

Received: 2021.01.17

Accepted: 2021.05.06

Published: 2021.05.26

Cytotoxicity and Antiplasmodial Properties of Different *Hylocereus polyrhizus* Peel Extracts

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1,2 **Rudi Hendra**
B 1 **Rohimatul Khodijah**
BC 1 **Rianti Putri**
BC 2,3 **Riezki Amalia**
DF 1 **Yuli Haryani**
DEF 1 **Hilwan Yuda Teruna**
DEF 2,3 **Rizky Abdulah**

1 Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Riau, Pekanbaru, Indonesia
2 Center of Excellence in Pharmaceutical Care Innovation, Universitas Padjadjaran, Bandung, Indonesia
3 Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia

Corresponding Author: Rudi Hendra, e-mail: rudi.hendra@lecturer.unri.ac.id

Source of support: This study was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through national competitive grant research, contract no. 392/UN.19.5.1.3/PT.01.03/2020

Background: Dragon fruit (*Hylocereus polyrhizus*) is one of the most common fruits in tropical countries, including Indonesia. The unique deep purple-colored pulp of the fruit is eaten whole and consumed as juice. However, the inedible thick peel is wasted, causing environmental issues. In this study, the toxic, cytotoxic, and antiplasmodium activity from various extract of *H. polyrhizus* peels were examined.

Material/Methods: We evaluated the cytotoxicity and antiplasmodial properties of the various peel extracts by using different organic solvents. The extraction of the peels was conducted using maceration to obtain pigment, *n*-hexane, dichloromethane, and ethyl acetate extracts. The toxicity of the extract was assessed using the brine shrimp lethality test, followed by WST assay to test in vitro cytotoxic properties and in vitro antiplasmodial properties in 2 *Plasmodium falciparum* strains (3D7 and W2).

Results: The *n*-hexane, dichloromethane, and ethyl acetate extracts depicted various levels of activity, whereas the pigment extract did not show any activities. However, dichloromethane demonstrated a high toxicity level with LC₅₀ of 10.32±0.13 µg/mL and a weak cytotoxic level against SK-OV-3 cell lines (IC₅₀ of 560.86±0.63 µg/mL). Moreover, the dichloromethane and *n*-hexane extracts showed high and promising antiplasmodial activity with IC₅₀ 2.13±0.42 and 6.51±0.49 µg/mL, respectively.

Conclusions: The dichloromethane extract demonstrated high antiplasmodial activity. Our observations have elucidated the cytotoxic and antiplasmodial activity of the peel of dragon fruits and can be used as a foundation for further research into the isolation and bioactivity of secondary metabolites.

Keywords: Antimalarials • Cytotoxicity Tests, Immunologic • Plant Extracts • Toxicity Tests

Full-text PDF: <https://www.basic.medscimonit.com/abstract/index/idArt/931118>



2249



1



2



29



Background

Hylocereus polyrhizus, commonly known as dragon fruit or pitaya, is a vine cacti belonging to the Cactoideae cactus sub-family [1]. This species' genus comes from Central and South America and has been an important crop in Southeast Asia since it was introduced through the Philippines in the sixteenth century. The Mayan people used the fruit as a diuretic and wound disinfectant and for the treatment of heart disease, tumors, hypoglycemia, and dysentery [2]. In the last 2 decades, it has been reported that the fruits from this genus possess antioxidant, antiproliferative [3], and antimicrobial pharmacological activity [4].

Dragon fruit's exotic aesthetic appearance, with its enticing deep purple-colored pulp, makes the fruit highly appealing in the European, U.S., and Asian markets. The deep purple color of the pulp comes from a group of pigments known as nitrogen-containing betalains [5]. Wybraniec et al fully characterized 3 major betalains along with 7 minor betalains present in the pulp and peel of *H. polyrhizus* [1,6]. Also, high levels of the essential fatty acids linoleic acid and linolenic acid have been identified in the seeds [7]. The steroids β -amyrin and γ -sitosterol were identified in the peel with gas chromatography and mass spectrometry and were quantified to have a value of 15.87% and 9.35%, respectively [8].

Despite the unique red color and shape of this plant, it is one of the most well-known fruits in tropical countries, including Indonesia, where it is consumed in beverages and for dessert. The thickness of the peel (22% of its total weight) causes environmental problems with waste since it is not used optimally. The pigment extract from this fruit peel demonstrated good free radical-scavenging activity by DPPH assay in our previous study, with 159.6 $\mu\text{g}/\text{mL}$ of half-maximal inhibitory concentration (IC_{50}) [9]. Apart from its antioxidant properties, Vijayakumar et al reported that *H. polyrhizus* peel extract exhibited a high sun protection factor value of 35.02 ± 0.39 at 1.00 mg/mL and broad-spectrum UVA and UVB photoprotection, indicating that this species is highly capable of substituting synthetic sunscreen agents as a natural active ingredient in the cosmetic industry [10]. Additionally, betalain pigments extracted from the species were used as a coloring agent in a lipstick formulation study [11].

Moreover, the pigment and ethyl acetate extracts inhibited 60% to 75% of growth for *Candida albicans* at 500 $\mu\text{g}/\text{mL}$ and 65% to 100% inhibition at 200 $\mu\text{g}/\text{mL}$ for *Streptococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Vibrio alginolyticus* [12,13]. Further research will be conducted on the biological activities and secondary metabolites derived from the peel of *H. polyrhizus*, as well as on the relationship between the isolated compounds and their biological activities. To investigate

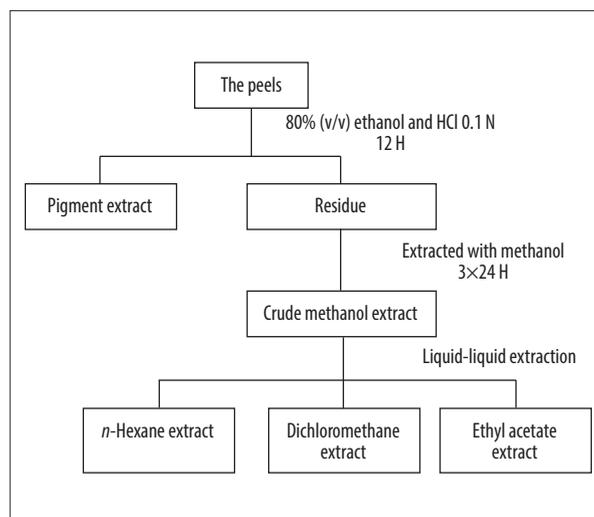


Figure 1. Extraction methods.

the bioactive extracts and type of secondary metabolites (polar, semipolar, and nonpolar), the species are extracted with different organic solvents. Therefore, the aim of this study was to evaluate the cytotoxic and antiplasmodial properties of the various peel extracts of this species by using different organic solvents.

Material and Method

Extraction

Dragon fruit was obtained from a traditional market in Pekanbaru City, Indonesia. The fresh peels were cleaned, cut into small pieces, blended, and kept in the refrigerator until use. The extraction was divided into 2 steps, pigment and non-pigment extractions, as reported by Hendra et al (Figure 1) [9,12,13]. The extraction was conducted using the organic solvents ethanol, *n*-hexane, dichloromethane, and ethyl acetate.

Toxicity Assay

The level of toxicity of the extracts was calculated using the brine shrimp lethality test (BSLT). The test method was performed by preparing 10 vials, each filled with 2 mL of seawater, and a 2-fold dilution was produced to generate a number of concentrations. An aliquot (0.1 mL) with approximately 10 nauplii were added to each vial and allowed to sit for 24 h. The vials were observed and dead larvae were counted after 24 h. Dimethyl sulfoxide (DMSO) was used as a negative control. Based on the mortality percentage, the concentration that led to 50% mortality (LC_{50}) was determined by the use of the graph of median mortality percentage vs log of concentration [14,15]. The assay was done in triplicate, and the data were reported as mean \pm standard deviation.

Table 1. Toxicity, cytotoxicity, and antiplasmodial activity of peels of *H. polyrhizus* extracts.

Extract	Yield (% g/g fresh sample)	Toxicity (LC ₅₀ ; µg/mL)	Cytotoxicity (IC ₅₀ ; µg/mL)		Antiplasmodial (IC ₅₀ ; µg/mL)
			SK-OV-3	HeLa	
Pigment	0.82	>1000	>1000	>1000	>100
<i>n</i> -Hexane	1.04	23.6±0.40	>1000	>1000	6.51±0.49
Dichloromethane	1.02	10.32±0.13	560.86±0.63	>1000	2.13±0.42
Ethyl acetate	2.26	148.93±0.57	>1000	>1000	>100

SK-OV-3 – ovarian cancer cell line; HeLa – cervical cancer cell line. The biological activities were tested in triplicate and reported as mean±standard deviation.

Cytotoxicity (MTS) assay

Ovarian cancer cell line (SK-OV-3) and cervical cancer cell line (HeLa) cells were obtained from the collection of the Department of Pharmacology and Clinical Pharmacy, Universitas Padjadjaran. The cells were grown in Dulbecco's modified Eagle's medium (Gibco, Paisley, UK) containing 10% of heat-inactivated fetal bovine serum (Gibco, Paisley, UK) and 1% penicillin-streptomycin solution (Gibco, Paisley, UK). The cytotoxicity assay was performed in 96-well plates, and cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂. Cells with 70% to 80% confluency were refreshed with new serum-free medium, further incubated for 4 h, and then treated with extracts in the amounts of 7.8125 µg/mL, 15.625 µg/mL, 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL. After 24 h of incubation, reagent cell counting kit-8 (Dojindo, Rockville, MD, USA) was added, and the mixtures were incubated for 2 h. DMSO was used as a negative control to avoid false results and the standard drug cisplatin was used as a positive control. The absorbance of the cell suspensions was measured using a Tecan Infinite spectrophotometer (Tecan, Grodig, Austria) at 450 nm [16]. The assay was done in triplicate and the data were reported as mean±standard deviation.

In Vitro Antiplasmodial Assays

Chloroquine-sensitive 3D7 and chloroquine-resistant W2 *Plasmodium falciparum* strains were used in this assay. A total of 1 mg of the sample was dissolved in 100 µL DMSO (stock solution, concentration of 10 000 µg/mL), and a serial dilution was made from the stock solution. The parasites used in this assay were synchronous parasites (Ring stage) with ±1% parasitaemia. A total of 2 µL of test solution with different concentrations was taken and added to each well of a 96-well plate. Next, 198 µL of parasite was added, so that the final concentrations of test material were 100 µg/mL, 10 µg/mL, 1 µg/mL, 0.1 µg/mL, and 0.01 µg/mL. The plate was placed in a chamber with mixed gas (O₂ 5%, CO₂ 5%, N₂ 90%). The plate was then incubated at 37°C for 48 h. The culture was then harvested,

and a thin layer of blood was formed with 20% Giemsa staining. The blood test was performed by counting the number of infected erythrocytes for every 1000 normal erythrocytes under a microscope. DMSO was used as a negative control to avoid false results and Artemisinin was used as a positive control (standard drug). Data were then used to assess the percentage of growth and the percentage of inhibition.

Based on the percentage of inhibition results, statistical analysis was performed with a probit analysis using SPSS version 20 to determine the IC₅₀ value or concentration of the test material that could inhibit the growth of the parasite by as much as 50% [17]. The assay was done in triplicate and the data were reported as mean ± standard deviation.

Statistical Analysis

SPSS version 20 was used for all analyses. Assays were performed in triplicate and data were reported as mean±standard deviation.

Results

In this present study, the fresh peels of *H. polyrhizus* were extracted with various organic solvents. The yields of the extracts are presented in **Table 1**. The ethyl acetate extract showed the highest yield, followed by *n*-hexane, dichloromethane, and pigment extracts, with values of 2.26%, 1.04%, 1.02%, and 0.82%, respectively. The toxicity, cytotoxicity, and antiplasmodial activity were tested in triplicate. The findings showed that the extracts exhibited varying levels of toxicity, cytotoxicity, and antiplasmodial activity, as shown in **Table 1**.

To investigate the pigment compounds in the extract, the extracts were analyzed using high-performance liquid chromatography (HPLC). The results are shown in **Figure 2**.

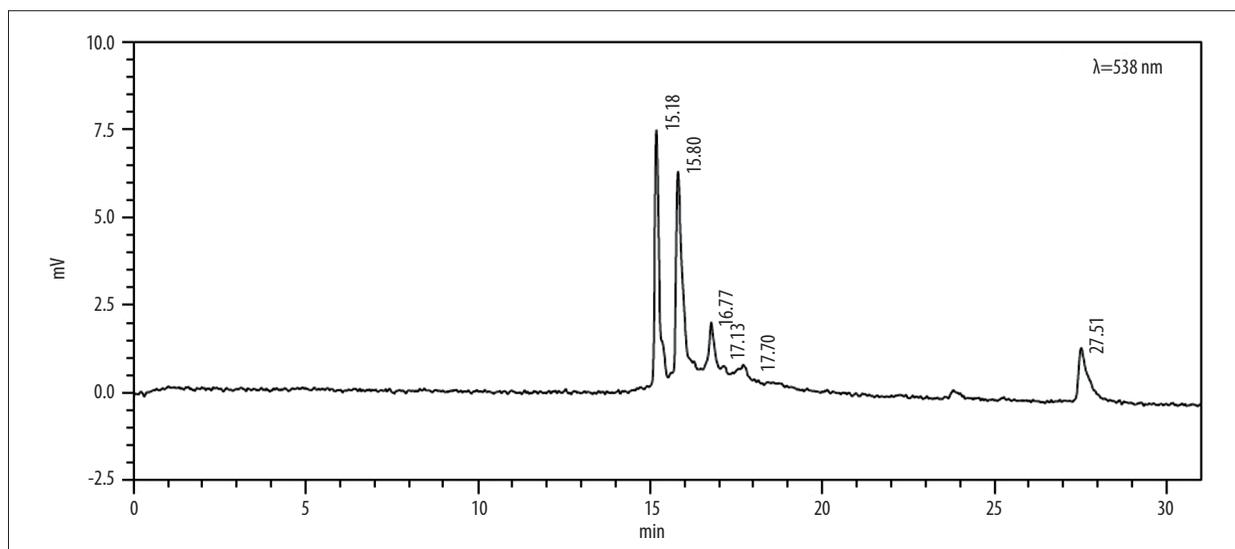


Figure 2. High-performance liquid chromatography profile of betalainins from dragon fruit peel; λ : 538 nm.

Discussion

In this present study, the fresh peels of *H. polyrhizus* were tested for their cytotoxicity and antiplasmodial activity. The extraction process was divided into 2 steps, betalains (pigment) and non-pigment extractions. To obtain pigment extract, the peels were macerated with 80% ethanol and HCl 0.1 N. This extraction method is commonly used to obtain betalains, and aqueous ethanol with slight extraction acidification improves betacyanine stability and prevents polyphenoloxidation [18]. HPLC analysis was conducted to measure the betalains in the pigment extracts (Figure 2) using a method similar to that previously described by Wybraniec et al in their report on chromatogram patterns [1,9]. Also, the non-pigment compounds were extracted after the pigment extract was obtained. The peel residues were macerated with methanol, which was followed by sonication. This technique increases the yield of the compounds extracted from the non-pigment components and enhances the solvent used for the extraction diffusion into the cells so that the desired compounds are dissolved [19].

The toxicity levels were measured using the BSLT, a simple, high-performance toxicity test for bioactive substances or extracts that is based on the ability of test compounds to kill shrimp (*Artemia salina*), a simple zoological organism [20]. In the study done by Meyer et al, plant extracts demonstrated toxicity of $LC_{50} < 1000$ ppm, while the extracts were regarded as highly toxic to the sage shrimp, with $LC_{50} < 30$ ppm [21]. The present study showed that the extracts exhibited varying levels of toxicity. The extract of dichloromethane was highly toxic, followed by that of *n*-hexane, ethyl acetate, and pigment extracts with LC_{50} values of 10.32 ± 0.13 $\mu\text{g/mL}$, 23.6 ± 0.40 $\mu\text{g/mL}$, 148.93 ± 0.57 $\mu\text{g/mL}$, and >1000 $\mu\text{g/mL}$, respectively (Table 1). Brine shrimp toxicity predicted cytotoxicity and parasite activity.

We also found a strong association between BSLT toxicity and cytotoxicity to the human nasopharyngeal carcinoma (9 KB) cell line and other solid tumors, as well as in vivo in murine leukemia. The BSLT detects strong anticancer activity but is limited in its predictive ability to differentiate between strong to moderate and poor anticancer compounds. Therefore, the BSLT provides a fast, initial screening for powerful cytotoxins and enables a finer degree of discrimination in cancer [22]. Hence, in the present study, we further investigated the cytotoxicity and antiplasmodial activity of the extracts.

After the BSLT study, the potency of the extracts as anticancer properties was determined and the cytotoxic activity in SK-OV-3 and HeLa cell lines was examined using the MTS assay. Studies have shown that the in vitro cytotoxicity MTS assay is a practical way to evaluate cell viability. The key features of this test are its ease of use, precision, and rapid toxicity result. The MTS assay may also be a valuable method in assessing risk to human health if adequate sensitivity and specificity of the test are demonstrated [23]. Table 1 shows the various cytotoxicity levels of the extracts in SK-OV-3 and HeLa cell lines. None of the extracts showed toxicity against HeLa cell lines. However, the extracts of dichloromethane showed weak toxicity to SK-OV-3, with an IC_{50} value of 560.86 ± 0.63 $\mu\text{g/mL}$; this finding is in agreement with that of the U.S. National Cancer Institute [24]. In the present study, the peel extracts showed weak activity against both cancer cell lines. However, Luo et al reported that the peel extracts inhibited different cancer cells (PC3, Bcap-37, and MGC-803 cell lines), with IC_{50} values ranging from 450 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$ [8]. Surprisingly, the pigment (betalain) extract did not display cytotoxicity toward either cell line. However, in another study, betalain extracts from cactus pear fruit (*Opuntia ficus-indica*) applied to the SK-OV-3 ovary cell line induced a significant increase in apoptosis and inhibition of cell growth [25].

In the present study, all 4 extracts were found to have various levels of activity against chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum* (Table 1). Pigment and ethyl acetate extracts did not show activity, while *n*-hexane and dichloromethane did show activity. The level of activity observed with dichloromethane was about 3 times higher than that of *n*-hexane, with IC_{50} 2.13 ± 0.42 and IC_{50} 6.51 ± 0.49 $\mu\text{g/mL}$, respectively. Extract activities were classified into 4 groups according to their IC_{50} values, in accordance with the WHO guidelines and fundamental requirements of antiparasitic drug discovery, as follows: high activity ($IC_{50} \leq 5$ $\mu\text{g/mL}$); promising activity (5 $\mu\text{g/mL} < IC_{50} \leq 15$ $\mu\text{g/mL}$); moderate activity (15 $\mu\text{g/mL} < IC_{50} \leq 50$ $\mu\text{g/mL}$); and poor activity ($IC_{50} > 50$ $\mu\text{g/mL}$) [17,26]. The dichloromethane and *n*-hexane extracts can therefore be classified as having high and promising antiplasmodial activity, respectively.

Regarding the chemical composition of the peel of this species, Lou et al (2018) identified 2 major phytosterols, β -amyirin and β -sitosterol [8], which could be responsible for the cytotoxicity and antiplasmodial activity of the extract. Isolated β -amyirin from *Dendropanax morbifera* was reported to have cytotoxicity against SK-OV-3 cell lines and antiplasmodial activity, with an IC_{50} of 5.29 and IC_{50} of 38.36 $\mu\text{g/mL}$, respectively [27]. Further, β -sitosterol was analyzed for its cytotoxicity and antiplasmodial activity against SK-OV-3, and the results showed that it exhibited antiplasmodial activity with an IC_{50} of 8.20 $\mu\text{g/mL}$ and no activity to inhibit SK-OV-3 cells [28,29]. The present study is therefore the first analysis of the antiplasmodial activity of *H. polyrhizus* peel. We will investigate the secondary metabolites responsible for this activity in future studies.

Conclusions

This research has shown the promising cytotoxicity and antiplasmodial activity of *H. polyrhizus* peel. The dichloromethane extract demonstrated high antiplasmodial activity. The findings provide a starting point for further research into the isolation and bioactivity of secondary metabolites in the extract of *H. polyrhizus*.

Acknowledgements

We would like to thank the Center of Research and Community Development, University of Riau and Ministry of Research, Technology and Higher Education of the Republic of Indonesia, and Ministry of Research, Technology and Higher Education of the Republic of Indonesia for their support.

References:

- Wybraniec S, Platzner I, Geresh S, et al. Betacyanins from vine cactus *Hylocereus polyrhizus*. *Phytochemistry*. 2001;58(8): 1209-12.
- Hernández YDO, Salazar JAC. Pitahaya (*Hylocereus spp.*): A short review. *Comun Sci*. 2012; 3(4):220-37
- Wu L-C, Hsu H-W, Chen Y-C, et al. Antioxidant and antiproliferative activities of red pitaya. *Food Chem*. 2006;95(2):319-27
- Nurmahani M, Osman A, Hamid AA, et al. Antibacterial property of *Hylocereus polyrhizus* and *Hylocereus undatus* peel extracts. *Int Food Res J*. 2012;19(1):77
- Rebecca O, Boyce A, Chandran S. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *Afr J Biotechnol*. 2010;9(10):1450-54
- Wybraniec S, Nowak-Wydra B, Mitka K, et al. Minor betalains in fruits of *Hylocereus species*. *Phytochemistry*. 2007;68(2): 251-59.
- Ariffin AA, Bakar J, Tan CP, et al. Essential fatty acids of pitaya (dragon fruit) seed oil. *Food Chem*. 2009;114(2):561-64
- Luo H, Cai Y, Peng Z, et al. Chemical composition and in vitro evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. *Chem Cent J*. 2014;8(1):1
- Hendra R, Masdeatresia L, Abdulah R, Haryani Y, editors. Red dragon peel (*Hylocereus polyrhizus*) as antioxidant source. AIP Conference Proceedings; 2020: AIP Publishing LLC
- Vijayakumar R, Abd Gani SS, Zaidan UH, et al. Exploring the potential use of *Hylocereus polyrhizus* peels as a source of cosmeceutical sunscreen agent for its antioxidant and photoprotective properties. *Evid Based Complement Alternat Med*. 2020;2020:7520736
- Azwanida N, Normasarah N, Afandi A. Utilization and evaluation of betalain pigment from Red dragon fruit (*Hylocereus polyrhizus*) as a natural colorant for lipstick. *Jurnal Teknologi*. 2014;69(6):3326
- Hendra R, Masdeatresia L, Abdulah R, Haryani Y, editors. Antibacterial activity of red dragon peel (*Hylocereus polyrhizus*) pigment. IOP Conf Ser: IOP Publishing. *J Physic*; 2019
- Hendra R, Masdeatresia L, Almurdani M, et al. editors. Antifungal activity of red dragon peel (*Hylocereus polyrhizus*). IOP Conf Ser: IOP Publishing. *Mater Sci Eng*; 2020:833012014
- Jasril J, Teruna HY, Aisyah A, Nurlaili N, Hendra R. Microwave assisted synthesis and evaluation of toxicity and antioxidant activity of pyrazoline derivatives. *Indones J Chem*. 2019;19(3):583-91
- Syahmi ARM, Vijayarathna S, Sasidharan S, et al. Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis Jacq.* (oil palm leaf) methanol extract. *Molecules*. 2010;15(11):8111-21
- Rachmawati H, Sundari S, Nabila N, et al. Orf239342 from the mushroom *Agaricus bisporus* is a mannose binding protein. *Biochem Biophys Res Commun*. 2019;515(1):99-103
- Jansen O, Tits M, Angenot L, et al. Anti-plasmodial activity of *Dicoma tomentosa (Asteraceae)* and identification of urospermal A-15-O-acetate as the main active compound. *Malar J*. 2012;11(1):289
- Strack D, Vogt T, Schliemann W. Recent advances in betalain research. *Phytochemistry*. 2003;62(3):247-69
- Liang Y-Z, Xie P, Chan K. Quality control of herbal medicines. *J Chromatogr B*. 2004;812(1-2):53-70
- Wu C. An important player in brine shrimp lethality bioassay: The solvent. *J Adv Pharm Technol Res*. 2014;5(1):57
- Meyer B, Ferrigni N, Putnam J, et al. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med*. 1982;45(1):31-34
- Ghisalberti EL. Detection and isolation of bioactive natural products. In: Colegate SM, Molyneux RJ, editors. *Bioactive natural products: Detection, isolation, and structural determination*. Second ed. Florida: CRC Press; 2008;18
- Malich G, Markovic B, Winder C. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*. 1997;124(3):179-92
- Widiyastuti Y, Sholikhah IYM, Haryanti S, editors. Cytotoxic activities of ethanolic and dichloromethane extract of leaves, stems, and flowers of Jarong [*Stachytarpheta jamaicensis* (L.) Vahl.] on HeLa and T47D cancer cell line. AIP Conference Proceedings; 2019: AIP Publishing LLC; 2019;2202(1)10.1063/1.5141714.
- Zou D, Brewer M, Garcia F, et al. Cactus pear: A natural product in cancer chemoprevention. *Nutr J*. 2005;4(1):1-12
- Fidock DA, Rosenthal PJ, Croft SL, et al. Antimalarial drug discovery: Efficacy models for compound screening. *Nat Rev Drug Discov*. 2004;3(6):509-20
- Chung IM, Kim MY, Park SD, et al. In vitro evaluation of the antiplasmodial activity of *Dendropanax morbifera* against chloroquine sensitive strains of *Plasmodium falciparum*. *Phytother Res*. 2009;23(11):1634-37
- Kim KH, Choi SU, Kim CS, Lee KR. Cytotoxic steroids from the trunk of *Berberis koreana*. *Biosci Biotechnol Biochem*. 2012;76(4):825-27
- Mwangi E, Keriko J, Machocho A, et al. Antiprotozoal activity and cytotoxicity of metabolites from leaves of *Teclea trichocarpa*. *J Med Plants Res*. 2010;4(9):726-31