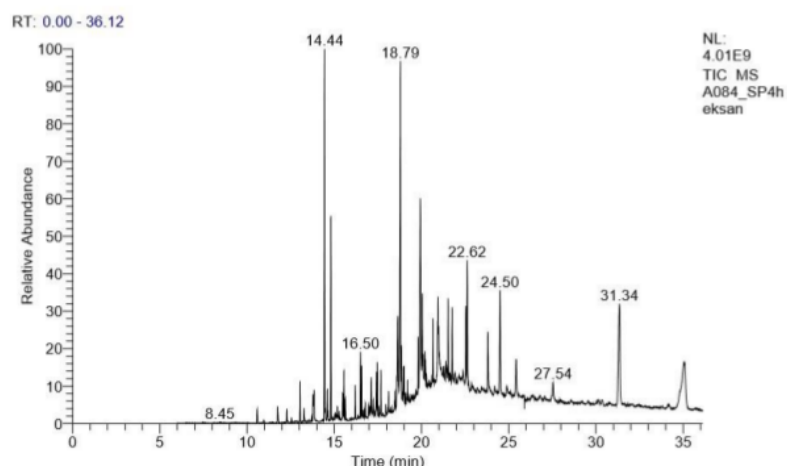


Figure 4. Chromatogram result of peak chemical compounds in *Periophthalmodon schlosseri*.
(Source: LHP, 2018)

3.8 Bioactive compounds in the *Periophthalmodon Schlosseri* extract

Bioactive compounds detected from *Periophthalmodon schlosseri* extract including steroids, carotenoids and terpenoids. Bioactive steroid compounds were detected as many as 11 types of compounds with a total of 21 compounds; carotenoid group compounds detected as many as 3 types of compounds with a total of 6 compounds; while terpenoid group compounds were detected only 1 type of compound with a total of 1 compound. The details of *Periophthalmodon schlosseri* extract elucidated in **Table 4**.

Table 4. The amount of bioactive compounds in the *Periophthalmodon schlosseri* extract

No.	Compound Names	Compound Classes	Total Numbers
1.	<i>D-Limonene</i>	Terpenoids	1
2.	<i>Cholestan-4-ol, 2-methylene-</i>	Steroids	4
3.	<i>Cholest-5-en-3-ol (3α)-, tetradecanoate</i>	Steroids	1
4.	<i>Cholesta-3,5-diene</i>	Steroids	1
5.	<i>Cholesteryl benzoate</i>	Steroids	1
6.	<i>Rhodopin</i>	Carotenoids	3
7.	<i>Azafrin</i>	Carotenoids	1
8.	<i>17.alfa.,21α-28,30-Bisnorhopane</i>	Steroids	2
9.	2 <i>Cholesterol</i>	Steroids	2
10.	<i>26-Nor-5-cholesten-3α-ol-25-one</i>	Steroids	1
11.	<i>Cholestane-3,5-diol, 5-acetate</i>	Steroids	1
12.	<i>Campesterol</i>	Steroids	1
13.	<i>Ergost-5-en-3-ol</i>	Steroids	1
14.	8 <i>Astaxanthin</i>	Carotenoids	2
15.	<i>Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-</i>	Steroids	6

The steroid compounds in *Periophthalmodon schlosseri* extract are named Cholestan-3-ol, 2-methylene-, Cholest-5-en-3-ol (3 α)-, tetradecane, Cholesta-3,5-diene, Cholesteryl benzoate, 17.alfa., 21 α -28, 30-Bisnorhopane, Cholesterol, 26-Nor-5 β -cholesten-3 α -ol-25-one, Cholestane-3,5-diol, 5-acetate, Campesterol, Ergost-5-en-3-ol, and Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-. According to Salempa and Muharram (2016), steroids are natural compounds which consist of a carbon skeleton and consist of three circles of six peridro phenanthrene and fused into one circle of five. Saturated-click hydrocarbons, which have a circumference system consisting of 17 carbon atoms.

D-Limonene is the only the group of terpenoid compounds which detected from *Periophthalmodon schlosseri* extract. This compound was also detected in samples of *Periophthalmus chrysospilos* extract and *Boleophthalmus dussumieri* extract. According to Sun (2007), D-Limonene is a monocyclic terpenoids with a lemon-like odor and is a major constituent of several essential oils of Citrus sp. (orange, lemon, mandarin, lime and grapefruit). D-Limonene is widely used as an additive (additive) there is perfume, soap, food, gum and drinks. This compound is also listed in the Code of Federal Regulation as a material that is known to be safe or generally recognized as safe (GRAS) as a flavoring agent.

The group of carotenoid compounds detected include rhodopin, azafrin and astaxanthin. Rhodopin compounds were detected in all samples with a sufficient number of total compounds in each sample. Astaxanthin compounds were also detected from the flesh extracts of *Boleophthalmus boddarti* and *Boleophthalmus dussumieri* with a total of 1 compound each in each sample. While azafrin compounds were only detected in *Periophthalmodon schlosseri* extract. According to Gopalakrishnan and Kalaiarasi (2012), azafrin is a compound of the carotenoid ester group with the molecular formula C₂₈H₄₀O₄. Azafrin compounds include bioactive compounds that have the potential as antioxidants, anti-inflammatory, anticancer, anti-criticism, natural dyes, eye pain medications, and as compounds that can reduce hypertension.

3.9 Physical and Chemical Properties of the Environment at Sampling Locations

The main point of sampling mudskippers based on the GPS is in the coordinates S 02° 21'57.5 "E 104° 54'00.1". The environment measurements of the physical and chemical properties were carried out at the main point of sampling and obtained as presented in Table 5.

Table 5. Physical and chemical properties of the environment at the sampling location

Parameter	Measurement Time		
	March	April Morning	April Afternoon
Soil moisture (%)	100	100	100
Soil pH	6.5	6.5	6.5
Water pH	6.82	7.32	6.7
Waters salinity (%)	0.1	0	0

The measurement of physical and chemical properties of the environment was carried out at 3 different times. The first measurements were made in late March precisely in the afternoon and obtained soil moisture data of 100%, soil pH of 6.5, water pH of 6.82, and water salinity of 0.1 ‰. The second measurement was carried out in April morning and 100% of soil moisture was obtained, soil pH was 6.5, water pH was 7.32, and water salinity was 0 ‰. The third measurement was carried out in the afternoon of April and obtained 100% soil moisture data, a soil pH of 6.5, a water pH of 6.7, and water salinity of 0 ‰.

392 Based on the data of the physical and chemical properties of the environment, it can be
393 seen that the soil moisture at the main sampling point shows 100% at 3 times measurements with
394 different times. This shows that the sampling location has a substrate in the form of mud which is
395 always wet. According to Kurnia et al. (2006), soil moisture is one of the key variables in the
396 hydrological process that plays an important role in determining the availability of water as a very
397 fundamental element in the life of living things. 100% soil moisture indicates that the soil is
398 saturated with water.

399 Water salinities at 3 measurements with different times showed the numbers 0.1 ‰, 0 ‰
400 and 0 ‰. This shows that the waters around the sampling location tend to be tasteless. According
401 to Masuda et al. (1984) and Redjeki (2013), the gudder is an amphidromous demersal fish that is
402 able to tolerate freshwater and sea and live on muddy and sandy bottom and spawn in estuarine
403 areas.

404 4. Conclusions

405 The bioactive compound analysis of *Periophthalmus chrysospilos* extract consist of
406 compounds the natural names of steroids, terpenoids, carotenoids, cannabinoids, and alkaloids. All
407 these hormones which was found as many as 10 types with a total of 24 compounds in
408 *Periophthalmus chrysospilos* extract. *Boleophthalmus boddarti* extract was natural names of steroids,
409 carotenoids, terpenoids, bufadilenoid, and carotatoksin. There are 24 types of compounds which
410 were detected with a total of 44 compounds in *Boleophthalmus boddarti* extract. *Boleophthalmus*
411 *dussumieri* extract has bioactive compounds from the groups of steroids, carotenoids, terpenoids,
412 and carotatoksin. *Boleophthalmus dussumieri* extract has as many as 11 types of compounds with
413 a total of 26 compounds. *Periophthalmodon schlosseri* detected bioactive compounds from groups
414 and or with the natural names of steroids, carotenoids and terpenoids. All hormones which was
415 detected as many as 11 types of compounds with a total of 21 compounds. The higher
416 concentrations of bioactive compounds were found in mudskippers that those are possibly
417 consumed for inflammatory disease, enuresis in children and allergy.

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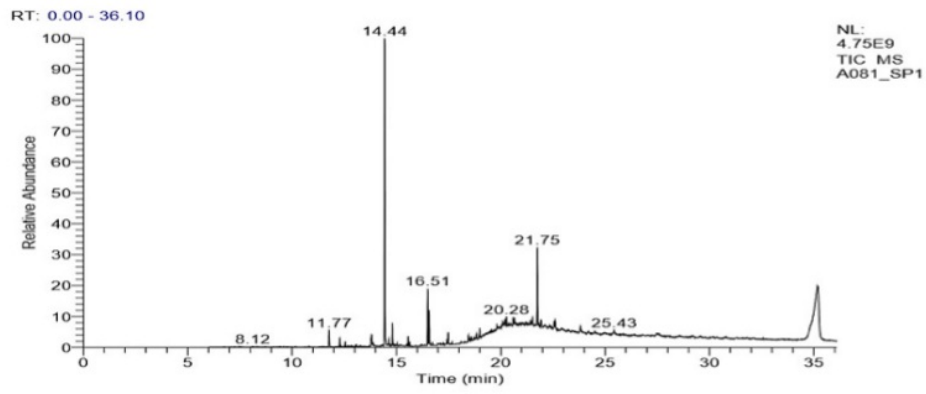


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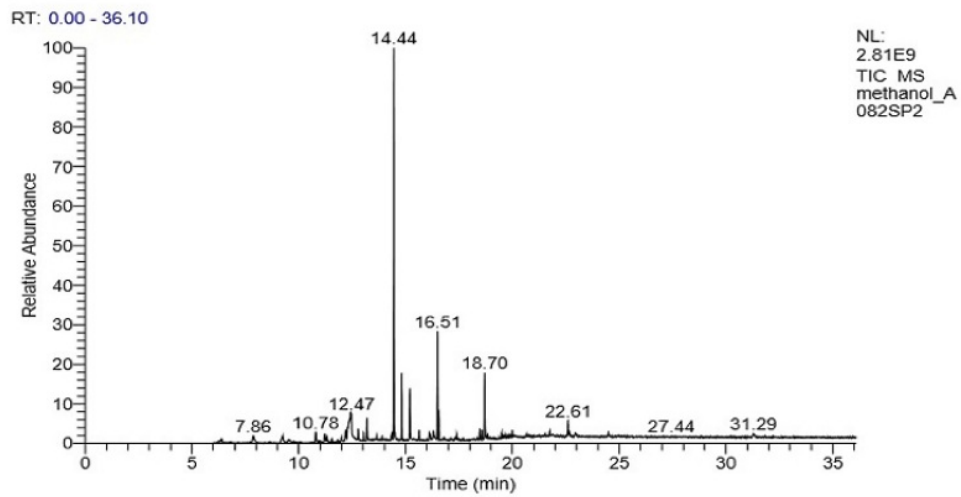


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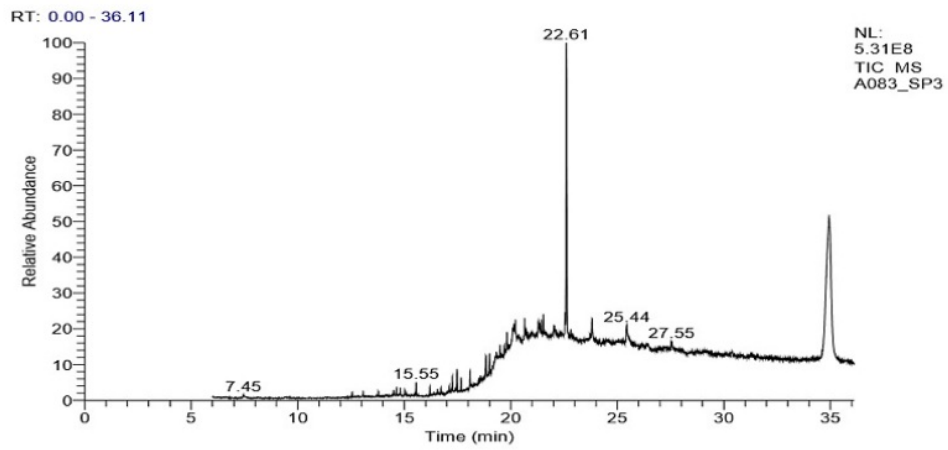


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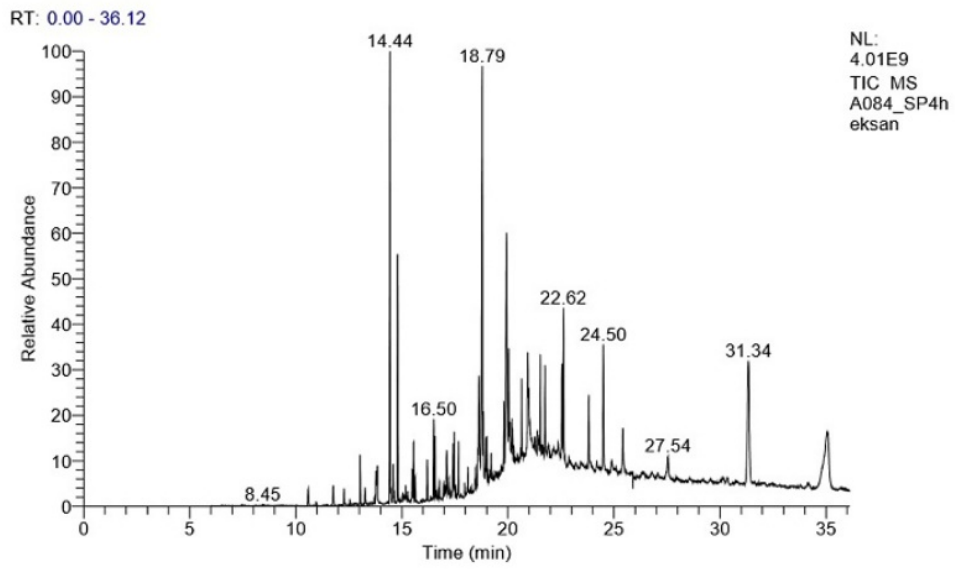


Figure 5[Download source file \(1.31 MB\)](#)

No.	Compound Names	Compound Classes	Total Numbers
1.	<i>D-Limonene</i>	Terpenoids	1
2.	<i>Limonene</i>	Terpenoids	1
3.	<i>Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15, 16-dimethoxy-</i> <i>(Aspidospermidin)</i>	Alkaloids	1
4.	<i>Choltesan-3-ol, 2-methylene-</i>	Steroids	6
5.	<i>Azulene, 1,4-dimethyl-7-(1-methylethyl)-</i>	Terpenoids	1
6.	<i>17.alpha.,21.alpha.-28, 30-Bisnorhopane</i>	Steroids	1
7.	<i>4-Androsten-6.alpha.-ol-3,17-dione</i>	Steroids	3
8.	<i>3,9-Epoxypregn-16-ene-14,20-diol, 7,11,18-triacetoxy-3-methoxy-</i>	Steroids	1
9.	<i>Cholestan-26-oic acid, 3,7,12-trihydroxy-</i>	Steroids	1
10.	<i>Rhodopin</i>	Carotenoids	1
11.	<i>Cannabinol, trifluoroacetate</i>	Cannabinoids	4
12.	<i>Cholestan-3,5-diol, 5-acetate</i>	Steroids	2
13.	<i>Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime</i>	Steroids	1
14.	<i>9,19-Cyclolanostan-3-ol, 24,24-epoxymethanoacetate</i>	Terpenoids	1
15.	<i>8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.alpha.-methoxy-4,4-dimethyl-</i>	Steroids	11
16.	<i>Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-</i>	Steroids	10
17.	<i>2-Phenanthrenecarboxylic acid, 1-(1,3-dithian-2-ylmethyl)-7-hydroxy-2,4b-dimethyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-,methyl ester</i>	Steroids	1

Figure 6

[Download source file \(1.68 MB\)](#)

No.	Compound Names	Compound Classes	Total Numbers
1.	<i>Curan-17-oic acid, 19,20-dihydroxy-, methyl ester</i>	Steroids	2
2.	<i>Cholestan-3-ol, 2-methylene-</i>	Steroids	12
3.	<i>Citronellol epoxide</i>	Terpenoids	1
4.	<i>Falcarinol</i>	Carotatoxin (secondary cancer anti metabolites)	1
5.	<i>Cholestan-3-one, cyclic 1,2-ethanediyl aetal</i>	Steroids	1
6.	<i>..psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy</i>	Carotenoids	8
7.	<i>Androsten-4-en-11-ol-3,17-dione, 9-thiocyanato-</i>	Steroids	2
8.	<i>Androsten-5-en-17-one-3,11-bis(trimethylsilyloxy)-, O-(phenylmethyl)oxime</i>	Steroids	1
9.	<i>Prosta-5,13-dien-1-oic acid, 9,11,15-tris(trimethylsilyloxy)-, trimethylsilyl ester</i>	Steroids	6
10.	<i>Rhodoxanthin</i>	Carotenoids	2
11.	<i>Rhodopin</i>	Carotenoids	2
12.	<i>5α-Ergost-24-en-26-oic acid, 5,6α-epoxy-4α, 18,22-trihydroxy-3-methoxy-1-ox o-, β-lactone, diacetate,</i>	Steroids	1
13.	<i>Ethyl iso-allocholate</i>	Steroids	1
14.	<i>Gamabufotalin</i>	Bufadienolide (steroid aglycones in frogs)	1
15.	<i>Betamethasone acetate</i>	Corticosteroid	2
16.	<i>Cholestan-26-oic acid, 3,7,12-trihydroxy-</i>	Steroids	2
17.	<i>..psi.,.psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1- methoxy-2-ox o</i>	Carotenoids	1
18.	<i>..psi.,.psi.-Carotene, 3,3',4,4'-tetrahydro-1',2'-dihydro-1-hydroxy-1'methoxy</i>	Carotenoids	1
19.	<i>Prosta-5,13-dien-1-oic acid, 9,11,15-tris(trimethylsilyloxy)-, trimethylsilyl ester</i>	Steroids	2
20.	<i>17α-Acetoxy-1',1'-dicarboethoxy-1α,2α-dihydrocy cloprop[1,2]-5α-androst-1-en-3-one</i>	Steroids	2
21.	<i>Cholesterol</i>	Cholesterol	1
22.	<i>26-Nor-5-cholesten-3α-ol-25-one</i>	Steroids	1
23.	<i>17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1Hcyclopenta[a]phenanthren-3-ol</i>	Steroids	1
24.	<i>Androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione</i>	Steroids	1

Figure 7[Download source file \(1.36 MB\)](#)

No.	Compound Names	Compound Classes	Total Numbers
1.	<i>Limonene</i>	Terpenoids	1
2.	<i>D-Limonene</i>	Terpenoids	1
3.	<i>Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)</i>	Steroids	2
4.	<i>Rhodopin</i>	Carotenoids	5
5.	<i>Ç-Sitosterol</i>	Steroids	1
6.	<i>Spirost-8-en-11-one, 3-hydroxy</i>	Steroids	13
7.	<i>Cholest-5-en-3-ol, 24-propylidene-</i>	Steroids	2
8.	<i>Stigmasta-5,24(28)-dien-3-ol</i>	Steroids	1
9.	<i>..psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy</i>	Carotenoids	4
10.	<i>5α-Ergost-24-en-26-oic acid, 5,6α-epoxy-4α, 18,22-trihydroxy-3-methoxy-1-ox o-, β-lactone, diacetate,</i>	Steroids	1
11.	<i>..psi.,psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-ox o</i>	Carotenoids	1
12.	<i>5-Chloro-6beta-nitro-5alpha-cholestan-3-one</i>	Steroids	1
13.	<i>Prosta-5,13-dien-1-oic acid, 9,11,15-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester</i>	Steroids	1
14.	<i>Cholest-4-ene, 3α-(methoxymethoxy)-</i>	Steroids	1
15.	<i>Rhodoxanthin</i>	Carotenoids	1
16.	<i>4'-Apo-á,psi.-carotenoic acid</i>	Carotenoids	1
17.	<i>Astaxanthin</i>	Carotenoids	1
18.	<i>Androst-7-ene-6,17-dione, 2,3,14-trihydroxy</i>	Steroids	2
19.	<i>Prosta-5-en-1-oic acid, 9,11,15-tris[(trimethylsilyl)oxy]-, methylsilyl ester</i>	Steroids	1
20.	<i>Falcarinol</i>	Carotatoxin	1

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No.	Compound Names	Compound Classes	Total Numbers
1.	<i>D-Limonene</i>	Terpenoids	1
2.	<i>Cholestan-4-ol, 2-methylene-</i>	Steroids	4
3.	<i>Cholest-5-en-3-ol (3β)-, tetradecanoate</i>	Steroids	1
4.	<i>Cholesta-3,5-diene</i>	Steroids	1
5.	<i>Cholesteryl benzoate</i>	Steroids	1
6.	<i>Rhodopin</i>	Carotenoids	3
7.	<i>Azafrin</i>	Carotenoids	1
8.	<i>17.alfa.,21α-28,30-Bisnorhopane</i>	Steroids	2
9.	<i>Cholesterol</i>	Steroids	2
10.	<i>26-Nor-5-cholesten-3β-ol-25-one</i>	Steroids	1
11.	<i>Cholestane-3,5-diol, 5-acetate</i>	Steroids	1
12.	<i>Campesterol</i>	Steroids	1
13.	<i>Ergost-5-en-3--ol</i>	Steroids	1
14.	<i>Astaxanthin</i>	Carotenoids	2
15.	<i>Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-</i>	Steroids	6

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