Macromitrium Brid. is a large genus of pantropical mosses consisting of xerophytic epiphytes. One member of this genus, *M. orthostichum* Nees ex Schwägr. is restricted in Southeast Asia up to Papua New Guinea was recorded in Luzon, Mindoro and Mindanao islands in the Philippines [1]. Like other members of *Macromitrium*, this species is distinguished from other mosses by having creeping prostrate stems that give rise to rigid branches that terminate into sporophytes [2]. The plant of *Macromitrium orthostichum*, as in most members of *Macromitrium*, have some shades of reddish brown with leaves variously twisted and contorted around the branches. *Macromitrium orthostichum* differs from other *Macromitrium* species by having papillose rhizoids arising along the basal leaf margins [3].

There is no report on the chemical constituents and biological activities of *Macromitrium orthostichum*. Herein, the isolation of zeorin (1), atranorin (2) and a mixture of β-sitosterol (3) and stigmasterol (4) (Fig. 1) from the dichloromethane extract of *Macromitrium orthostichum* is reported. To the best of our knowledge, this is the first report on the isolation of compounds 1-4 from *Macromitrium orthostichum*.

**Keywords:** *Macromitrium orthostichum*, Orthotrichaceae, Zeorin, Atranorin, β-Sitosterol, Stigmasterol.

**EXPERIMENTAL**

NMR spectra were recorded on a Varian VNMR5 spectro-meter in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C 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 F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

**Sample collection:** The Philippine specimen chosen for present investigation was gathered from a tree branch on a mossy forest at Mount Natib's Peak, Morong, Bataan, Philippines on 19th December 2017. The specimen was collected and authenti-cated as *Macromitrium orthostichum* Nees ex Schwägr. by one of the authors (VCL).

**General isolation procedure:** A glass column 12 inches in height with 0.5 inches in internal diameter was used for the fractionation of the crude extract. Fractions of 10 mL volumes were collected and monitored by thin layer chromatography. Fractions containing spots with similar Rf values were combined and rechromatographed using the appropriate solvent. Final purification was carried out using Pasteur pipette as the column, collecting 1 mL fractions. TLC-pure isolates were combined,
Isolation of chemical constituents of *Macromitrium orthostichum*: The freeze-dried *Macromitrium orthostichum* (17.81 g) was ground in a blender, soaked in CH$_2$Cl$_2$ for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.1723 g) which was chromatographed by gradient elution using increasing proportions of acetone in CH$_2$Cl$_2$ at 10% increment. The fraction eluted with 40% acetone in CH$_2$Cl$_2$ was rechromatographed using the same solvent to afford compound 1 (0.7 mg) after washing with petroleum ether. The fractions eluted with 15% ethyl acetate in petroleum ether were combined and rechromatographed using 10% increment. The fraction eluted with CH$_3$CN:Et$_2$O:CH$_2$Cl$_2$ (0.5:0.5:9, v/v) was rechromatographed using the same solvent to yield compound 2 (0.3 mg) after washing with petroleum ether.

** Zeorin (1): **$^1$H NMR (600 MHz, CDCl$_3$): δ 0.74 (3H, s, H-28), 0.85 (3H, s, H-25), 0.96 (3H, s, H-27), 1.00 (3H, s, H-26), 1.02 (3H, s, H-23), 1.13 (3H, s, H-24), 1.16 (3H, s, H-29), 1.19 (3H, s, H-30), 3.94 (1H, dt, J = 4.2, 10.8 Hz, H-6). $^{13}$C NMR (150 MHz, CDCl$_3$): δ 40.33 (C-1), 18.50 (C-2), 43.79 (C-3), 33.60 (C-4), 61.07 (C-5), 69.30 (C-6), 45.48 (C-7), 42.85 (C-8), 49.41 (C-9), 39.33 (C-10), 21.03 (C-11), 23.98 (C-12), 49.77 (C-13), 41.86 (C-14), 34.32 (C-15), 21.90 (C-16), 53.94 (C-17), 43.99 (C-18), 41.21 (C-19), 26.58 (C-20), 51.05 (C-21), 73.90 (C-22), 36.73 (C-23), 22.10 (C-24), 17.11 (C-25), 18.25 (C-26), 17.05 (C-27), 16.07 (C-28), 28.75 (C-29), 30.87 (C-30).

** Atranorin (2): **$^1$H NMR (600 MHz, CDCl$_3$): δ 0.68 (3H, s, H-5), 10.34 (1H, s, H-8), 2.67 (3H, s, H-9), 6.50 (1H, s, H-6′), 2.07 (3H, s, H-8′), 2.53 (3H, s, H-9′), 3.97 (3H, s, OCH$_3$) 12.48 (1H, s, 2-OH), 12.53 (1H, s, 4-OH), 11.92 (1H, s, 3′-OH).

** β-Sitosterol (3): **$^1$H NMR (600 MHz, CDCl$_3$): δ 3.50 (1H, m, H-3), 5.35 (1H, d, J = 4.8 Hz, H-5), 0.66 (3H, s, H-18), 0.99 (3H, s, H-19), 0.93 (3H, d, J = 6.6 Hz, H-21), 0.84 (3H, d, J = 6.6 Hz, H-26), 0.83 (3H, d, J = 6.0 Hz, H-27), 0.86 (3H, t, J = 6.0 Hz, H-29).

** Stigmasterol (4): **$^1$H NMR (600 MHz, CDCl$_3$): δ 3.50 (1H, m, H-3), 5.33 (1H, d, J = 4.8 Hz, H-5), 0.68 (3H, s, H-18), 0.99 (3H, s, H-19), 1.01 (3H, d, J = 6.6 Hz, H-21), 5.13 (1H, dd, J = 8.4, 15.6 Hz, H-22), 5.00 (1H, dd, J = 8.4, 15.0, H-23), 0.84 (3H, d, J = 6.6 Hz, H-26), 0.83 (3H, d, J = 6.0 Hz, H-27), 0.80 (3H, t, J = 6.0 Hz, H-29).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Macromitrium orthostichum* afforded zeorin (1), atranorin (2) and a mixture of β-sitosterol (3) and stigmasterol (4) in about 1:1 ratio. The structure of compound 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data with those reported in the literature [4]. The NMR data of compound 2 were in accordance with those reported in the literature for atranorin [4]; compound 3 for β-sitosterol [5] and compound 4 for stigmasterol [5]. The 1:1 ratio of compounds 3 and 4 was deduced from the intensities and integrations of the $^1$H NMR resonances for olefinic protons at δ 5.35 (d, J = 4.8 Hz, H-5) and methyl protons at δ 0.66 (s) for compound 3 and olefinic protons at δ 5.35 (d, J = 4.8 Hz, H-5) and methyl protons at δ 0.68 (s) for compound 4 [5].

Although there is no reported biological activity for *M. orthostichum*, the compounds isolated from the plant were reported to possess diverse activities. Zeorin (1) and atranorin (2) exhibited anti-diabetic and antioxidant activities [6]. Compound 1 exhibited strong antibacterial and antifungal activities [7]. On the other hand, compound 2 showed antiproliferative action against malignant cell lines [8], possessed antinociceptive

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![Fig. 1. Chemical structures of zeorin (1), atranorin (2), β-sitosterol (3) and stigmasterol (4) from the dichloromethane extract of *M. orthostichum*.](image-url)
effects [9,10], exhibited antibiotic action against M. aurum [11], inhibited leukotriene B4 synthesis in leukocytes [12], and modulated the wound healing process [13]. β-Sitosterol (3) inhibited the growth of human breast MCF-7 and MDA-MB231 adenocarcinoma cells [14]; was effective for the treatment of benign prostatic hyperplasia [15]; showed potential regarding the publication of this article. A research grant from the De La Salle University Science Foundation through the URCO is gratefully acknowledged.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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