Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Aug 18; 10(A):1285-1289.
https://doi.org/10.3889/oamjms.2022.10035
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Biochemistry*

Anti-Mitoticpotential Identification of Nyale *(Eunice sp.)* in The Tourism Area of Kuta Beach, Lombok Island, West Nusa Tenggara

Putu Dedy Arjita[1]*, Rozikin Rozikin[1], Gede Angga Adnyana[1], Ayu Anulus[1], Sukandriani Utami[1], Aris Widiyanto[2], Santy Irene Putri[3]

[1]Department of Medicine, Faculty of Medicine, Universitas Islam Al-Azhar, West Nusa Tenggara, Indonesia; [2]Nursing Study Program, School of Health Sciences Mamba'ul 'Ulum Surakarta, Surakarta, Indonesia; [3]Midwifery Study Program, Universitas Tunggadewi, Tunggadewi Malang, Indonesia

Edited by: Slavica Hristomanova-Mitkovska
Citation: Arjita IPD, Rozikin R, Adnyana IGA, Anulus A, Utami S, Widiyanto A, Putri SI. Anti-Mitoticpotential Identification of Nyale (Eunice sp.) in The Tourism Area of Kuta Beach, Lombok Island, West Nusa Tenggara. Open Access Maced J Med Sci. 2022 Aug 18; 10(A):1285-1289. https://doi.org/10.3889/oamjms.2022.10035
Keywords: Anti-mitotic; *Eunice* sp.; Lombok Island; Marine Worm; Nyale
***Correspondence:** I. Putu Dedy Arjita, Faculty of Medicine, Universitas Islam Al-Azhar, West Nusa Tenggara, Indonesia. E-mail: iputudedyarjita@unizar.ac.id
Received: 30-Jun-2022
Revised: 01-Jul-2022
Accepted: 08-Aug-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: BauNyale festival is a Lombok tradition that originated in the Kuta beach tourism area in Central Lombok. The locals of Lombok Island gathered in this location to harvest marine worms known as Nyale (*Eunice* sp.). The ability of marine worms to act as anticancer agents has received little attention in Indonesia.

AIM: This study aimed to examine the biomolecules compounds of Nyale as antimitotic candidates.

METHODS: This was a preliminary study using post-test only with a control group design. The sample of this study was Nyale (*Eunice* sp.), and sea urchin (*Tripneustes ventricosus*) collected from Kuta beach, Central Lombok. The Nyale was extracted with 96% ethanol as the solvent. Gas Chromatography-Mass Spectrometry (GCMS) was used to determine the content of bioactive compounds. Sea urchin embryos were divided into four treatment groups (control; 10 mg, 100 mg, and 1000 mg; 1000 mg of Nyale extract). After 2 h of fertilization incubation, the number of cell division phases (2–32 cells) was counted. The data were analyzed using ANOVA.

RESULTS: The results of the GCMS test revealed that there were ten different compounds in the Nyale ethanol extract. There was a tendency for cleavage when testing the anti-mitotic potential of Nyale extract in each group. There was a significant difference in the percentage of cell changes in all treatments (control, 10 mg, 100 mg, and 1000 mg) ($p < 0.005$).

CONCLUSION: Bioactive compounds found in marine worms (nyale) influence the percentage of cell division (anti-mitotic) in sea urchin embryos.

Introduction

As an archipelagic country, Indonesia is surrounded by a vast coastal area with a wide variety of marine species. The nyale worm is a unique marine biota and is believed by the people of Lombok to have good health benefits that are often consumed by the people of Lombok, even every year the "BauNyale festival" tradition is held, a tradition in the Kuta beach area of central Lombok, people are very interested in this festival, where they harvest the Nyale sea worms. Judging from the morphological characteristics. This worm is thought to be a member of the genus Polychaeta, which includes four species: *Eunice* sp., *Lysidice* sp., *Neanthes* sp., and *Aphrodite* sp. [1].

Several marine biotas, including sponges, molluscs, cyanobacteria, and marine worms, have been investigated for anticancer content [2]. Biomolecules produced by marine biota organisms as a defense mechanism against predators or other competitors by inhibiting their mitotic cell division [3]. They cannot develop known to have high potential in the health sector, one of which is anti-cancer [4]. This is because living conditions in the sea cause biota organisms to produce biomolecules as a defense mechanism against predators or other competitors by inhibiting their mitotic cell division so that they cannot develop [5].

Marine worms from the Polychaeta class Hermione hystrix have antimitotic-cytotoxic activity against sea urchin embryos Paracentrotus Lividus, indicating that they may have antimitotic activity [6]. The discovery of compounds that have antimitotic activity, such as alkaloids, has increased the potential of marine worms as anticancer drugs. Ritterazines and Cephalostatin alkaloids from Cephalodiscusgilchristi, a marine worm, have antimitotic activity against human tumors [7]. Furthermore, other marine worms such as Paranemertes peregrine contain anticancer alkaloids. Other evidence suggests that the alkaloid content of indoles, pyrroles, pyrazines, quinolines, and pyridoacridines found in marine invertebrates has anticancer activity [8].

Tubulin is a protein component that is very important for microtubules [9]. This component plays

a vital role in cell division, intracellular transport, cell motility, and maintenance [10]. During mitosis, microtubules play an important role in spindle formation and separation. Tubulin inhibition mechanism is one of the targets to prevent the development of cancer cells and as a cancer target therapy [11]. Sea urchin embryos can be used to study the development of cell biology. Processes from sea urchins can be carried out efficiently to carry out fertilization, treatment, embryogenesis and larger embryo sizes so that optical observations can be more manageable [12] that they can be used as a model for studying the antimitotic effect.

Public consumption of nyale worms is high, but the ability of the Nyale sea worm (Eunicesp.) as an anticancer agent needs to be identified, especially along the coast of Lombok. Therefore, it is necessary to conduct preliminary research to determine whether biomolecules can act as antimitotics that can be used as anticancer candidates.

Methods

Study design

This was a preliminary study using post-test only with a control group design. The samples of Nyale (*Eunice* sp.) and sea urchin (*Tripneustes ventricosus*) were collected from Kuta, Central Lombok, and West Nusa Tenggara. The samples were examined for morphological image data selection and retrieval at the Laboratory, Faculty of Medicine, Al-Azhar Islamic University. The Nyale was extracted and tested at the Laboratory of Analytical Chemistry, Mataram University. The antimitotic test of the Nyale extract was performed in the Laboratory of Biomedical Research Unit at Mataram General Hospitals.

Extraction

The maceration method with ethanol solvent was used to extract Eunice sp. specimens. *Eunice* sp. dried samples were finely ground in a mortar and macerated in 96% ethanol for 24 h until everything was completely submerged. After that, the extract and residue were separated, and the residue was extracted 3 times with ethanol until it was colourless. A rotary vacuum evaporator was then used to combine and concentrate the filtrate.

Test of bioactive compounds

The quantitative and qualitative test of Nyale (Eunice sp.) extracts was gas mass spectrophotometry, called Gas Chromatography-Mass Spectrometry (GCMS). The number of compounds contained in the extract was indicated by the number of peaks in GCMS chromatography. In contrast, the types of compounds present were interpreted based on the spectral data of each peak using the literature approach method in the GCMS databases.

Test of anti-mitotic

An anti-mitotic test was performed at the cellular level using sea urchin embryos collected from the coastal waters of Sire, North Lombok. The gonads of male and female sea urchins were induced by injecting 1 ml of 10% KCl into them. The sperm cells had milky white color, and the ovum cells had golden yellow color. Both of them were kept in separate beakers. Fertilization took place in a beaker containing 50 ml of protozoa-free seawater, with 1 ml of sperm and 4 ml of ovum cells being fertilized. The embryos were incubated for 2–3 h.

The incubation was carried out at 140°C or 150°C, followed by homogenization to avoid precipitation, increase the presence of contact barriers, and ensure efficient sample distribution. Each ethanol extract of Nyale worms was weighed at a concentration of 1000 g/ml before being mixed with 10 ml of seawater as a stock. Pipette 10,100 and 1000 L of the stock solution into an Eppendorf tube containing 890 L and 800 L of protozoa-free seawater, respectively, and then add 100 L of a solution containing 100 L of zygotes with 10,100, and 1000 g/mL.

Furthermore, homogenization was interspersed at a temperature of 15–20^{o}C. After 3 h of incubation, observations on cell division inhibition were made. There was a drop of formalin added. After a 2–3-h incubation period, cleavage inhibition was observed under light microscopy in random populations. The outcome was given as a percentage (number of dividing cells divided by the total number of ovum cells times one hundred). Each treatment group was replicated 6 times.

Data analysis

Morphology and anti-mitotic compounds of Nyale extract were analyzed descriptively. The data on anti-mitotic compound tests on the embryo of sea urchins were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level.

Ethical approval

Research ethics in this study include approval sheets, anonymity, confidentiality, and ethical feasibility. Ethical feasibility in this study came from the Health Research Ethics Committee in the Faculty of Medicine of Al-Azhar Islamic University (18/EC/FK-06/UNIZAR/V/2020).

Results

Nyale extract

The extract's results were then analyzed using the GC-MS method to determine the various substances present [13]. The results of the GCMS test revealed that there were ten different compounds in the Nyale ethanol extract, with one compound not found in the library. Table 1 shows the compounds in question.

Table 1: Compounds in Nyale ethanol extract

No	Compounds Name	Molecular formula	Amount of substance (%)
1	Tetradecanoic acid (CAS) Myristic acid	$C_{14}H_{28}O_2$	8.62
2	RT: 11.540	-	5,68
3	Pentadecanoic acid (CAS) Pentadecylic acid	$C_{15}H_{30}O_2$	2.99
4	Hexadecanoic acid, methyl ester (CAS), Methyl palmitate	$C_{17}H_{34}O_2$	3.69
5	OCTADEC-9-ENOIC ACID	$C_{18}H_{34}O_2$	11.82
6	Hexadecanoic acid (CAS) Palmitic acid	$C_{16}H_{32}O_2$	12.16
7	Hexadecanoic acid (CAS) Palmitic acid	$C_{16}H_{32}O_2$	4.05
8	Heptadecanoic acid (CAS) Margaric acid	$C_{17}H_{34}O_2$	6.77
9	HEPTADECENE-(8)-CARBONIC ACID-(1)	$C_{18}H_{34}O_2$	36.66
10	Octadecanoic acid (CAS) Stearic acid	$C_{18}H_{36}O_2$	7.57

Anti-mitotic test of nyale extract

Four groups were tested: One control group, three groups with different concentrations (10, 100, and 1000 g), and three with different concentrations (10, 100, and 1000 g). The test was repeated 6 times in each group. The anti-mitotic potential of Nyale extract was determined by comparing the number of cells in various stages of cell division, starting with two compartments, 4 cells, eight cells, 16 cells, and 32 cells. The observations began with the fertilization of sea urchin eggs, which took 1.5 h until the eggs were fully fertilized (>90%).

The test lasted 2 h after the sea urchin eggs were fertilized. The percentage of cell division in each group is presented as the result of observations in each group. According to the findings, when testing the anti-mitotic potential of Nyale extract in each group, there was a tendency for cleavage to occur. Figures 1a-d illustrate this.

Statistical Results

There was a difference in the percentage of cell changes in all treatments (control, 10 mg, 100 mg, and 1000 mg), and they were statistically significant ($p<0.005$).

Discussion

Nyale extraction

Nyale (*Eunice* sp.) are marine worms that appear in coastal areas around the island of Lombok, West Nusa Tenggara, at certain times. At the time of its appearance, residents usually gather to collect Nyale in the area; this activity is known as the bauNyale festival [1]. Nyale belongs to the class Polychaeta which generally lives in the sea. This marine worm from the Polychaeta class is known to have a high protein content (54.72%) [14], [15]. Local people believe that Nyale has various health benefits, so they often take Nyale to be dried, resold, or eaten directly (Figure 2).

The extraction of Nyale worms was done using the maceration method, which used 70% ethanol as a solvent. The freeze-drying method is used to dry fresh Nyale that has been separated from impurities. The freeze-dry drying method retains more of the material's constituents, and the drying process is faster than traditional drying methods [16].

The highest compound found in the Nyale extract was HEPTADECENE-(8)-CARBONIC ACID-(1) (36.66%). These compounds have not been thoroughly researched as anti-cancer agents. This study aimed to map the compounds found in nyale's ethanol extract, not to test (each compound) for its anti-mitotic ability against sea urchin embryo cells. This was included as the limitation of this study.

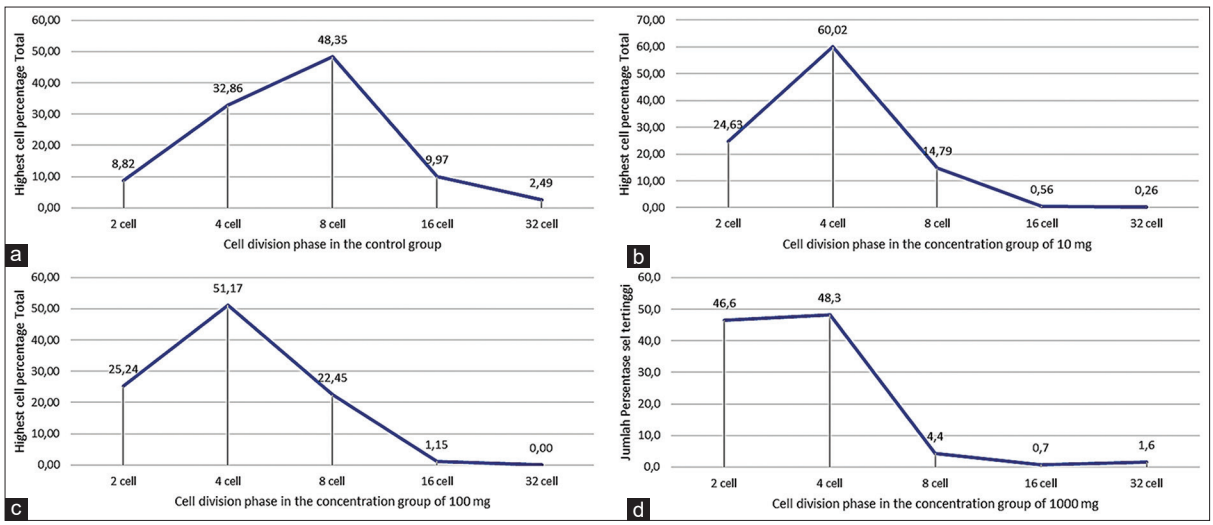

Figure 1: (a-d) Graph of Cell Division in the Control; 10 mg; 100 mg and 1000 mg group

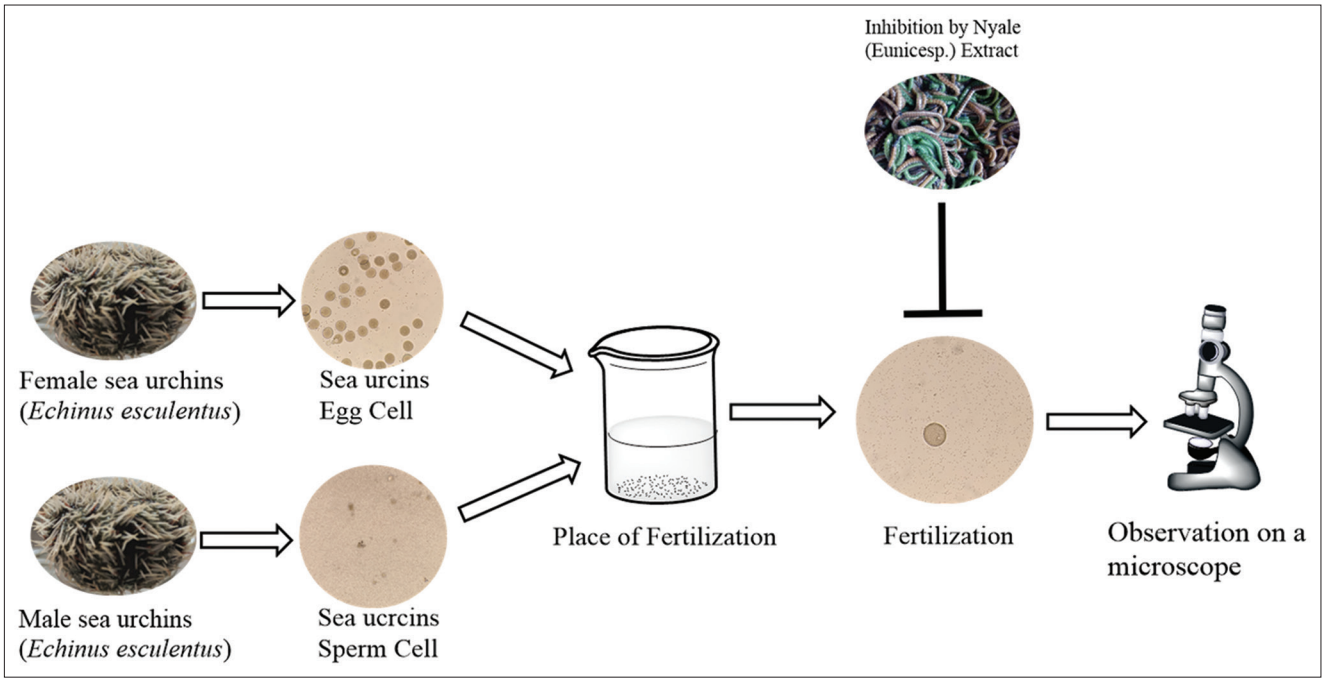

Figure 2: Nyale (Eunice sp.) research scheme. Egg cells and sperms cells from sea urchins will be fertilized, then nyale extract will be given, and cell division will be observed under a microscope.

Anti-mitotic test of nyale extract

The phase of cell division from 2-cell to 8-cell normally occurred in the control group, and at one point, an exponential occurred. Furthermore, in the 4-cell division phase, the treatment group with 10 mg, 100 mg, and 1000 mg Nyale ethanol extract showed inhibition.

The control group's ability to divide began to decline in the 8-cell division phase, whereas the treatment group's ability to divide began to decrease in the 4-cell division phase. This suggests that using Nyale ethanol extract to treat sea urchin zygote cell division could be effective (anti-mitotic). The 10 mg and 100 mg treatment groups showed the most inhibition in the 4-cell phase, while the 1000 mg treatment group showed inhibition in both the 2-cell and 4-cell phases.

The percentage of cell division in each treatment group was tested using ANOVA with a 95% confidence level to see if the percentage difference was significant. The significance level in each group was less than 0.05, indicating a significant difference in the percentage of cell changes in all treatments, according to the results of the ANOVA test (Table 2) (control, 10 mg, 100 mg, and 1000 mg). In other words, bioactive compounds found in marine worms (nyale) from the Lombok region significantly impact the percentage of cell division/anti-mitosis in sea urchin embryos.

Table 2: Analysis results of ANOVA test

Number of cells	Mean				p-value
	Control	10 mg	100 mg	1000 mg	
2	8.82	24.63	25.24	46.60	<0.001
4	32.86	60.02	51.17	48.31	0.003
8	48.34	14.78	22.44	4.35	<0.001
16	9.97	0.55	1.14	0.74	<0.001
32	2.49	0.25	<0.01	1.60	0.035

The ability and function of proliferative activity by bioactive compounds in Nyale extract can be inhibited by ethanol extract of Nyale by slowing down anti-proliferative activity through induction of cell cycle arrest.

This is in line with Ngoi Liew (2021) findings, which claim that compounds like quercetin have anti-proliferative activity by inducing cell cycle arrest through several molecular targets, including p21, cyclin, cyclin-dependent kinase, pRb, p53, and topoisomerase II [17]. According to Henriques, Silva [18], antimitotic drugs activate the spindle assembly checkpoint by interfering with microtubule formation and chromosomal segregation, causing mitotic termination. Apoptosis occurs when these compounds interfere with proper microtubule attachment, causing cells to die [19].

Conclusion

Based on the findings of this study, it can be concluded that each group can inhibit cell division at specific stages, indicating that the ethanol extract of nyale has anti-mitotic potential. For further research, it is necessary to optimize the techniques and methods for testing potential anti-cancer compounds so that the specific bioactive compounds and their effects on the growth and development of cancer cells can be determined with certainty. This study had several flaws as well. The bioactive compounds in the ethanol extract of Nyale were not isolated or separated in this study, so the specific bioactive compounds that play a role in

inhibiting the mitosis division of the sea urchin zygote cannot be determined with certainty. The division of sea urchin embryos was observed only at the end (post-test only), that is, 2 h after the testing process began, so that the initial picture of each group could not be described optimally and the final results could not be compared.

References

1. Bachtiar I, Odani S. Revisiting the Spawning Pattern of Nyale Worms (Eunicidae) Using the Metonic Cycle. ILMU KELAUTAN Indones J Marine Sci. 2021;26(2):87-94. https://doi.org/10.14710/ik.ijms.26.2.87-94
2. Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep M. Marine Natural Products.Nat. Prod. Rep. 2022;35:8-53.
3. Noble K, Rohaj A, Abegglen LM, Schiffman JD. Cancer therapeutics inspired by defense mechanisms in the animal kingdom. Evolutionary Applications. 2020;13(7):1681-700. https://doi.org/10.1111/eva.12963
4. Wang E, Sorolla MA, Krishnan PD, Sorolla AJ. From seabed to bedside: A review on promising marine anticancer compounds. Biomolecules. 2020;10(2):248. https://doi.org/10.3390/biom10020248
5. Joshi D, Mankodi P. Malacology and Pharmacology: An Integrated Approach with Special Emphasis on Marine Realm. Marine Niche: Applications in Pharmaceutical Sciences. Singapore: Springer; 2020. p. 255-64.
6. Coutinho MC, Teixeira VL, Santos CS. A review of “Polychaeta” chemicals and their possible ecological role. J Chem Ecol. 2018;44:72-94. http://doi.org/10.1007/s10886-017-0915-z
   PMid:29273953
7. Huigens RW 3rd, Brummel BR, Tenneti S, Garrison AT, Xiao T. Pyrazine and phenazine heterocycles: Platforms for total synthesis and drug discovery. Molecules. 2022;27(3):1112. http://doi.org/10.3390/molecules27031112
   PMid:35164376
8. Matulja D, Wittine K, Malatesti N, Laclef S, Turks M, Markovic MK, *et al*. Marine natural products with high anticancer activities. Curr Med Chem. 2020;27(8):1243-307. http://doi.org/10.2174/0929867327666200113154115
   PMid:31931690
9. Coulup SK, Georg GI. Revisiting microtubule targeting agents: α-Tubulin and the pironetin binding site as unexplored targets for cancer therapeutics. Bioorg Med Chem Lett. 2019;29(15):1865-73. http://doi.org/10.1016/j.bmcl.2019.05.042
   PMid:31130264
10. Roll-Mecak A. How cells exploit tubulin diversity to build functional cellular microtubule mosaics. Curr Opin Cell Biol. 2019;56:102-8. http://doi.org/10.1016/j.ceb.2018.10.009
    PMid:30466050
11. Haider K, Rahaman S, Yar MS, Kamal A. Tubulin inhibitors as novel anticancer agents: an overview on patents (2013-2018). Expert Opin Ther Pat. 2019;29(8):623-41. http://doi.org/10.1080/13543776.2019.1648433
    PMid:31353978
12. Babic T, Dinic J, Buric SS, Hadzic S, Pesic M, Radojkovic D, *et al*. Comparative toxicity evaluation of targeted anticancer therapeutics in embryonic zebrafish and sea urchin models. Acta Biol Hung. 2018;69(4):395-410. http://doi.org/10.1556/018.69.2018.4.3
    PMid:30587022
13. Leary PE, Kammrath BW, Lattman KJ, Beals GL. Deploying portable gas chromatography-mass spectrometry (GC-MS) to military users for the identification of toxic chemical agents in theater. Appl Spectrosc. 2019;73(8):841-58. http://doi.org/10.1177/0003702819849499
    PMid:31008649
14. Wibowo ES, Yuwono E, Sukardi P, Siregar AS. Survival rate, growth, and chemical content of Dendronereis pinnaticirris (Polychaeta, Nereidae) in maintenance with different feeds and substrates. Indones J Mar Sci. 2020;25(2):75-84.
15. Pamungkas J. Species richness and macronutrient content of wawo worms (Polychaeta, Annelida) from Ambonese waters, Maluku, Indonesia. Biodivers Data J. 2015;3:e4251. http://doi.org/10.3897/BDJ.3.e4251
    PMid:25829856
16. Hariyadi P. Freeze drying technology: for better quality and flavor of dried products. Foodreview Indones. 2013;8(2):52-7.
17. Ngoi NY, Liew AQ, Chong SJ, Davids MS, Clement MV, Pervaiz S. The redox-senescence axis and its therapeutic targeting. Redox Biol. 2021;45:102032. http://doi.org/10.1016/j.redox.2021.102032
    PMid:34147844
18. Henriques AC, Silva PM, Sarmento B, Bousbaa H. Antagonizing the spindle assembly checkpoint silencing enhances paclitaxel and Navitoclax-mediated apoptosis with distinct mechanistic. Sci Rep. 2021;11(1):4139. http://doi.org/10.1038/s41598-021-83743-7
    PMid:33603057
19. Alhussan A, Chithrani DB. Microtubule Targeting in Cancer Treatment. Organelle and Molecular Targeting. Boca Raton, FL: CRC Press; 2021. p. 403-20.