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Original Article

TOPOISOMERASE INHIBITOR ACTIVITY OF JAMU GENDONG PAHITAN (BITTER HERB) USING MECHANISM-BASED YEAST BIOASSAY

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ABSTRACT

Objective: This research was conducted to screen the anticancer activity of bitter herbs that contains *Andrographis paniculata* (Brum. f) leaves (AP) and *Tinospora crispa* L. stems (TC) in form of fresh materials and extracts using a mechanism-based yeast bioassay.

Methods: Samples tested by mechanism-based yeast bioassay (MBYB) were single extract, mixed extract, and jamu gendong pahitan from a traditional market and made in the laboratory. Fresh sample of jamu gendong pahitan from the market and a single extract (AP and TC) was tested at one dose. While fresh jamu gendong pahitan made in the laboratory and the mixed extract (AP: TC) was tested at three different doses, doses 1 (3:10), dose 2 (1:1), and dose 3 (10:3). The leaves and stems were extracted by 70% ethanol for 3x24 h, each day the solvent was changed then every macerate was evaporated using a rotavapor and water bath. By this MBYB method, noted that the active sample must have an IC_{12} value of<8000µg/ml, so all the samples or doses were tested using final concentration varying at around 8,000; 4,000; 2,000; 1,000; 500, 250, and 125µg/ml.

Results: The percentage yield of *Andrographis paniculata* (Brum. f) leaves was 11.2% and *Tinospora crispa* L. stems was 19.%. The activity assay for jamu gendong pahitan from the traditional market was inactive as a topoisomerase inhibitor (IC_{12}>8000µg/ml). Samples showed topoisomerase I inhibitor activity were jamu gendong pahitan made in laboratory doses 1 and 2. While samples showed topoisomerase I and II inhibitor activities were jamu gendong pahitan made in laboratory dose 3, single and mixed extracts.

Conclusion: The fresh material of jamu gendong pahitan (bitter herbs) bought from the market is inactive, while the fresh material of samples of jamu gendong pahitan made in laboratory doses 1 and 2 have topoisomerase I inhibitor activity. Based on the $IC_{12,}$ value, it is known that the sample that gave the best activity was the mixed extract of bitter herbs dose 3 that contain extract of *A. paniculata* and *T. crispa* (10:3), with IC_{12} values in strains 1138, 1140, and 1353 were 926.28±173, 576.75±42, and 865.5±135µg/ml respectively.

Keywords: Topoisomerase inhibitor, Jamu gendong, Mechanism-based yeast bioassay, Pahitan, Bitter herbs

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INTRODUCTION

Jamu gendong is a traditional medicine in Indonesian culture that is sold by carrying it on the back of women using a shawl. The ingredients are made from roots, stems, leaves, and flowers, which are brewed with hot water and then filtered and drunk [1]. Jamu gendong pahitan (bitter herbs) is a type of black potion made from *Andrographis paniculata* (Brum. f) leaves and *Tinospora crispa* L. stems. This potion has many functions; for instance, it prevents allergy, itch, and acne as anti-inflammation [2, 3]. Moreover, pahitan could be used as an immunomodulator, stabilization of blood glucose, antioxidant, and antiproliferation in cancer treatment [4-6]. *A. paniculata* leaves have compounds such as andrographolide, neoandrographolid, chlorogenic acid, caffeic acid, tannins, saponins, and flavonoids [7]. In HT-29 cancer cell line, it allows apoptosis and inhibits the cell cycle that depends on its doses [8]. The andrographolide compound has activity as an anticancer through inhibition of NF-kb and multiple lipid-dependent pathways in the lungs, ovary, and breast cancer [9, 10]. In addition, *T. crispa* L. stems contain tinocropside, columbine, berberine, apigenin, and pycroretoside glycoside for allowing apoptosis, decreasing blood glucose levels, and NF-Kb gene expression in breast cancer cells [11, 12].

Genetic disease grows more significantly from tumors that could move across cells or tissues named cancer. In cancer disease, there is a gene mutase from protooncogen, DNA repair, and suppressor genes. Chemotherapy targeting topoisomerase enzyme is one of the options for the treatment of cancer. Topoisomerase enzyme has two types, namely topoisomerase I and II. The topoisomerase I enzyme is relaxing one strand of supercoiled DNA, while the topoisomerase II enzyme is relaxing two strands of supercoiled DNA. The inhibition process is initiating stable covalent bound enzyme-DNA; therefore, the DNA diminishes. This process inhibits anti-apoptosis (Bcl-xl and Bcl-2) expression, hence the cancer cells not growing up [13]. The process targeting this enzyme was studied in yeast as a biological model system due to its biochemical close to mammalian cells, therefore, it could be used for evaluating anticancer agents. Each component of this bitter herb has been reported to have activity as anticancer. However, the composition of these plants as bitter herb ingredients has never been reported. Therefore, this research was conducted to screen the anticancer activity of bitter herbs in fresh material and extract forms using a mechanism-based yeast bioassay.

MATERIALS AND METHODS

Andrographis paniculata (Brum. f) leaves *Tinospora crispa* L. stems was identified at Herbarium Jatinangor of Universitas Padjadjaran with document number 38/HB/10/2022 and 22/HB/10/2022 respectively. 70% ethanol (technical grade), Distilled water, Dimethyl sulfoxyde (Merck), agar bacteriological (Himedia), peptone (Condalab), dextrose (Condalab), yeast extract (Condalab), *potato dextrose agar* (PDA, Condalab), *potato dextrose broth* (PDB, Condalab), and sodium chloride (Merck). *Saccharomyces cerevisiae* strain wild, 1140, 1138, 1353 is similar with previous research [14-16].

Preparation of jamu gendong pahitan

Fresh jamu gendong pahitan was collected from six traditional market in Bandung city West Java, Indonesia. Before collection, we make some short survey. Every seller was asked questions about ingredients, how to make it, and indications of it. Based on the interviews, we compare the results and choose one seller with the standard formula that represents the other seller. Then, the other sample was jamu gendong made in the laboratory, dependent on a chosen formula from the interview. All ingredients were grinded, brewed with water at 70 °C and filtrated it [17].

Extraction

A. paniculata (Brum. f) leaves and *T. crispa* L. stems were sorted, chopped into small pieces then dried in an oven for 24-48 h. Extraction of dried leaves and stems was done by ethanol maceration for 3x24 h. The macerate was filtrated and concentrated with a rotary evaporator temperature at 60 ℃. The concentrated extract was evaporated using a water bath [18].

Activity test with mechanism-based yeast bioassay

This method was done according to Zuhrotun *et al.* [14-16] that which the *S. cerevisiae* strain wild was cultured in PDB. Whereas *S. cerevisiae* strain mutants 1140, 1138, and 1353 were cultured in YPDB and then left for 24 h in an incubator. The suspension was mixed with a sterile 0.9% saline solution and measured with spectrophotometry UV-Vis at 600 nm until the transmittance value was 80%. The inoculum 1 ml was mixed with 20 ml of medium YPDA in a petri dish and left for a minute. Medium YPDA and YPDB were made from a mixture of 1 g of yeast extract (Y), 2 g of peptone (P) and 2 g of dextrose (D) in agar (A) media or broth media. The medium agar was perforated 7 wells, then add 50µl of sample. Extract samples at variation of concentrations were dissolved in a mixture of dimethyl sulfoxide with methanol (1:1). Petri dishes were incubated at 30 ℃ for 36-48 h. The diameter of the inhibition zone that produced then convert to dose-response curves, where axis x was the sample concentration (µg/ml) and y was the diameter of the inhibition zone. Equation of linear regression can be built from the curve. From that equation, we can calculate IC_{12} value of each sample. The active samples showed an IC_{12} value<8000 µg/ml, which means a concentration that produce an inhibition zone of 12 mm around the well. The bioassay was done in three replicates with variation of sample concentration were around 8000, 4000, 2000, 1000, 500, 250, 125 µg/ml. A topoisomerase I inhibitor is defined as to sample against *S. cerevisiae* strain 1140 and a topoisomerase II inhibitor is defined as to sample against *S. cerevisiae* strain 1353.

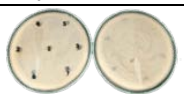
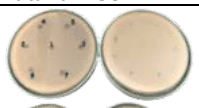
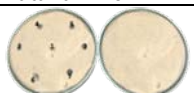
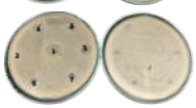
RESULTS AND DISCUSSION

Each plant material is extracted by cold method, namely the maceration method, because it is easier and the chemical compounds are not damaged [19]. The solvent used is 70% ethanol because of its general and can extract a wide range of polarity from polar and non-polar compounds, more quickly and safely and can inhibit the growth of other microbes [20, 21]. The yield obtained for A. paniculata extract was 11.2%, and for T. crispa was 19.1%. These results were compared with the Indonesian Herbal Pharmacopoeia for A. paniculata extract>9.6% and T. crispa extract>18.5% [18], that showed all plant yields were good because they met the requirements.

This yeast bioassay method was carried out using a wild strain as a negative control so that the yeast continues to grow because there is a DNA repair mechanism. In contrast, the mutant strain is a sample-specific marker that has activity as a topoisomerase inhibitor because it does not have a DNA repair mechanism. The tested samples were jamu gendong pahitan bought from the market, jamu gendong pahitan made in the laboratory, single extracts, and mixed extract of its jamu gendong pahitan components. All samples were suspended with solvent mixture methanol: DMSO (1:1). Methanol is used because it is polar, while DMSO, as a universal solvent, can dissolve polar and non-polar compounds and does not affect the test results [22].

Fresh material of jamu gendong pahitan bought from traditional market was tested in one dose, as it made by the seller. According to the survey, known that jamu gendong pahitan is made from ½ handheld of *A. paniculata* leaves (around 3 g, AP) and ½ medium size of *T. crispa* stem (around 10 g, TC). So, the fresh material of jamu gendong pahitan made in laboratory was tested using mixed AP: TC (3:10) as dose 1, (10:10 or 1:1) as dose 2, and (10:3) as dose 3.

Table 1: Agar plate profile of jamu gendong buy at the traditional market and made at the laboratory

Jamu gendong	Dose	*Saccharomyces cerevisiae*			
		Wild	Mutant 1138	Mutant 1140	Mutant 1353
Traditional market	-				
Laboratory	1				
	2				
	3				

Notes: All assays were done in three replications (n = 3)

Table 2: Inhibition zone of jamu gendong made in the laboratory

Concentration (µg/ml)	Samples	Inhibition zone (mm)			
		SC Wild	SC1140	SC1353	SC1138
16,086	Dose 1 (AP: TC = 3:10)	-	14.37±0.4	10.1±0.0	14.8±0.39
8043		-	13.33±0.25	9.47±0.15	12.28±0.6
4021.5		-	12.2±0.36	7.93±0.4	10.43±0.76
2010.8		-	-	7.53±0.05	-
1005.4		-	-	6.97±0.15	-
502.7		-	-	6.4±0.17	-
251.3		-	-	6.03±0.06	-
14,542	Dose 2 (AP: TC = 1:1)	-	14.17±0.42	11.27±0.15	15.33±0.06
7271		-	13.07±0.57	10.3±0.1	13.57±0.06
3635.5		-	12.6±0.88	9.43±0.25	12.03±0.45
1817.8		-	10.27±0.55	8.23±0.11	11.13±0.25
908.9		-	-	7.57±0.46	8.17±1.91

Concentration (µg/ml)	Samples	Inhibition zone (mm)			
		SC Wild	SC1140	SC1353	SC1138
454.4		-	-	6.63±0.23	-
227.2		-	-	6.1±0.17	-
10,888	Dose 3 (AP: TC =	-	15.4±0.36	12.8±0.17	15.67±0.21
5444	10:3)	-	13.23±0.81	11.37±0.12	14.03±0.11
2722		-	11.83±1.50	9.83±0.65	12.23±0.50
1361		-	10.17±2.74	8.17±0.23	11.7±0.1
680.5		-	-	7.9±0.3	7.2±2.75
340.3		-	-	7.3±0.1	-
170.1		-	-	6.77±0.68	-

Notes: All assays were done in three replications (n = 3)

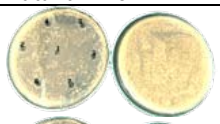
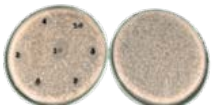
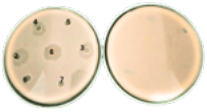
To know the concentration (mg/ml) of each fresh material jamu gendong pahitan bought from the market and made in the laboratory, we put 5 ml of each sample and then dried it in the oven. By this research, showed that the concentration of jamu gendong pahitan from the market was 28,434 µg/ml. while the concentration of jamu gendong made in laboratory doses 1, 2, and 3 were 16,086; 14,542; and 10,888 µg/ml, respectively. These concentrations are used as the highest concentration in bioassay.

Samples of jamu gendong pahitan bought from the market did not produce any inhibition zone against wild and mutant *S. cerevisiae* strains (1138, 1140, and 1353) as it showed that no inhibition zones produced (table 1). Preliminary survey data showed that most of seller made jamu gendong pahitan using boiling method as extraction process, so this could be damaged the active compound. Although the concentration of this samples is higher than jamu gendong pahitan made in the laboratory but still inactive. While the bioassay result of jamu gendong pahitan made in the laboratory showed that this sample is active as topoisomerase inhibitor (table 1 and 2).

The single extract of AP and TC was tested using one dose while the mixed extract (AP: TC) was tested at three doses similar with the fresh material made in laboratory, (3:10) as dose 1, (10:10 or 1:1) as dose 2, and (10:3) as dose 3. Each dose of these samples was made at variation of concentration were 8000, 4000, 2000, 1000, 500, 250, and 125 µg/ml.

Table 3 and 4 is the result of the activity test of single and mixed extract of jamu gendong pahitan (Bitter herb). From table 1, it is known that the single and mixed extract of bitter herbs were active as topoisomerase inhibitor, shown by the inhibition zone that produced. By the size of the inhibition zone, known that a single extract AP in more higher than TC, means more active as a topoisomerase inhibitor. This is implied in the activity of the mixed extract that showed dose 3 produced the largest zone of inhibition for all strains, where this dose contain higher AP than TC (10:3).

Table 3: Agar plate profile of single and mixed extract of jamu gendong

Extract	Dose	*Saccharomyces cerevisiae*			
		Wild	Mutant 1138	Mutant 1140	Mutant 1353
A. paniculata (AP)	-				
T. crispa (TC)	-				
Mixed (AP: TC)	1 (3:10)				
	2 (1:1)				
	3 (10:3)				

Notes: All assays were done in three replications (n = 3)

Table 4: Inhibition zone of single and mixed extract of jamu gendong

Concentration (µg/ml)	Samples	Inhibition zone (mm)			
		SC Wild	SC1140	SC1353	SC1138
8000	*Andrographis*	-	21.43±0.73	11.6±0.3	20.47±0.75
4000	*paniculata (AP)*	-	17.13±0.47	10.53±0.57	19.47±0.75
2000		-	15.83±1.36	9.6±0.26	15.7±0.72
1000		-	12.8±0.98	9.0±0.56	14.47±0.75

Concentration (µg/ml)	Samples	Inhibition zone (mm)			
		SC Wild	SC1140	SC1353	SC1138
500		-	10.1±3.57	-	12.1±0.36
250		-	-	-	10.53±0.35
125		-	-	-	-
8000	*Tinospora crispa (TC)*	-	16.73±0.15	14.56±0.31	16.77±0.21
4000		-	15.07±0.92	12.8±0.44	14.1±0.17
2000		-	13.7±0.17	11.0±0.61	13.37±0.15
1000		-	11.7±0.53	9.5±0.3	11.37±0.71
500		-	7.4±2.42	-	-
250		-	-	-	-
125		-	-	-	-
8000	Dose 1 (AP: TC = 3:10)	-	16.87±0.23	14.53±0.45	15.2±0.1
4000		-	14.57±0.06	13.17±0.31	13.6±0.36
2000		-	13.53±0.32	12.5±0.1	12.7±0.17
1000		-	10.27±0.35	10.57±0.12	11.67±0.06
500		-	-	9.27±0.40	-
250		-	-	-	-
125		-	-	-	-
8000	Dose 2 (AP: TC = 1:1)	-	19.97±1.1	15.67±0.06	15.93±0.06
4000		-	17.2±1.47	14.6±0.3	14.83±0.06
2000		-	14.53±0.95	13.2±0.1	12.8±0.26
1000		-	12.43±0.9	11.9±0.17	11.33±0.35
500		-	-	9.77±0.12	10.23±0.21
250		-	-	-	-
125		-	-	-	-
8000	Dose 3 (AP: TC = 10:3)	-	19.17±0.15	20.03±0.51	20.07±0.92
4000		-	18.87±0.35	17.13±0.15	18.0±0.23
2000		-	17.2±0.44	15.27±0.49	14.67±1.11
1000		-	14.07±0.75	13.07±0.31	11.8±1.01
500		-	11.73±0.31	11.3±0.44	9.6±0.36
250		-	8.8±0.2	7.6±0.37	6.7±1.21
125		-	-	-	6.9±1.56

Notes: All assays were done in three replications (n = 3)

Every data in table 3 is used to make dose-response curves and equation of linear regression, so the IC_{12} value is then can be calculated. The IC_{12} value indicates the results of the topoisomerase inhibitor activity test. A sample is declared active as a topoisomerase inhibitor if it fulfills one of these conditions, namely inhibiting one or more strains, has an IC_{12} value<8000µg/ml, or an IC_{12} value equal to or at least three times greater than S. cerevisiae strain 1138 [14]. Table 5 and 6 shows the IC_{12} values of all samples of jamu gendong pahitan.

Tabel 5: IC_{12} value of jamu gendong buy in the market and made in the laboratory

Jamu gendong	Dose	IC_{12} (µg/ml)				Inhibitor topoisomerase
		Wild	SC 1140	SC 1353	SC 1138	
Traditional market	1	-	-	-	-	-
Laboratory (AP: PC)	1 (3:10)	-	4176±282	>8000	5842±240	Top I
	2 (1:1)	-	4752±759	>8000	3810±410	Top I
	3 (10:3)	-	2179±334	7806±755	2039±185	Top I and II

Notes: All IC12 values were calculated from three linear regression equation

Tabel 6: IC_{12} value of single and mixed extracts

Extract	Dose	IC_{12} (µg/ml)				Topoisomerase Inhibitor
		Wild	SC 1140	SC 1353	SC 1138	
A. paniculata	-	-	648±47	1073±148	465±71	Top I and II
T. crispa	-	-	1101±200	2888±556	1342±213	Top I and II
Mixed	1 (3:10)	-	1441±162	1940±157	1342±103	Top I and II
	2 (1:1)	-	1067±149	1216±133	1296±91	Top I and II
	3 (10:3)	-	577±42	866±135	926±173	Top I and II

Notes: All IC12 values were calculated from three linear regression equation

Table 5 showed that all samples of jamu gendong bitter herbs was inactive to *S. cerevisiae* wild strains. Hence, these samples were not toxic to the human body. The IC_{12} values of jamu gendong bought from the market can't be calculated because there are no inhibition zones, which means inactive in inhibiting the topoisomerase enzyme I and II. From table 5, also showed that bitter herbs made in the laboratory have activity as topoisomerase I inhibitor (dose 1 and 2), and are active as topoisomerase I and II inhibitors (dose 3). From this data, we can conclude that dose 3 is the best formula for fresh material bitter herbs. The preparation of this potion suggested using a brewing technique at 70 °C to maintain the active compounds, especially those that are thermolabile in the materials (fresh leaves and stems) [23].

Table 6 showed that single and mixed extracts of jamu gendong pahitan were active as topoisomerase I and II inhibitors. The lower IC_{12} values mean the more active sample. By the IC_{12} value of a single extract, known that *A. paniculata* extract is more active than *T. crispa*. Activity of *A. paniculata* extract supported by its chemical contents such as androgafolide compounds that have an activity to inhibit cell proliferation in breast, colon, lung, prostate, leukemia, and ovarian cancer through the mechanism of increasing proapoptotic expression, namely Bax, caspase 3 and 9, as well as reducing the expression of antiapoptosis, namely bcl-2 [24]. This type of cancer has overexpression of topoisomerase I [25, 26]. This is following the results of the study above that *A. paniculata* leaf extract tends to have activity as an inhibitor of topoisomerase I.

In line with this research result, *T. crispa* extract reported has cytotoxic activity with an IC_{50} value of 314µg/ml, and apoptosis induction was 28.9% at 25 µg/ml of concentration [27]. In addition, *T. crispa* can also be lowering NF-kB gene expression at negative triple of breast cancer cell line and inhibit the cell at IC_{50} value of 30.64 µg/ml [11, 12].

The most active sample is mixed extract dose 3 that contains AP: TC (10:3). If all of the samples above were compared to the positive controls tested by Zuhrotun *et al.* [14], namely camptothecin as a topoisomerase I inhibitor drug with IC_{12} values in strains 1140, 1353, and 1138 were 432±140; 2828±494; and 95±53µg/ml respectively, the IC_{12} value of the mixed extract of bitter herbs dose 3 is still higher, mean it was not as good as camptothecin. But it still has the potency as a natural anticancer agent by inhibiting the topoisomerase I and II enzymes.

This research reported for the first time the activity of *A. paniculata* and *T. crispa* as a potion or in Indonesian peoples known as jamu gendong pahitan (bitter herbs) as a topoisomerase inhibitor. This finding can increase the potential usage of jamu gendong pahitan not only for prevent allergy, itch, and acne (anti-inflammation) as its traditional usage but also has a good prospect develop as natural anticancer agent.

CONCLUSION

It can be conclude seen that fresh material of jamu gendong pahitan (bitter herbs) bought from the market is inactive, while the fresh material of samples of jamu gendong pahitan made in laboratory doses 1 and 2 have topoisomerase I inhibitor activity. If seen from the IC_{12} value, the mixed extract dose 3, that contain extract of *A. paniculata* and *T. crispa* (10:3), has the best activity with IC_{12} values in strains 1138, 1140, and 1353 were 926.28±173, 576.75±42, and 865.5±135µg/ml respectively.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors assert that they do not possess any conflict of interest.

REFERENCES

1. Torri MC. Knowledge and risk perceptions of traditional Jamu medicine among urban consumers. Eur J Med Plants. 2013;3(1):25-39. doi: 10.9734/EJMP/2013/1813.
2. Isnawati DL, Sumarno. Minuman jamu tradisional sebagai kearifan lokal masyarakat di kerajaan majapahit pada abad ke-14 masehi. J Pendidikan Sejarah Indones. 2021;11(2):1-10.
3. Moelyono MW. Etnofarmasi. Bandung: Deepublish; 2017.
4. Yadav R, Yadav N, Kharya MD. Immunomodulation potential of Andrographis paniculata and Tinospora cordifolia methanolic extracts in combination forms. Int J Pharmacol Res. 2016;6(1):29-41.
5. Mussard E, Cesaro A, Lespessailles E, Legrain B, Berteina Raboin S, Toumi H. Andrographolide, a natural antioxidant: an update. Antioxidants. 2019;8(12):1-22. doi: 10.3390/antiox8120571.
6. Polu PR, Nayanbhirama U, Khan S, Maheswari R. Assessment of free radical scavenging and anti-proliferative activities of tinospora cordifolia Miers (Willd). BMC Complement Altern Med. 2017;17(1):1-12. doi: 10.1186/s12906-017-1953-3.
7. Kementrian Kesehatan RI. Formularium obat Herbal asli indonesia. Jakarta: Kemenkes RI; 2016.
8. Khan I, Khan F, Farooqui A, Ansari IA. Andrographolide exhibits anticancer potential against human colon cancer cells by inducing cell cycle arrest and programmed cell death via augmentation of intracellular reactive oxygen species level. Nutr Cancer. 2018;70(5):787-803. doi: 10.1080/01635581.2018.1470649.
9. Naomi R, Bahari H, Ong ZY, Keong YY, Embong H, Rajandram R. Mechanisms of natural extracts of Andrographis paniculata that target lipid-dependent cancer pathways: a view from the signaling pathway. Int J Mol Sci. 2022;23(11):1-15. doi: 10.3390/ijms23115972.
10. Paul S, Roy D, Pati S, Sa G. The adroitness of andrographolide as a natural weapon against colorectal cancer. Front Pharmacol. 2021;12:1-14. doi: 10.3389/fphar.2021.731492.
11. Mutiah R, Azizah LN, Annisa R, Listyana A. Profile of anticancer activities of Brotowali (Tinospora crispa L.) plants of various regions in east Java. J Pharm Sci. 2020;16(2):68-77.
12. Rashidi R, Ibahim MJ, Hamid H. Tinospora crispa extract enhances cisplatin-induced apoptosis in triple-negative breast cancer cells. Regeneration Med. 2016;4(2):1-10.
13. Srivastava M, Raghavan SC. DNA double-strand break repair inhibitors as cancer therapeutics. Chemistry & Biology. 2015;22(1):17-29. doi: 10.1016/j.chembiol.2014.11.013.
14. Zuhrotun A, Suganda AG, Wirasutisna KR, Wibowo MS. Isolation of the bioactive compound of michelia champaca L. bark and its activity test using mechanism-based yeast bioassay. Asian J Pharm Clin Res. 2016;9(5):158-61. doi: 10.22159/ajpcr.2016.v9i5.12856.
15. Zuhrotun A, Wiwiek I, Frederick A, Yuni EH, Imam AW. Topoisomerase inhibitor compound isolated from n-hexane fraction of yellow champaca stem bark (Michelia champaca L.). J Pharm Sci Res; 2018. p. 8-13.
16. Zuhrotun A, Hadisaputri YE, Alexander F, Suseno R, Wicaksono IA. Topoisomerase inhibitors activity of Yellow champaka (michelia champaca L.) bark extracts and fractions and its liriodenine contents. Int J App Pharm. 2022;14(4):151-4. doi: 10.22159/ijap.2022.v14s4.PP37.
17. Yuliantari NWA, Widarta IWR, Permana IDGM. The influence of time and temperature on flavonoid content and antioxidant activity of sirsak leaf (Annona muricata L.) using ultrasonic. J Food Sci Technol. 2017;4(1):35-42.
18. Kemenkes RI. Farmakope herbal Indonesia II. Vol. II. Jakarta: Kementrian Kesehatan Indonesia; 2017.
19. Haeria H, Andi TUP. Penentuan kadar flavonoid total dan aktivitas antioksidan ekstrak etanol daun bidara (Ziziphus spina-christi L.). J Pharm Sci. 2016;1(2):57-61.
20. Suharsanti R, Wibowo FXS. Uji aktivas antijamur ekstrak etanol daun som jawa terhadap pertumbuhan Candida albicans Untuk Menjamin Mutu Penggunaan sebagai obat Herbal Antikeputihan. Media Farmasi Indones. 2016;11(2):1067-74.
21. Yulianti W, Ayuningtyas G, Martini R, Resmeiliana I. Pengaruh Metode Ekstraksi dan Polaritas Pelarut Terhadap Kadar Fenolik Total Daun Kersen (Muntingia calabura L). J Sains Terapan. 2020;10(2):41-9. doi: 10.29244/jstsv.10.2.41-9.
22. Rahmi M, Putri DH. The antimicrobial activity of DMSO as a natural extract solvent. Serambi Biol. 2020;5(2):56-68.
23. Che Sulaiman IS, Basri M, Fard Masoumi HR, Chee WJ, Ashari SE, Ismail M. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of clinacanthus nutans lindau leaves by response surface methodology. Chem Cent J. 2017;11(1):1-11. doi: 10.1186/s13065-017-0285-1.
24. Pearngam P, Kumkate S, Okada S, Janvilisri T. Andrographolide inhibits cholangiocarcinoma cell migration by down-regulation

of claudin-1 via the p-38 signaling pathway. Front Pharmacol. 2019;10(827):1-13. doi: 10.3389/fphar.2019.00827.

25. Heestand GM, Schwaederle M, Gatalica Z, Arguello D, Kurzrock R. Topoisomerase expression and amplification in solid tumours: analysis of 24,262 patients. Eur J Cancer. 2017;83:80-7. doi: 10.1016/j.ejca.2017.06.019.
26. Tesauro C, Simonsen AK, Andersen MB, Petersen KW, Kristoffersen EL, Algreen L. Topoisomerase I activity and sensitivity to camptothecin in breast cancer-derived cells: a comparative study. BMC Cancer. 2019;19(1):1-15. doi: 10.1186/s12885-019-6371-0.
27. Harwoko W. Penapisan Senyawa Antikanker dari batang brotowali (Tinospora crispa L.) dan uji aktivitas sitotoksik pada kultur sel WiDr screening of anticancer compounds from brotowali (Tinospora crispa L.) bark and cytotoxic activity on WiDr cells culture. Acta Pharm Indones. 2018;6(2):51-9.