PHYTOCHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY FROM *Scoparia dulcis* LINN OF INDONESIA ORIGIN

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ABSTRACT

*Scoparia dulcis* Linn is a widely distributed perennial in tropical and subtropical regions, including Indonesia. Its pharmacological properties include cytotoxic, antiproliferative, antitumor, antiviral, anticancer, antibacterial, antifungal, antidiabetic, anti-inflammatory, and antioxidant activities. Therefore, the purpose of this research is to determine the cytotoxic effects of phytochemical constituents of *Scoparia dulcis* Linn grown in Indonesia. In the process, 6-methoxy benzoxazole-2-(3H)-one was separated from soluble fraction ethyl acetate (FEA) of *Scoparia dulcis* ethanolic extract. Furthermore, extensive NMR studies were used to determine the compound structure. The cytotoxic activities of these compounds against MCF-7 and T47D breast cancer cell lines were also tested. The results showed that 6-methoxy benzoxazole-2-(3H)-one had no significant effect on this cancer cell. It was concluded that this compound could be developed to be another biological activity test; hence, it is used as a lead compound.

Keywords: Cytotoxic, *Scoparia dulcis*, MCF-7, T47D, Phytochemical Constituents

INTRODUCTION

*Scoparia dulcis* Linn (fam. Scrophulariaceae) grows in subtropical and tropical regions, including Indonesia, one of the megadiverse countries rich in biodiversity. A source of infinitely numerous biomolecules of organic compounds known as biodiversity occurs due to the Indonesian archipelago's geographical location between two continents and two oceans, with a climate characterized by two distinct conditions, namely high rainfall, and year-round heat. This condition stimulates the production of secondary metabolite compounds. *S. dulcis* Linn has traditionally been used to treat jaundice, stomach ache, blennorhagia, fever, cancer, wounds, skin rash, and tuberculosis.\(^1\) Otherwise, it is used in India and Taiwan to treat DM (diabetes mellitus) and hypertension, respectively.\(^2\) Several groups of compounds were isolated from *S. dulcis* Linn and showed various biological activity, including diterpenes, triterpenes, flavonoids, and benzoxazinoids. However, not all active components of secondary metabolites from plants can be used as therapeutic agents, because of their non-optimal activity, lack of specificity, and absence of biologically functional groups.\(^3\) Furthermore, the isolation, structural determination, and cytotoxic activity of these compounds were reported in this study. The thick extracts, fractions, and active compounds were tested on two breast cancer cells, MCF-7, and T47D. Although an extensive NMR investigation entirely determined the pure compound structure and has been examined for cytotoxicity, the structure of this compound can be developed for further biological activity tests, allowing it to be used as a lead compound.

EXPERIMENTAL

The above ground parts of *S. dulcis* Linn were collected in June 2020 in Kalasan, Yogyakarta, Indonesia. The plant specimen was identified in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.
General Procedure
The UV spectrophotometer, FTIR spectrophotometer, and JEOL Resonance Delta 2 JNM_ECS 400 nuclear magnetic resonance (NMR) spectrometer were used. Additionally, 400 and 100 MHz (1H and 13C), COSY, DEPT, HSQC, and HMBC spectra, were measured by Bruker JNM-ECS 400 instruments. Chromatography was performed using Kieselgel silica gel 60 GF254 (Merk, Darmstadt, Germany).

Detection Method
Extraction and Separation
The plant's dry aboveground portions (6,000g) were soaked in 96% ethanol (EtOH, 3x7.5L, 24 h each) at room temperature, and the dry ethanol extract was colored gum green bold (339.73g). Subsequently, the extract was triturated with n-hexane to yield soluble fractions of n-hexane (FNH) (69.75g) and ethyl acetate, the soluble fraction of ethyl acetate (FEA) (23.52g), and an insoluble fraction of n-hexane and ethyl acetate (Precipitate) (124,21). Vacuum liquid chromatography was used to fractionate the dissolved ethyl acetate using n-hexane-ethyl acetate (from 100%; 97:3; 90:10 to 0:100%) and MeOH with increasing polarity. Each 100 mL was collected, and up to 18 fractions were obtained in total. The first 7 fractions also known as (FEA.7) were obtained by crystallization. Afterward, it was recrystallized, and the preparative TLC (n-hexane: ethyl acetate 2:1) yielded yellowish-white powder crystals, such as 6-methoxy benzoazazole-2-(3H)-one (23mg).

Cytotoxicity Assay
The cytotoxicity of the extracted ethanol, FEA, FNH, and 6 methoxy benzoxazole-2-(3H)-one was tested using, MCF-7 and T47D breast cancer cells. The MTT assay procedures described by 4 were used to calculate the percentage (%) of cell viability. Furthermore, the dilution series of 100, 50, 25, 12.5, 6.25 µg/mL of the pure compound, FEA, FNH, ethanolic extract, and control were tested against MCF-7 and T47D cell lines. Three replicate analyses were performed for every concentration, and the percentage (%) of cell viability was estimated for each of the concentrations. Doxorubicin was used as a positive control, and cancer cells were cultured for confluent growth using RPMI-1640 and DMEEM complete medium. The cell lines were grown to confluence in an incubator by being treated with 5% CO2 in a humidified atmosphere. At 37°C for 2 minutes, the cell was treated with 500µL of 0.025% Trypsin in PBS/0.5mM and transferred to EDTA solution to T flasks under aseptic conditions. Afterward, a stock solution of the pure compound, FEA, FNH, and the ethanolic extract was produced at a concentration of 100,000µg/mL in PBS. After 24 hours, samples were added to the grown cell and incubated. Finally, the percentage (%) of cell viability and IC50 values were calculated using linear regression and the excel program (Microsoft Inc, USA).

RESULTS AND DISCUSSION
The cytotoxic activity of ethanol extract from aerial parts of S. dulcis Linn, soluble fraction of n-hexane (FNH), soluble fraction of ethyl acetate (FEA), and an insoluble fraction of n-hexane and ethyl acetate (Precipitate) are shown in Table-1. The National Cancer Institute (NCI), the extract has strong anticancer activity if IC50 < 30, moderate 30 < IC50 < 100, and inactive IC50 > 100µg/mL. Thus, ethanol extract of S. dulcis is not potent as an anticancer. 5

Table-1: Cytotoxic Activity against MCF-7 and T47D Cell Lines

| Sample                  | IC50 (µg/mL) |
|-------------------------|--------------|
|                         | MCF-7        | T47D         |
| Ethanol extract         | 2,261.54±0.17| 693.5±0.03   |
| FNH                     | 248.69±0.07  | 128.8±0.02   |
| FEA                     | 725.83±0.06  | 114.6±0.03   |
| Precipitate/insoluble   | 1,700±0.05   | 1,031.8±0.03 |
| 6-methoxy benzoazazole-2-(3H)-one | 24,265±0.06 | 2,242.6±0.06 |

IC50: Half maximal inhibitory concentration, Inhibitory concentration that causes 50% cell death to breast cancer MCF-7 and T47D cell line, with value mean IC50±SD.

After purification, the FEA fraction was isolated and 6-methoxy benzoazazole-2-(3H)-one was obtained. The structure of this compound is known using a variety of spectroscopic investigations, including UV,
GC-MS (gas chromatography-mass spectroscopy), IR, 1D, and 2D NMR. $C_{8}H_{7}NO_{3}$ was represented using ESI-MS data and 1H, 13C NMR, and DEPT spectrum, as well as COSY, HMBC, and HMQC. The ESI-MS data show a fragment with a mass of 165 m/z (M+), whereas the UV spectroscopic data indicate a wavelength of 292 nm. On fragment IR spectroscopy, a carbonyl group is detected at 1.621,52 cm$^{-1}$, CH-stretching = 2,367.64 cm$^{-1}$, and NH = 3,618.29 cm$^{-1}$. According to 1H spectroscopic data for $C_{8}H_{7}NO_{3}$, this compound (Table 2) shows that the DEPT-135 and $^{13}$C NMR spectra exhibited three methine resonance at C-4, C-5, and C-7, one methyl at C-10 was obtained from methoxy. This compound was classified as a Benzoxazolinone, a benzoxazinoid compound discovered in Poaceae, Acanthaceae, Ranunculaceae, and Scrophulariaceae. Benzoxazinoids have been isolated from natural sources$^{6}$, $^{7}$, $^{8}$, $^{9}$. According to to$^{10}$, the 6-methoxy benzoxazole-2-(3H)-one compound (figure.1) in the NH group does not bind to the OH molecule. However, several benzoxazinoids compounds in the N group, such as 2,4 dihydroxy-1,4-benzoxazine-3-one, bind to the OH group and thus significantly increase cytotoxic and antiproliferative effects. This is congruent with the study conducted by$^{11}$, which states that 2,4 dihydroxy-1,4-benzoxazine-3-one compounds have cytotoxic effects against HepG2 liver cancer cells at a concentration of 10-100 µg/mL and human prostate cancer cells DU-145 at a concentration of 100 µg/mL$^{10}$. When this compound is bound to O-glycosylated, its inhibitory effect against cancer cells in vitro is lost$^{10}$. According to to$^{12}$ 7-methoxy-2H-1,4-benzoxazine-3(2H)-one 2-O-hexopyranoside compound did not exhibit cancer cell activity in vitro. Additionally, the results of this study showed that 6-methoxy benzoxazole-2-(3H)-one compound (figure.1) lacked cytotoxic action against the MCF-7 and T47D cell line at a concentration ranging from 6.25µg/mL to 200µg/mL.

**CONCLUSION**

The 6-methoxybenzoxazole-2-(3H)-one shows no inhibitory activity against breast cancer line cells MCF-7 and T47D in vitro. Therefore, the presence of the N-OH group in increasing cytotoxic and antiproliferative activity is essential.

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