

Cytotoxicity of Butyrospermol and Sitosterone from the Stem Bark of *Syzygium aqueum* *by Alfinda Novi Kristanti*

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Cytotoxicity of Butyrospermol and Sitosterone from the Stem Bark of *Syzygium aqueum*Ei E. Aung^{1,2}, Alfinda N. Kristanti^{3,4*}, Nanik S. Aminah^{3,4}, Yoshiaki Takaya⁵, Rico Ramadhan³¹Ph.D. Student of Mathematics and Natural Sciences, Faculty of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, Jl. Mulyorejo-60115, Surabaya, Indonesia²Department of Chemistry, Yadanarbon University, Amarapura-05063, Mandalay, Myanmar³Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Komplek Kampus C Unair, Jl. Mulyorejo-60115, Surabaya, Indonesia⁴Biotechnology of Tropical Medicinal Plants Research Group, Universitas Airlangga, Surabaya, Indonesia⁵Faculty of Pharmacy, Meijo University, Tempaku-468-8502, Nagoya, Japan

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ABSTRACT

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Syzygium aqueum is a public large species of *Syzygium* genus in Myrtaceae family and it is native to Indonesia and Malaysia. Various parts of *S. aqueum* have been widely known to be used for traditional medicine, therefore this plant is documented as a medicinal plant. The isolation of some phytochemical compounds from the stem bark of *Syzygium aqueum* and their cytotoxicity were determined. The extraction and separation of phytochemical compounds in the stem bark of *S. aqueum* was carried out using standard methods. The structures of isolated compounds were determined by UV, FTIR and NMR. The analysis of all spectra obtained and comparison with literature data led to the conclusion that the isolated compounds were steroids, namely butyrospermol and sitosterone. The cytotoxicity of the isolated compounds was then evaluated against HeLa and T47D cell lines by the MTT method and A549 cell lines by the XTT method.

Keyword: *Syzygium aqueum*, Butyrospermol, Sitosterone, HeLa, MTT assay, XTT assay.

Introduction

Cancer is a worldwide health problem leading to high mortality and morbidity rates in children and adults.¹ Mortality cases are increasing all over the world and Weasam Kooti *et al.* reported that the deaths due to cancer will rise up to 13.1 million by 2030.² In the aim of numerous studies, multiple researchers have described plant-derived known and unknown bioactive compounds from several plants for the treatment of cancer.³⁻⁵ The bioactive compounds refer to phytochemical constituents from various parts of medicinal plants such as flowers, pulps, seeds, leaves, barks, etc.⁶ The phytochemicals from medicinal plants have low side effects and high effectiveness.⁷ *Syzygium* in Myrtaceae family is a genus of flowering plants which comprises about 1100-1200 species and it has high levels of diversity.^{8,9} Several public large species in this genus are cultivated and they are used as traditional medicine.¹⁰⁻¹¹ Phytochemicals and bioactivities have been widely studied from many species in this genus.¹² One of public large species, *Syzygium aqueum*, is also known as *Eugenia aquea* and it is native to Malaysia and Indonesia.^{11,13} The various parts of the plant are applied for traditional medicine and this plant is well documented as a medicinal plant.¹⁴ The previous researchers reported tannin and flavonoid compounds from the leaf of *S. aqueum*.¹⁵⁻¹⁶ Moreover, antidiabetic¹⁵ and cytotoxicity on MCF-cell line¹⁷ were evaluated from some of these flavonoids. Cytotoxicity against MCF-7, MDAMB-231 and HS-27 cancer cell lines was tested on aqueous and methanol extracts of *S. aqueum* fruits.¹⁸

Cervical cancer (HeLa) and breast cancer (T47D) are common in women. Breast cancer is the second leading mortality after cervical cancer.^{19,20} Lung cancer (A549) also has a high incidence a leading cause cancer death worldwide both in men and women.²¹⁻²³ Cytotoxicity test on HeLa, T47D and A549 cancer cell lines of isolated compounds from *S. aqueum* (various parts) have not been reported by any researchers. Therefore, the focus of this study is to isolate phytochemical constituents from stem bark of *S. aqueum*, and then followed by cytotoxicity test against on the important cancer cell lines (HeLa, T47D and A549) using MTT and XTT assay. From this study, two steroids, namely Butyrospermol and Sitosterone, were isolated successfully. The structure of these compounds were presented in Figure-1. These two compounds were then subject to cytotoxicity evaluation.

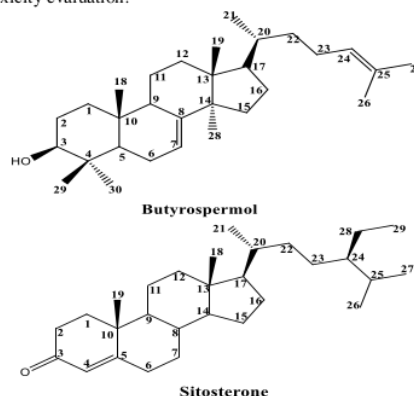


Figure 1: Chemical structures of butyrospermol and sitosterone of *S. aqueum*

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Materials and Methods

General experimental procedures

For Analytical TLC (Thin Layer Chromatography), a pre-coated Silica gel 60 F₂₅₄ (25 Aluminum sheets 20×20 cm, Merck) was used. Silica gel 60 (700-200 mesh ASTM) was used for Column chromatography. UV-Vis spectra were measured on UV-Vis spectrophotometer (Shimadzu). Tracer-100 FT-IR spectrophotometer (Shimadzu) was applied for obtaining FT-TR (Fourier Transform Infrared spectroscopy) spectra which was measured in cm⁻¹. ¹H- and ¹³C-NMR (Nuclear Magnetic Resonance) were recorded on BRUKER 600 Hz with Tetramethylsilane (TMS). Chemical shift was given in δ (ppm) and coupling constants (*J*) were expressed in hertz (Hz). HR-DART-MS was accomplished with a JEOL JMS HX-110 mass spectrometer. ELISA (Enzyme-Linked Immunosorbent Assay) reader was used for measuring cytotoxicity.

Plant material

The stem bark of *S. aqueum* was obtained from Wage, Taman, Sidarjo, East Java, Indonesia on September 5th, 2018 and the voucher specimen (UA-MSa050918) was deposited at the Herbarium of Universitas Airlangga. Laboratory of Biosystematic, Department of Biology, Faculty of Science and Technology, Universitas Airlangga.

Extraction and Separation

The stem bark powder (2 kg) was macerated in methanol (40 L) at room temperature for 3 days. After that, the methanol extract was evaporated using evaporator (Buchi Rotary). The extract (450 g) was partitioned with n-hexane (6 L) in triplicate. N-hexane extract (50 g) was separated utilizing silica gel chromatography with n-hexane:ethyl acetate (6:4, 3 L) as eluent. The similar fractions were combined yielding 19 fractions (SA-1 to SA-19). Fraction SA-6 (800 mg) was re-chromatographed with eluent n-hexane:dichloromethane (6:4, 1 L) which yielded 3 fractions (SA-6-1 to SA-6-3). Butyrospermol (58 mg) was obtained from fraction-1 (SA-6-1) and sitosterone (51 mg) was gotten from fraction-3 (SA-6-3).

Cytotoxicity of isolated compounds

Cell culture

Three cancer cell lines, HeLa, T47D and A459 were obtained from American Type Culture Collection (ATCC). Cells were cultured at 37°C and 5% CO₂ for 24 h and 100% humidity in medium supplemented with 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin.

MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method was used. HeLa and T47D cells were cultured in a 96-well plate at 213 × 10³ cell/well density. The cells were then treated with 100 μL of the prepared compounds with various concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50 and 100 μg/mL) for 24 h. A 100 μL of MTT solution (50 mg in 10 mL of PBS) was added, followed by incubation in CO₂ incubator for 4 h. The amount of viable cells was visualized by the formation of purple colour due to formation of formazan crystals. The formed formazan that was proportional to the total number of viable cells was calculated using spectrophotometer at 560 nm. The number of viable cells was calculated using the following formula:

$$\% \text{ viable cells} = \frac{\text{treatment absorbance} - \text{control medium absorbance}}{\text{negative control absorbance} - \text{control medium absorbance}} \times 100\%$$

IC₅₀ was obtained after statistical analysis using SPSS program. Doxorubicin was used as a positive control in this assay. The test was conducted in triplicate.

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XTT assay

XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide) assay method was carried out. The XTT method is basically the same as MTT. The only difference lies in the tetrazolium salt used. The XTT method used 1 mg/mL XTT reagent (4 mg in 4 mL DMEM/MEM/RPMI-1640 culture medium) added with 10 μL of PMS. The absorbance was determined at 450 nm.

Results and Discussion

Butyrospermol was collected as pink oil. UV spectrum in MeOH appeared at 239 nm. DART-MS: m/z 427.3934 [M+H]⁺ suitable for molecular formula C₃₀H₅₀O. FTIR spectra showed the bands at 3385 cm⁻¹ (-OH), 2927-2929 cm⁻¹ (sp³ C-H stretching), 1668 cm⁻¹ (C=C), 1452 cm⁻¹ and 1064 (C-O). ¹H-NMR δ_H (ppm): 0.75 (3H, s), 0.81 (3H, s), 0.86 (3H, s), 0.89 (3H, d, *J* = 6.5 Hz), 0.97 (6H, s), 1.04 (2H, m), 1.14 (1H, m), 1.31 (1H, m), 1.40 (1H, m), 1.44 (1H, m), 1.50 (4H, m), 1.61 (3H, s), 1.64 (3H, m), 1.67 (1H, m), 1.69 (3H, s), 1.79 (1H, m), 1.87 (1H, m), 1.94 (2H, m), 1.97 (1H, m), 2.04 (1H, m), 2.15 (1H, m), 2.21 (1H, m), 3.25 (1H, dd, *J* = 3.9, 11.5 Hz), 5.10 (1H, m), 5.26 (1H, m). The ¹³C-NMR δ_C (ppm): 37.2 (C-1), 27.7 (C-2), 79.3 (C-3), 38.9 (C-4), 50.6 (C-5), 23.9 (C-6), 117.8 (C-7), 145.9 (C-8), 48.9 (C-9), 35.0 (C-10), 18.1 (C-11), 33.8 (C-12), 43.5 (C-13), 51.2 (C-14), 34.0 (C-15), 28.2 (C-16), 52.9 (C-17), 22.0 (C-18), 13.1 (C-19), 35.9 (C-20), 18.3 (C-21), 36.1 (C-22), 25.0 (C-23), 125.1 (C-24), 130.9 (C-25), 17.6 (C-26), 25.7 (C-27), 27.6 (C-28), 14.7 (C-29), 27.3 (C-30). ¹H-NMR δ_H: the signals at 5.26 ppm and 5.10 ppm belonged to protons of olefinic (C-7 & C-24) and confirmed by HSQC. And then, the carbon and proton (δ_C 79.29 ppm & δ_H 3.35 ppm) at position C-3 appeared in downfield region because it was connected to electronegative group (-OH). COSY and HMBC spectra were linked to confirm the structure (Figure 2). The analysis of NMR spectra is presented in Table 1. This NMR data was also compared with literature.

Sitosterone was obtained as pink oil. DART-MS: m/z 411.3632 [M-H]⁻ suitable for molecular formula of C₂₉H₄₈O. FTIR spectra showed some bands at 2926-2848 cm⁻¹ (sp³ C-H stretching), 1678 cm⁻¹ (C=O), 1618 cm⁻¹ (C=C). ¹H-NMR δ_H (ppm): 0.72 (3H, m), 0.82 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.0 Hz), 0.84 (3H, t, *J* = 6.0 Hz), 0.92 (5H, m), 1.02 (3H, m), 1.11 (1H, m), 1.16 (3H, m), 1.18 (3H, s), 1.26 (2H, m), 1.32 (1H, m), 1.36 (1H, m), 1.44 (1H, m), 1.52 (1H, m), 1.53 (1H, m), 1.61 (2H, m), 1.67 (1H, m), 1.71 (1H, m), 1.85 (3H, m), 2.02 (2H, m), 2.27 (2H, m), 2.35 (1H, m), 2.41 (1H, m), 5.73 (1H, s). ¹³C-NMR δ_C (ppm): 35.7 (C-1), 34.0 (C-2), 199.6 (C-3), 123.7 (C-4), 171.6 (C-5), 33.9 (C-6), 32.9 (C-7), 36.1 (C-8), 53.8 (C-9), 38.6 (C-10), 21.0 (C-11), 39.6 (C-12), 42.2 (C-13), 56.0 (C-14), 24.2 (C-15), 28.2 (C-16), 55.9 (C-17), 12.0 (C-18), 17.4 (C-19), 35.7 (C-20), 18.7 (C-21), 32.1 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.0 (C-27), 23.1 (C-28), 11.8 (C-29). The signal at chemical shift δ_C 199.6 ppm was a signal belonging to C=O. COSY and HMBC correlations were joined for structure confirmation (Figure 2). The analysis of NMR spectra was presented in Table 2. This NMR data was also compared with literature.

The cytotoxicity evaluation of isolated compounds was determined against HeLa, T47D and A549 cancer cell lines (Table 3). Butyrospermol (43.59 ± 0.393 μg/mL) was moderately active against HeLa cell line and it was less cytotoxic on T47D and A549 cell lines. Moreover, sitosterone (29.96 ± 0.0422 μg/mL) also had moderate toxicity against A549 cell line using XTT assay. In literature, pure compound is considered as highly toxic (≤ 4 μg/mL), toxic (≤ 20 μg/mL), moderately toxic (20-100 μg/mL) and non-toxic (> 100 μg/mL).^{27,28} In XTT assay, orange-coloured formazan is water soluble and its intensity can be measured with a spectrophotometer. However, the purple needle-shaped crystals or formazan needles in the cells are insoluble in water and the formazan has to be dissolved in DMSO or isopropanol for MTT assay before measuring the absorbance.²⁹

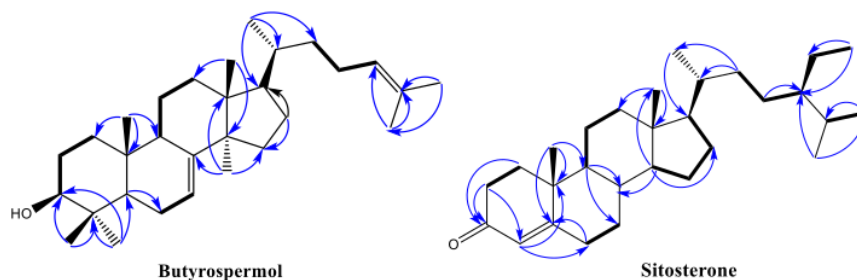


Figure 2: () HMBC and () COSY correlations of Butyrospermol and Sitosterone

Table 1: NMR Spectra Data of Butyrospermol Recorded in CDCl₃ / 600 Hz

Position	DEPT	Butyrospermol		Literature ²⁴⁻²⁵	
		δ_c (ppm)	δ_H (ppm) (mult, J in Hz)	δ_c (ppm)	δ_H (ppm) (mult, J in Hz)
1	CH ₂	37.2	1.67 (m), 1.14 (m)	37.5	-
2	CH ₂	27.7	1.64 (m)	27.7	-
3	CH	79.3	3.25 (dd, J = 3.9, 11.5 Hz)	79.3	3.24 (m)
4	C	38.9	-	38.9	-
5	CH	50.6	1.31 (m)	50.6	-
6	CH ₂	23.9	1.97 (m), 2.15 (m)	23.9	-
7	CH	117.8	5.26 (m)	117.8	5.30 (dd, J = 3.5 Hz)
8	C	145.9	-	145.9	-
9	CH	48.9	2.21 (m)	48.9	-
10	C	35.0	-	35.0	-
11	CH ₂	18.1	1.50 (m)	18.2	-
12	CH ₂	33.8	1.79 (m), 1.64 (m)	33.8	-
13	C	43.5	-	43.5	-
14	C	51.2	-	51.3	-
15	CH ₂	34.0	1.44 (m), 1.50 (m)	33.9	-
16	CH ₂	28.2	1.94 (m)	28.5	-
17	CH	52.9	1.50 (m)	52.2	-
18	CH ₃	22.0	0.81 (s)	22.1	0.81 (s)
19	CH ₃	13.1	0.75 (s)	13.1	0.74 (s)
20	CH	35.9	1.40 (m)	35.8	-
21	CH ₃	18.3	0.89 (d, J = 6.5 Hz)	18.6	0.84 (brs)
22	CH ₂	36.1	1.04 (m)	35.2	-
23	CH ₂	25.0	2.04 (m) 1.87 (m)	25.4	-
24	CH	125.1	5.10 (m)	125.1	5.10 (dd, J = 6.8, 7.2 Hz)
25	C	130.9	-	130.9	-
26	CH ₃	17.6	1.61 (s)	17.7	1.61 (s)
27	CH ₃	25.7	1.69 (s)	25.7	1.68 (s)
28	CH ₃	27.6	0.97 (s)	27.6	0.97 (s)
29	CH ₃	14.7	0.86 (s)	14.7	0.86 (s)
30	CH ₃	27.3	0.97 (s)	27.3	0.97 (s)

Table 2: NMR Spectra Data of Sitosterone Recorded in CDCl₃ / 600 Hz

Position	DEPT	20 Sitosterone		Literature ²⁶	
		δ_C (ppm)	δ_H (ppm) (mult, J in Hz)	δ_C (ppm)	δ_H (ppm) (mult, J in Hz)
1	CH ₂	35.7	2.02 (m), 1.71 (m)	35.8	-
2	CH ₂	34.0	2.41 (m), 1.32 (m)	34.1	-
3	C	199.6	-	199.8	-
4	CH	123.7	5.73 (s)	123.8	5.72 (s)
5	C	171.6	-	171.8	-
6	CH ₂	33.9	2.35 (m), 1.02 (m)	33.1	-
7	CH ₂	32.9	2.27 (m)	32.2	-
8	CH	36.1	1.36 (m)	35.7	-
9	CH	53.8	0.92 (m)	53.9	-
10	C	38.6	-	38.7	-
11	CH ₂	21.0	1.52 (m), 1.44 (m)	19.9	-
12	CH ₂	39.6	2.02 (m), 1.16 (m)	39.7	-
13	C	42.2	-	42.5	-
14	CH	56.0	1.11 (m)	56.0	-
15	CH ₂	24.2	1.61 (m)	24.3	-
16	CH ₂	28.2	1.85 (m)	28.3	-
17	CH	55.9	1.02 (m)	56.1	-
18	CH ₃	12.0	0.72 (s)	12.1	0.74 (s)
19	CH ₃	17.4	1.18 (s)	17.5	1.18 (s)
20	CH	35.7	1.53 (m)	36.2	-
21	CH ₃	18.7	0.92 (m)	19.9	0.91 (d, J = 6.5 Hz)
22	CH ₂	32.1	1.85(m), 1.02 (m)	34.0	-
23	CH ₂	26.1	1.16 (m)	26.2	-
24	CH	45.9	0.92 (m)	45.9	-
25	CH	29.2	1.67 (m)	29.3	-
CH ₂	CH ₃	19.8	0.83 (d, J = 6.0 Hz)	21.1	0.83 (d, J = 6.8 Hz)
27	CH ₃	19.0	0.82 (d, J = 6.8 Hz)	19.1	0.80 (d, J = 6.4 Hz)
28	CH ₂	23.1	1.26 (m)	23.1	-
29	CH ₃	11.8	0.84 (t, J = 6.0 Hz)	12.1	0.84 (t, J = 7.2 Hz)

Table 3: Cytotoxicity (IC₅₀) data of Butyrospermol and Sitosterone against HeLa, T47D and A549

	IC ₅₀ (µg/mL)		
	MTT assay		XTT assay
	HeLa	T47D	A549
Butyrospermol	43.59 ± 0.393	419.05 ± 0.246	354.85 ± 0.017
Sitosterone	ND*	ND*	29.96 ± 0.0422
Doxorubicin	2.67 ± 0.247	0.035 ± 0.012	ND*

*ND = not determined

Conclusion

In the current study, two steroids, butyrospermol and sitosterone, were isolated from the stem bark of *S. aqueum*. These two steroids were reported to have been isolated from this species for the first time. They were evaluated for their cytotoxicity on different cell lines with MTT assay and XTT assay. Both compounds have moderate cytotoxicity on different cancer cell lines.

Conflict of interest

The Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Barliana MI, Diantini A, Subarnas A, Abdulah R, Izumi T. Inhibition of phosphorylated c - Jun NH (2)-terminal kinase by 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone isolated from *Eugenia aquea* Burm f. leaves in Jurkat T-cells. *Pharmacogn Mag.* 2017; 13(Suppl 3):S573-S577.
2. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, Marzoumi HZ. Effective medicinal plant in cancer treatment, Part 2: Review Study. *J. Evid Based Complementary Altern Med.* 2017; 22(4):982-995
3. Greenwell M and Rahman PKSM. Medicinal plants: their use in anticancer treatment. *Int J Pharm Sci.* 2015; 6(10):4103-4112.
4. Nahata A. Anticancer agents: a review of relevant information on important herbal drugs. *Int J Clin Pharmacol Toxicol.* 2017; 6(2):250-255.
5. Roy A, Attre T, Bharadvaja N. Anticancer agent from medicinal plants: a review. In: Tiezzi A, Karpinski TM (Eds.). *New aspects in medicinal plants and pharmacognosy*, Poznan, Poland. JBBBooks; 2017. 54-73 p.
6. Hemavathy A, Shanthi P, Sowndharya C, Thiripura SS, Priyadarshini K. Extraction and isolation of bioactive compounds from a therapeutic medicinal plant - *Wrightia tinctoria* (Roxb.) R. *Br Int J Pharmacogn Phytochem Res.* 2019; 11(3):199-204.
7. Tyagi N, Sharma GN, Shrivastava B, Saxena P, Kumar N. Medicinal plants: used in Anti-cancer treatment. *Int J Res Dev Pharm Life Sci.* 2017; 6(5):2732-2739.
8. Memon AH, Ismail Z, Al-Suede FSR, Aisha FAF, Hamil MSR, Saeed MAA, Laghari M, Majid AMSA. Isolation, characterization, crystal structure elucidation of two flavanones and simultaneous RP-HPLC determination of five major compounds from *Syzygium campanulatum* Korth. *Molecules.* 2015; 20:14212-14233.
9. Nigam V, Nigam R, Singh A. Distribution and medicinal properties of *Syzygium* species. *Curr Res Pharm Sci.* 2012; 02:73-80.
10. Abera B, Adane L, Mamo F. Phytochemical investigation the root extract of *Syzygium guineense* and isolation of 2,3,23- trihydroxymethyl oleanate. *J Pharmacogn Phytochem.* 2018; 7(2):3104-3111.
11. Mudiana D. *Syzygium* diversity in Gunung Baung, East Java, Indonesia. *Biodiversitas.* 2016; 17(2):733-740.
12. Sobeh M, Mahmoud MF, Petruk G, Rezaq S, Ashour ML, Youssef FS, El-Shazly AM, Monti DM, Abdel-Naim AB, Wink M. *Syzygium aqueum*: A Polyphenol-rich leaf Extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. *Front Pharmacol.* 2018; 9:1-14.
13. Manaharana T, Chakravarthi S, Radhakrishnan AK, Palanisamy UD. In vivo toxicity evaluation of a standardized extract of *Syzygium aqueum* leaf. *Toxicol Rep.* 2014; 1:718-725.
14. Palanisamy UD, Ling LT, Manaharan T, Sivapalan V, Subramaniam T, Helme MH, Masilamani T. Standardized extract of *Syzygium aqueum*: a safe cosmetic ingredient. *Int J Cosmet Sci.* 2011; 33:269-275.
15. Manaharan T, Appleton D, Cheng HMC, Palanisamy UD. Flavonoids isolated from *Syzygium aqueum* leaf extract as potential antihyperglycaemic agents. *Food Chem.* 2012; 132(4):1802-1807.
16. Nonaka G, Aiko Y, Aritake K, Nishioka I. Samarangenins A and B, Novel proanthocyanidins with doubly bonded structures, from *Syzygium samarangense* and *S. aqueum*. *Chem Pharm Bull.* 1992; 40(10):2671-2673.
17. Subarnas A, Diantini A, Abdulah R, Auhrotun A, Hadisaputri YE, Puspitasari IM, Yamazaki C, Kuwano H, Koyama H. Apoptosis induced in MCF-7 human breast cancer cells by 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone isolated from *Eugenia aquea* Burm f. leaves. *Oncol Lett.* 2015; 9:2303-2306.
18. Rabeta MS, Chan S, Neda GD, Lam KL, Ong MT. Anticancer effect of underutilized fruits. *Int Food Res J.* 2013; 20(2):551-556.
19. Tunjung WAS and Sayekti PR. Apoptosis induction on human breast cancer T47D cell line by extracts of *Ancorina* sp. [version 2; peer review: 2 approved with reservations]. *F1000Research.* 2019; 8(168):1-15.
20. Mutiah R, Listiyana A, Suryadinata A, Annisa R, Hakim A, Anggraini W, Susilowati R. Activity of inhibit the cell cycle and induct apoptosis in HeLa cancer cell with combination of Sabrang onion (*Eleutherine palmifolia* (L.) Merr) and Starfruit Mistletoe (*Macrosolen cochinchinensis* (Lour.) Tiegh). *J Appl Pharm.* 2018; 8(10):122-128.
21. Amaani R and Dwira S. Phytochemical content an in vitro toxicity of *Glycine soja* ethanol extract on the A549 lung cancer line cell. *IOP Conf. Series: J Phy: Conf Series.* 2018; 1073(032042):1-9.
22. Nair RR, Bhaskar D, Jabbar KK, Amresh N, Sathyamurthy B. Invitro studies on the effect of singiber officinale methanol extract in lung cancer A549 cell lines. *World J Pharm Res.* 2017; 6(15):1097-1114.
23. Shettar AK, Madagi SB, Vedamurthy AB. Phytochemical screening and in-vitro antioxidant and antiproliferative activity of aqueous leaf extract of *ximtenia americana* against non-small cell lung cancer. *Cancer Surg.* 2018; 3(1):1-8.
24. Zarea S, Ghaedi M, Miri R, Heiling S, Asadollahi M, Baldwin IT, Jassbi AR. Phytochemical Investigation on *Euphorbia macrostegia* (Persian wood spurge). *Iran J Pharm Res.* 2015; 14(1):243-249.
25. Wu J, Li M, Xiao Z, Zhou Y. Butyrospermol fatty acid esters from the fruit of a Chinese mangrove *Xylocarpus granatum*. *Z. Naturforsch., C, J Biosci.* 2006; 61(11):1447-1449.
26. Liang XU, Xiao-han WANG, Rui-ya LUO, Shi-qiong LU, Ze-jian GUO, Ming-an WANG, Yang LIU, Li-gang ZHOU. Secondary metabolites of rice sheath blight pathogen *Rhizoctonia solani* Kühn and their biological activities. *J Integr Agric.* 2015; 14(1):80-87.

27. Sriwiryajan S, Ninpesh T, Sukpondmav Y, Nasomyon T, Graidist P. Cytotoxicity screening of plants of genus *Piper* in breast cancer cell lines. Trop J Pharm Res. 2014; 13(6):921-928.
28. Fikroh RA, Matsjeh S, Anwar C. Synthesis and anticancer activity of (E)-2'-hydroxy-2-bromo-4,5-dimethoxychalcone against breast cancer (MCF-7) cell line. Molekul. 2020; 15(1):34-39.
29. Aslantürk OS. In vitro cytotoxicity and cell viability assays: disadvantages in vitro cytotoxicity and viability assays: principles, advantages and disadvantages, chapter 1; 2018. 4 p.

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