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Research Article

Characterization of Onchidiid Slug (*Onchidium typhae*) West Kalimantan Waters as Antibacterials and Antifungal

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Abstract

Onchidiid slug (*Onchidium typhae*) is a nudibranch that coastal communities in West Kalimantan have widely used as wounds. The study aims to characterize the West Kalimantan water *O. typhae* as antibacterial and antifungal. The study of *O. typhae* was carried out in several stages: preparation and optimization, extraction by Quinn method, characterization and identification of bioactive compounds, and antibacterial and antifungal assay using the microdilution method. The result of the proximate test showed that *O. typhae* powder contains high protein, namely 67.68%. Phytochemical screening results from methanol, ethyl acetate, and chloroform extracts contain alkaloids and amino acids. Methanol, chloroform, and ethyl acetate extract 1% of *O. typhae* showed inhibitory activity against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. The most significant inhibition value was indicated by chloroform extract 1%, where the inhibition value against *S. aureus*, *E. coli*, and *C. albicans* was 82±0.01%; 85.8±0.01%; 85±0.01%, respectively. From these results, *O. typhae* powder can be developed as a wound medicine through its antibacterial and antifungal activity.

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INTRODUCTION

West Kalimantan, part of a group of islands in Indonesia, is rich in animal and biological diversity. With the variety of its population, West Kalimantan will also be rich in traditional knowledge in using natural resources as medicine or healthy food preparations. The coastal communities of West Kalimantan use many natural ingredients, especially their aquatic products, as medicine. For example, the onchidiid slug (*Onchidium typhae*) has been used as a medicine for wounds/ulcers. *Onchidium typhae* is known to have the ability to produce secondary metabolites that are toxic to predators and some chemical compounds obtained from their food[1,2].

Onchidium genus (Onchidiidae family) is treated as a commodity with high economic value in waters along the Indo-Pacific coast due to its high nutritional and medicinal value. They are considered high-grade food due to their high protein and low-fat characteristics. This commodity has an aphrodisiac effect, digestive function, anticytotoxic activity *in vitro*, and antineoplastic *in vivo*[3-5]. According to a traditional Chinese medicine book, consuming fresh meat can maintain and improve as a cure for liver cirrhosis[6].

This research is essential because the wealth of marine biological resources and traditional knowledge that has been carried out empirically are abundant. However, exploration and exploitation are still minimal, even though marine natural resources have been proven to source various active ingredients with great potential as medicine[7]. Based on the literature

review, non-polar and polar active ingredients have promising pharmacological activities such as antioxidant activity[8]. In addition, reports of antibacterial activity from *O. typhae* have not been exploited much.

A wound is a form of tissue damage to the skin caused by physical contact (with a heat source), medical action, or changes in physiological conditions. The body naturally heals through sustainable bio-cellular and biochemical activities. The wound healing process is divided into five stages, including the stages of homeostasis, inflammation, migration, proliferation, and maturation[9-11]. Long-healed wounds are characterized by wounds that do not heal after 12 weeks. This condition is referred to as a chronic wound caused by infection. Infection can occur when bacteria get into an open wound. When an injury becomes infected, the body does more to fight off the infection than heal the wound. This condition can hinder wound healing[12,13]. *Staphylococcus aureus* and *Escherichia coli* are the bacteria that most often infect wounds. *Staphylococcus aureus* and *E. coli* are clinically relevant pathogens due to antibiotic resistance[14,15]. They are non-motile, non-sporing, facultatively anaerobic, catalase-positive, and oxidase-negative[16]. This study was conducted to explore the potential of *O. typhae*, commonly found on the coast of West Kalimantan, as an antibacterial. In addition, this study was also carried out to characterize the added value of *O. typhae* commodity.

MATERIALS AND METHODS

Materials

This study material used *O. typhae* with a length ranging from 4-6 cm in fresh conditions collected from the coast of Sambas, West Kalimantan (**Figure 1**). The sample was determined with specimen No. 023/A/LB/F.MIPA/UNTAN/2021 at the Biology laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. An antibacterial assay was performed using *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922), for the antifungal assay was performed using *Candida albicans* (ATCC 102310). Other materials were 1% DMSO, NaCl, 0.5 McFarland standard, sterile distilled water, Brain Heart Infusion (BHI) media, phosphate buffer saline (PBS) solution, and 1% crystals violet. The equipment used at the sample preparation stage was glassware for extraction, sieves, rotary evaporator, chopper, grinder, scales, refrigerator, vortex, and oven. The instrument used in the antibacterial assay was Laminar Air Flow (LAF), incubator (Moderna), micropipette (Socorex), multichannel micropipette (Socorex), microplate flat-bottom polystyrene 96 well (Iwaki), microtiter plate reader (Optic Ivymen System 2100-C, Spain), spectrophotometer UV Genesys 10 UV Scanning, 335903 (Thermo Scientific Spectronic, US), autoclave, and analytical balance (AB204-5, Switzerland).

Figure 1. *Onchidium typhae*

Methods

This research has passed the ethical clearance with No.700/UN22.9/PG/2022. It is critical to ensure that the research has complied with the principles of respect for the person, benefit and non-maleficence, and the principle of justice.

Extraction of Onchidium typhae

Extraction of active ingredients from *O. typhae* used was a multi-level extraction based on Quinn's method and was modified[17]. Modifications were made to the maceration time, 3 x 24 hours. The solvents used were chloroform (non-polar), ethyl acetate (semi-polar), and methanol (polar). Fifty grams of dry *O. typhae* powder and 100 mL of chloroform solvent were added until submerged, macerated at room temperature for 3x24 hours, then filtered (filtrate 1). The residue was added with ethyl acetate until submerged and macerated for 3 x 24 hours at room temperature, then filtered (filtrate 2). The remaining residue was added with methanol until submerged and macerated for 3 x 24 hours at room temperature, then filtered (filtrate 3). The filtrate 1, 2, and 3 were evaporated to obtain a crude extract. From the crude extract and then tested to identify *O. typhae* bioactive compounds. *Onchidium typhae* bioactive compounds were identified on alkaloids, steroids, saponins, carbohydrates, reducing sugars, peptide compounds, and free amino acids.

Proximate analysis of Onchidium typhae

A perfect *O. typhae* powder was obtained from the preparation under control, starting from fresh samples, cleaning from mud to the boiling process with constant stirring to ensure that the mucus was wholly removed. The drying of *O. typhae* samples was carried out at a temperature of 60°C for three days. The method provided a moisture content of <5%. Proximate analysis of *O. typhae* was carried out on powder samples. Proximate analysis was carried out using the Association of Official Analytical Chemists (AOAC) methods[18] in the Center Food and Nutrition Study laboratory, Universitas Gadjah Mada.

Bacterial strains

Staphylococcus aureus, *E. coli*, and *C. albicans* were grown within 24 hours at 37°C in BHI media. The optical density (OD) 600 of the microbial culture was adjusted to 0.1 (equivalent to the 0.5 McFarland standard - 1.5 x 10^8 CFU/mL) and then diluted in a new growth medium to 0.01 OD600[16].

Antibacterial and antifungal assay

The antibacterial and antifungal assay was carried out using the microdilution method. The assay was carried out with test compounds: 1; 0.5; 0.25; and 0.125% w/v on microplate 96 wells. The control for the antibacterial assay used was chloramphenicol 1% w/v, while the antifungal assay used fluconazole 1% w/v. The microplate was then incubated for 24 hours at 37°C — the percent inhibition was determined by observing the clarity of the solution[19,20]. Microplate absorbance reading process using a microplate reader at a wavelength of 595 nm[16,19].

RESULTS AND DISCUSSION

The sample preparation results obtained good *O. typhae* powder with less than 5% moisture content. *Onchidium typhae* powder was followed by proximate assay and extraction. The modified Quinn's method was chosen in the extraction process because it effectively extracts active compounds with different polarity levels. The crude extract obtained using chloroform, ethyl acetate, and methanol solvents were 8.7%, 0.25%, and 10%, respectively. Each extract obtained was then subjected to phytochemical screening. Phytochemical screening results can be seen in **Table I.**

Table I. Phytochemical screening of *Onchidium typhae*

Compound class test	Reagent	Result		
		Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	Wagner	+	-	+
	Mayer	-	-	-
	Dragendorff	+	-	+
Steroids & triterpenoids	Liebermann-Burchard	+	-	-
Saponins	Distilled water	+	-	-
Tannins	$FeCl_3$	-	-	-
Flavonoids	Mg and Cl ribbon	-	-	-
Reduction sugar	Benedict	-	-	-
Free amino acids	Ninhydrin	+	+	+

The test results showed that the chloroform extract contained alkaloids, steroids, saponins, and free amino acids, as shown in **Table I**. However, different results were shown in the methanol extract. It was shown that methanol extract does not contain saponins, steroids, and triterpenoids. These findings are in line with previous studies on the Onchidiidae family where polypropionate and its derivatives were found, amides and depsipeptides, terpenoids, and other types of compounds have been isolated from the dominant chemical constituents in the genus of Onchidium[3,4,6]. Previous research on the species *Onchidium sp*., a polypropionate compound with a pyrone ring main skeleton and several asymmetric centers like ilikonapyrone (**1**), is responsible for its biological activity. Ilikonapyrone is the first polypropionate secondary metabolite of this genus, followed by five ilikonapyrone-based derivatives (**2-6**), which were also isolated from the mixture of esters (**Figure 2**)[1,21].

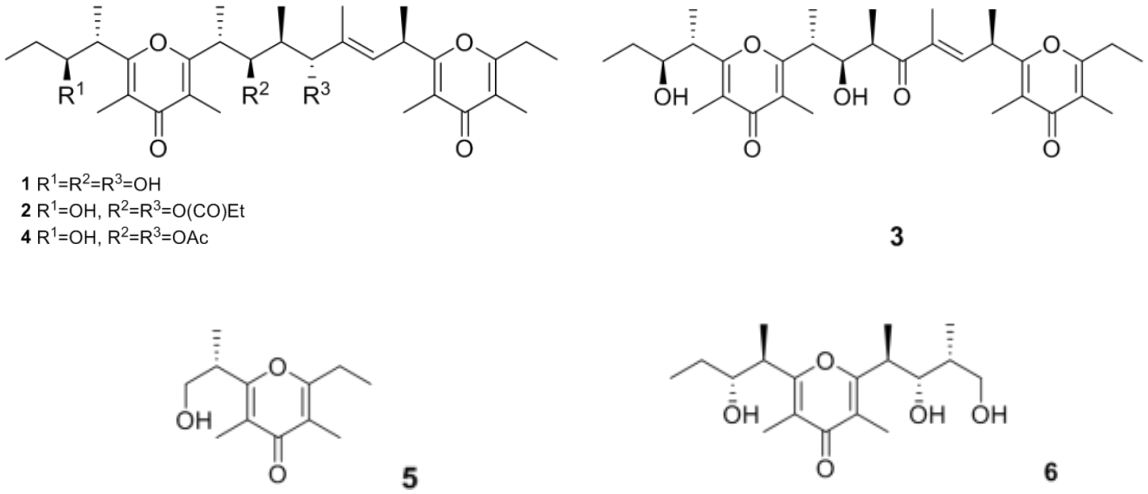

Figure 2. The structures polypropionate and its derivatives compounds[6]

Proximate analysis was carried out on *O. typhae* powder. Proximate analysis is carried out to determine the food's nutritional content, such as protein, carbohydrates, fat, and fiber. The results of the proximate analysis showed as shown in **Table II**. *Onchidium typhae* were rich in protein (67.88%) but a low number in fat (3.17%). The water, ash, carbohydrate, and crude fiber percentages were 4.37, 7.76, 14.55, and 0.65, respectively. Some literature explains that the high protein nutrient in food can be a supportive therapy in treating wounds, even postoperative wounds[22-24]. In other literature, steroid content such as squalene which is widely contained in Mollusca is known to have antioxidant activity[25].

Table II. Proximate analysis of *Onchidium typhae*

Test	Rate (%)
Water	4.37
Ash	7.76
Fat	3.17
Protein	67.88
Carbohydrate	14,55
Crude Fiber	0,65

Staphylococcus aureus, *E. coli*, and *C. albicans* were grown within 24 hours at 37°C in BHI media. Inhibition activity was carried out by observing the clarity of the solution—absorbance readings using a microplate reader at a wavelength of 595 nm. The antibacterial and antifungal activity assay results on 1% methanol, chloroform, and ethyl acetate extracts showed inhibitory activity against *S. aureus*, *E. coli*, and *C. albicans*. The most significant inhibition value was shown by 1% chloroform extract, where the inhibition value against *S. aureus*, *E. coli*, and *C. albicans* was 82±0.01%; 85.8±0.01%; 85±0.01%, respectively. The antibacterial and antifungal activity assay results are shown in **Table III**.

Table III. Antibacterial and antifungal result of *Onchidium typhae*

Crude extract	Percentage inhibitory (%)		
	S. aureus	*E. coli*	*C. albicans*
Chloroform 1%	82	85.5	84.9
Ethyl acetate 1%	77	85	86
Methanol 1%	73	67	74
Chloramphenicol 1%	85.2	86	-
Fluconazole 1%	-	-	85.3

Note: (-) the assay was not carried out

Recently, a new compound from the polyketides group known as penisclerotiorin A and penidepsidone A known to be responsible for antimicrobial activity (**Figure 3**)[26]. Onchidal isolated from another Onchidiid genus has shown the inhibition against *S. aureus*. The minimum inhibitory concentration was between 0.21 and 0.63 µg/mL, implying that *O. typhae* were as potent as antibacterial. These findings are in line with this study[27,28].

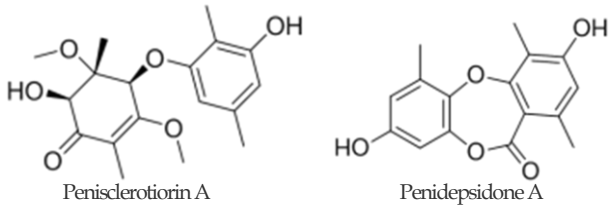

Figure 3. Structure of polyketides grup as antibacterial

CONCLUSION

Onchidium typhae extract has antibacterial and antifungal activity, especially in 1% chloroform extract against *S. aureus*, *E. coli*, and *C. albicans* with microdilution test.

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AUTHORS' CONTRIBUTION

Bambang Wijianto: research team leader and coordinator, validation, and article writing. **Hasyrul Hamzah**: antibacterial and antifungal assay, and article writing. **Annisa Larasati Nurhidayah**: sampling and phytochemical screening testing. **Guci Intan Kemuning**: extracting and determination of *O. typhae*. **Riyadh Aqilsya Amaryl Dyas**: extracting of *O. typhae*.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The author declares there is no conflict of interest and equivalent.

REFERENCES

1. Chen DL, Zheng W, Feng J, Liu YY, Wang XB. Muscle nutrition composition analysis of three Onchidium species from Hainan Island. Mar Sci. 2017; 41:104-9. doi:10.11759/hykx20170420001

2. Fisch KM, Hertzer C, Böhringer N, Wuisan ZA, Schillo D, Bara R, et al. The Potential of Indonesian Heterobranchs Found around Bunaken Island for the Production of Bioactive Compounds. Mar Drugs. 2017;15(12):384. doi:10.3390/md15120384

3. Shishui H, Xuqian W, Weiming T, Hongkai W, Yongxu C. Biochemical compositon and nutritional evaluation in the muscle of different size Onchidum struma. Oceanol Limnol Sin. 2012;43:43. doi:10.11693/hyhz201204012012

4. Rodriguez J, Riguera R, Debitus C. The natural polypropionate-derived esters of the mollusk Onchidium sp. J Org Chem. 1992; 57(17):4624-32. doi:10.1021/jo00043a018

5. Sun B, Chen C, Shen H, Zhang K, Zhou N, Qian J. Species diversity of Onchidiidae (Eupulmonata: Heterobranchia) on the mainland of China based on molecular data. Molluscan Res. 2014;34(1):62–70. doi:10.1080/13235818.2013.868860

6. Wang B, Chen D, Yu M, Liu Y, Liu P, Zhang X. A Review on Metabolites from Onchidium Genus: Chemistry and Bioactivity. Chem Biodivers. 2021;18(2):e2000580. doi:10.1002/cbdv.202000580

7. Khalifa SAM, Elias N, Farag MA, Chen L, Saeed A, Hegazy MEF, et al. Marine Natural Products: A Source of Novel Anticancer Drugs. Mar Drugs. 2019;17(9):491. doi:10.3390/md17090491

8. Chakraborty K, Joy M. High-value compounds from the molluscs of marine and estuarine ecosystems as prospective functional food ingredients: An overview. Food Res Int. 2020;137:109637. doi:10.1016/j.foodres.2020.109637

9. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. Pharmaceutics. 2020;12(8):735. doi:10.3390/pharmaceutics12080735

10. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. Physiol Rev. 2019;99(1):665-706. doi:10.1152/physrev.00067.2017

11. Gonzalez ACdO, Costa TF, Andrade ZdA, Medrado ARAP. Wound healing - A literature review. An Bras Dermatol. 2016;91(5):614-20. doi:10.1590/abd1806-4841.20164741

12. Iqbal A, Jan A, Wajid MA, Tariq S. Management of Chronic Non-healing Wounds by Hirudotherapy. World J Plast Surg. 2017;6(1):9-17.

13. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Adv Wound Care. 2015;4(9):560-82. doi:10.1089/wound.2015.0635

14. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, et al. Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. Antibiotics. 2021;10(10):1162. doi:10.3390/antibiotics10101162

15. Ekawati ER, Husnul SN, Herawati D. Identifikasi kuman Pada Pus dari Luka Infeksi Kulit. Jq SainHealth. 2018;2(1):31-5. doi:10.51804/jsh.v2i1.174.31-35

16. Hamzah H, Siregar KA, Nurwijayanto A, Wahyuningrum R, Sari S. Effectiveness of Oxalis corniculata L. Ethanol Extract against Mono-Species of Biofilm Staphylococcus aureus. Borneo J Pharm. 2021;4(3):184-91. doi:10.33084/bjop.v4i3.2418

17. Salam AM, Lyles JT, Quave CL. Methods in the Extraction and Chemical Analysis of Medicinal Plants. In: Albuquerque U, de Lucena R, Cruz da Cunha L, Alves R, editors. Methods and Techniques in Ethnobiology and Ethnoecology. Springer Protocols Handbooks. New York (US): Humana Press; 2019. p. 257-83. doi:10.1007/978-1-4939-8919-5_17

18. Helrich K, editor. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Ed. Virginia (US): Association of Official Analytical Chemist; 1990.

19. Wijianto B, Ritmaleni, Purnomo H, Nurrochmad A. Curcumin mono-carbonyl analogs as potent antibacterial compounds: synthesis, biological evaluation and docking simulation study. Rasayan J Chem. 2020; 13(2):1153–65. doi:10.31788/RJC.2020.1325554

20. Wijianto B, Ritmaleni R, Purnomo H, Nurrochmad A. In silico and in vitro assay of HGV analogue as antibacterial. Int J Pharm Pharm Sci. 2019;11(3):78–85. doi:10.22159/ijpps.2019v11i3.30581

21. Carbone M, Ciavatta ML, Wang JR, Cirillo I, Mathieu V, Kiss R, et al. Extending the record of bis-γ-pyrone polypropionates from marine pulmonate mollusks. J Nat Prod. 2013;76(11):2065-73. doi:10.1021/np400483c

22. Barchitta M, Maugeri A, Favara G, Lio RMS, Evola G, Agodi A et al. Nutrition and Wound Healing: An Overview Focusing on the Beneficial Effects of Curcumin. Int J Mol Sci. 2019;20(5):1119. doi:10.3390/ijms20051119

23. Darmawati D, Hasna C, Fitri A, Munira D. The Effectiveness of High Protein Nutrient to the Post Sectio Caesarea Healing Process. J Medica Vet. 2019;13(2):192-9. doi:10.21157/j.med.vet..v13i2.14090

24. Molnar JA, Underdown MJ, Clark WA. Nutrition and Chronic Wounds. Adv Wound Care. 2014;3(11):663-81. doi:10.1089/wound.2014.0530

25. Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 2009;14(1):540-54. doi:10.3390/molecules14010540

26. Zhao M, Ruan Q, Pan W, Tang Y, Zhao Z, Cui H. New polyketides and diterpenoid derivatives from the fungus Penicillium sclerotiorum GZU-XW03-2 and their anti-inflammatory activity. Fitoterapia. 2020;143:104561. doi:10.1016/j.fitote.2020.104561

27. Ciavatta ML, Lopez-Gresa MP, Gavagnin M, Nicoletti R, Manzo E, Mollo E, et al. Cytosporin-related compounds from the marine-derived fungus Eutypella scoparia. Tetrahedron. 2008;64(22):5365-9. doi:10.1016/j.tet.2008.03.016

28. Ireland C, Faulkner DJ. The defensive secretion of the opisthobranch mollusc Onchidella binneyi. Bioorg Chem. 1978;7(2):125-31. doi:10.1016/0045-2068(78)90043-3