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Isolation, Identification and Biological Activities of B-Sitosterol from *Hedyotis Corymbosa*

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Abstrack :Purification of methanol extract from Hedyotiscorymbose resulted in the isolation of one compound namely β-sitosterol. Chemical structures were established mainly by using ^{1}H and ^{13}C NMR data and by comparing current NMR data with those reported in the literature. This compound is known and has been isolated from other plants species but is being reported from this plant species for the first time. The anticancer effect of this compound on MCF-7 and MDA-MB-231 breast cancer cells also have been studied and showed that this compound has the potential effect to inhibit breast cancer cells.

Keyword :Purification of methanol extract from Hedyotiscorymbose resulted in the isolation of one compound

INTRODUCTION

Pearl grass (Hedyotiscorymbosa (L.) Lam) from the family Rubiaceae has been reported to have some properties traditionally as an anti-inflammatory, anticancer, and several other diseases (Dalimunthe,2005). Scientific studies on pearl grass show that this plant contains several compounds such as geniposidic acid, oleanolic acid, geniposida, and ursulat acid (Permadi, 2005). These compounds have been scientifically tested and proven to be oleanolic acid and ursulat acid can inhibit cancer growth and repair injuries due to radiation, whereas genoposide and genoposide acids have the potential as antitumor ingredients and have radioprotective activity (Lin et al., 2004).

Preliminary research on antioxidant and anticancer activities from pearl grass extracts has been carried out and from the results of this study it is known that pearl grass extracts contain high antioxidants and can kill breast cancer cells (Susi Endrini, 2011). In vivo studies have also been carried out to determine the anti-inflammatory activity of pearl grass extracts and the results are also very promising (data not yet published).

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In this study, pearl grass has been isolated, purified and their biological activities on breast cancer cells have been studied.

MATERIAL AND METHODS

Preparation of extracts

The dried powdered leaves (1kg) were soaked in methanol for 72 hours using the maceration technique. It was then collectively concentrated in a rotary evaporator under reduced pressure, at less than 40 °C and kept in a desiccator at room temperature. The dry methanol extract was obtained (20.5 gr).

Fractionation of crude extracts and isolation of active constituents

Fractionation of plant extracts was carried out using repetitive positive pressure column chromatography (PPCC) over silica gel GF254 (particle size 5–40 m) (Merck) eluting with solvents of increasing polarity namely n-hexane, ethyl acetate, and methanol. Samples were applied either as solutions or as dry powders (sample dissolved in solvent was mixed with silica and the solvent evaporated to leave a dry powder). The fractions collected were monitored using pre-coated silica (silica gel GF254) TLC plates (Merck) and similar fractions were combined and concentrated in vacuo (Newton et al., 2002).

The isolation procedure in this study uses the guided bioactivity isolation method. The stages of isolation are divided into two namely fractionation and purification. In the fractionation step, methanol extract is fractionated using vacuum liquid chromatography (KCV) using silica gel stationary phase and mobile phase combination of n-hexane and ethyl acetate solvent. In this fractionation a mixture of n-hexane, ethyl acetate is used in various ratios, which is 8: 2 (1 time); 7: 3 (1 time); 6: 4 (1 time); 1: 1 (2 times); 6.5: 3.5 (2 times); 8: 2 (1 time); (EtOAc) 100% (1 time); and (MeOH) 100% (1 time) produces 10 fractions namely H1 (4.52 g), H2 (3.13 g), H3 (2.43 g), H4 (1.09g), H5 (3.6 g), H6 (1.43 gr), H7 (0.47 g), H8 (2.79 gr), H9 (3.71 gr), and H10 (2.21 gr). The fractionation results are evaporated and then monitored by TLC to find out the results of the separation. Fractions that have the same spot can be combined. The fractions obtained from KCV were tested cytotoxically against MCF-7 cells andMDA_MB-231 cell lines. The active fraction is purified by chromatography radial to get pure compounds. The purity test

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was performed by TLC and LC-MS tests. Then the pure compound of the isolated structure is determined.

Identification

Determination of the molecular structure of the pure compound from the isolation was determined by mass spectroscopy analysis by ionizing Electrospray Ionization (ESI) and Nuclear Magnetic Resonance (NMR) spectroscopy including ^{1}H, ^{13}C, HSQC, and HMBC.

Cell culture

MCF-7 cells (Human breast carcinoma cell line) were grown in RPMI-1640 medium (Sigma, Germany) supplemented with 10% fetal bovine serum (FBS) (Sigma, Germany), 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma, Germany). Cells were incubated in a humidified incubator containing 5% CO2 at 37 ºC. At 80% confluence, cells were rinsed with PBS/0.5%EDTA and harvested from 25cm2 flasks using 0.25 % trypsin/ EDTA solution (Gibco, U.K). Then, cells were sub-cultured into 75cm2 flasks, 96-well plates (Nunc, Denmark) according to experiments. The experiments were performed in triplicate.

MTS cell proliferation/viability assay

MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] is a tetrazolium compound that can be bioreduced by metabolically active cells to a soluble formazan product. The quantity of formazan produced is indicative of the number of viable cells in culture and can be determined colorimetrically by recording the change in absorbance at 490 nm. This assay was performed according to the manufacturer's recommendations for the Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega, WI, USA). Briefly, Complete Minimal Essential Media (Hyclone) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone) and 2 mM L-glutamine (Gibco), 100 U/mL penicillin (Gibco), and 100 µg/mL streptomycin (Gibco) and was dispensed into a 96-well plate. NPs were added to each well to achieve final concentrations of 1, 10, or 100 mg/L. The kit reagents were then prepared, added to the plate, and incubated for an additional 2 h (37°C, 5% CO2). Absorbance (490 nm) was recorded using a microplate reader (WALLAC 1420, PerkinElmer, MA, USA).

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RESULTS AND DISCUSSION

In this paper, we report the isolation of one compound, a sterol β-Sitosterol (figure 1). This compound has earlier been isolated from other plant species but being reported from H.corymbosa for the first time. Isolation of the compound was effected through chromatographic technique and their structures established based on NMR spectroscopic data together comparison with other existing data (Table 1). This compound is soluble in chloroform and are white crystalline in nature.

Compound 1 was identified using 1H and 13C spectra and confirmed using mass spectral data (m/z): 397 [M-OH]-. Signals in the 1H NMR spectrum were observed mainly in the up field region. The spectra exhibited only two signals with high chemical shifts values; the first one resonated in the olefinic region and the other one was observed alittle upfield region. The olefinic signal at δ 5.3 (1H, br d, J = 4.8 Hz) appeared to be characteristic of the sterols, and it was assigned to H-6 proton in the β–sitosterol chemical skeleton. The 1H-NMR spectra of a compound also exhibiteda signal corresponding to the proton connected to the C-3 hydroxyl group which appeared as a multiplet at δ 3.50 (1H, m). Six other proton signals were evident which include four secondary methyl groups (δH 0.91, 0.82, 0.81 and 0.79 all doublets with J = 6.6, 7.2, 6.4 and 6.4 Hz respectively) and two tertiary methyl groups (δH0.66 and 0.99. The 13C NMR spectra exhibited 29 carbon signals, characteristic of phytosterols. These data were in agreement with the structure of β-sitosterol. The NMR data of Table 1 is in agreement with the published values (Sai et al., 2012; Nygo et al., 2016).

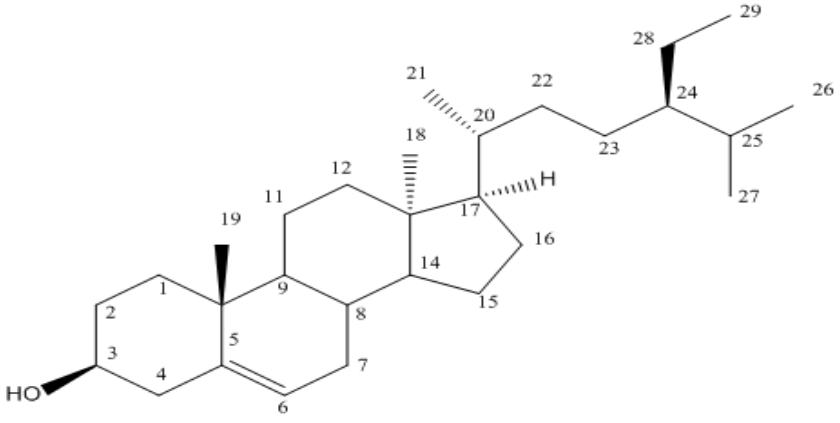

***Figure 1*. Molecular Structure of β–sitosterol**

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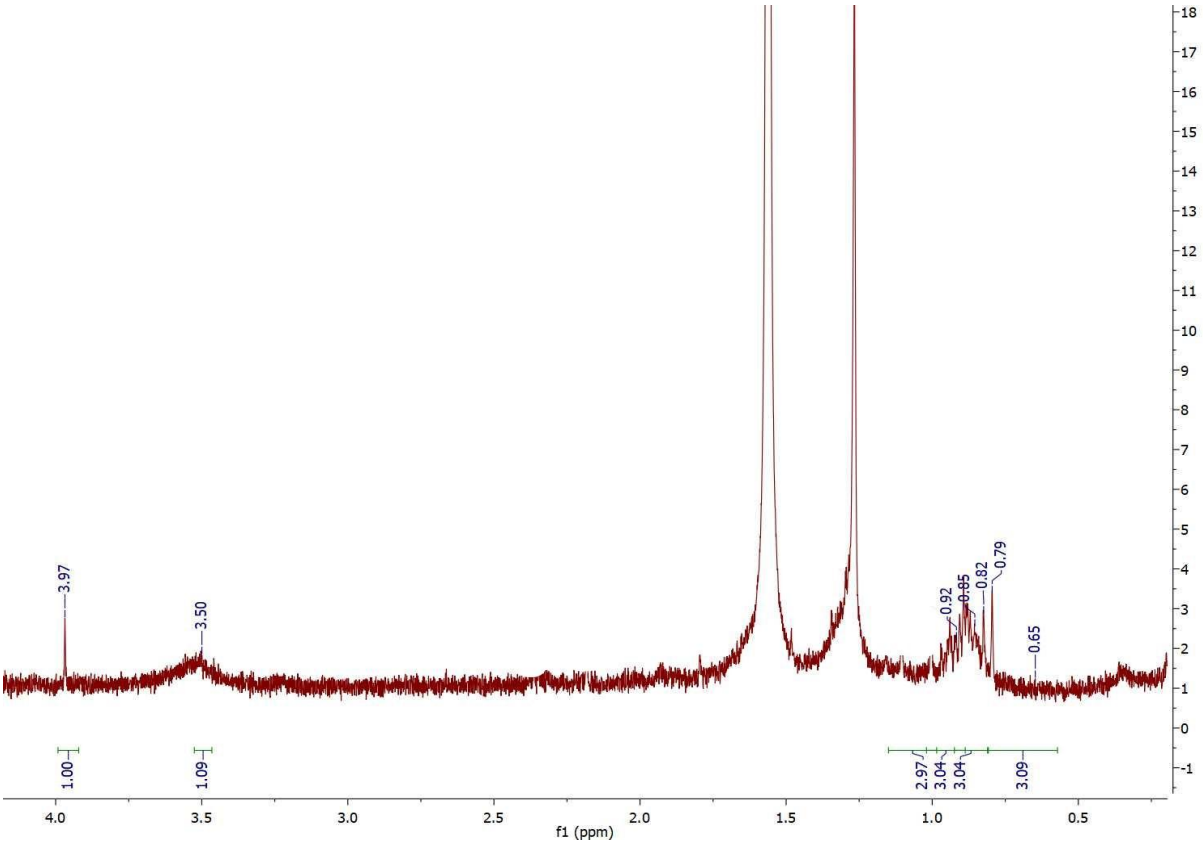

***Figure 2*. Molecular Structure of β–sitosterol**

Table 1:^{13}C NMR data for β-sitosteroland from the literature

No C	β-sitosterol Experimental	Literature
3	3,50 (m, 1H)	3,50 (m, 1H)
5		5,33 (t, 1H)
19	0,92 (d, 3H)	0,91 (d, 3H)
24	0,85 (t, 3H)	0,82 (t, 3H)
26	0,82 (d, 3H)	0,81 (d, 3H)
27	0,79 (d, 3H)	0,79 (d, 3H)
28	0,65 (s, 3H)	0,66 (s, 3H)

β-Sitosterol :White powder (4 mg); mp: 128-130°C; 1H NMR (CDCl3, 600 MHz): ; ^{13}C NMR (CDCl3, 600 MHz): ; MS (*m*/*z*): 397 $[M\text{-}OH]^-$

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Cytotoxic effects of the compound on MCF-7 and MDA-MB-231 cell lines

The results showed that the compound had a potent cytotoxic effect on MCF-7and MDA-MB-231 cells (Figure 3 and 4). The IC50 values of below 100 ug/mL showed the potential effect ofpure compound.

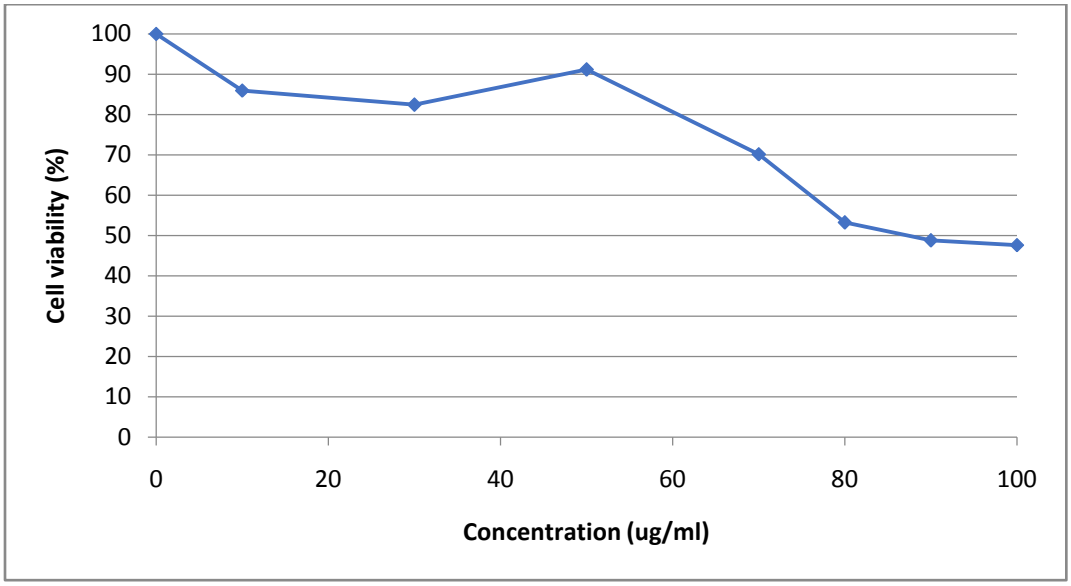

***Figure 3*.Cytotoxic effect of compound on MCF-7. IC50-value : 90.48 ug/mL**

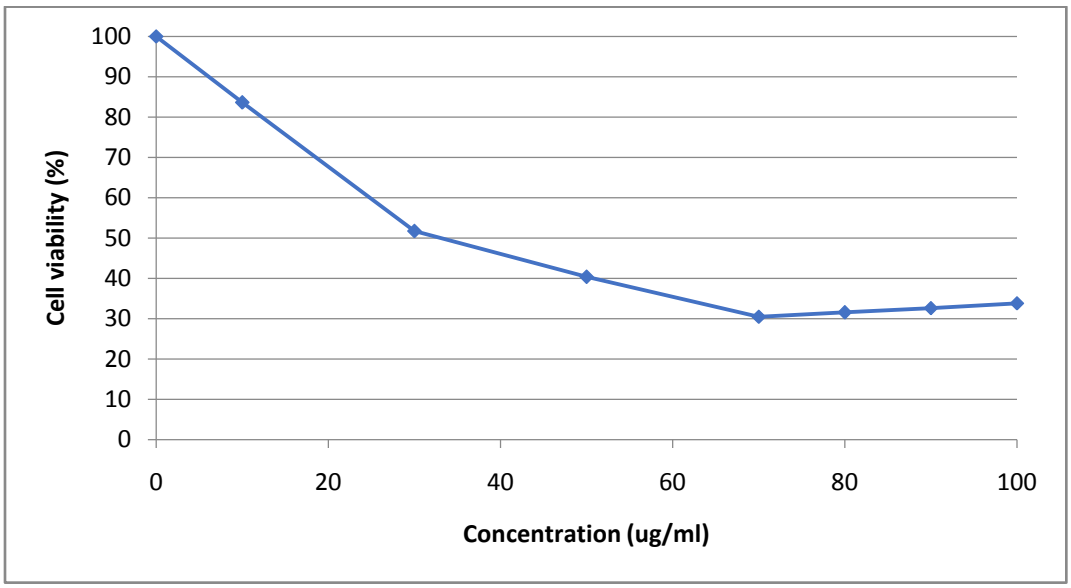

***Figure 4*.Cytotoxic effect of compound on MCF-7. IC50-value : 30.51 ug/mL**

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CONCLUSION

One compound, a sterol was isolated for the first time from H.corymbosa. The structures of the isolated compound were identified as β-sitosterol based on ^{1}H and ^{13}C NMR spectroscopic data and by comparing it to those reported in the literature. The anticancer effect of an isolated compound has been studied on MCF-7 and MDA-MB-231breaset cancer cell ines. This compound has shown potent to inhibit breast cancer cell lines MCF-7 and MDA-MB-231 with an IC50 value of 90.48ug/mL and 30.51 respectively

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REFERENCES

[1]. Azwanida, NN., A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants, 2015.4(3); .3-8.

[2]. Dalimarta S. Traditional Remedy for the Treatment of Hepatitis. New York: Sower Self Reliance, 2005.

[3]. Harbone JB. Phytochemical methods; a guide to modern techniques of plant analysis.3rd edition. 1998. Chapman & Hall. Thomson Science, 24 Boundary Row, London SE18HN, UK.

[4]. Lin, C.C., Ng, LT., Yang, JJ. Antioxidant activity of extracts of Peh-Hue- Juwa-hi-CaoinaCellFreeSystem.TheAmericanJournalofChineseMedicine, 2004; 32 (3) :339-349.

[5]. Mosmann, T. Rapid colorimetric assay for cellular growth and survival : Application to proliferation and cytotoxicity assay. J. Immunol. Methods. 1983; 65 : 55- 63.

[6]. Newton, SM., Lau, C., Gurcha, SS., Besra, GS. and Wright, CW., The evaluation of forty-three plant species for *in vitro*antimycobacterial activities; isolation of active constituents from *Psoraleacorylifolia* and *Sanguinariacanadensis*. Journal of ethnopharmacology, 2002; 79(1), pp.57- 67.

[7]. Permadi A. 2006. Urine facilitating Medicinal Plants.London : Sower Self Reliance.

[8]. Rhodes, A. and Yip, C., Comparison of breast cancer in Indonesia and Malaysia–a clinico-pathological study between Dharmais Cancer Centre Jakarta and University Malaya Medical Centre, Kuala Lumpur. Asian Pacific Journal of Cancer Prevention, 2011;12, pp.2943-6.

[9]. Sai V, Chaturvedula P, & Prakash I. Isolation of Stigmasterol and β- Sitosterol from the dichloromethane extract of *Rubussuavissimus*. International current pharmaceutical journal. 2012; 1(9): 239–242.

[10]. Nyigo VA, Peter X, Mabiki F, Malebo HM, Mdegela RH, Fouche G. Isolation and identification of euphol and β-sitosterol from the dichloromethane extracts of *Synadeniumglaucescens*. The Journal of Phytopharmacology 2016;5(3):100- 104.