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Research Article
LC-MS/MS Profiling and Antimicrobial Evaluation of Marine Alkaloids from *Pseudoceratina purpurea*

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Abstract

Background and Objective: Alkaloids are a group of naturally occurring compounds with diverse pharmacological properties, including their use as antibacterial and antifungal agents such as chloramphenicol and nystatin (CY and NY). This study aimed to investigate the alkaloid profile and antimicrobial potential of the marine sponge *Pseudoceratina purpurea* collected from Southeast Sulawesi. **Materials and Methods:** Methanol extraction was performed to obtain the methanol extract (ME), which was then fractionated using n-hexane to yield the n-hexane extract and methanol extract residue (MER). Alkaloid isolation was conducted on the MER to obtain the alkaloid isolate (AI). The AI was partially analyzed using UPLC-HRMS for compound identification, while the remaining portion was tested for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. **Results:** A total of 117 alkaloid compounds were identified and classified into seven subclasses: Indole (1.8%), isoquinoline (4.14%), lipid (32.66%), proto (52.90%), purine (2.02%), pyridine (6.39%) and steroidal alkaloids (0.1%). The AI demonstrated strong antimicrobial activity, with minimum inhibitory concentration (MIC) values of 4±0.064 ppm against *E. coli*, 8±0.09 ppm against *S. aureus* and 4±0.05 ppm against *C. albicans*. **Conclusion:** These findings suggest that the alkaloid isolate from *P. purpurea* possesses promising broad-spectrum antimicrobial properties and may serve as a potential candidate for the development of novel antimicrobial agents.

Key words: *Pseudoceratina purpurea*, Southeast Sulawesi, alkaloids, antibacterial, antifungal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alkaloids are a group of secondary metabolites that play a major role in medicine. Some alkaloids that have been commercialized include vincristine and vinblastine from *Catharanthus roseus* as anti-leukemia[1], chloramphenicol (CY) is isolated from *Streptomyces venezuelae* and functions as an antibacterial (antibiotic)[2] and *Streptomyces noursei* produced nystatin, which has antifungal property (antibiotic)[3] and also quinine comes from *Cinchona officinalis* as anti-malarial[4]. Apart from these activities, some alkaloids are also useful as nerve drugs, including cocaine from *Erythroxylon coca*[5] and morphine (*Papaver somniferum*), which were useful as an analgesic agent[6].

Some alkaloids from marine that are used as medicine include Manzamine A and C found from sponge *Haliclona* sp., which are active as anti-cancer, anti-inflammation and neuroprotective[7]. Caulerpin was isolated from seaweed, *Caulerpa* sp., has anti-inflammatory and anti-oxidant activities that can help reduce the symptoms of neurological diseases such as Alzheimer's and Parkinson's[8]. Tetrodotoxin (from seashells, *Tetrodotoxin* sp.) has anti-pain and anti-inflammatory properties, which can help reduce the symptoms of nerve diseases such as neuropathy[9].

The results of phytochemical screening of several sponges in Southeast Sulawesi waters show diverse and interesting chemical content and biological activities, including *Callyspongia* sp., *Clathria* sp., *Melophlus sarasinorum* and *Xestospongia* sp.[10]. One of the sponges that grows abundantly in the waters of Southeast Sulawesi is *Pseudoceratina purpurea* (Fig. 1). Research reports on the chemical and pharmaceutical aspects of *P. purpurea* from various places in the world showed different chemical contents and interesting biological activities, including from The South China Sea[11], Thailand[12], the coastal waters of Singapore[13] and Japan[14-16].

The above description displayed that chemical study, especially alkaloids and biological activities of *P. purpurea* from Southeast Sulawesi, Indonesia, has not been reported. This information is initial information for further study of *P. purpurea*, such as alkaloid isolation, determination of the structure and their biological activities, so that it can increase the value of the benefits and economic value of this species. The objective of this study was to identify and characterize the alkaloid compounds present in the marine sponge *Pseudoceratina purpurea* using UPLC-HRMS analysis and to evaluate their antimicrobial activity against a panel of pathogenic microorganisms. The study aimed to explore the potential of marine-derived alkaloids as novel antimicrobial agents.

MATERIALS AND METHODS

Study area: This research was conducted from April, 2024 to August, 2024. Alkaloid extraction and isolation at the Chemistry Laboratory of Bina Husada Kendari Polytechnic, antimicrobial activity test at the Pharmacy Laboratory, Faculty of Pharmacy, Halu Oleo University, while secondary metabolite analysis was carried out at Biotek Rekayasa Indonesia.

Sample and preparation: The sampling of the marine sponge of *P. purpurea* was conducted in the waters of Soropia, Konawe Regency, Southeast Sulawesi, using diving equipment (Fig. 1). A small portion of the sponge was carefully cut without damaging the entire organism to ensure the preservation of the population's regeneration. The samples collected (1 kg) were stored in sterile containers and kept in a cool condition during transportation to the laboratory to prevent the degradation of active compounds. The sponge *P. purpurea* was then cleaned of debris, sand and other organisms before being cut into small pieces to maximize contact with the solvent during the extraction process[10].

Extraction and isolation of alkaloids: The extraction was performed using the maceration method, in which the sponge was soaked in methanol (Merck, Germany) at room temperature with continuous stirring for 3×24 hrs to ensure optimal dissolution of bioactive compounds. The mixture was then filtered and the liquid extract was concentrated using a vacuum rotary evaporator (Büchi R-300, Büchi Labortechnik AG, Switzerland) at 55°C to remove the solvent, yielding a crude extract (8.7 g) containing bioactive compounds. Alkaloid isolation began with the fractionation of the crude extract in n-hexane. The fraction containing alkaloids was dissolved in a dilute hydrochloric acid solution (Merck, Germany) (0.1-0.5 M) with stirring for 1-2 hrs to form water-soluble alkaloid salts. The acidic solution containing the alkaloid salts was neutralized using a 1 M sodium hydroxide (Merck, Germany) solution until the pH reached 9-10, causing free alkaloids to precipitate. The precipitated free alkaloids were extracted using chloroform (Merck, Germany) (in a 1:1 ratio to the solution volume). This process was repeated 2-3 times to ensure maximum alkaloid extraction. The organic layer was collected and evaporated using a rotary evaporator at 55°C to obtain the alkaloid isolate (0.62 g)[17].

Fig. 1: Sponge *Pseudoceratina purpurea*

Identification of alkaloids: The identification of alkaloids followed the method outlined by Narváez *et al.*[18] with slight modifications. The analysis was conducted using an Ultra-High-Performance Liquid Chromatography coupled with Quadrupole-Orbitrap High-Resolution Mass Spectrometry (UHPLC-Q-Orbitrap HRMS; Thermo Scientific, USA), equipped with an Accucore C18 column (100×2.1 mm, 1.5 µm particle size). The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a gradient elution program: 0-3 min (5-20% B), 3-20 min (20-40% B), 20-26 min (40-75% B), 26-28 min (75-95% B), 28-30 min (95% B), 30.00-30.01 min (95-5% B) and 30.01-35 min (5% B). The flow rate was set to 0.2 mL/min, with an injection volume of 2.0 µL.

Mass spectrometry (MS) ionization was performed using electrospray ionization (ESI) in both positive and negative ionization modes, covering an m/z range of 100-1500 Da. The parameters were adjusted as follows: Capillary temperature at 320°C, spray voltage at 3.8 kV, sheath gas flow rate at 15 mL/min, auxiliary gas flow rate at 3 mL/min, resolving power at 70,000 FWHM and a full MS/dd MS2 scan type. Data from the UHPLC-Q-Orbitrap HRMS analysis were processed and interpreted using Compound Discoverer 2.2 software to tentatively identify metabolites[19].

Antimicrobial activity: Antimicrobial activity testing was conducted using the well diffusion and microdilution methods based on previous studies[20-22]. Cultures of *E. coli*, *S. aureus* and the fungus *Candida albicans* were prepared and standardized to 0.5 McFarland, approximately equivalent to 1.5×10 CFU/mL. Mueller Hinton broth (MHB) was used as the medium for bacteria, while sabouraud dextrose agar (SDA) was used for fungi. The media were sterilized via autoclaving at 121°C, 2 atm, for 20 min.

Well diffusion assay: The medium, maintained at 45-50°C, was poured into sterile petri dishes and allowed to solidify. The surface of the agar was inoculated with a suspension of the test microorganisms. Wells of 6 mm diameter were created in the inoculated agar using a cork borer. Each well was filled with 100 µL of the test sample at concentrations ranging from 50 to 200 µg/mL. Chloramphenicol (CY) was used as the positive control for antibacterial activity, while nystatin (NY) was used for antifungal activity. The petri dishes were incubated at 37°C for 18-24 hrs. Antimicrobial activity was evaluated by observing the clear zones around the wells, measured using a vernier caliper.

Microdilution assay: Each well of a 96-well plate was filled with 100 µL of MHB. The tested fractions, including the positive control antibiotic, were serially diluted within the plate to achieve concentrations ranging from 1 to 512 µg/mL. Negative controls consisted of media without bacteria, while growth controls contained bacteria without the test compounds. To each well, except the negative control, 10 µL of bacterial suspension was added to achieve a final concentration of 10 CFU/mL. The plates were incubated at 37°C for 18-24 hrs. After incubation, absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 625 nm. The lowest absorbance value, equal to the negative control, indicated no bacterial growth and was considered the minimum inhibitory concentration (MIC).

RESULTS

Total alkaloid content: The total alkaloid content of the *P. purpurea* sponge was found to be approximately 0.062%, as shown in Table 1. This result indicates the presence of a measurable amount of alkaloids within the sample, though varying when compared to other sponge species.

Alkaloid profiling by UPLC-HRMS: The UPLC-HRMS analysis of the alkaloid isolate (AI) in Fig. 2 show a chromatogram indicating the presence of various compounds with differing peak intensities across a retention time range of 0 to 20 min. The distribution pattern of the peaks from the beginning to the end reflects the diversity of compounds in the sample, with varying polarity and molecular masses, highlighting the complexity of the chemical components in the analyzed extract. Alkaloid Isolate of *P. purpurea* revealed the presence of seven classes of alkaloids with detailed structures and concentrations of each identified alkaloid are presented in Table 2.

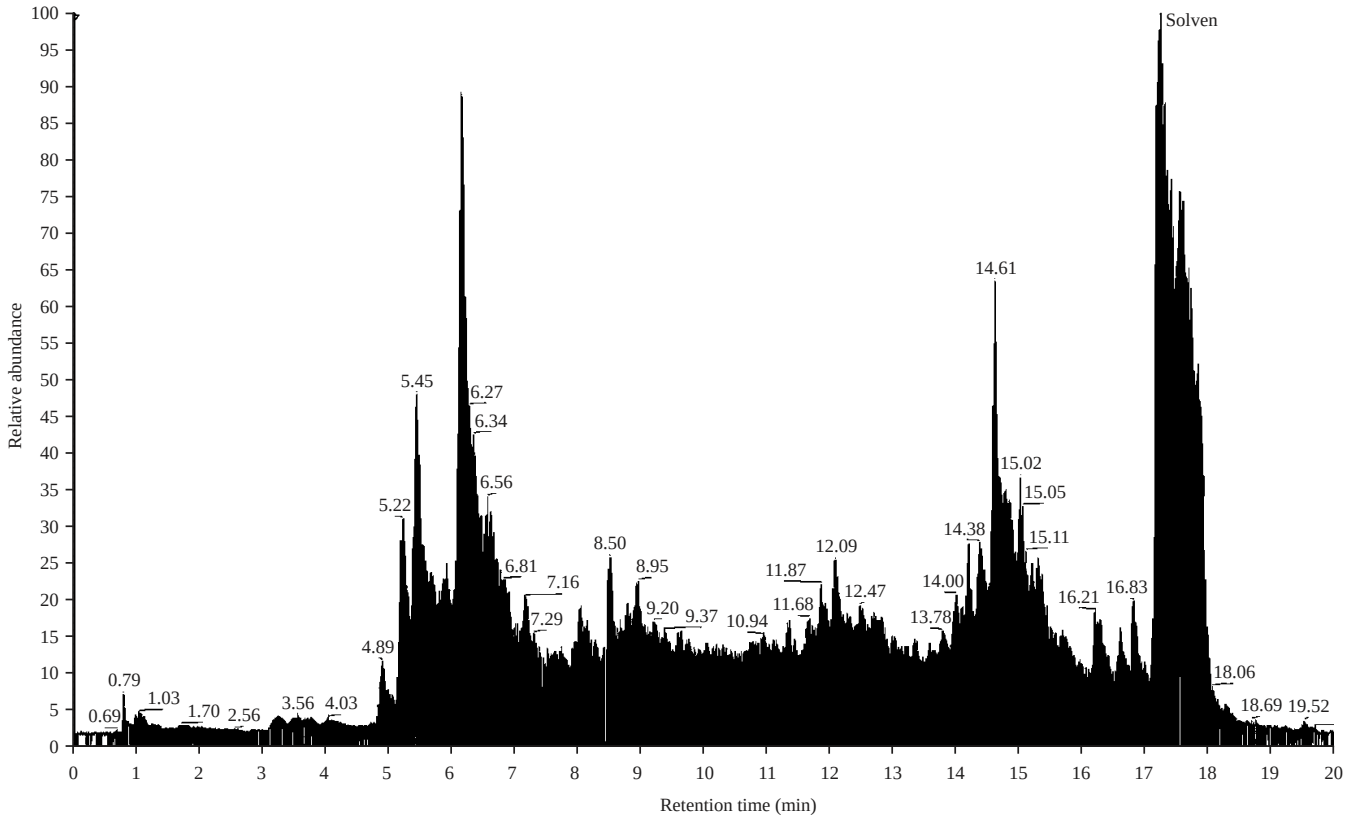

Fig. 2: Total ion chromatogram (TIC) in mass spectrometry of *P. purpurea* alkaloids

Table 1: Alkaloids from *P. purpurea*

Number	Sample (g)	Methanol extract (ME) (g)	Alkaloids isolate (AI) (g)
1	1000	8.7	0.62

The first group identified was indole alkaloids, contributing a total of 1.80% of the overall alkaloid content. The most dominant compound in this group was Indole-3-acetic acid, which appeared at a retention time of 5.262 min, with a calculated mass of 175.06316, a peak area of 206,455,168.47 and a relative concentration of 0.80%. Other notable compounds included Heptaphylline, detected at 11.070 min with a content of 0.27% and N-2-Ethylhexyl bicycloheptene dicarboximide and (2α)-12-Methoxyibogamine, each contributing 0.27 and 0.11%, respectively. The molecular structures of the indole alkaloids can be seen in Fig. 3.

The isoquinoline alkaloid group followed, with a total relative content of 4.14%. Among the fifteen compounds detected in this group, Norbuprenorphine was the most abundant, with a retention time of 12.497 min, a mass of 413.25603, a peak area of 335,077,583.19 and a content of 1.30%. The (-)-Lycodine was also significantly present at 7.649 min with a content of 0.99%, along with *Lemobiline* detected at 7.795 min and contributing 0.42%. Representative isoquinoline alkaloid structures are presented in Fig. 4.

The lipid alkaloids represented the second most abundant group, with a cumulative content of 32.66%. The major constituent was *Oleamide*, which appeared at a retention time of 14.615 min, with a calculated mass of 281.271, an extremely high peak area of 4,512,063,030.33 and the highest relative content of 17.50%. Other significant compounds included *Hexadecanamide*, detected at 14.195 min with a content of 6.53% and *Stearamide*, appearing at 15.312 min with a content of 2.79%. The corresponding lipid alkaloid structures are depicted in Fig. 5.

The protoalkaloid class accounted for the highest overall content among all groups, reaching 52.90%. The predominant compound in this class was N-phenethyl acetamide, detected at 6.157 min with a mass of 163.09946, an exceptionally large peak area of 10,119,043,630.90 and a relative content of 39.25%. Other major contributors were Napropamide-M, detected at 8.962 min with a content of 1.99% and Venlafaxine, at 10.795 min with a content of 1.07%. The chemical structures of the major protoalkaloids are illustrated in Fig. 6.

Table 2: Marine alkaloids from *P. purpurea* base on HRMS analysis

Number of structure	RT (min)	Calculated mass weight	Formula	Compound	Area	Content (%)	Database source
Indole alkaloids							
1	5.100	249.11118	$C_{12}H_{15}N_3O_3$	Oxibendazole	21898958.24	0.08	ChemSpider
2	5.262	175.06316	$C_{10}H_9NO_2$	Indole-3-acetic acid	206455168.47	0.80	mzCloud
3	7.823	310.2043	$C_{20}H_{26}N_2O$	(2alpha)-12-Methoxyibogamine	28790325.32	0.11	ChemSpider
4	9.105	246.1368	$C_{14}H_{18}N_2O_2$	Hypaphorine	30485237.01	0.12	ChemSpider
5	9.354	275.18852	$C_{17}H_{25}NO_2$	N-2-Ethylhexyl bicycloheptenedicarboximide	68426576.25	0.27	mzCloud
6	10.250	294.13674	$C_{18}H_{18}N_2O_2$	11-(4-Hydroxyphenyl)-3-methyl-6,7,8,9-tetrahydropyridazino[1,2-a] indazol-10-ium-1-olate	6676317.36	0.03	ChemSpider
7	11.070	279.12566	$C_{18}H_{17}NO_2$	Heptaphylline	69729909.38	0.27	mzCloud
8	16.253	496.43846	$C_{33}H_{56}N_2O$	N-[2-(1H-Indol-3-yl) ethyl] tricosanamide	30944157.76	0.12	ChemSpider
Total indole alkaloids					463406649.79	1.80	
Isoquinoline alkaloids							
1	5.030	221.14125	$C_{13}H_{19}NO_2$	Carnegine	34628999.69	0.13	ChemSpider
2	6.745	244.15738	$C_{15}H_{20}N_2O$	3,4,5,6-Tetradehydrospartein-2-one	71850969.29	0.28	ChemSpider
3	7.225	159.06845	$C_{10}H_9NO$	2-Methyl-4-quinolinol	40547794.76	0.16	ChemSpider
4	7.283	278.14198	$C_{18}H_{18}N_2O$	Proquazone	34475032.84	0.13	ChemSpider
5	7.649	242.17827	$C_{16}H_{22}N_2$	(-)-Lycodine	254864118.33	0.99	ChemSpider
6	7.795	243.12592	$C_{15}H_{17}NO_2$	Lemobiline	108579322.75	0.42	ChemSpider
7	8.524	310.16762	$C_{19}H_{22}N_2O_2$	(8alpha,9R)-Cinchonan-6',9-diol	21018039.74	0.08	ChemSpider
8	8.808	275.11568	$C_{15}H_{17}NO_4$	6-Hydroxy-2-(2-hydroxy-2-propanyl)-9-methyl-3,9-dihydrofuro[2,3-b] quinolin-4(2H)-one	19110913.54	0.07	ChemSpider
9	9.066	304.19371	$C_{21}H_{24}N_2$	Quinupramine	6236276.62	0.02	mzCloud
10	9.575	247.19338	$C_{16}H_{25}NO$	Lycopodine	21063604.81	0.08	ChemSpider
11	9.934	280.12108	$C_{17}H_{16}N_2O_2$	Cyclopeptine	9460725.45	0.04	ChemSpider
12	10.094	259.16826	$C_{15}H_{21}N_3O$	Primaquine	9367198.78	0.04	mzCloud
13	11.894	467.30327	$C_{29}H_{41}NO_4$	Buprenorphine	67639968.24	0.26	ChemSpider
14	12.497	413.25603	$C_{25}H_{35}NO_4$	Norbuprenorphine	335077583.19	1.30	ChemSpider
15	17.280	189.04192	$C_{10}H_7NO_3$	Kynurenic acid	32746906.58	0.13	ChemSpider
Total isoquinoline alkaloids					1066667454.62	4.14	
Lipid alkaloids							
1	3.892	187.15701	$C_{10}H_{21}NO_2$	N-Hydroxydecanamide	43251758.79	0.17	ChemSpider
2	5.036	201.17261	$C_{11}H_{23}NO_2$	11-Aminoundecanoic acid	23561299.88	0.09	ChemSpider
3	7.552	341.2563	$C_{19}H_{35}NO_4$	trans-2-Dodecenoylcarnitine	40544485.66	0.16	ChemSpider
4	8.762	171.16228	$C_{10}H_{21}NO$	Decanamide	182302697.52	0.71	mzCloud
5	10.505	289.20403	$C_{18}H_{27}NO_2$	Hydroxy-Î³-isosanshool	18235033.05	0.07	ChemSpider
6	10.621	317.29256	$C_{18}H_{39}NO_3$	Phytosphingosine	18832357.71	0.07	ChemSpider
7	11.151	325.29766	$C_{20}H_{39}NO_2$	Oleoylethanolamide	18527596.10	0.07	ChemSpider
8	11.664	199.19328	$C_{12}H_{25}NO$	Lauramide	366803691.85	1.42	ChemSpider
9	12.994	227.2246	$C_{14}H_{29}NO$	Myristamide	59507639.46	0.23	ChemSpider
10	13.556	299.28203	$C_{18}H_{37}NO_2$	Palmitoylethanolamide	21201788.84	0.08	mzCloud
11	13.961	325.2972	$C_{20}H_{39}NO_2$	Oleoylethanolamide	6713106.92	0.03	mzCloud
12	14.195	255.25578	$C_{16}H_{33}NO$	Hexadecanamide	1682842780.76	6.53	mzCloud
13	14.291	269.27153	$C_{17}H_{35}NO$	Capsi-amide	62362800.11	0.24	ChemSpider
14	14.615	281.271	$C_{18}H_{35}NO$	Oleamide	4512063030.33	17.50	mzCloud
15	15.312	283.28646	$C_{18}H_{37}NO$	Stearamide	720126636.82	2.79	mzCloud
16	15.727	323.31807	$C_{21}H_{41}NO$	1-(14-Methylhexadecanoyl) pyrrolidine	165708923.79	0.64	ChemSpider
17	16.616	337.33368	$C_{22}H_{43}NO$	Erucamide	450221671.13	1.75	mzCloud
18	16.627	339.34899	$C_{22}H_{45}NO$	Docosanamide	27149283.98	0.11	mzCloud
Total lipid alkaloids					8419956582.70	32.66	

Table 2: Continue

Number of structure	RT (min)	Calculated mass weight	Formula	Compound	Area	Content (%)	Database source
Proto alkaloids							
1	2.161	179.09442	$C_{10}H_{13}NO_2$	3,4-Methylenedioxyamphetamine	46221978.82	0.18	ChemSpider
2	4.231	207.12575	$C_{12}H_{17}NO_2$	1,3-Benzodioxolyl-N-methylbutanamine	13157204.86	0.05	mzCloud
3	5.517	197.1201	$C_{14}H_{15}N$	Dibenzylamine	119867824.49	0.46	mzCloud
4	5.938	235.15743	$C_{14}H_{21}NO_2$	Phenol, 3-(2-(1,1-dimethylethyl)-3-methyl-5-oxazolidinyl)-	43856059.40	0.17	ChemSpider
5	6.071	207.12575	$C_{12}H_{17}NO_2$	4-(2,2,3-Trimethyl-5-oxazolidinyl) phenol	31411087.31	0.12	ChemSpider
6	6.157	163.09946	$C_{10}H_{13}NO$	N-phenethylacetamide	10119043630.90	39.25	ChemSpider
7	6.209	222.13664	$C_{12}H_{18}N_2O_2$	3-Hydroxymonoethylglycinexylidide	20482951.24	0.08	ChemSpider
8	6.795	185.21424	$C_{12}H_{27}N$	Tributylamine	146221587.36	0.57	mzCloud
9	7.165	272.1888	$C_{17}H_{24}N_2O$	Pilsicainide	25112693.21	0.10	ChemSpider
10	7.278	225.13643	$C_{12}H_{19}NO_3$	Terbutaline	13624786.01	0.05	mzCloud
11	7.333	207.08939	$C_{11}H_{13}NO_3$	Methylone	14309179.41	0.06	ChemSpider
12	7.495	209.14144	$C_{12}H_{19}NO_2$	Octyl cyanoacrylate	138310825.65	0.54	ChemSpider
13	7.597	276.2202	$C_{17}H_{28}N_2O$	Etidocaine	83059558.72	0.32	ChemSpider
14	7.637	185.14156	$C_{10}H_{19}NO_2$	Atagabalin	15034019.63	0.06	ChemSpider
15	7.675	320.20976	$C_{18}H_{28}N_2O_3$	Iprovalicarb	24001118.44	0.09	ChemSpider
16	8.051	264.14723	$C_{14}H_{20}N_2O_3$	Vorinostat	11487458.53	0.04	ChemSpider
17	8.353	215.13097	$C_{14}H_{17}NO$	6-[(dimethylamino)methylidene]-6,7,8,9-tetrahydro-5H-benzo[a]cyclohepten-5-one	11536909.11	0.04	mzCloud
18	8.548	235.15743	$C_{14}H_{21}NO_2$	(1R,2R)-2-(Aminomethyl)-1-(3-methoxyphenyl) cyclohexanol	58179670.56	0.23	ChemSpider
19	8.789	231.12569	$C_{14}H_{17}NO_2$	3-(phenethylethanimidoyl) tetrahydrofuran-2-one	35916175.49	0.14	mzCloud
20	8.962	271.15701	$C_{17}H_{21}NO_2$	Napropamide-M	512237053.31	1.99	ChemSpider
21	9.059	277.20403	$C_{17}H_{27}NO_2$	Venlafaxine	31920610.48	0.12	ChemSpider
22	9.148	257.1415	$C_{16}H_{19}NO_2$	Medifoxamine	19841443.56	0.08	ChemSpider
23	9.367	229.14639	$C_{15}H_{19}NO$	N1-bicyclo [2.2.2] oct-2-ylbenzamide	12212212.40	0.05	mzCloud
24	9.498	195.1257	$C_{11}H_{17}NO_2$	4-(2-Aminopropoxy)-3,5-dimethylphenol	14561230.36	0.06	ChemSpider
25	9.618	245.14145	$C_{15}H_{19}NO_2$	Tasimelteon	166529973.04	0.65	ChemSpider
26	9.634	249.17253	$C_{15}H_{23}NO_2$	N-Desmethyltramadol	14304509.53	0.06	ChemSpider
27	9.855	239.13088	$C_{16}H_{17}NO$	Diphenamid	19601013.92	0.08	ChemSpider
28	10.795	277.20403	$C_{17}H_{27}NO_2$	Venlafaxine	277017701.20	1.07	ChemSpider
29	10.928	282.17287	$C_{18}H_{22}N_2O$	2-hydroxydesipramine	204870932.62	0.79	ChemSpider
30	11.052	234.17298	$C_{14}H_{22}N_2O$	Lidocaine	26492802.28	0.10	ChemSpider
31	11.101	274.16817	$C_{16}H_{22}N_2O_2$	Allyxycarb	21988081.95	0.09	ChemSpider
32	11.275	241.27667	$C_{16}H_{35}N$	Bis(2-ethylhexyl) amine	12782063.86	0.05	mzCloud
33	11.573	255.16213	$C_{17}H_{21}NO$	Tofenacin	30427229.59	0.12	ChemSpider
34	11.673	255.16213	$C_{17}H_{21}NO$	Atomoxetine	135917063.31	0.53	ChemSpider
35	11.682	339.21919	$C_{22}H_{29}N_2O_2$	Propoxyphene	33278231.17	0.13	ChemSpider
36	12.387	203.1306	$C_{13}H_{17}NO$	α-Pyrrolidinopropiophenone	39611809.99	0.15	ChemSpider
37	14.097	417.32343	$C_{26}H_{43}NO_3$	N-Vanillyl oleamide	971782528.47	3.77	ChemSpider
38	14.281	303.25563	$C_{20}H_{33}NO$	cis-Fenpropimorph	44982558.11	0.17	ChemSpider
39	15.648	281.21437	$C_{20}H_{27}N$	Alverine	14033349.92	0.05	mzCloud
40	18.306	393.33882	$C_{28}H_{43}N$	bis(4-Octylphenyl) amine	63569173.20	0.25	ChemSpider
Total proto alkaloids					13638796291.42	52.90	

Table 2: Continue

Number of structure	RT (min)	Calculated mass weight	Formula	Compound	Area	Content (%)	Database source
Purine alkaloids							
1	3.568	196.12091	$C_{10}H_{16}N_2O_2$	3-(propan-2-yl)-octahydropyrrolo[1,2-a] pyrazine-1,4-dione	226262047.34	0.88	mzCloud
2	5.859	244.12096	$C_{14}H_{16}N_2O_2$	Cyclo(phenylalanyl-prolyl)	253761061.93	0.98	mzCloud
3	6.174	194.17816	$C_{12}H_{22}N_2$	3-Nonyl-1H-pyrazole	12494336.23	0.05	ChemSpider
4	13.239	253.24043	$C_{16}H_{31}NO$	1-Dodecyl-2-pyrrolidinone	8305031.98	0.03	mzCloud
5	14.728	592.26708	$C_{35}H_{36}N_4O_5$	Pheophorbide A	18702645.15	0.07	ChemSpider
Total purine alkaloids					519525122.63	2.02	
Pyridine alkaloids							
1	7.216	217.11009	$C_{13}H_{15}NO_2$	Glutethimide	34468140.76	0.13	ChemSpider
2	7.296	218.1055	$C_{12}H_{14}N_2O_2$	Primidone	15417367.63	0.06	ChemSpider
3	7.457	250.20448	$C_{15}H_{26}N_2O$	Leontiformine	46718222.30	0.18	ChemSpider
4	7.511	259.12093	$C_{15}H_{17}NO_3$	Ilepcimide	17783503.93	0.07	ChemSpider
5	7.647	223.1572	$C_{13}H_{21}NO_2$	4-Methyl-6-[(1-methyl-2-piperidinyl) methyl]-5,6-dihydro-2H-pyran-2-one	88036453.36	0.34	ChemSpider
6	7.718	203.0582	$C_{11}H_9NO_3$	Anibine	11339200.23	0.04	ChemSpider
7	7.739	252.16251	$C_{17}H_{20}N_2$	2-(1-Adamantyl) imidazo[1,2-a] pyridine	104204946.84	0.40	mzCloud
8	7.877	191.09453	$C_{11}H_{13}NO_2$	Feninetramide	17698593.54	0.07	ChemSpider
9	8.335	256.12145	$C_{15}H_{16}N_2O_2$	Ancymidol	21282874.95	0.08	ChemSpider
10	8.432	201.11508	$C_{13}H_{15}NO$	6-Methyl-5-(5-methyl-2-furyl)-2,3-dihydro-1H-pyrrolizine	19730749.75	0.08	ChemSpider
11	8.550	290.17803	$C_{20}H_{22}N_2$	Azatadine	110098577.30	0.43	ChemSpider
12	8.793	237.17257	$C_{14}H_{23}NO_2$	Piroctone	354018141.07	1.37	ChemSpider
13	9.368	211.15698	$C_{12}H_{21}NO_2$	Elaeokanine C	269702881.95	1.05	ChemSpider
14	9.676	281.17784	$C_{19}H_{23}NO$	Diphenylpyraline	17934705.16	0.07	ChemSpider
15	10.020	260.15239	$C_{15}H_{20}N_2O_2$	Fenspiride	27299009.30	0.11	mzCloud
16	10.024	233.14145	$C_{14}H_{19}NO_2$	Methylphenidate	14657862.65	0.06	ChemSpider
17	10.226	231.12569	$C_{14}H_{17}NO_2$	Indeloxazine	14928033.82	0.06	ChemSpider
18	10.270	243.16223	$C_{16}H_{21}NO$	(6E,8Z)-9-(7-Methyl-3H-azepin-2-yl)-6,8-nonadien-3-one	14363893.99	0.06	ChemSpider
19	10.367	225.11505	$C_{15}H_{15}NO$	Navenone A	34396941.24	0.13	ChemSpider
20	10.776	277.27657	$C_{19}H_{35}N$	Perhexiline	14610927.33	0.06	ChemSpider
21	10.789	229.14639	$C_{15}H_{19}NO$	(2E,4Z,6E,8E)-1-(3,4-Dihydro-1(2H)-pyridinyl)-2,4,6,8-decatetraene-1-one	23258179.85	0.09	ChemSpider
22	10.899	316.15706	$C_{13}H_{24}N_4O_3S$	Bupirimate	175297647.95	0.68	mzCloud
23	10.963	233.17766	$C_{15}H_{23}NO$	Meptazinol	10330773.59	0.04	ChemSpider
24	13.814	301.23995	$C_{20}H_{31}NO$	Trihexyphenidyl	17593183.97	0.07	ChemSpider
25	15.083	293.27114	$C_{19}H_{35}NO$	4-[(1S)-1-Cyclohexyl-2-(2-piperidinyl) ethyl] cyclohexanol	31118788.54	0.12	ChemSpider
26	15.303	297.30239	$C_{19}H_{39}NO$	Tridemorph	22271477.02	0.09	mzCloud
27	16.225	359.31801	$C_{24}H_{41}NO$	1-(6-Nonyl-3-pyridinyl)-1-decanone	95112821.00	0.37	ChemSpider
28	16.895	373.33379	$C_{25}H_{43}NO$	(2E,4E,16Z)-1-(1-Piperidinyl)-2,4,16-icosatrien-1-one	22801301.74	0.09	ChemSpider
Total pyridine alkaloids					1646475200.76	6.39	
Steroid alkaloids							
1	10.080	312.21971	$C_{20}H_{28}N_2O$	[3,2-c] Pyrazole-androst-4-en-17β-ol	8147361.23	0.03	mzCloud
2	11.076	337.20339	$C_{22}H_{27}NO_2$	Danazol	9786065.04	0.04	mzCloud
3	17.088	387.34941	$C_{26}H_{45}NO$	25-Azacholesterol	7390827.66	0.03	ChemSpider
Total steroid alkaloids					25324253.93	0.10	
Alkaloids total					25780151555.86	100.00	

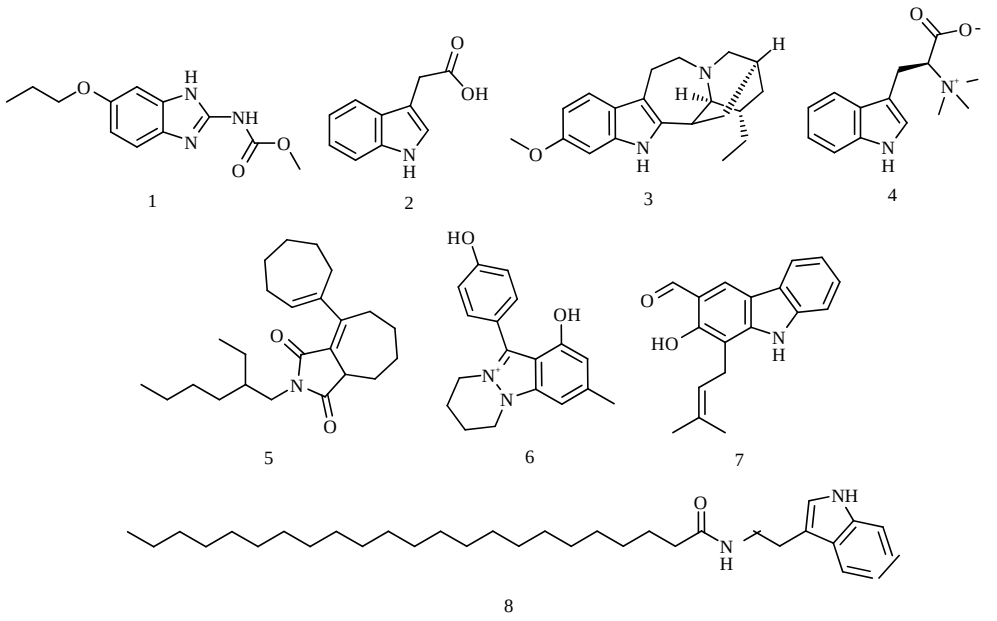

Fig. 3: Structure of indole-alkaloids

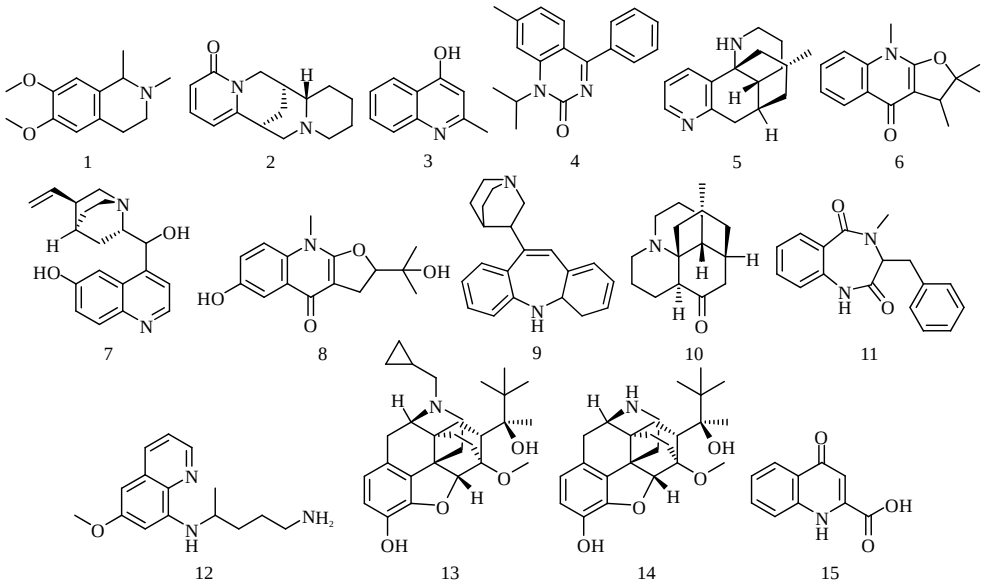

Fig. 4: Structure of isoquinoline-alkaloids

In the purine alkaloid class, a total content of 2.02% was observed. The major constituents were Cyclo(phenylalanyl-prolyl), detected at 5.859 min with a content of 0.98% and 3-(propan-2-yl)-octahydropyrrolo[1,2-a]pyrazine-1,4-dione, found at 3.568 min with a content of 0.88%. Purine alkaloid structures identified from the extract are shown in Fig. 7.

The pyridine alkaloids accounted for 6.39% of the total alkaloids detected. Within this group, Piroctone emerged as the leading compound, with a retention time of 8.793 min and a content of 1.37%, followed by Elaeokanine C at 9.368 min with a content of 1.05%. Other active compounds, such as Azatadine and 2-(1-Adamantyl) imidazo[1,2-a]pyridine, also contributed 0.43 and 0.40%, respectively. Figure 8 displays the structural formulas of the pyridine alkaloids.

The steroid alkaloid group was the least abundant, comprising only 0.10% of the total alkaloids detected. Among the few identified compounds, Danazol appeared at 11.076 min with a content of 0.04% and 25-Azacholesterol was found at 17.088 min with a content of 0.03%. The steroid alkaloid structures are presented in Fig. 9.

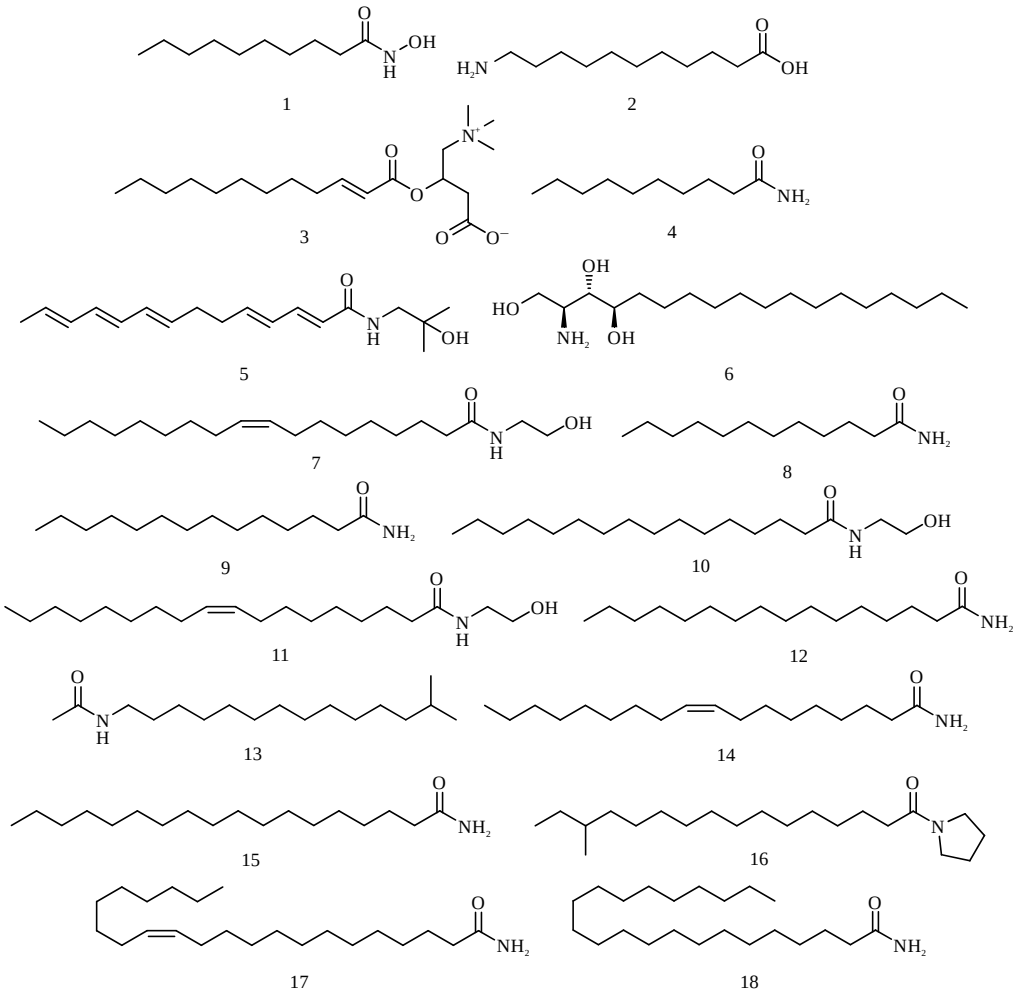

Fig. 5: Structure of lipid-alkaloids

The overall composition of each class of alkaloids is illustrated in Fig. 10. In the percentage distribution of seven types of marine alkaloids in the sample, proto alkaloids (52.90%) and lipid alkaloids (32.66%) are the dominant components. Other alkaloid types, such as pyridine, isoquinoline, purine, indole and steroid alkaloids, show much lower percentages, each below 7%. These results indicate that proto and lipid alkaloids are the major compounds potentially contributing to the sample's bioactive properties, making them important targets for further exploration.

Antimicrobial activity: The antimicrobial activity of the AI and ME of *P. purpurea* was assessed using both the well diffusion and microdilution methods. In the well diffusion assay, both AI and ME exhibited moderate zones of inhibition against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. In contrast, the positive controls (CY) for bacteria and (NY) for fungi demonstrated significantly larger inhibition zones, indicating higher antimicrobial potency. The inhibition zone diameter data of ME, AI and positive controls against three tested microorganisms in Fig. 11 show that AI exhibits higher antimicrobial activity compared to ME, as indicated by consistently larger inhibition zones at all concentrations. The activity of AI is even comparable to that of the positive controls, particularly against *E. coli* and *S. aureus*. This is further supported by the results of the minimum inhibitory concentration (MIC) test in Fig. 12, which demonstrate that AI has significantly lower MIC values than ME, indicating stronger antimicrobial potential. The MIC values of AI are also comparable to those of CH and NY as positive controls. Overall, these results suggest that the alkaloid isolate from the marine sample possesses strong potential as an antimicrobial agent against Gram-positive bacteria, Gram-negative bacteria and fungi.

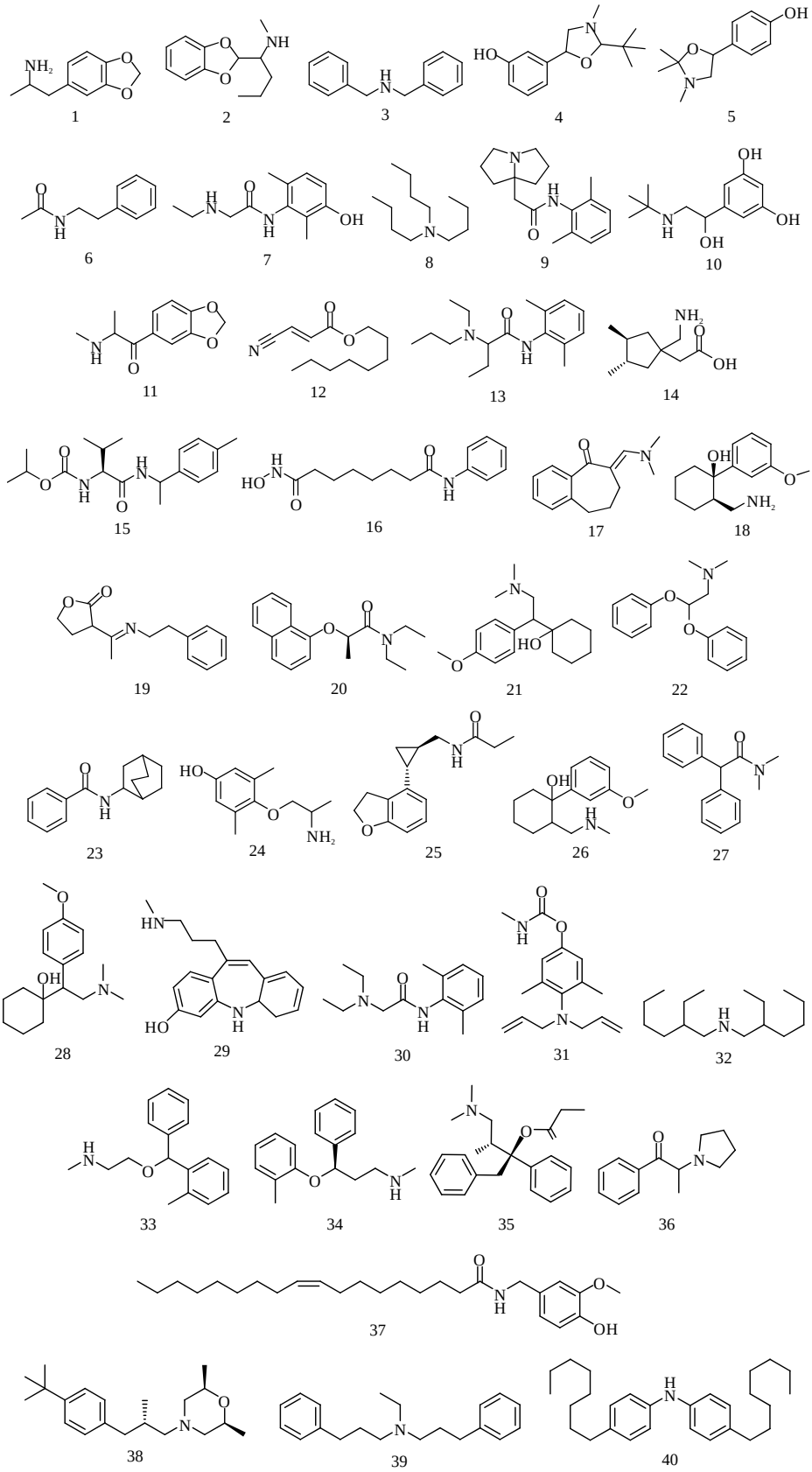

Fig. 6: Structure of proto-alkaloids

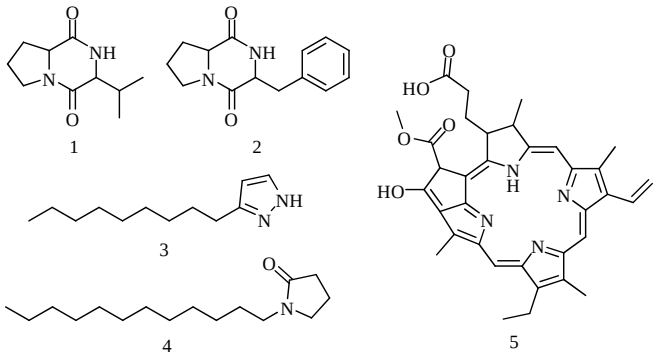

Fig. 7: Structure of purine-alkaloids

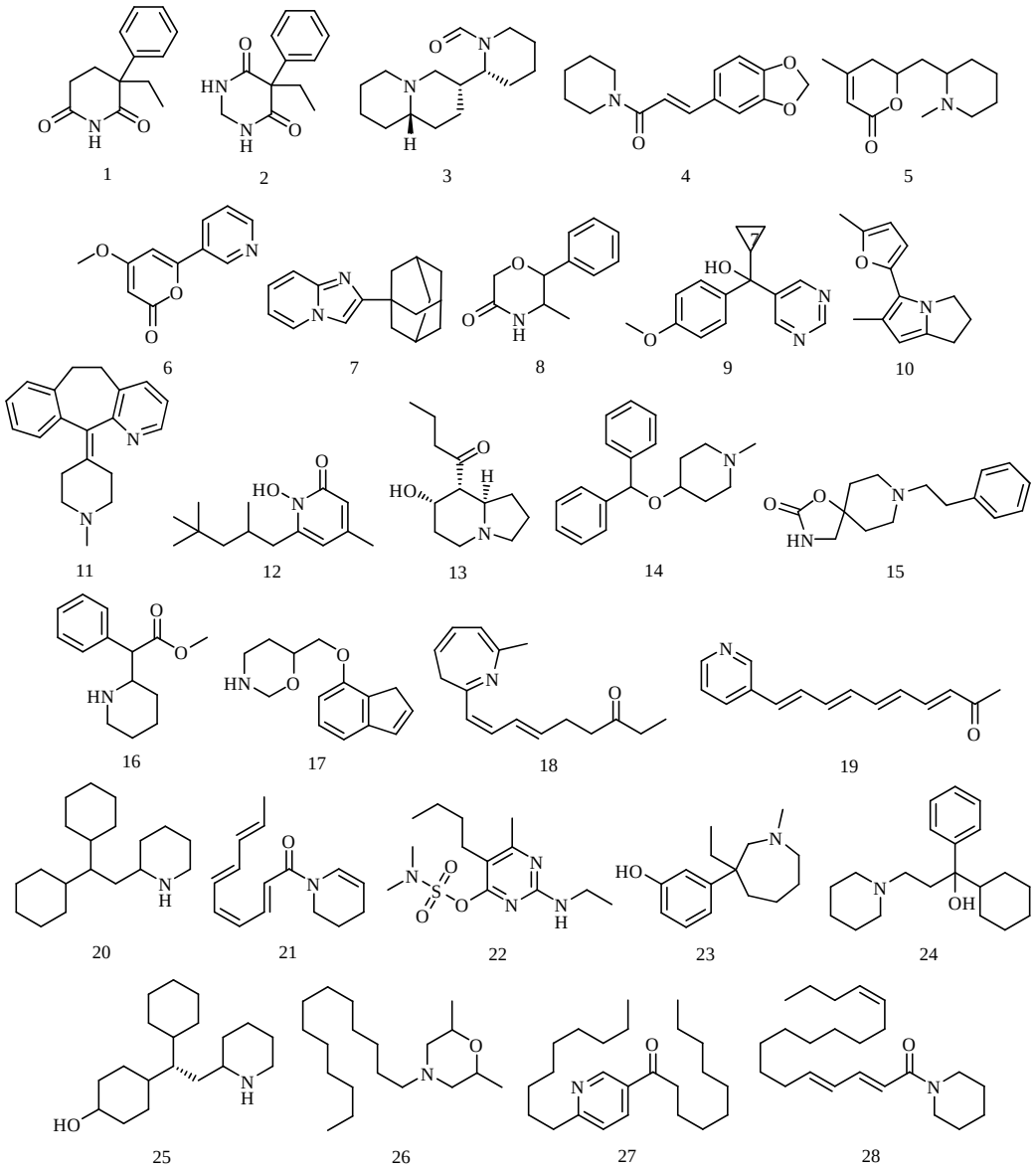

Fig. 8: Structure of pyridine-alkaloids

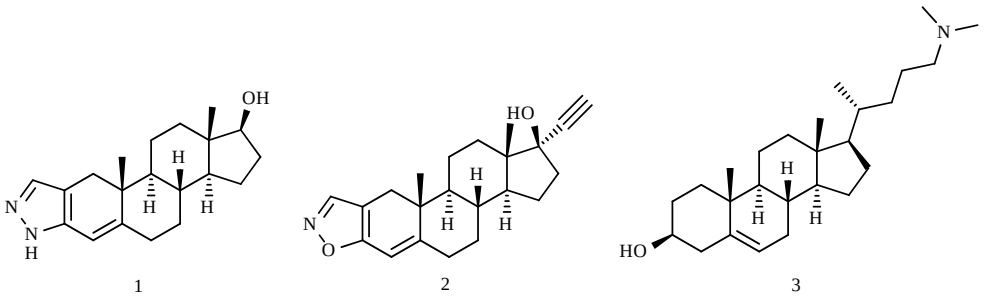

Fig. 9: Structure of steroid-alkaloids

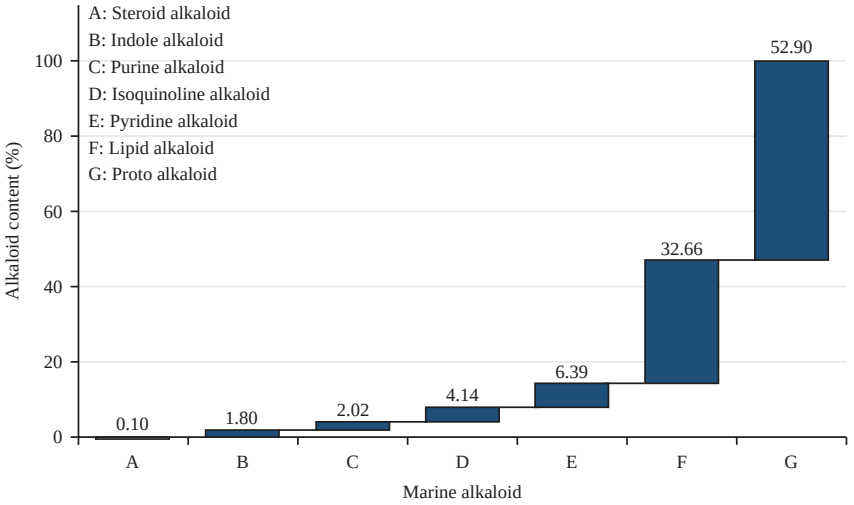

Fig. 10: Marine alkaloids concentration in the *P. purpurea* sponge

A: Steroid alkaloid, B: Indole alkaloid, C: Purine alkaloid, D: Isoquinoline alkaloid, E: Pyridine alkaloid, F: Lipid alkaloid and G: Proto alkaloid

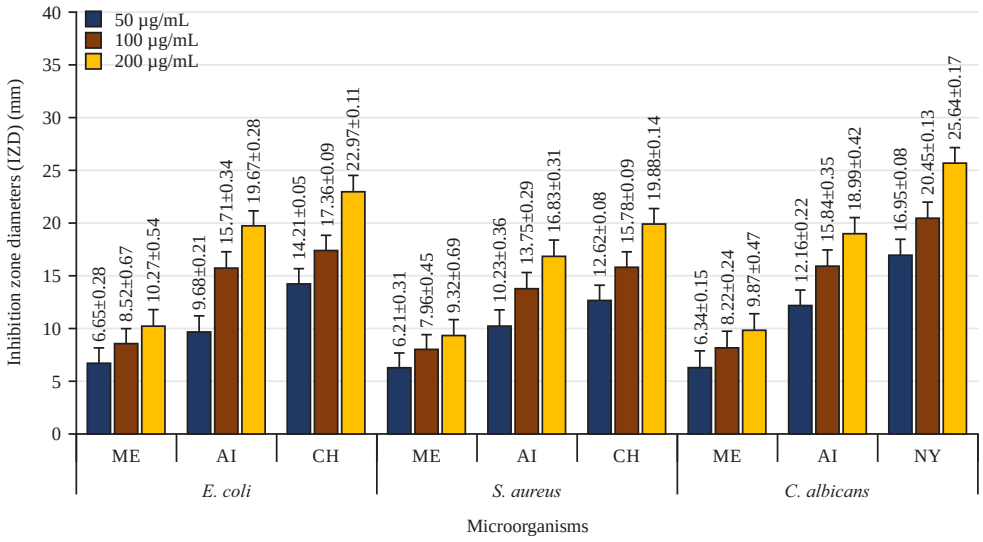

Fig. 11: Inhibition zone diameters (IZD)

ME: Methanol extract, CH: Chloramphenicol, AI: Alkaloid isolate and NY: Nystatin

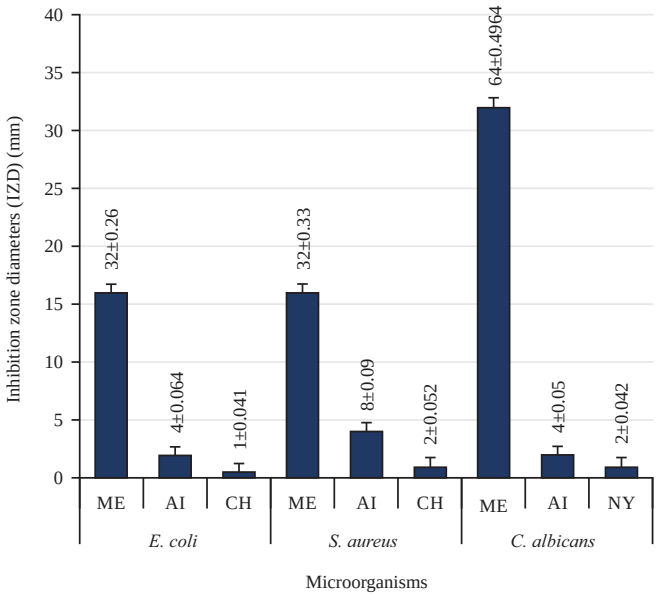

Fig. 12: Minimum inhibitory concentrations (MIC)
ME: Methanol extract, CH: Chloramphenicol, AI: Alkaloid isolate and NY: Nystatin

DISCUSSION

Marine sponges are widely recognized as a rich source of bioactive compounds, including alkaloids with antimicrobial, antitumor and cytotoxic properties. The alkaloid content in sponges is known to vary significantly depending on species, environmental habitat and extraction methods. For example, *Agelas* sponges from Okinawa yield only 0.00052% of bromopyrrole alkaloids[23], while *Aplysina cavernicola* from the Mediterranean Sea may contain up to 10% brominated alkaloids[24]. On the other hand, the sponge *Agelas oroides* exhibits alkaloid contents consisting of linear pyrrole alkaloids, fused cyclic pyrrole alkaloids, dimeric pyrrole alkaloids and terpenoid alkaloids at 95.1, 0.7, 0.4 and 0.6%, respectively[25].

Compared to these references, the 0.062% alkaloid content in *P. purpurea* reflects a moderate yield but still suggests a promising potential as a source of marine-derived alkaloids. The diversity of alkaloids observed in this study supports the importance of using metabolomic profiling techniques such as UPLC-HRMS to reveal the chemical richness of marine organisms.

Interestingly, although proto and lipid alkaloids were the most abundant in *P. purpurea*, the alkaloid classes with documented antimicrobial properties, namely indole and pyridine alkaloids were relatively minor components (8.29% total in AI). Indole alkaloids such as Manzamine A, Dragmacidin and Bromotryptamine have been shown to possess strong antimicrobial activity via mechanisms involving membrane disruption[26-29]. Pyridine alkaloids like Haliclamine A have also demonstrated activity against both Gram-positive and Gram-negative bacteria[30].

Therefore, the modest antimicrobial activity observed in the AI and ME samples as reflected by the IZD and MIC values, is likely linked to the low concentrations of these bioactive alkaloid classes. This hypothesis is supported by the greater activity of the positive control compounds (CY and NY), which are themselves alkaloids. Despite this, the detection of such a wide range of alkaloid scaffolds, especially proto and lipid types, opens the door for further investigation into their potential biological activity beyond antimicrobial properties.

CONCLUSION

Pseudoceratina purpurea sponge contains 0.062% of alkaloids which consists of 117 compounds and classified as 7 subclasses of alkaloids which are indole (1.8%), isoquinoline (4.14%), lipid (32.66%), proto (52.90%), purine (2.02%), pyridine (6.39%) and steroid alkaloids (0.1%). The antimicrobial potency of alkaloid isolate (AI) revealed that it is a strong category with MIC 4±0.064 ppm towards *E. coli*, a strong category against *S. aureus* with MIC 8±0.09 ppm and a strong category towards *C. albicans* with MIC 4±0.05 ppm. The existence of diverse alkaloid structural frameworks reinforces the potential of *P. purpurea* as a source for the development of new therapeutic agents based on marine resources.

SIGNIFICANCE STATEMENT

This study highlights the chemical richness and antimicrobial potential of alkaloid isolates derived from the marine sponge *P. purpurea*. The total alkaloid yield of 0.062% indicates a moderate but significant presence of alkaloid compounds. The UPLC-HRMS profiling revealed a diverse array of seven alkaloid classes, with proto and lipid alkaloids being the most abundant. Although the known antimicrobial alkaloid classes (indole and pyridine) were present in relatively low amounts, both the alkaloid isolate (AI) and methanol extract (ME) demonstrated measurable antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*, with AI exhibiting stronger effects supported by larger inhibition zones and lower MIC values. These findings suggest that while proto and lipid alkaloids may contribute to the observed bioactivity, their full pharmacological potential remains to be explored. The presence of structurally diverse alkaloid scaffolds underscores the promise of *P. purpurea* as a source of novel bioactive compounds. Future research should focus on the isolation, structural elucidation and mechanism-of-action studies of individual alkaloids, particularly targeting their antimicrobial, anticancer, or anti-inflammatory potentials, which may pave the way for new marine-derived therapeutic agents.

ACKNOWLEDGMENTS

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