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Original Article

Chemical Constituents of *Cymodocea rotundata* Asch. and Schweinf

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ABSTRACT

Introduction: *Cymodocea rotundata* Asch. and Schweinf, a widespread seagrass with reported antimicrobial activity, was investigated for its chemical constituents. **Methods:** The compounds were isolated by silica gel chromatography and identified by NMR spectroscopy. **Results:** This study has led to the isolation of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**1**), chlorophyll a (**2**) and a mixture of β-sitosterol (**3a**) and stigmasterol (**3b**) in about 1:1 ratio from the dichloromethane extract of *C. rotundata*. **Conclusion:** This is the first report on the isolation of **1**-**3b** from *C. rotundata*. Compounds **2**-**3b** were reported to exhibit antibacterial activity and may be partly responsible for the reported antimicrobial activity of the *C. rotundata* extract.

Key words: *Cymodocea rotundata,* Cymodoceaceae, β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters, Chlorophyll , β-sitosterol, Stigmasterol.

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INTRODUCTION

Cymodocea rotundata is a widespread seagrass that occurs in shallow water, on sand-mud in sheltered coves or bays, lagoons, mouth of rivers and coral reefs throughout the Philippines.[1] It has a wide Indo-Pacific distribution and is relatively common within its range.[2] *C. rotundata* extracts exhibited predominant growth inhibitory activity against UTI bacteria[3] and human pathogens.[4,5] Phytochemical analysis of *C. rotundata* extracts indicated the presence of tannins, saponins, resins, proteins, acidic compounds, reducing sugars, terpenoids, cardiac glycosides and alkaloids.[4] Another study reported that *C. rotundata* contained vitamin C (28.43 mg g^{-1}) and p-coumaric acid. *C. rotundata* extracts gave a total antioxidant activity of 6.65 mg ascorbic acid equivalent/g and was found to be a potent DPPH radical scavenger (70.30%).[6]

This study is part of our research on the chemical constituents of seagrasses found in the Philippines. We earlier reported the isolation of bis(2-ethylhexyl) phthalate, chlorophyll a, β-sitosterol and stigmasterol from *Cymodocea serrulata*.[7] We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters (**1**), chlorophyll a (**2**) and a mixture of β-sitosterol (**3a**) and stigmasterol (**3b**) from *C. rotundata*. To the best of our knowledge, this is the first report on the isolation of **1**-**3b** from *C. rotundata*.

MATERIALS AND METHODS

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

Samples of the leaves of *Cymodocea rotundata* Asch. and Schweinf. were collected from the seagrass meadow of Caramoan, Camarines Sur Philippines in September 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 6 inches in height and 0.25 inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents from the Leaves of C. rotundata

The air-dried *C. rotundata* (50 g) leaves were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.3 g) which was chromato-

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graphed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 10% EtOAc in petroleum ether to afford **2** (4 mg) after washing with petroleum ether, followed by Et_2O. The more polar fractions were combined and rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield a mixture of **3a** and **3b** (6 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **1** (3 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *C. rotundata* has led to the isolation of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**1**), chlorophyll a (**2**) and a mixture of β-sitosterol (**3a**) and stigmasterol (**3b**). The NMR data of **1** are in accordance with the data reported in the literature for β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters;[8] **2** for chlorophyll a;[9] **3a** for β-sitosterol;[10,11] and **3b** for stigmasterol.[10,11]

Although no biological activity tests were conducted on the isolated compounds, literature search revealed that **2-3b** exhibited antibacterial activities. Chlorophyll and its derivatives were reported to exhibit wound healing properties[12] and antibacterial activities.[13] β-Sitosterol (**3a**) and stigmasterol (**3b**) were also reported to possess antibacterial activities.[14,15,16,17]

CONCLUSION

This is the first report on the isolation of **1-3b** from *C. rotundata*. Compounds **2-3b** were reported to exhibit antibacterial activity and may be partly responsible for the reported antimicrobial property of the *C. rotundata* extract.

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ABBREVIATIONS

EtOAc: ethyl acetate; **Et2O:** diethyl ether.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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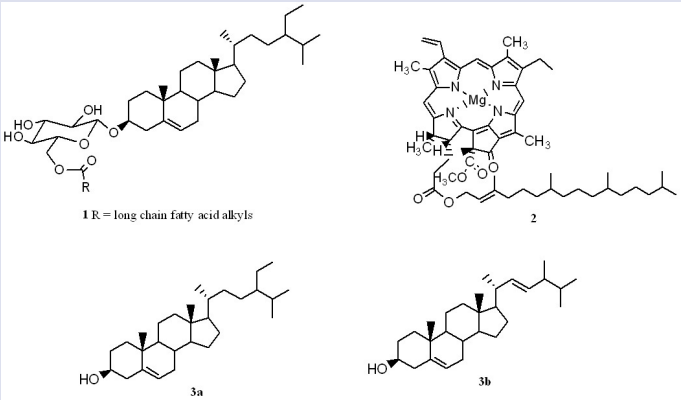
GRAPHICAL ABSTRACT



SUMMARY

- The dichloromethane extract of *C. rotundata* afforded β-sitosteryl-3β-glucopyranoside-6′-O-fatty acid esters **(1)**, chlorophyll a **(2)** and a mixture of β-sitosterol **(3a)** and stigmasterol **(3b)**. The structures of **1-3b** were identified by NMR spectroscopy. Compounds **2-3b** were reported to exhibit antibacterial activity which may be partly responsible for the reported antimicrobial property of the *C. rotundata* extract.

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