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Beta-sitosterol glycoside from Paraboea leuserensis and cytotoxicity test against MCF-7 human breast cancer cells

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Abstract

Background: *Paraboea leuserensis*, a plant endemic to the Leuser Mountain in the provinces of Aceh and North Sumatra, has been traditionally used for medicinal purposes by chewing or boiling, addressing conditions such as stomachaches and providing stamina enhancement. This study aimed to isolate secondary metabolite compounds from *Paraboea leuserensis* and conduct in vitro anticancer tests.
Method: The ethyl acetate extract was isolated through column chromatography, yielding beta-sitosterol glycoside, and was tested for bioactivity against human breast cancer cells MCF-7 using the MTT method.
Results: The compound demonstrated activity against breast cancer cells (MCF-7) with an IC_{50} value of 24.83 mg/L.
Conclusion: *Paraboea leuserensis* exhibits potential anticancer activity. The isolation of beta-sitosterol glycoside from the plant and its demonstrated activity against MCF-7 human breast cancer cells suggest a promising avenue for further exploration of the plant's anticancer properties.

Keywords: *Paraboea lauserensis*, beta-sitosterol glycoside, anticancer, MCF-7.

INTRODUCTION

Cancer is a significant health concern in Indonesia and is the second leading cause of death, following cardiovascular disorders. Indonesia has the greatest incidence of breast cancer cases, making it the most prevalent form of cancer in the country. Additionally, breast cancer ranks among the primary causes of cancer-related fatalities. Based on the 2020 Globocan data, there were 68,858 newly diagnosed instances of breast cancer in Indonesia, accounting for 16.6% of the total 396,914 new cancer cases. Concurrently, the mortality rate from breast cancer surpassed 22,000 cases.

Herbal medicine is one of the complementary and alternative treatments in healthcare outside of conventional medicine (1). Herbal medicine involves utilizing various parts of plants, such as seeds, barks, roots, leaves, or flowers, for their therapeutic properties (2). The most common reasons for using traditional medicine are its affordability, alignment with patients' preferences to avoid concerns about the side effects of synthetic drugs, the desire for personal healthcare, and enabling greater access to public health information (3).

Beta-sitosterol glycoside (daucosterol) was previously reported to be present in the plant *Pabarobea glutinous* (4). The compound beta-sitosterol glycoside is a saponin found in various medicinal plants. Beta-sitosterol glycoside exhibits known anticancer properties by affecting various signaling pathways. These include promoting the expression of pro-apoptotic proteins Bax and Bcl2, reducing the Bcl-2/Bax ratio, inhibiting the PI3K/Akt pathway, enhancing the regulation of the phosphatase and tensin homolog (PTEN) gene, and interfering with tumor cell development and cell cycle progression (5).

Paraboea is a member of the family Gesneriaceae, which is believed to consist of approximately 3,500 species among 147-150 genera. These species are primarily found in tropical and subtropical locations, however there are a few exceptions in temperate conditions (6). *Paraboea leuserensis* is an

endemic plant of Mount Leuser, situated in two provinces, Aceh and North Sumatra, covering the regencies of Dairi, Karo, and Langkat. Locally, *Paraboea leuserensis* is known by the Karo people as "Gagatan Harimau." This shrub has been utilized as a medicinal plant, traditionally chewed for treating conditions such as stomach ailments and as a stamina booster (7,8). Scientific studies on the plant's secondary metabolites with potential anticancer properties have not been reported. The aim of this study is to discover and separate secondary metabolite molecules from plants that possess the capability to impede the proliferation of breast cancer cells, particularly MCF-7 cells.

METHOD

Materials and Instrumentals

Rotary evaporator Heidolph WB 2000, 96-well plate, 37°C incubator with 5% CO_2, laminar air flow safety cabinet, and ELISA reader were used in the characterization process. Equipment employed for characterization includes FTIR spectrophotometer and NMR. Materials used include *Paraboe leuserensis*, ethyl acetate, silica gel 60 (70-230 Mesh Merck), TLC plates (silica gel 60 F254 Merck), MCF-7 human cancer cells, fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), Dulbecco's Modified Eagle Medium (DMEM), and reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical, St. Louis, MO).

Isolation

This Vacuum column chromatography was the method used to separate secondary metabolite chemicals from the ethyl acetate extract of *Paraboea leuserensis*, with silica gel serving as the stationary phase. The process of isolating components from the ethyl acetate extract (50g) was carried out utilizing the vacuum liquid chromatography (VLC) technique. From the VLC results, with a mobile phase of hexane-ethyl acetate ranging from 100:0 to 0:100 and continued with the ratio of ethyl acetate-methanol up to 0:100, fractions with similar spot patterns were combined. The combined fractions were then evaporated, resulting in 8 combined fractions. The 7th fraction was further isolated using column chromatography, and the separation was achieved using eluent ethyl acetate-methanol 2:8, showing a single spot pattern on TLC plate with hexane-ethyl acetate 1:9 as the eluent. The process of column chromatography was employed to achieve more separation until the isolated chemical was produced as a white amorphous powder weighing 20 mg. Subsequently, the product was subjected to characterization utilizing FTIR, 1H NMR, and 13C NMR techniques.

Cytotoxicity Test Against MCF-7 Breast Cancer Cells Using MTT

The cytotoxicity test against breast cancer cells begins with the preparation of the culture medium inside a laminar air flow safety cabinet. The medium used is Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) as nutrition and 1% penicillin-streptomycin as antibacterial and antifungal agents, and then stored in a refrigerator at 4°C until use.

MCF-7 human breast cancer cells, pre-seeded in a 96-well plate at a density of $2x10^4$ cells/well, are incubated for 24 hours at 37°C in a 5% CO2 incubator, observed under a microscope, and then the medium is removed and washed with PBS. Subsequently, 100 µL of DMEM containing 20,000 cells is added to each test well and positive control (medium and cells) wells.

The test solution, which has been dissolved in DMSO, is applied to each well at doses of 100, 50, 25, 12.5, and 6.25 µg/mL, with four replicates. The cells are subsequently placed in an incubator for a duration of 48 hours. Following the incubation period, the medium is extracted and 100 µL of a solution containing 0.5 mg/mL of MTT is introduced into each well. This is followed by a 4-hour incubation at a temperature of 37°C. The MTT solution is thereafter discarded, and the resulting formazan crystals are dissolved utilizing DMSO. The measurement of absorbance is conducted using an ELISA reader at a wavelength of 595 nm. The

intensity of the produced formazan is directly linked to cell viability.

The acquired absorbance data is subsequently transformed into the percentage of cell viability utilizing the formula:

$$\% = \frac{(\text{Sample absorbance}) - (\text{negative control absorbance})}{(\text{Positive control absorbance}) - (\text{Negative control absorbance})} \times 100\%$$

Explanation:
Sample absorbance: test sample + cells + medium
Negative control absorbance: medium
Positive control absorbance: medium + cells

The variation of the logarithm of the concentration of the test sample with the percentage of cell viability is presented in the form of a graph, and the IC_{50} value is determined using the GraphPad software.

RESULTS

FTIR

The isolated compound obtained was further analyzed with FTIR. The infrared spectrum of the isolated compound shows the presence of functional groups contained within the compound.

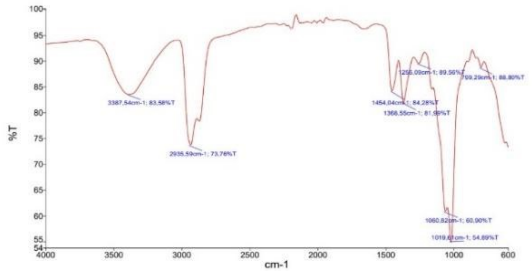

Figure 1. The FTIR spectrum of beta-sitosterol glycoside

In Figure 1, there are several IR absorption regions in the spectrum of the isolated compound. The broad absorption band at 3387.54 cm^{-1} indicates the stretching vibration of O-H groups in the range of 3800-2700 cm^{-1}. The absorption band at 2935.59 cm^{-1} corresponds to the stretching vibration of C-H alkane (3000-2850 cm^{-1}) (Pavia et al., 2014), reinforced by the presence of methylene groups with the bending vibration of $–CH_2–$ at 1454.04 cm^{-1}. The geminal dimethyl group $–CH(CH_3)_2$ at 1368.55 cm^{-1} and 1256 cm^{-1} is characteristic of triterpenoid/steroid compounds. Sharp bands at 1060 and 1019 cm^{-1} are attributed to the ether linkage (C-O-C) present in the formed glycoside. The absorption band at 799 cm^{-1} is associated with the –C=C– group. Based on the interpretation of the FTIR spectrum data and the presence of various functional groups, the isolated compound is tentatively identified as belonging to the glycosidic steroid group (9,10).

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NMR

Further analysis was conducted using Nuclear Magnetic Resonance (NMR) spectroscopy to determine the number of carbon (C) and hydrogen (H) atoms using 13C NMR and 1H NMR, allowing for the prediction of the structure of the isolated compound. NMR data analysis was performed using MestReNova 12.0 software. The 1H NMR spectrum (700 MHz, DMSO) of the isolated compound showed similarity to the spectrum of beta-sitosterol glycoside. The 1H NMR spectrum of the isolation results indicated a signal at δ 5.33, identifying the presence of an olefin proton at position H-6 in the structure. Shifts at δ 2.9-4.14 can characterize glucose protons at positions 2'-6'-H. The doublet peak at δ 4.22 represents protons from the glycoside group –CH. Chemical shifts from 0.65 to 0.96 suggest the presence of methyl protons (-CH3: H-18, H-19, H-21, H-26, H-27, and H-29). The single peak at δ 0.65 indicates the methyl proton at H-18, and a doublet peak is observed at δ 0.90 for the H-21 proton.

The 13C-NMR spectrum (176 MHz, DMSO) of the isolated compound indicated 35 carbon signals. Six of them are carbon atoms from the sugar group, and 29 carbons belong to the aglycone group. Carbon signals from the sugar group were observed at shifts of 100.81 (C-1'), 73.52 (C-2'), 76.80 (C-3'), 70.16 (C-4'), 76.77 (C-5'), and 61.15 (C-6'). Signals from the aglycone group were observed at chemical shifts of 36.87 (C-1), 29.31 (C-2), 77.00 (C-3), 38.35 (C-4), 140.51

(C-5), 121.27 (C-6), 31.48 (C-7), 31.43 (C-8), 50.63 (C-9), 36.26 (C-10), 20.64 (C-11), 40.00 (C-12), 41.91 (C-13), 56.23 (C-14), 23.91 (C-15), 28.76 (C-16), 55.47 (C-17), 11.83 (C-18), 19.15 (C-19), 35.52 (C-20), 18.99 (C-21), 33.39 (C-22), 25.48 (C-23), 45.19 (C-24), 28.41 (C-25), 19.77 (C-26), 18.67 (C-27), 22.66 (C-28), and 11.72 (C-29) ppm. The chemical shifts of 140.51 and 121.27 indicate the presence of two carbon atoms in the diene group. Signals observed in the downfield region include 6 methyl (CH_3) carbons at δ 11.72, 11.83, 18.67, 18.99, 19.15, and 19.77.

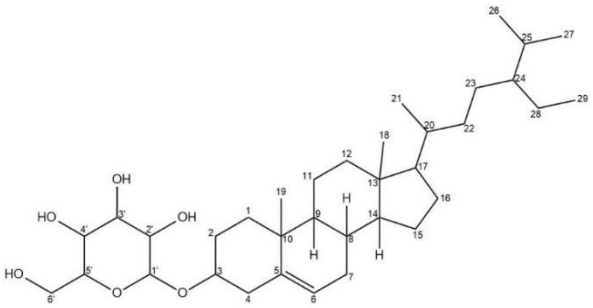

Figure 2. The structure of beta-sitosterol glycoside

Based on the obtained data, it is predicted that the isolated compound is beta-sitosterol glycoside (Figure 2), as indicated by chemical shifts compared with previous literature (11,12).

Potential Cytotoxicity Against MCF-7 Human Breast Cancer Cells

The results of cytotoxicity testing of hexane, ethyl acetate, methanol extracts, and beta-sitosterol glycoside compound from *P. leuserensis* using the MTT method against MCF-7 human breast cancer cells. MTT(3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) is a tetrazolium salt commonly used to determine cell viability by measuring the reduction of the salt into formazan crystals (13). The conversion of MTT to formazan is facilitated by the mitochondrial enzyme succinate dehydrogenase, which is found in cells that are actively involved in metabolism. The formazan crystals that are produced are not soluble and build up within living cells, enabling their measurement (14). The percentage viability values provide information about the cells that survive after exposure to the test samples. The higher the % viability, the more cells are alive (15).

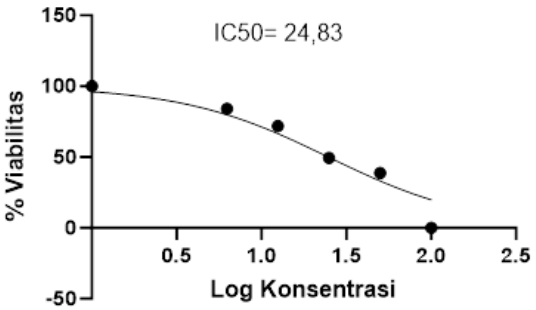

Figure 3. Relationship between the log concentration of the test sample and % cell viability

The cytotoxicity assay conducted on the beta-sitosterol glycoside molecule derived from *P.leuserensis* exhibited a dose-dependent reduction in the viability of breast cancer cells. Figure 3 shows a decrease in the number of live cells as the concentration increases. The beta-sitosterol glycoside compound from Paraboea leuserensis against MCF-7 human breast cells exhibits strong cytotoxicity properties with an IC_{50} value (half-maximal inhibitory concentration) less than 50 µg/mL, specifically with an IC_{50} of 24.83 mg/L for the beta-sitosterol glycoside compound.

CONCLUSIONS

Based on the structural elucidation of the isolated compound from the ethyl acetate extract through spectroscopic analysis and literature comparison, the isolated compound is identified as beta-sitosterol glycoside. It exhibits cytotoxic activity against MCF-7 human breast cancer cells with an IC_{50} of 24.83 mg/L.

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REFERENCES

1. Delima, Widowati L, Siswoyo H, Nurhayati, Sampurno OD, Halim FS. The Pattern of Herbal Medicine Prescribed by Medical Doctor for 10 Health Problems in Several Cities of Indonesia (Analysis of Jamu Registry 2016 and 2018 Database). In: Proceedings of the 4th International Symposium on Health Research (ISHR 2019). Paris, France: Atlantis Press; 2020.
2. Maqbool M, Amin Dar M, Gani I, Mir SA. Herbal Medicines As An Alternative Source Of Therapp: A Review. Article in World Journal of Pharmacy and Pharmaceutical Sciences [Internet]. 2019;8(2):374–80.
3. Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton: CRC Press/Taylor & Francis; 2011.
4. Wang XQ, Peng Y, Peng B, Xiao PG, Liu Y. Chemical Constituents of Paraboea glutinosa. Chem Nat Compd. 2014 Nov 30;50(5):952–4.
5. Omari N El, Jaouadi I, Lahyaoui M, Benali T, Taha D, Bakrim S, et al. Natural Sources, Pharmacological Properties, and Health Benefits of Daucosterol: Versatility of Actions. Vol. 12, Applied Sciences (Switzerland). MDPI; 2022.
6. Puglisi C, Yao TL, Milne R, Möller M, Middleton DJ. Generic recircumscription in the Loxocarpinae (Gesneriaceae), as inferred by phylogenetic and morphological data. Taxon. 2016 May 3;65(2):277–92.
7. Ginting KB, Purwoko A, Simanjuntak J. Kearifan Lokal dalam Pengelolaan Hutan Di Desa Serdang Kecamatan Barusjahe, Kabupaten Karo. Peronema Forestry Science Journal. 2015;4(4):186–99.
8. Purba EC, Nisyawati, Silalahi M. The ethnomedicine of the Batak Karo people of Merdeka sub-district, North Sumatra, Indonesia. International Journal of Biological Research. 2016 Aug 29;4(2):181.
9. López-Salazar H, Camacho-Díaz BH, Ávila-Reyes SV, Pérez-García MD, González-Cortazar M, Arenas Ocampo ML, et al. Identification and quantification of β-Sitosterol β-d-Glucoside of an ethanolic extract obtained by microwave-assisted extraction from Agave angustifolia Haw. Molecules. 2019;24(21).
10. Katja DG, Harneti D, Mayanti T, Nurlelasari, Maharani R, Shiono Y, et al. Cytotoxic Steroids From The Stembak of Chisocheton celebicus KOORD. Jurnal Kimia Valensi. 2019 Nov 1;5(2):143–8.
11. Madeleine DT, Noël NJ, Theodore AA de, Abdoulaye H, Emmanuel T, Laurent S, et al. Antioxidant Activity and Chemical Constituents from Stem Bark of Ficus abutilifolia. Miq (Moraceae). European J Med Plants. 2020 Aug 31;48–59.
12. Peshin T, Kar H. Isolation and Characterization of β-Sitosterol-3-O-β-D-glucoside from the Extract of the Flowers of Viola odorata. Br J Pharm Res. 2017 Jan 10;16(4):1–8.
13. Stockert JC, Horobin RW, Colombo LL, Blázquez-Castro A. Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Vol. 120, Acta Histochemica. Elsevier GmbH; 2018. p. 159–67.
14. Momeni RH, Hussein Abnosi M, Eskandari N. Quantitative evaluation of human sperm viability using MTT assay: A laboratory study. International Journal of Reproductive BioMedicine (IJRM). 2020 Nov 22;
15. Ulia RV, Suryati, Santoni A. Cytotoxic Potential of Essential Oil Isolated from Semambu (Clibadium surinamese L) Leaves Against T47D Breast and HeLa Cervical Cancer Cells. Molekul. 2023 Jul 10;18(2):289–99.