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.

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. According to a traditional Chinese medicine book, consuming fresh meat can maintain and improve liver cirrhosis.

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. Based on the literature...
review, non-polar and polar active ingredients have promising pharmacological activities such as antioxidant activity. 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. 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. 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. They are non-motile, non-sporing, facultatively anaerobic, catalase-positive, and oxidase-negative. 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 Ilymen System 2100-C, Spain), spectrophotometer UV Genesys 10 UV Scanning, 335903 (Thermo Scientific Spectronic, US), autoclave, and analytical balance (AB204-5, Switzerland).

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\(^7\). 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 3 x 24 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\(^8\) 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*

<table>
<thead>
<tr>
<th>Compound class test</th>
<th>Reagent</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Wagner</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dragendorff</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids &amp; triterpenoids</td>
<td>Liebermann-Burchard</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Distilled water</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl(_2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Mg and Cl ribbon</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reduction sugar</td>
<td>Benedict</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Free amino acids</td>
<td>Ninhydrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
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. 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).

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. In other literature, steroid content such as squalene which is widely contained in Mollusca is known to have antioxidant activity.

<table>
<thead>
<tr>
<th>Test</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>4.37</td>
</tr>
<tr>
<td>Ash</td>
<td>7.76</td>
</tr>
<tr>
<td>Fat</td>
<td>3.17</td>
</tr>
<tr>
<td>Protein</td>
<td>67.88</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>14.55</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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%; and 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

<table>
<thead>
<tr>
<th>Crude extract</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform 1%</td>
<td>82</td>
<td>85.5</td>
<td>84.9</td>
</tr>
<tr>
<td>Ethyl acetate 1%</td>
<td>77</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>Methanol 1%</td>
<td>73</td>
<td>67</td>
<td>74</td>
</tr>
<tr>
<td>Chloramphenicol 1%</td>
<td>85.2</td>
<td>86</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole 1%</td>
<td>-</td>
<td>-</td>
<td>85.3</td>
</tr>
</tbody>
</table>

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)\textsuperscript{26}. Onchidal isolated from another Onchidiid genus has shown the inhibition against \textit{S. aureus}. The minimum inhibitory concentration was between 0.21 and 0.63 \(\mu\)g/mL, implying that \textit{O. typhae} were as potent as antibacterial. These findings are in line with this study\textsuperscript{27,28}.

![Figure 3. Structure of polyketides group as antibacterial](image)

CONCLUSION

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

ACKNOWLEDGMENT

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AUTHORS’ CONTRIBUTION

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

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

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


