International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 4, 2022

Original Article

# TOPOISOMERASE INHIBITORS ACTIVITY OF YELLOW CHAMPAKA (*MICHELIA CHAMPACA* L.) BARK EXTRACTS AND FRACTIONS AND ITS LIRIODENINE CONTENTS

**ADE ZUHROTUN[1*], YUNI ELSA HADISAPUTRI[1], FREDERICK ALEXANDER[1], RUDY SUSENO[1], IMAM ADI WICAKSONO[2]**

[1]Biological Pharmacy Department, [2]Pharmacology and Clinical Pharmacy Department, Faculty of Pharmacy, Universitas Padjadjaran, Jalan Ir. Soekarno Km. 21 Jatinangor, Sumedang West Java, Indonesia 45363
*Email:ade.zuhrotun@unpad.ac.id

*Received: 17 Jul 2022, Revised and Accepted: 04 Sep 2022*

**ABSTRACT**

**Objective:** This research is aimed to investigate the activity of various extracts and fractions of yellow champaka (*Michelia champaca* L.) by mechanism-based yeast bioassay (MBYB) against the mutant yeasts. Liriodenin content, an active compound in *M. champaca*, will analyze to know the correlation with this assay.

**Methods:** The bark of *M. champaca* was extracted by maceration and graded maceration using n-hexane, ethyl acetate, and methanol. The methanol extract was fractionated using water, n-hexane and ethyl acetate in the liquid-liquid extraction process. Each extract and fractions were tested *in vitro* by mechanism-based yeast bioassay for their topoisomerase inhibitor activity. Liriodenine content of all samples was analyzed by thin layer chromatography (TLC) densitometry method.

**Results:** The yeast bioassay results showed that all extracts were active as topoisomerase inhibitors ($IC_{12}$ values under 8000 µg/ml) except MGM. Liriodenine can be used as a marker of active compound in ethyl acetate samples that were EAM, EAGM and EAF with Pearson analysis value-0.887 (P=0.153) and these samples were relatively more active than others.

**Conclusion:** This research showed that various extracts and fractions of yellow champaka bark have topoisomerase inhibitor's activity. Liriodenine can be used as a marker of the active compound so yellow champak bark is a potent natural agents for breast cancer.

**Keywords:** Yellow champaka, *Michelia champaca* L., Topoisomerase inhibitors, Mechanism-based yeast bioassay


DOI: https://dx.doi.org/10.22159/ijap.2022.v14s4.PP37 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

## INTRODUCTION

The cancer prevalence in the world is increasing from 14.1 million in 2012 to 18.1 million in 2018. These numbers always increase year by year [1, 2]. Breast cancer is the second highest cancer cases in worldwide, and the percentage of death caused by this disease is 6.6% [2]. While in Indonesia, nationally, cancer cases reported increases from 1.4 ‰ in 2013 to 1.8‰ in [3]. Breast cancer is one of the most common cancer cases for Indonesian women [4].

Cancer treatment is generally taken by surgery, radiation, and chemotherapy or a combination of all. Every patient has different cancer treatments depended on various types of cancer cells, tumor size, severity, and individual tolerance to drug side effects. Besides, the risk of drug resistance and toxic effects make cancer treatment more difficult [5]. Therefore still needed to search the new anticancer agents with low toxicity and more safety.

The prospect of finding sources for natural anticancer is now more promising, proven by the National Cancer Institute has released 68 of 488 were plant-based anticancer drugs used in single or combination therapy [6]. While in Indonesia, as many as 16 of 74 anticancer drugs in circulation today is a plant-derived medicine [7].

*Michelia champaca* L. is well known in Indonesia as yellow champaka is a potential plant to be developed as natural anticancer agents that support by some research results. Parthenolide isolated from the ethanol extract of *M. champaca* bark has cytotoxic activity toward the human epidermoid carcinoma of the nasopharynx test system (KB) with $IC_{50}$ value is 2.3 µg/ml [8]. Methanolic extract of *M. champaca* flower was active against Ehrlich Ascites carcinoma cell line [EAC] with $IC_{50}$ value is 147,5 µg/ml [9]. This yellow champak is one of the most active plant extracts from 23 Indonesian plants species that screened for their anticancer properties [10]. Liriodenine, an aporphine alkaloid isolated from ethyl acetate fraction of yellow champaka bark was proven active both as topoisomerase I and II inhibitors. This activity is one of the anticancer drug mechanisms [11].

This research is aimed to find out the prospect of yellow champaka bark as a natural agent for breast cancer. The bark was extracted and fractionated by using several solvents and each sample was then tested *in vitro* using a mechanism-based yeast bioassay. Chemical content and activity correlation between these assays were statistically analyzed using Liriodenine as the standard of topoisomerase inhibitors.

## MATERIALS AND METHODS

### Materials

Yellow champaka bark, n-hexane (Merck), chloroform (Merck), ethyl acetate (Merck), methanol, aqua distillate (chemical laboratory), dimethyl sulfoxide (DMSO, Merck), precoated plate silica gel GF254 (Merck), agar bacteriological (Oxoid), peptone (Oxoid), dextrose (Oxoid), yeast extract (Oxoid), and sodium chloride (Merck), camptothecin (Sigma) and Liriodenine (isolated by previous research with 95.25% of purity) [11].

### Sample preparations

A bark sample was collected from the campus area of Universitas Padjadjaran, Jantinangor, on July 2017. This material was identified as *Michelia champaca* L. or yellow champak in Herbarium Jatinangor at Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (No. 420/HB/07/2017). The fresh bark was sorted, coarsely cut, and dried.

An amount of 500 g of powdered yellow champak bark was extracted by graded and normal maceration procedures as follow. Firstly, the bark was macerated using n-hexane at room temperature; after 24 h the solvent was replaced by fresh n-hexane. This process was repeated until n-hexane filtrate was clear. Secondly, the rest of crude bark then air-dried and macerated using ethyl acetate with the same conditions. Finally, after the ethyl acetate filtrate was clear and the rest of the bark was air dried, the maceration process continued using methanol with the same

conditions. This graded maceration yield n-hexane extract (nHM), ethyl acetate extract (EAGM), and methanol extract (MGM) were 0.92, 3.18, and 11.36% respectively.

In a different container, a normal maceration process was done to 300 g of powdered yellow champak bark using ethyl acetate and methanol at room temperature for 3x24 h with solvent replacement every 24 h. This maceration yielded ethyl acetate extract (EAM), and methanol extract (MM) was 3.98% and 21.16%, respectively. After that, 80 g of MM was dissolved in water and extracted by n-hexane and ethyl acetate, respectively using a separating funnel. This fractionation process yielded n-hexane fraction (nHF), ethyl acetate fraction (EAF), and water fraction (WF) were 10.75, 14.75, and 50.77 %, respectively.

**Mechanism-based yeast bioassay**

Yeast *Saccharomyces cerevisiae* mutant strains SC1140, SC1353, and SC1138 were available at Biological Pharmacy Department. The yeasts were cultured in yeast peptone dextrose (YPD) broth and incubated at 30 °C for 36-48 h. Normal yeast *S. cerevisiae* was used as a control that was cultured in potato dextrose broth (PDB) and incubated at room temperature for 18-24 h. The broth was suspended in sterile 0.9% saline solution until the transmittance was 80% at 600 nm produced inoculums for assays.

The procedures of this assay were described in a previous study [11], in brief, were prepared agar plate using yeast peptone dextrose (YPD) agar 20 ml and yeast inoculums 1 ml, homogenized. Samples 50 µl in dimethyl sulfoxide-methanol (1:1) with a variation of concentration (500-8000 µg/ml) were poured into wells on the plate that made using perforator 6 mm diameter then incubated at 30 °C for 36-48 h. Camptothecin was prepared using the same condition in a variation of concentration (15.63-1000 µg/ml). The inhibition zone that was produced were measured and calculated by regression analysis to determine $IC_{12}$ value. This value means a required sample concentration (in µg/ml) that produced an inhibition zone of 12 mm around a well. The active extract should have values under 8000 µg/ml, while active fractions are under 4000 µg/ml. Samples that active against *S. cerevisiae* strain 1140 means a topoisomerase I inhibitor and the samples that active against *S. cerevisiae* strain 1353 means a topoisomerase II inhibitor.

**Liriodenine content analysis by thin layer chromatography (TLC) Densitometry**

Condition and analysis of TLC were adapted in [11, 12]. Liriodenine were prepared at range 80-140 µg/ml, extracts at 25,000 µg/ml, and fractions at 50,000 µg/ml of concentrations. All samples applied 5 µl at pre-coated plate silica, develop in chloroform-methanol (9:1) as mobile phase and analyze at 414 nm using TLC densitometer. Validation of this analysis was evaluated by linearity, accuracy, precision (repeatability and intermediate precision), limit of detection (LOD) and limit of quantification (LOQ).

**RESULTS**

The yeast bioassay results of yellow champak extracts and fractions can be seen in table 1. The validation result of TLC densitometry method can be seen in fig. 1 and table 2, while liriodenine content in each sample is in table 3.

**Table 1: The yeast bioassay results of yellow champak extracts and fractions**

| Samples | $IC_{12}$ value (µg/ml) | | |
|---|---|---|---|
| | **SC1140** | **SC1353** | **SC1138** |
| n-Hexane extract (nHM) | 7951.55±458.48 | 6813.09±494.17 | 2822.57±428.52 |
| n-Hexane fraction (nHF) | 680.04±575.70 | 1039.34±34.244 | 567.13±326.60 |
| Ethyl acetate extract (EAM) | 5157.59±1057.04 | 1890.04±442.77 | 4418.01±730.30 |
| Ethyl acetate extract (EAGM) | 7525.28±876.67 | 0.00±0.00 | 6250.15±364.21 |
| Ethyl acetate Fraction (EAF) | 246.63±53.46 | 651.53±226.12 | 2315.19±831.79 |
| Methanol extract (MM) | 3619.20±502.50 | 4566.4±589.65 | 1452.77±492.39 |
| Methanol extract (MGM) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Water fraction (WF) | 2442.55±185.45 | 1801,99±246,99 | 2525.40±721.39 |
| Camptothecin (CPT) | 195.20±52.24 | 0.00±0.00 | 2519.53 |
| Liriodenine (Lir)* | 22.15±1.71 | 24.76±0.56 | 7.02±1.85 |

Note: (0.00±0.00) = not active; *previously research [11]

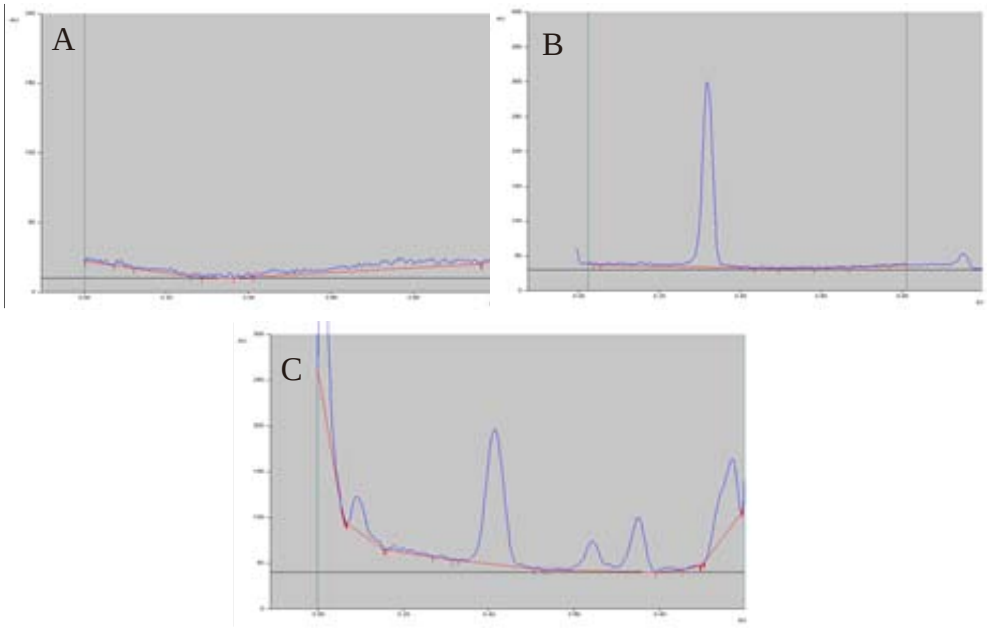


**Fig. 1: Specificity of spectrum blank (A), liriodenine (B), and extract samples (C)**

**Table 2: Liriodenine analysis validation of yellow champak extracts and fractions**

| Parameters | Results |
|---|---|
| Linearity | y = 10392x–2255.1 |
| $R^2$ | $R^2$ = 0.9474 |
| Accuracy (%) | 79.48-90.93 |
| Precicion (RSD) | 1.39±0.14 |
| LOD | 0.0709 µg/spot |
| LOQ | 0.2151 µg/spot |

**Table 3: Liriodenine content in yellow champak extracts and fractions**

| Samples | Liriodenine (%w/w) |
|---|---|
| n-Hexane extract (nH) | 0.00±0.00 |
| n-Hexane fraction (nHF) | 0.035±0.00 |
| Ethyl acetate extract (EAM) | 0.0093±0.00 |
| Ethyl acetate extract (EAGM) | 0.0075±0.00 |
| Ethyl acetate Fraction (EAF) | 0.39±0.04 |
| Methanol extract (MM) | 0.0064±0.00 |
| Methanol extract (MGM) | 0.0057±0.00 |
| Water fraction (WF) | 0.00±0.00 |

## DISCUSSION

In yeast bioassay, normal yeast was used as a control, besides mutant strains. The results showed that this normal yeast could be grown at media tested by extracts, while the mutant strains showed inhibition zones. The inhibition zone form after incubation indicates that samples or extracts contain or are a DNA-damaging compound or topoisomerase inhibitor (anticancer substance) [13].

Table 1 showed that all extracts were active as topoisomerase inhibitors ($IC_{12}$ values under 8000 µg/ml) except methanol extract (MGM). All fractions tested were active as topoisomerase inhibitors ($IC_{12}$ values under 4000 µg/ml). This assay revealed the potency of yellow champak bark as natural anticancer with the mechanism of topoisomerase inhibitors. By $IC_{12}$ values showed that nHM, nHF, EAM, EAF, MM, and WF were active against all mutant strain mean these extracts and fractions were active as topoisomerase I and II inhibitors. EAGM showed activity against 1140 and 1138 mutant strains mean this extract was active as topoisomerase I inhibitor.

One-way analysis of variants (ANOVA) using SPSS v.22 to all these $IC_{12}$ values showed significance to SC1140, SC1353, and SC1138 were 0.744, 0.848, and 0.072, respectively ($\alpha$=0.05). It means that mutant strain SC1138 is the most significant activity as a topoisomerase inhibitor. This result fits with the fact that SC 1138 is *rad52* mutant yeast that deletion of DNA double-strand break repair and meiotic recombination pathway**.** The activity of this mutant is represented by topoisomerase I and II inhibitors [13].

Validation is done to ensure the method used is valid and the data obtained can be trusted truthfully. The profile of TLC spectrum meets the requirement because the instrument can distinguish blanks without analytes and analytes in the sample (fig. 1). In general, the TLC densitometry method used is valid with parameters that can be seen in table 2 and liriodenine content in table 3. It can saw that all samples that are active as topoisomerase inhibitors and contain liriodenine except nH and WF. The higher liriodenine level lies in ethyl acetate samples i. e EAM, EAGM and EAF. Analysis of Pearson correlation show value-0.887 (*p* value =0.153) that categorized high correlation [14]. It means that liriodenine level of those ethyl acetate samples is correlated with their activity as topoisomerase inhibitors and a higher liriodenine level will associate with a lower $IC_{12}$ value (SC1138) or, the more active samples. So Liriodenine can be used as a marker of active compound in ethyl acetate samples. But, in nH and WF there must be other compounds except for liriodenine that also acts as topoisomerase inhibitors and further research is needed to find out what the compounds are.

Camptothecin is an anticancer agent derived from *Camptotheca acuminata* Decne family Cornaceae, which acts as a topoisomerase I inhibitor. Camptothecin is no longer used clinically because of its toxic properties and low water solubility. Its derivate compounds, topotecan, and irinotecan, have been used in the treatment of solid tumors [15]. Liriodenine was isolated from yellow champak bark in previous research and is active both as topoisomerase I and inhibitors [11]. Liriodenine proves as the strongest inhibitor to A549 human lung adenocarcinoma cells and MDA-MB-231 human breast adenocarcinoma cells [16].

This research finds out that various extracts and fractions of yellow champaka bark are active as topoisomerase inhibitors or DNA damaging agents. This mechanism is similar to the activity of *Valeriana jatamansi* Jones fraction that induces cell death via DNA damage in human breast cancer cells [17]. It can be concluded that yellow champaka bark is a potent natural agents for breast cancer. Other research that reported the activity of *M. champaca* related to cell line only covered to KB cell and EAC cell [8, 9].

## CONCLUSION

*In vitro* study using mechanism-based yeast bioassay showed that various extracts and fractions of yellow champak bark have activity as topoisomerase inhibitors. In general, the active samples as topoisomerase inhibitors contain liriodenine and moreover, in ethyl acetate samples, liriodenine can be used as a marker of the active compound. By this research conclude that yellow champak bark is potent natural agent for breast cancer.

## ACKNOWLEDGMENT

The authors convey grateful thanks to the Rector of Universitas Padjadjaran and Director of Research, Community service and Innovation for the Hibah Internal Unpad grant batch II contract no 872/UN.6.3.1/lT/2017 and 750r/UN6. O/PM/2018.

## FUNDING

Hibah Internal Unpad grant batch II contract no 872/UN.6.3.1/lT/2017 and 750r/UN6. O/PM/2018.

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

## REFERENCES

1. Forman D, Ferlay J. The global and regional burden of cancer. In: Stewart BW, Wild CP, editors. World cancer report. Geneva: WHO Press; 2014. p. 16-53.
2. International Agency for Research on Cancer (IARC). Fact Sheet IARC: 2019.
3. Ministry of Health Republic of Indonesia. Main research of national basic of Health Research 2018. The agency of Research and Development. Jakarta: Ministry of Health RI; 2019.
4. Ministry of Health Republic of Indonesia, Stop Cancer. Center of Data and Information, Jakarta: Ministry of Health RI; 2015.
5. Lyon L. Diagnosis and treatment of cancer. In: Bozzone DM. editor. The biology of cancers. New York: Chelsea house Publishers; 2007. p. 11-131.
6. National Cancer Institute (NCI). A to Z list of anticancer drugs. Available from: https://www.cancer.gov/about-cancer/treatment/drugs. [Last accessed on 10 Oct 2016]
7. The Indonesian Pharmacists Association. Information of Indonesian drug Spesialite Volume 50. Antineoplastic, Immunosuppressant and drug for Paliatic. Jakarta: PT ISFI publisher; 2016.
8. Hoffmann JJ, Torrance SJ, Widehopf RM, Cole JR. Cytotoxic agents from Michelia champaca and Talauma ovata: parthenolide and costunolide. J Pharm Sci. 1977;66(6):883-4. doi: 10.1002/jps.2600660642, PMID 889597.
9. Ananthi T, Chitra M, Aruna B. *In vitro* anticancer activity of Michelia champaca L. flowers against Ehrlich ascites carcinoma cell line. Int J Pharm Biol Sci. 2014;5(4):357-63.

10. Zuhrotun A, Suganda AG, Wirasutisna KR, Wibowo MS. Anticancer screening of selected apocynaceae, simaroubaceae and magnoliaceae of Indonesian plants using mechanism-based yeast bioassay. Int J Pharm Sci Rev Res. 2015;35(2):90-4.
11. 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.
12. Mongkolrat S, Palanuvej C, Ruangrungsi N. Thin layer chromatography and image analysis of selected liriodenine bearing plants in Thailand. J Health Res. 2013;27(2):67-72.
13. Gunatilaka AAL, Kingston DGI. DNA-damaging natural product with potential anticancer activity. In: Atta-ur-Rahman. editor. Studies in natural products chemistry: structure and chemistry (part F). Amsterdam: Elsevier Science BV; 1998. p. 457-505.
14. Mukaka MM. Statistics Corner: A guide to the appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71. PMID 23638278.
15. Cragg GM, Kingston DGI, Newman DJ. Anticancer agents from natural products. Camptothecin and its Analogs. Boca Raton: CRC Press. 2005.
16. Yeh YT, Huang JC, Kuo PL, Chen CY. Bioactive Constituents from Michelia champaca. Nat Prod Commun. 2011;6(9):1251-2. PMID 21941890.
17. Zhu Z, Shen W, Tian S, Yang B, Zhao H. F3, a novel active fraction of Valeriana jatamansi Jones induces cell death via DNA damage in human breast cancer cells. Phytomedicine. 2019;57:245-54. doi: 10.1016/j.phymed.2018.12.041, PMID 30797986.