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Determination of Secondary Compounds in Extracts from Catfish (Pangasius sp)

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ABSTRACT

Catfish, scientifically known as Pangasius sp., is a common inhabitant of Indonesian freshwater, including regions such as Sumatera and Kalimantan. In South Sumatera, it is frequently used as an ingredient in traditional dishes like pindang and brengkes. This investigation aimed to analyze the presence of secondary metabolites in Pangasius sp. The research employed a descriptive approach, utilizing extracts from various parts of the catfish, including the skin, bones, and meat. The extracts used were extracts from various solvents such as ethanol, ethyl acetate, and n-hexane, obtained from the maceration process and the infusion process for aquadest. The results provided information that when using ethanol, the skin contains alkaloids, saponins, and steroids; the bone contains flavonoids and saponins,; the meat contains alkaloids, saponins, and flavonoids. Moreover, when ethyl acetate was used, the skin and the bone contain alkaloids; while the meat contains flavonoids and steroids. In n-hexane, the skin contains alkaloids and steroids, the bone contains alkaloids; and the meat contains saponins and terpenoids. In water, the skin contains alkaloids, saponins, tannins, and steroids; the bone contains alkaloids; and the meat contain alkaloids, flavonoids, and saponins. It was clearly seen that Pangasius sp. has secondary metabolites of alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids.

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1. Introduction

Catfish (*Pangasius sp.*) is a fish that lives in freshwater and is often found in Indonesian waters, especially Sumatra and Kalimantan. This fish is also one of the leading commodities of freshwater fish, widely used in industrial-scale development [1]. In the province of South Sumatra, this fish is often found in the waters of the Musi River basin. This fish is easy to get, has relatively fast growth, and is easy to cultivate [2]. This fish is also one of the typical culinary favorites from South Sumatra, namely *pindang* and *brengkes*.

The distinctive features of this catfish are that it does not have scales, does not have many thorns, has a grayish-white color, and the body is flat and smooth. The head of the catfish is balanced, has a proportional width, and has slightly downward eyes. The mouth is relatively wide at the corners of the mouth, it has two pairs of whiskers, which function as a sense of touch when swimming or looking for food. The two pairs of whiskers are the hallmark of this catfish [3].

Pangasius sp is a local catfish species resulting from crossing Siamese catfish (*Pangasius hypophthalmus*) with djambal catfish (*Pangasius djambal*). The results of this cross led to the

emergence of the Pangasius sp species with superior characteristics, significant body weight, white flesh, low-fat content, and relatively fast development. Pangasius of this type have an oval head shape, slightly flattened, round and large eyes, a slightly tapered snout, and fine teeth. This fish has a pair of fins on its chest with fins on its tail in a shape resembling the letter V. The back of this type of fish is muddy green, while the belly is silver [4].

There are two types of metabolism in living things: primary and secondary. Primary metabolism is the main thing that is very important for the life of an organism to produce energy, such as photosynthesis and respiration. Secondary metabolites are generally used as a source of antioxidants, antibacterials, anti-inflammatories, etc. The content of catfish's primary metabolites namely protein, carbohydrates, and fat. Siamese catfish meat has a protein content of 12.94-17.52%, 1.09% fat content, and 0.89-1.23% sodium minerals. [6]. Catfish also contains glycogen 0.181 mg/100 mL, cholesterol 282.48 mg/dL, triglycerides 307.77 mg/dL, High-Density Lipoprotein (HDL) 94.42 mg/dL, Low-Density Lipoprotein (LDL) 42.55 mg /dL, and plasma protein [5].

The secondary metabolite content of catfish (*Pangasius sp*) is unknown. Active compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids are widely used to supplement medical needs. This study aims to determine the content of secondary metabolites of catfish (*Pangasius sp*.) that can be used in the health sector.

2. Methodology

This type of research was a descriptive study using extracts from catfish's skin, bones, and flesh (*Pangasius sp*). The research was conducted at the Basic Chemistry Laboratory, Faculty of Medicine, Madang Campus, Palembang. The research subjects were dead catfish separated from their skin, bones, flesh, and digestive organs. This catfish species has been identified and authenticated at the Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya.

Maserator (Lahnpan, China), beaker, dropper pipette, petri dish, test tube, Whatman filter paper number 40, blender, thermometer (Omron, Indonesia). The main ingredients used were catfish skin, bones, and meat, taken from catfish cultivation in the Jakabaring area of Palembang, Indonesia. Other ingredients are n-hexane solvent, ethyl acetate, ethanol, distilled water, chloroform test material, magnesium powder and concentrated hydrochloric acid, 1% ferritricloride, glacial CH3COOH, concentrated citric acid, ammonia, 2 N HCl, Wagner reagent, Dragendorff reagent, reagent Mayer, which was purchased at PT. Brataco Chemicala, Indonesia.

The samples came from fresh catfish's skin, bones, and meat (Pangasius sp). The catfish's skin, bones, and meat are separated and washed thoroughly under running water. The three parts of the catfish are cut into small pieces, placed in a separate cup, and then mashed using a blender.

The skin, bones, and mashed catfish meat were placed in four containers. Then, n-hexane solvent, ethyl acetate solvent, ethanol solvent, and aquadest infusion method were added to each container that had been prepared until it was submerged by the solvent and left for three days at room temperature. The mixture was heated to 90°C and allowed to stand for 15 minutes. The mixture was filtered using Whatman filter paper number 40. The results of the liquid filtrate were evaporated to obtain a concentrated extract then a phytochemical analysis.

2.1. Phytochemical Identification

Examination of Alkaloid

Examine alkaloids in extracts from the skin, bones, and meat of catfish by adding 0.1 gram with 5 drops of chloroform and then adding a few drops of ammonia. Then, the chloroform fraction was separated and acidified with a few drops of concentrated sulfuric acid. The acid fraction was taken and then added with Wagner, Mayer, and Dragendorff reagents. The alkaloid content would be indicated by forming a brown precipitate in Wagner's reagent, a white precipitate in Mayer's reagent, and an orange precipitate in Dragendorff's reagent.

Examination of Flavonoids

Examination of flavonoids in 1 ml of *Pangasius sp*. Extraction was done by adding 1 gram of magnesium powder and 10 mL of concentrated hydrochloric acid. The reddish, orange, or yellow formation indicated that the extract contains flavonoids.

Examination of Tannins

This examination was done by adding 1 gram of extract with 10 mL of distilled water, heating it to 1000 C, allowing it to cool, and then adding 5 mL of 1% ferric chloride. The presence of active tannins was indicated by a change in color to dark blue.

Examination of Saponins

Examination of saponins in catfish extract was carried out using a foam test, namely, the extract was mixed in hot water. Saponin content was indicated by the formation of stable foam for 10 minutes.

Examination of Terpenoid

Terpenoid examination was performed by adding 1 mL of glacial acetic acid and 1 mL of concentrated H2SO4. The appearance of a red color indicated the presence of terpenoids.

Examination of Steroid

A steroid examination was performed by adding 1 mL of glacial acetic acid and 1 mL of concentrated H2SO4. The appearance of a blue or purple color indicated steroid content.

2.2. Data collection

The results of phytochemical tests for secondary metabolites of extracts from the skin, bones, and meat of catfish were listed in the data collection table on the study results.

3. Result and Discussion

3.1. Preparation of Receptor Structures and Ligands

The results of the secondary metabolite phytochemical tests of the skin, bone, and meat extracts of catfish can be seen in Tables 1, 2, and 3

Table 1. Secondary metabolite test results of catfish skin extract (*Pangasius sp)*

No.	Phytochemical screening	Skin extract of *Pangasius sp.*			
		ethanol	ethyl acetate	n-hexane	aquadest
1	Alkaloid				
	- *Wagner* reaction	-	-	+	-
	- *Mayer* reaction	+	+	+	+
	- *Dragendorff* reaction	+	+	-	+
2	Flavonoids	-	-	-	-
3	Saponins	+	-	-	+
4	Tannins	-	-	-	+
5	Terpenoid	-	-	-	-
6	Steroid	+	-	+	+

[a] (+) = detected, (-) = undetected

Table 1 shows that catfish skin extract contains alkaloids and saponins in ethanol solvent, alkaloids in ethyl acetate solvent, and alkaloids and steroids in n-hexane solvent. In distilled water, the metabolites obtained are alkaloids, saponins, tannins, and steroids.

Table 2 shows that in ethanol solvent, catfish bone extract contains flavonoids and saponins, and alkaloids in ethyl acetate, n-hexane, and distilled water.

Table 2. Test results for secondary metabolites of catfish bone extract (*Pangasius sp*)

No.	Phytochemical screening	Bone extract of *Pangasius sp.*			
		ethanol	ethyl acetate	n-hexane	aquadest
1	Alkaloid				
	- *Wagner* reaction	-	+	+	-
	- *Mayer* reaction	-	+	+	+
	- *Dragendorff* reaction	-	-	+	+
2	Flavonoids	+	-	-	-
3	Saponins	+	-	-	-
4	Tannins	-	-	-	-
5	Terpenoid	-	-	-	-
6	Steroid	-	-	-	-

[b.] (+) = detected, (-) = undetected

Table 3 shows that in ethanol solvent, catfish meat extract contains alkaloids, flavonoids, and saponins, contains flavonoids and steroids in ethyl acetate solvent, contains saponins and terpenoids in n-hexane solvent, and contains alkaloids, flavonoids, and saponins in distilled water.

Table 3. Test results for secondary metabolites of catfish meat extract (*Pangasius sp)*

No.	Phytochemical screening	Meat extract of *Pangasius sp.*			
		ethanol	ethyl acetate	n-hexane	aquadest
1	Alkaloid				
	- *Wagner* reaction	+	-	-	-
	- *Mayer* reaction	+	-	-	-
	- *Dragendorff* reaction	+	-	-	+
2	Flavonoids	+	+	-	+
3	Saponins	+	-	+	+
4	Tannins	-	-	-	-
5	Terpenoid	-	-	+	-
6	Steroid	-	+	-	-

[c.] (+) = detected, (-) = undetected

The above research showed that catfish extract contains active substances of alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. These ingredients can be used in the health sector. Alkaloids have antibacterial and antiviral effects [6].

Flavonoids are often widely used to maintain a healthy body. These compounds are often used in various pharmaceutical, cosmetic, nutritional, and drug industries. Flavonoids have antioxidant, antibacterial, anti-inflammatory, antimutagenic, and anticarcinogenic effects [7]. Antioxidant defense mechanisms in the body include activating the enzymes superoxide dismutase, catalase, and glutathione peroxidase. These enzymes play an essential role in the protective function of cells and indirectly maintain the balance of reactive oxygen species [8,9].

Saponins have antibacterial, antifungal, and immune booster effects. Saponins can lyse the cell wall of *Enterococcus faecalis* [10]. As antifungals, saponins work by reducing the adhesion ability of *Candida albicans*, inhibiting the production of fungal exopolysaccharides, which produce biofilms, and suppressing the growth of fungal hyphae [11]. Saponins can stimulate the cell-mediated immune system in the body and increase the body's antibodies [12].

Tannins are a group of polyphenolic biomolecules that can bind to proteins and various organic compounds such as amino acids. Tannins have anti-oxidant, anti-cancer, anti-allergic, anti-inflammatory, anti-heretic, and antimicrobial activities [13]. Tannins have a high degree of hydroxylation, so they play an active role as antioxidants. Tannins block the formation of carcinogens and slow tumor growth. In addition, tannins also have antimutagenic activity against aflatoxin B, 2-amino fluorene, and benzo-alpha-pyrene [14]. Tannins also effectively kill parasites and bacteria, such as Penicillium spp., S. aureus, C. botulinum, and H. pylori [13].

Terpenoids are a secondary metabolite compound that is often found in plants. Terpenoids can inhibit the production of nitric oxide (NO), interleukin-6 (IL-6), and tumor

necrosis factor-alpha (TNF-α), which are induced by lipopolysaccharide in the inflammatory process [15]. Terpenoids also have activity in inhibiting the formation of biofilms from *C. albicans*. Besides that, terpenoids also inhibit the growth of aerobic and anaerobic bacteria [16]. In the case of malaria, terpenoids act as antimalarials by binding to parasite proteins on the plasmodium, so protein activation is inhibited and causes death in the plasmodium [17]. For steroids, compounds have long been used as anti-inflammatories. Steroids can inhibit inflammatory mediators [18].

4. Conclusion

Based on the results of this study, it was concluded that catfish (*Pangasius sp.*) extract contains alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids.

References

[1] K. Mahyuddin, "*Panduan lengkap agribisnis patin*", Jakarta: Penebar Swadaya, 2011.

[2] S. Bahri, "Pengamatan jenis-jenis ikan di perairan sungai Musi Sumatera Selatan," *Buletin Teknik Litkayasa,* vol. 4, no. 1, pp. 9-12, 2007, doi: 10.15578/btl.5.1.2007.1-4

[3] A. Septimesy, D. Jubaedah, A. D. Sasanti, "Pertumbuhan dan kelangsungan hidup ikan patin (*Pangasius sp.)* di sistem resirkulasi dengan padat tebar berbeda," *Jurnal Akuakultur Rawa Indonesia,* vol. 4, no. 1, pp. 1-8, 2016, doi: 10.36706/jari.v4i1.4417

[4] G. Sandipan, "*Pangasius pangasius* (Hamilton, 1822), a threatened fish of Indian subcontinent," *Journal of Aquaculture Research and Development,* vol. 7, no. 2, pp. 1-3, 2016. [Online]. Available: www.cabdirect.org/cabdirect/abstract/20163136423

[5] H. Arfah, A. O. Sudrajat, M. A. Suprayudi, and M. Zairin, "Biochemical study of striped catfish Pangasianodon hypophthalmus broodstock induced by PMSG hormone + anti–dopamine and turmeric addition," *Jurnal Akuakultur Indonesia (E-journal)*, vol. 17, no. 2, pp. 191-197, Jul. 2018, doi: 10.19027/jai.17.2.191-198.

[6] T. P. T. Cushnie, B. Cushnie, and A. J. Lamb, "Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities," *International Journal of Antimicrobial Agents*, vol. 44, no. 5, pp. 377–386, Nov. 2014, doi: 10.1016/j.ijantimicag.2014.06.001.

[7] B. Arifin and S. A. Ibrahim, "Struktur, Bioaktivitas Dan Antioksidan Flavonoid," *Jurnal Zarah*, vol. 6, no. 1, pp. 21–29, Apr. 2018, doi: 10.31629/zarah.v6i1.313.

[8] A. Panche, A. D. Diwan, and S. Chandra, "Flavonoids: an overview," *Journal of Nutritional Science*, vol. 5, Jan. 2016, doi: 10.1017/jns.2016.41.

[9] M. Fazil, R. N. Suci, F Alfiah, D. N. Alam, G. Angelina, B. Situmeang, "Analisis senyawa alkaloid dan flavonoid dari ekstrak kitolod (*Isotoma longiflora*) dan uji aktivitasnya terhadap bakteri penyebab karies gigi," *Jurnal Itekima*,vol. 2, no. 1, pp. 73-83, 2017. [Online]. Available: http://stakc.ac.id/wp-content/uploads/2018/04/07-Muhamad-Fazil.pdf

[10] M. Arabski, A. Węgierek-Ciuk, G. Czerwonka, A. Lankoff, and W. Kaca, "Effects of Saponins against ClinicalE. coliStrains and Eukaryotic Cell Line," *Journal of Biomedicine and Biotechnology*, vol. 2012, pp. 1–6, Jan. 2012, doi: 10.1155/2012/286216.

[11] R. L. Vifta, S. Khotimah, and F. P. Luhurningtyas, "Uji Aktivitas Antifungi Ekstrak Etanol Biji Timun Suri (Cucumis melo L.) terhadap Pertumbuhan Candida albicans secara In Vitro," *Indonesian Journal of Pharmacy and Natural Product*, vol. 1, no. 1, pp. 10-17, Jul. 2018, doi: 10.35473/ijpnp.v1i1.29.

[12] T. K. Das, D. Banerjee, D. Chakraborty, M. C. Pakhira, B. Shrivastava, and R. C. Kuhad, "Saponin: Role in Animal system," *Veterinary World*, vol. 5, no. 4, pp. 248-254, Jan. 2012, doi: 10.5455/vetworld.2012.248-254.

[13] K. Sharma *et al.*, "Health effects, sources, utilization and safety of tannins: a critical review," *Toxin Reviews*, vol. 40, no. 4, pp. 432–444, Sep. 2019, doi: 10.1080/15569543.2019.1662813.

[14] E. Sieniawska, "Activities of Tannins – from in Vitro Studies to Clinical Trials," *Natural Product Communications*, vol. 10, no. 11, Nov. 2015, doi: 10.1177/1934578x1501001118.

[15] W. Yang, C. Xu, Y. Li, S. Guo, Z. Wang, and X. Yu, "Advances in pharmacological activities of terpenoids," *Natural Product Communications*, vol. 15, no. 3, pp. 1-13, Mar. 2020, doi: 10.1177/1934578x20903555.

[16] A. C. Guimarães *et al.*, "Antibacterial activity of terpenes and terpenoids present in essential oils," *Molecules*, vol. 24, no. 13, p. 2471, Jul. 2019, doi: 10.3390/molecules24132471.

[17] M. D. Astuti, T. Sriwinarti, and K. Mustikasari, "Isolation And Identification Of Terpenoid Compounds From N-Hexana Extract Of Permot Plant Bracts (Passiflora foetida L)," *Jurnal Ilmiah Berkala Sains Dan Terapan Kimia*, pp. 1-8, Oct. 2017, doi: 10.20527/jstk.v11i2.4041.

[18] W. Ericson-Neilsen, A. D. Kaye, "Steroids: pharmacology, complications, practice delivery issues. *The Ochsner Journal, 14(*2), 203-207, 2014. [Online]. Available: https://www.ochsnerjournal.org/content/14/2/203/tab-article-info