Sungkai (*Peronema canescens* Jack) Leaf Methanol Extract for Proliferation of *Saccharomyces cerevisiae* Yeast and Mice (*Mus muculus*)

Irena Francina Kelly Kamal[1], Hermansyah Hermansyah[2], Desnelli Desnelli[3], Muharni Muharni[4], Yosie Andriani[5]

{08031381924100@student.unsri.ac.id[1], hermansyah@unsri.ac.id[2], desnelli@unsri.ac.id[3], muharnikimia@unsri.ac.id[4]; yosie.hs@umt.edu.my[5]}

Undergratude Student of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Jalan Raya Palembang Prabumulih KM32, Indralaya 30662, South Sumatra, Indonesia[1]

Chemistry Dept., Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Jalan Raya Palembang Prabumulih KM32, Indralaya 30662, South Sumatra, Indonesia[2,3,4]

Institute of Climate Adapatation and Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu[5]

Corresponding author: hermansyah@unsri.ac.id

Abstract. Yeast proliferation is closely associated with cancer cells while leukocytes assay is to immunostimulatory activity. This research was aimed to know of sungkai extract in immunomodulator potency in mice (*Mus muculus*) and proliferation of *Saccharomyces cerevisiae* yeast. This research used phytochemistry screening and yeast and mice (*Mus musculus*) leukocytes assay. It was detected that methanol extract of Sungkai leaf contains terpene, steroid, alkaloid, saponin, and flavone. Yeast growth was observed on OD observation at λ = 600 nm, it was showed that there was no significant difference *S.cerevisiae* cell growth among in the absence and in the presence of various concentrations of Sungkai leaf extract. Mice leukocytes assay resulted in 15% concentration extract showing the highest content of leukocytes found in the mice tail, 9560 units, while negative control results in the lowest one, 5250 units. It was concluded that sungkai extract has potency as an immunomodulatory agent.

Keywords: sungkai, leukocytes *S. cerevisiae*, proliferation, immunomodulator.

1. Introduction

Sungkai is a plant commonly found in Borneo and Southern part of Sumatra in Indonesia. It is commonly used by locals to treat many illnesses, such as, fever, flu, and worm infection.

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Sungkai is also believed by many locals to be able to treat cancer and to improve immune system [1]. Several studies [1,2,3] have suggested that sungkai leaf extract, namely ethanol extract, has exhibited several bioactivivties i.e. immunomodulatory, antibacterial, and antiproliferative. Sungkai has several secondary metabolites that is also usually found in other plants, such as betulinic acid, stigmasterol, and quercetin [4,5]. Peronemin A_2, A_3, B_1, B_2, B_3, C_1, and D_1 was isolated from acetone extract [6].

Leukocytes is closely associated with immunomodulatory activity. Leukocytes has a very important role in mmune system as it works as a defence against pathogens. The increase of leukocytes count can indicate that there might be an infection in the body [7]. Leukocytes can be classified by the presence of enzyme granules in its cytoplasm under Giemsa or Leishman stain. There are granulocytes and agranulocytes leukocytes. Leukocytes were then differentiated into neutrophls, eosinophils, basophils, monocytes, and lymphocytes [8].

Yeast is one of the most versatile microorganisms that can be used in the research. Yeast is a eukaryotic mcroorganism that lives in various ecological environments [9]. *Saccharomyces cerevisiae (S.cerevisiae)* is one of the yeast that is used as bioassay in many tests as it is easy to be cultivated and inexpensive [10]. *S. cerevisiae* proliferation can be associated with cancer cells growth because of the similarity between *S. cerevisiae* proliferation and cancer cells growth [11]. Sungkai ethanol extract is known to have exhibited anticancer activity to HeLa cells [3] while other types of extract was not investigated. In this study, methanol extract of sungkai leaves is investigated for its bioactivity mainly on antiproliferative and immunomodulator activity.

2. Research Methods

2.1 Sample Collection and Extraction

Sungkai leaf was collected in Prabumulih, South Sumatera, Indonesia. 1.2 kg of sungkai leaf was collected and dried naturally to obtain dried leaves. Dried leaves was then grounded by using blender. Powder leaf was macerated using methanol (Merck) for 3x24 and evaporated using rotary evaporator.

2.2 Phytochemistry Screening [12, 13]

2.2.1 Flavonoid

1 g of sample was added to a test tube and heated while adding 10% NaOH (Merck) for 15 minutes. Color change to red or yellow indicates flavonoid compound.

2.2.2 Alkaloid

2 g of sample was added to test tube. 5 mL of 2 N HCl (Merck) was added and heated. The mixture was then cooled to room temperature. Dragendorff reagent was added and brown precipitate means that there is alkaloid compounds.

2.2.3 Terpene and Steroid

2 g of sample was added to test tube. 2 mL of ethyl acetate (Merck) was added and shaken. Ethyl acetate (Merck) layer was taken and dropped to a drip plate. It was left to dried. 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid was added. Red or yellow colour indicates terpene while green indicates steroid.

2.2.4 Saponin

1 g of sample was added to test tube then it was added with 10 mL of hot water. It was cooled and shaken for 10 seconds. Positive test was indicated by a foam layer that form for about 10 minutes.

2.2.5 Terpene and Steroid

1 g of sample was added to test tube and 10 mL of hot water was added and boiled for 5 minutes.3-4 drops of $FeCl_3$ was added. Greenish blue color indicates cathecol tannin while dark blue indicates pirogalol tannin.

2.3 Immunomodulator Test [14]

30 male mice weighing 20—50 g were used in this experiment. The mice were adapted to the environment for 1 week. Then, those mice were grouped into 5 different treatment groups (Table 1).

Table 1. Treatment group

No.	Groups	Treatment
1	Positive control	Stimuno 0,19 mg/30g BW
2	Negative control	CMC-Na 0,5%/30g BW
3	1st group	5% dosage extract/30g BW
4	2nd group	10% dosage extract/30g BW
5	3rd group	15% dosage extract/30g BW

The treatment was given for 7 days using gavage method and in the 8th day, mce blood was taken by making an incision to the tail of mice. Blood was stored in EDTA tube and diluted by using Turk solution. Leukocytes were counted with hemocytometer under micoscope with the magnification of 10x. Results were analyzed by using ANOVA Test and Duncan Test.

2.4 Yeast Proliferation Assay [15]

Saccharomyces cerevisiae was cultured on YPDA media and incubated for 24 hours. One colony of *S. cerevisiae* was taken and put to YPD broth. The broth was then incubated for another 24 hours. 1 mL of this broth was then added to 5 different erlenmeyer flask containing 100 mL of YPD broth. 2nd, 3rd, 4th, and 5th erlenmeyer flask contained 5 mL of 5%, 10%, 15%, and 20% extract respectively. The mixture was shaken, incubated, and observed on OD600 every 2 hours. Absorbance found was then converted into estimated yeast cells by multiplying with $1.5x10^7$ cells/mL. The data was analyzed using ANOVA Test.

3. Results and Discussion

3.1 Sample Preparation and Phytochemical Screening

Sungkai leaf was obtained and dried to 465 g. These dried leaves were then grounded into its powder form and macerated using methanol. Maceration is an extraction method that can be used to extract thermolabile compound [16]. 40.912 g of concentrated extract was obtained by evaporating methanol content from the extract. This result was tested for its secondary metabolite and resulting in the presence of secondary metabolites below (Table 2).

Table 2. Secondary metabolite present in the extract

No.	Groups	Results	Previous Research
1	Flavone	Positive	Positive
2	Alkaloid	Positive	Positive
3	Terpene and Steroid	Positive	Positive
4	Saponin	Positive	Positive
5	Tannin	Negative	Positive

This result was in accodance to what was done before [17] in which sungkai extract contains flavone, alkaloid, terpene, steroid, and saponin. Tannin, nonetheless, was not found in this extract. There are many reasons that could explain this including the difference in the plant habitat an also extraction method used for this experiment [18].

3.2 Imunomodulator Potency in Mice (*Mus musculus*)

200 microliter of blood was taken from the tail incision and observed in the counting chamber with microscope. Average leukocytes found in mice given 15% extract was 9560 cells/mm^3 meanwhile for the negative control group was only around 5250 cells/mm^3. It was found that the higher the concentration of extract given, the higher the leukocytes count (Figure 1). Figure 1 also shows that 10% concentration and 15% concentration gave a higher leukocytes count rather than positive control. This results in a higher leukocytes count compared to previous research using sungkai infusion [14], in which the 20% infusion gives 8670 cells/mm^3.

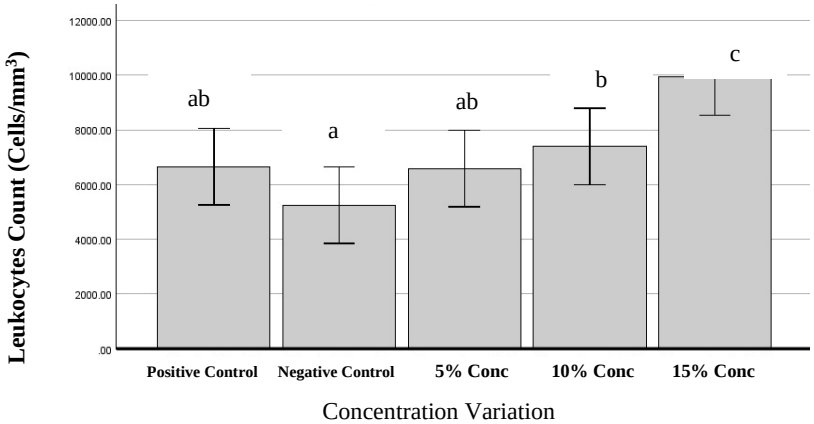

Fig. 1. Average leukocytes count for mice in groups

According to the data tested with ANOVA test (Table. 3.), sungkai methanol extract has a significant role in the increase of leukocytes with $p<0.05$. Duncan test (Fig. 1) was further done and was concluded that 15% concentration gives the highest difference from negative control. This shows that methanol extract of sungkai leaf has a great potency to be a source of immunostimulant agent.

Table 3. ANOVA Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46321750.000	4	11580437.500	3.884	.023
Within Groups	44721250.000	15	2981416.667		
Total	91043000.000	19			

The increase of leukocytes count was made possible because of the presence of flavone in sungkai methanol extract. Flavone is a group that displays many bioactivity, such as immunomodulator activity. The mechanism of how flavonoid can stimulate the immune system is not clear yet. Nevertheless, several compounds from flavone group isknown to have been able to increase NK (Natural Killer) cells. NK cells are part of leukocytes that work to kill disease infected cells and also cancer cells [19]. Flavone compounds are also known to have reduce the damage in cells caused by radiation [20].

3.3 *Saccharomyces cerevisiae* Proliferation

Sungkai leaf has been tested in silico for its antifungal activity towards *Candida albicans (C albicans*). It was concluded that it has the potential to work as an antifungal agent [21]. Sungkai extract was tested to see its inhibition eefect towards *S. cerevisae*. Data obtained from the spectophotometer was converted to estimated amount of cells (Fig. 2). The highest cell count was in the 16th hour, in which the media without any addition of the extract has 1.895 $x10^7$ cells/mL. In the same hour, yeast with 20% extract added was estimated around 1,721 $x10^7$ cells/mL. This data was tested wth ANOVA test and resulted in $p>0.05$ which means that there was no signifiicant difference for the growth of *S. cerevisiae* with or without the addition of sungkai extract.

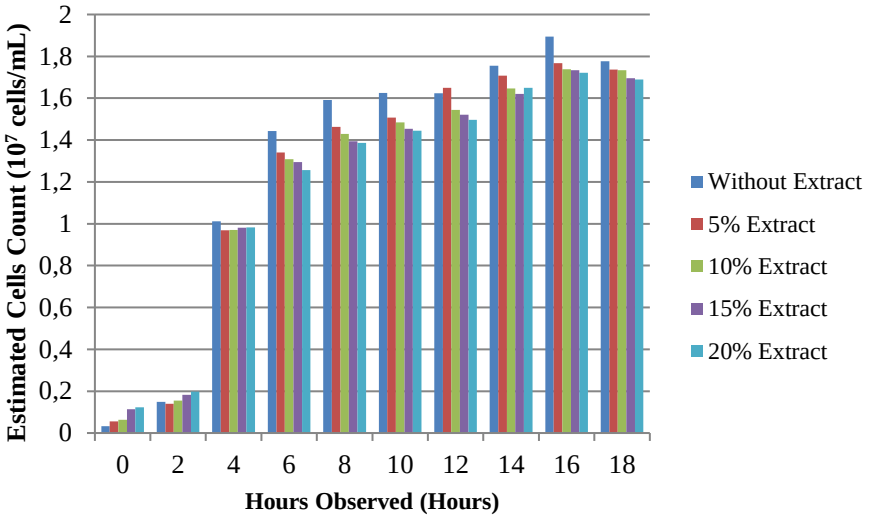

Fig. 2. Estimated cells count

Quercetin, one of compounds commonly found in sungkai, is known to exhibit antioxidant properties that could postpone the aging caused by reactive oxygen species (ROS) [22]. Oxidative stress, caused by ROS, can cause substantial damage towards many biomolecules, such as nucleic acid, protein, and also lipid. ROS can also mediate programmmed cell death [23]. This could also explain why there was no significant change in the growth of *S. cerevisiae*. The absence of tannin can also explain the lack of growth inhibition. Tannin is known as antifungal and anti microbial agent. Tannic acid, one of compound found in tannin group, can destroy the integrity of cell membrane, hence causing a leak of intracelluler organelle in a cell [24].

4. Conclusion

Methanol extract of sungkai leaf has a great potential for being an immunomodulator agent, specifically as immunostimulant agent while, it has little to no effect for *S. cerevisiae* proliferation. Therefore, it can not be said that sungkai leaf methanol extract has anti-proliferative activity. Further study is recommended to be done to understand and learn more about sungkai plant and its bioactivities.

References

[1] Yani AP, Putranto AMH: Examination of the sungkai's young leaf extract (*Peronema canescens*) as an antipyretic, immunity, anti plasmodium, and teratogenicity in mice (*Mus muculus*). Vol. 7. No. 1. pp: 30-34. International Journal of Science and Engineering, (2014)

[2] Fransisca D, Kahanjak DN, Frethernety A: Uji aktivitas antibakteri ekstrak etanol daun sungkai (*Peronema canescens* Jack.) terhadap pertumbuhan *Escherichia coli* dengan metode difusi cakram Kirby-Bauer. Vol. 4. No. 1. pp: 460-470. Jurnal Pengelolaan Lingkungan Berkelanjutan, (2020)

[3] Ibrahim A, Siswandono S, Bambang PEW: Anticancer activity of Peronema canescenss Jack leaves extracts againsts human cells: HT-29 and HeLa in vitro. Vol. 15. No. 10. pp: 4739-4745. Research Journal of Pharmacy and Technology, (2022)

[4] Muharni M, Ferlinahayati F, Yohandini H, Riyanti F: The anticholesterol activity of betulinic acid and stigmasterol isolated from the leaves of sungkai (*Peronema canescens* Jack). Vol. 13. No. 2. pp: 198-203. International Journal of Applied Pharmaceutics, (2021)
[5] Dillasamola D, Aldi Y, Kurniawan H, Jalius IM: Immunomodulator effect test of sungkai leaves (*Peronema canescens* Jack) ethanol extract using carbon clearance method. Proceedings of the 2nd International Conference on Contemporary Science and Clinical Pharmacy. Vol. 13. No. 6. pp: 1397-1407. (2021)
[6] Kitagawa I, Simanjuntak P, Hori K, Nagami N, Mahmud T, Shibuya H, Kobayashi M. Indonesian medicinal plants. VII. Seven new clerodane type diterpenods, Peronemins A_2, A_3, B_1, B_2, B_3, C_1, and D_1 from the leaves of *Peronema canescens* (Verbenaceae). Vol. 42. No. 5. pp: 1050-1055. Chemical & Pharmaceutical Bulletin, (1994)
[7] Fathima SJ, Khanum F: Blood cells and leukocytes culture - a short review. Vol. 1. No. 2. pp: 1-2. Open Access Blood Research & Transfusion Journal, (2017)
[8] Tigner A, Ibrahim SA, Murray IV: Histology, White Blood Cell. StatPearls [Internet]. (2022).
[9] Maicas S: The role of yeast in fermentation processes. Vol. 8. No. 8. pp: 1142. Microorganisms, (2020)
[10] Hermansyah H: *Saccharromyces cerevisiae* as model to identify mengkudu (*orinda cirtrifolia*) as an anticancer medicinal plants candidate with antiproliferative propoerties. The 5th Indonesia Biotechnology Conference (2012)
[11] Natter K, Kohlwein SD: Yeast and cancer cells – common principles in lpid metabolism. Vol. 1831. No. 2. pp: 314-326. Biochimica et Biophysica Acta, (2013)
[12] Mailuhu M, Runtuwene MRJ, Koleangan HSJ: Skrining fitokimia dan aktivitas antioksidan ekstrak metanol kulit batang soyogik (Saurauia bracteosa DC). Vol. 10. No. 1. pp: 1-6. Chemistry Progress, (2017)
[13] Muthmainnah B: Skrining fitokimia senyawa metabolit sekunder dari ekstrak etanol buah delima (Punica granatum L.) dengan metode uji warna. Vol. 12. No. 2. pp: 23-28. Media Farmasi, (2017)
[14] Rahman A, Rengganis GP, Prayuni S, Sari TN, Pratiwi PD, Pratama S: Pengaruh pemberian infusa daun sungkai (Peronema canescens Jack.) terhadap jumlah leukosit pada mencit. Vol. 7, No. 2. pp: 614-619. Journal of Healthcare Technology and Medicine, (2021)
[15] Mathi P, Nikhil K, Ambatipudi M, Roy P, Bokka VR, Botlagunta M: In-Vitro and In-Silico characterization of Sophora interrupta plant extract as an anticancer activity. Vol. 10. No. 3. pp: 144-151. Bioinformation, (2014)
[16] Abubakar RA, Haque M: Preparation of medicinal plants: basic extraction an fractionation procedures for experimental purposes. Vol. 12. No. 1. pp: 1-10. Journal of Pharmacy and Bioallied Sciences, (2020)
[17] Emilia I, Setiawan AA, Novianti D, Mutiara D, Rangga R: Skrining fitokimia ekstrak daun sungkai (*Peronema canescens* Jack.) secara infudansi dan maserasi. Vol. 5. No. 2. pp: 95-102. Jurnal Indobiosains, (2023)
[18] Ghasemzadeh A, Jaafar HZE, Bukhori MFM, Rahmat MH, Rahmat A: Assessment and comparison of phytochemical constituents and biological activities of bitter bean (*Parkia speciosa* Hassk.) collected from different locations in Malaysia. Vol. 12. No. 1. pp: 12. Chemistry Central Journal, (2018)
[19] Han L, Fu Q, Deng C, Luo L, Xiang T, Zhao H: Immunomodulatory potential of flavonoids for the tratment of autoimmune diseases and tumour. Vol. 95. No. 1. pp. e13106. Scandinavian Journal of Immunology, (2021)
[20] Boojar MMA: An overview of the cellular mechanisms of flavonoids radioprotective effects. Vol. 10. No. 1. pp: 13-19. Advanced Pharmaceutical Bulletin, (2020)
[21] Istiqomah N, Fatikasari S, Hutuba AH: Kajian in Silico Daun Sungkai (*Peronema canescens*) dalam Menghambat Enzim Lanosterol 14-a demethylase Jamur *Candida albicans*. Vol. 3. No. 1. pp: 131-142. Indonesian Journal of Pharmaceutical Education, (2023)
[22] Zahoor H, Watchaputi K, Hata J, Pabruprapap W, Suksamrarn A, Chua LS, Soontorngun N: Model yeast as a versatile tool to examine the antioxidant and anti aging potential of flavonoids, extracted from medicinal plants. Vol. 13. pp: 980066. Frontiers in Pharmacology, (2022)

[23] Farrugia G, Balzan R: Oxidative stress and progmed cell death in yeast. Vol. 2. Ed: 64. Frontiers in Oncology, (2012)
[24] Zhu C, Lei M, Andargie M, Zeng J, Li J: Antifungal activity and mechanism of action of tannic acid against Penicillium digitatum. Vol. 107. pp: 46-50. Physiological and Molecular Plant Pathology, (2019)