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Original Article

# STUDY OF ACTIVE ISOLATED COMPOUNDS FROM SUNGKAI LEAF (*PERONEMA CANESCENS* JACK) AS IMMUNOSTIMULANT FROM EXPOSURE OF THE SARS-COV-2 VIRUS ANTIGEN TO NATURAL KILLER CELLS

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**ABSTRACT**

**Objective**: SARS-CoV-2 is an illness that attacks the respiratory tract's alveoli lining cells. One approach to tackling it is to strengthen the immune system of the body, which has an organ specifically dedicated to defending against diseases. Among the key components of the body's immune system that defend against the SARS-CoV-2 virus are Natural Killer (NK) cells. Sungkai (*Peronema canescens* Jack) is a plant commonly used by communities to boost their immunity. The aim of this study was to examine the activity and characterization results of Sungkai leaves on Natural Killer cells.

**Method**: Sungkai powder was extracted using a 70% ethanol solvent and evaporated using a rotary evaporator. It was then fractionated using n-Hexane and evaporated using a rotary evaporator to obtain a thick fraction. The thick fraction was then chromatographed, resulting in two isolated compounds that were tested *in vivo* for their immunostimulatory activity using mice as test animals with ethical approval (405/UN.16.2/KEP-FK/2021).

**Results**: The study consisted of five groups, including a normal group and four other groups of white mice that had been exposed to the SARS-CoV-2 virus antigen Moderna vaccine 0.0013 ml.

**Conclusion**: The results showed that stigmasterol was more effective than Bis(2-ethylhexyl) phthalate in increasing Natural Killer cells' immunostimulant activity.

**Keywords**: SARS-CoV-2, Sungkai (*Peronema canescens* Jack), Natural Killer (NK), pthalate, Stigmasterol, Immunostimulant


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## INTRODUCTION

The mysterious Pneumonia case that was first reported in Wuhan, Hubei Province in early 2020 has shocked the world. The rapid spread of the disease has affected more than 190 countries worldwide with an unclear etiology. The case has spread to various provinces in China, Thailand, Japan, and South Korea, and there were 44 new cases recorded from December 31, 2019, to January 3, 2020 [1]. The disease was named Coronavirus Disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). The virus has spread worldwide and infected more than 214 million people as of August 2021. In the last two decades, there have been three coronavirus outbreaks, namely Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and COVID-19 [2]. In humans, SARS-CoV-2 mainly infects cells in the respiratory tract that line the alveoli. SARS-CoV-2 binds to receptors and enters the cell. The body contains an immune system that protects against diseases. The key component of the immune system is white blood cells that can spread throughout the body through blood vessels. A crucial component of the immune system in protecting the body against SARS-CoV-2 infection is natural killer cells [3].

The body contains an immune system that protects against diseases. The key to the immune system is white blood cells, which can spread throughout the body through blood vessels. The body's defense mechanisms can be enhanced with certain compounds that act as immunostimulants. Immunostimulants are generally defined as compounds that can enhance the body's defense mechanisms, both specific and non-specific, as well as cellular and humoral defense mechanisms. Therefore, the presence of chemical compounds that can enhance the activity of the immune system, which can be obtained from plants, is important. One of the important components of the immune system in protecting the body against SARS-CoV-2 virus infection is Natural Killer (NK) cells [4]. These cells protect the body against SARS-CoV-2 virus infection by producing a group of cytokines, such as interferon, granzyme, and perforin, as well as proinflammatory cytokines known as the cytokine storm. In addition, the body also protects itself through phagocytosis, regardless of the differences among foreign substances. The body's defense mechanisms can be enhanced with specific compounds through an immunomodulatory approach [5].

Natural Killer (NK) cells play a central role in antiviral immunity. They can lyse virus-infected host cells by inducing apoptosis through several different pathways. Specifically, they can release various proteins such as perforin and granzyme through exocytosis, and together, these molecules can induce apoptosis of target cells. Second, NK cells can kill targets by expressing FAS ligand (FasL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) as killer molecules and then initiate extrinsic apoptosis signaling pathways [6]. In addition, NK cells are capable of secreting pro-inflammatory cytokines, such as IFNy, TNF-a, GM-CSF, and chemokines. NK cells exhibit functional plasticity in the context of disease development by displaying pro-inflammatory or anti-inflammatory properties. Due to the reduced frequency of NK cells found in SARS-Cov-2 patients and the well-known antiviral role of NK cells, it is very intriguing to speculate that the recovery of NK cells and their function would be a good solution for SARS-Cov-2 patients [7].

The literature review on the extract of the *Peronema canescens* Jack plant found that a dose of 0.5625 mg/kgBW of *Peronema canescens* Jack increased leukocyte counts by 36% [8]. The ethanol extract from *Peronema canescens* Jack leaves had *in vivo* antiplasmodium activity, with an ED50 value of 102.88 mg/kgBW. N-hexane extract from *Peronema canescens* Jack leaves inhibited the growth of all test

bacteria at a concentration of 1 mg/ml. Other studies include research on the activity of hand antiseptic gel containing ethanolic fraction of *Peronema canescens* Jack leaves against several pathogenic bacteria, as well as studies on the potential of *Peronema canescens* Jack young leaves for immunity in Mus musculus [9]. A study on the antibacterial activity of ethanol extract from *Peronema canescens* Jack leaves against *Escherichia coli* using the Kirby-Bauer disk diffusion method [10]. The ethanol extract of *Peronema canescens* Jack bark did not have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, while the leaf extract and ethyl acetate and methanol fractions had Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus* at 1024 µg/ml, 1024 µg/ml, and 512 µg/ml, respectively. Against *Escherichia coli*, the extract and fraction had MIC and MBC of 512 µg/ml [11]. Bioautography results against *Staphylococcus aureus* and *Escherichia coli* showed that the compounds with antibacterial activity in the extract and fraction of *Peronema canescens* Jack leaves were likely to be alkaloids and flavonoids [12]. A dose of 500 mg/kg BW of ethanol extract from *Peronema canescens* Jack leaves could lower uric acid levels [13]. The n-hexane fraction of *Peronema canescens* Jack leaves could lower cholesterol levels, and asam betulinat was identified as the active compound from the triterpenoid group [14]. The secondary metabolite compounds in *Peronema canescens* Jack leaves, such as alkaloids, terpenoids, flavonoids, and tannins, which were found to have antibacterial properties [15]. The isolation of n-hexane extract from *Peronema canescens* Jack leaves yielded one compound, namely isolate B1, which was positively identified as a terpenoid compound based on chemical reactivity data. UV spectrum analysis showed a maximum wavelength of 207 nm, and IR spectrum analysis revealed that the active compound contained OH (hydroxyl),-CH-aliphatic, C=O (carbonyl), C-O (ketone), C=C-(cyclic or aromatic ester), and CH2 and CH3 (alkyl aliphatic) functional groups (Ningsih *et al.* 2013). The compounds with immunostimulant activity are polysaccharides, terpenoids, alkaloids, and polyphenols [16].

Based on the literature review of sungkai plant, there hasn't been any research found on the active isolate of sungkai leaves towards immunostimulant. Based on the above description, the researchers are interested in conducting research on the active isolate from sungkai leaves (*Peronema canescens* Jack) towards protecting against SARS-CoV-2 virus infection as an immunostimulant by observing the activity of NK cells.

## MATERIALS AND METHODS

### Materials

The materials used in this study are dried and ground leaves of sungkai (*Peronema canescens*, L.), Ethanol (Lichrosolv R (1.11727.2500), Methanol (EMSURER) (1.06009.2500), n-hexane, ethyl acetate, n-butanol, RAW 264.7 cells (CCRC UGM), CDCl3, Dulbecco's Modified Eagle Medium (DMEM) (Gibco), Fetal Bovine Serum (FBS) (Sigma), Phosphate-buffered saline (PBS) (Sigma), DMSO (Merck), wash buffer, lipopolysaccharide (eBioscience), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (eBioscience), dichloromethane (DCM), lipopolysaccharide (eBioscience), Silica gel 60 (Merck, 0.063-0.200 mm) (1.07734.1000), Vanillin (Merck) (8.18718.0100), Chloroform (EMSURE R (1.02445.2500), Silica gel 60 (Merck) (0.2-0.5 mm) (1.07733.1000), Mouse NK Cell ELISA kit (BT Lab), Mouse CD8 Cell ELISA kit (BT Lab), physiological NaCl, Na CMC, Moderna vaccine.

The equipment used in this study includes filter paper, rotary evaporator, a set of column chromatography equipment, vials, TLC Silica desgel 60 F254 chambers (Merck) (1.05554.0001), dropper pipettes, syringes, measuring glasses, animal scales, spatulas, oral needles, analytical balances, containers (bottles), mortars and pestles, surgical scissors, UV lamps 365 nm, incubator, a set of centrifuge equipment, centrifuge tubes, UV-Vis spectrophotometer (Shimadzu), IR spectrophotometer (Shimadzu) and BIO-RAD spectrophotometer, NMR500 MHZ Bruker Germany, FTIR Perking Elmer USA, UPLC MSMS Waters USA, FTIR Nicolet-iS50 (Thermo Scientific, USA), and the mass of the compound was recorded using TQD LCMS-Ultra Performance Liquid Chromatography (UPLC) (Waters, USA). 1-D and 2-D NMR 500 MHz Cryo-Probe from Bruker (USA).

### Extraction and isolation

Ethanol extract of Sungkai leaves was obtained by extracting the crude drug using the maceration method. The maceration method was chosen because it did not require a heating process that could damage thermolabile compounds. Ethanol 70% was used as a solvent for the maceration process because the crude drug used had a relatively low water content, so 70% ethanol wa needed, which was known to contain 30% water which functioning to break down cell walls so that the penetration of ethanol into the cells became faster and optimal (Dillasamola *et al.* 2022).

The maceration process was carried out with a ratio of crude drug to solvent of 1:10 in a brown bottle container. The crude drug that had been soaked in the solvent was stirred and then left at room temperature for 18 h with occasional stirring. After maceration, a filtration process was carried out, which was the process of separating the macerate from the residue using filter paper. This method was repeated 3 times. The obtained macerate was evaporated using a rotary evaporator. The evaporation process was carried out at a temperature of 45 °C and a pressure of 150 bar until a thick extract of Sungkai leaves was obtained (1).

After obtaining the thick extract of Sungkai leaves, the extract was fractionated using n-hexane, ethyl acetate, and n-butanol. 50 g of ethanol extract were dissolved in 600 ml of distilled water and then placed in a separating funnel with a closed valve. 200 ml of n-hexane were added to the extract solution and shaken until homogenous. The n-hexane fraction was separated from the aqueous fraction by opening the valve of the separating funnel and collected in an Erlenmeyer flask. The same procedure was repeated 2 times with the same solvent. The n-hexane fraction was evaporated using a rotary vacuum evaporator at a temperature of 40 °C to obtain the n-hexane extract. The remaining aqueous fraction from the n-hexane fraction was mixed with 200 ml of ethyl acetate solvent. The same process was carried out to obtain the ethyl acetate extract. Then, 200 ml of n-butanol was added to the remaining aqueous fraction from the ethyl acetate fraction, resulting in the n-butanol extract and the remaining aqueous fraction. The n-hexane, ethyl acetate, n-butanol, and remaining aqueous extracts were tested for immunostimulant activity using the ELISA method. The results show that the fractionation using n-hexane had higher immunostimulant activity compared to other fractionations in increasing the levels of NK cells (1).

Isolation of immunostimulant compounds from the active isolate of sungkai leaves obtained from n-hexane fractionation was carried out using column chromatography. The sungkai leaf isolate was chromatographed using a silica gel 60 (0.063-0.2 mm) stationary phase and an isocratic mobile phase of ethyl acetate: methanol. First, the silica slurry was prepared by mixing silica with the mobile phase, stirring it homogeneously, and then pouring it into a column that had been lined with cotton at the bottom. The silica slurry was packed by tapping the column to compact the silica gel. The sample was prepared by dissolving it in a solvent that could dissolve it, then mixing it with an equal amount of silica gel, and then evaporating the solvent until the sample and silica gel were dry. The sample was then added to the chromatography column and the column was eluted with a fixed mobile phase ratio (1).

The chromatography results were collected using vials and monitored with TLC plates and a UV lamp at 254 nm. Fractions with the same spot were combined. After obtaining the most active fraction, the purification process of the compound was carried out using column chromatography, where the process could be repeated 2-3 times until a single pure spot is obtained and identified through TLC plates (1).

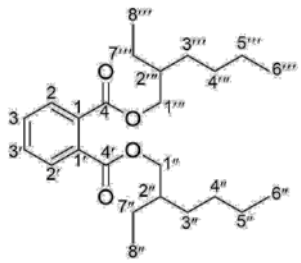
### Bis(2-ethylhexyl) phthalate (1)

Yellow oil; ESIMS $[M+Na]^+$ *m/z* 413.14; $^1$H NMR (Chloroform-d, 500 MHz) $\delta_H$: 7.63 (2H, *dd*, $^4J$ = 3.3, 5.7Hz, H-2,2`), 7.45 (2H, *dd*, $^4J$ = 3.3, 5.7Hz, H-3,3`), 4.15 (4H, *m*, H-1``, H-```), 1.61 (4H, *m*, H-2``, 2```), 1.28 (4H, *m*, H-3``, 3```), 1.25 (4H, *m*, H-4``, 4```), 1.23 (4H, *m*, H-5``, 5```), 0.82 (6H, *t*, $^3J$ = 7.2 Hz, H-6``, 6```), 1.35 (4H, *m*, H-7``, 7```), 0.86 (6H, *t*, $^3J$ = 7.45 Hz, H-8``, 8```); $^{13}$C NMR (Chloroform-d, 125 MHz) $\delta_H$: 132.43

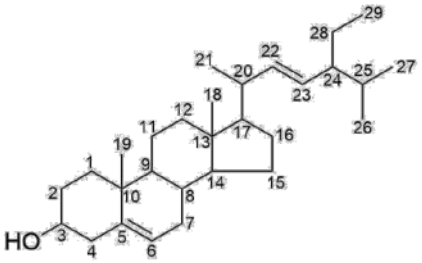
(C-1,1'), 128.77 (C-2,2'), 130.84 (C-3,3'), 167.72 (C-4,4'), 68.12 (C-1'',1'''), 38.71 (C-2'',2'''), 30.34 (C-3'',3'''), 22.94 (C-4'',4'''), 28.90 (C-5'',5'''), 14.01 (C-6'',6'''), 23.72 (C-7'',7'''), 10.93 (C-8'',8''').

**Stigmasterol (2)**

Colorless crystal; m. p 215-217° C; ESIMS $[M+H]^+$*m/z* 413; $^1$H NMR (Chloroform-d, 500 MHz) $\delta_H$: 1.84 (2H, *m*, H-1), 1.51 (2H, *m*, H-2), 3.51 (1H, *m*, H-3), 2.24 (2H, *dd*, 10.5, 2.1, H-4), 5.34 (1H, *d*, 5.4, H-6), 1.84 (2H, *m*, H-7), 1.93 (1H, *m*, H-8), 1.44 (1H, *m*, H-9), 1.47 (2H, *m*, H-11), 2.00 (2H, *dd*, 9.3, 3.0, H-12) 1.10 (1H, *m*, H-14), 1.55 (2H, *m*, H-15), 1.25 (2H, *m*, H-16), 1.07 (1H, *m*, H-17), 0.68 (3H, *s*, H-18), 0.99 (3H, *s*, H-19), 1.99 (1H, *m*, H-20), 0.91 (3H, *d*, 6.3, H-21), 4.99 (1H, *dd*, 15.2, 8.7, H-22), 5.14 (1H, *dd*, 15.2, 8.7, H-23), 1.93 (1H, *m*, H-24), 1.65 (1H, *m*, H-25), 0.81 (3H, *d*, 6.5, H-26), 0.79 (3H, *d*, 6.5, H-27), 1.25 (2H, *m*, H-28), 0.84 (3H, *t*, 3.3, H-29); [13]C NMR (Chloroform-d, 125 MHz) $\delta_H$: 37.0 (C-1), 31.7 (C-2), 71.5 (C-3), 42.0 (C-4), 140.5 (C-5), 121.5 (C-6), 31.4 (C-7), 31.6 (C-8), 49.9 (C-9), 36.3 (C-10), 20.9 (C-11), 39.5 (C-12), 42.1 (C-13), 56.5 (C-14), 24.1 (C-15), 28.0 (C-16), 55.8 (C-17), 11.6 (C-18), 18.8 (C-19), 40.3 (C-20), 18.6 (C-21), 138 (C-22), 129.0 (C-23), 51.0 (C-24), 28.9 (C-25), 19.6 (C-26), 19.2 (C-27), 22.8 (C-28), 11.7 (C-29).



**Bis(2-ethylhexyl) phthalate (1)**



**Stigmasterol (2)**

**Fig. 1: Chemical structure of isolated compounds**

**Immunostimulant assay**

The test was conducted *in vivo* using healthy BALB/c mice weighing 20-25 g, totaling 45 individuals and randomly divided into groups of 5. The treatment was administered once a day in the evening; for group I, the isolated fraction of sungkai leaf at 1 mg/kgbw was given for 7 d until day 7 of vaccination, and on day 8, evaluation was performed. For group II, mice were vaccinated on the first day and then given a fraction at a dose of 10 mg/kgbw for 7 d. For group III, the fraction was given 3 d before vaccination, and on day 4, the mice were vaccinated, followed by a fraction at a dose of 100 mg/kgbw, 4 d after vaccination. Group IV consisted of mice that only received the Moderna Covid vaccine 0,0013 ml on day 1 and Na CMC 0,5% for 6 d, without treatment with the isolated sungkai leaf fraction. For group V, each mouse received 1 mg/kgbw of the isolated sungkai leaf fraction for 7 d without vaccination, and for group VI, only the vaccine was administered on day 1, followed by Na. CMC for 3 d. Before the treatment was administered, the experimental animals were acclimatized for 7 d. The treatment process lasted for 8 d, and on the morning of day 9, an evaluation was performed. Blood was collected by guillotine (neck artery), then centrifuged for 30 min at a speed of 3000 rpm to obtain serum (1). The serum was then used to test NK cell levels using the ELISA method [17].

Blood was collected using guillotine method (from the neck artery). The collected blood was then centrifuged at 3000 rpm for 30 min to obtain serum. The serum was then used to test the NK cell levels using the ELISA method. 50 µl of standard solution was added to the wells for the standard. For the sample, 40 µl of the sample solution and 10 µl of NK antibody were added to the wells. Then, 50 µl of streptavidin-HRV was added to the wells containing the standard and the sample (except for the blank well). The plate was mixed thoroughly and sealed. The plate was then incubated at 37 °C for 60 min. The sealer was removed and all wells were washed with 0.35 µl of wash buffer five times and left for 30 seconds to 1 min each time. Then, 50 µl of substrate solution A was added to all wells, followed by 50 µl of substrate solution B. The plate was sealed with a new sealer and incubated in the dark at 37 °C for 10 min. After that, 50 µl of stop solution was added to each well, which immediately changed the blue color in the wells to yellow. The optical density (OD value) was measured using a microplate reader at a wavelength of 450 nm within 10 min after adding the stop solution [17].

**ETHICAL APPROVAL**

Ethical approval for the test animals was obtained from the Faculty of Medicine Ethics Committee of Universitas Andalas, with approval number 405/UN.16.2/KEP-FK/2021.

**RESULTS AND DISCUSSION**

**Bis(2-ethylhexyl) phthalate (1)**

The compound under analysis consists of 24 carbon atoms, 38 hydrogen atoms, and 4 oxygen atoms. There are three carbon atoms in the aromatic region and one carbonyl carbon of the ester group (C-4,4', 167.72 ppm), while the rest are non-cyclic aliphatic carbon atoms. The aromatic ring in this compound belongs to the benzene type and consists of 2 symmetric quaternary carbon atoms (C-1,1 132.43 ppm) and two symmetric methine groups (C-2,2, 128.77; 7.63 ppm and C-3,3`, 130.84; 7.45 ppm), where both methine groups are positioned meta to each other (J = 3.5, 5.5 Hz).

Overall, the position of each carbon and hydrogen atom in this compound is confirmed by HMBC experiments. Based on the HMBC data, it is known that the carbonyl carbon is directly attached to the aromatic ring via C-4. The aliphatic chain in this compound is attached to the ester oxygen atom, starting from C-1,1``` (68.12; 4.15 ppm) and stretching to C-6,6````. There are two methyl groups (C-6,6``` and C-8,8) and five methylene groups (C-1``,1, C-3,3```, C-4,4, C-5``,5, and C-7,7```). The methine group C-2,2``` is a chiral carbon atom because there is an ethyl group attached to it.

**Stigmasterol (2)**

Compound 2 was obtained as colourless needle crystal. There is only one absorbance peak observed in the UV spectrum at 204.03 nm. This indicates the absence of conjugated double bond in the molecule. The FTIR spectral data shows the stretching vibration band of O-H at 3431 $cm^{-1}$, C-O bend at 1052 $cm^{-1}$ and the out-of plane bending vibration of O-H at 803 $cm^{-1}$. The peaks of stretching $Csp^3$-H appear at 2958, 2937 and 2869 $cm^{-1}$, while the bending vibrations of $Csp^3$-H of the methyl groups give absorption at 1464 (asymmetrical) and 1380 $cm^{-1}$ (symmetrical). An absorbance peak of symmetrical stretching C=C at 1645 $cm^{-1}$ indicates the existence of olefinic group in this compound The structure of 2 is further determined using $^{13}$C and $^1$H NMR spectral data. The carbon experiment shows that this compound contains 29 carbons consisting of six methyls, nine methylenes, 11 methines, and three quarternary carbons. A methine carbon at $\delta_C$ 71.5 (C-3) indicates a carbon attached to an oxygen atom (oxymethine), which reveals the existence of a hydroxyl group. The presence of two pairs of highly deshielded carbons at $\delta_C$ 140.5 (C-5), 121.5 (C-6), 138.1 (C-22), and 129.0 (C-23) suggests that there are two double bonds in the molecule. The DBE value of six suggests that this compound is a tetracyclic compound containing two double bonds. The $^1$H NMR experiment of 2 (fig. 1) displays information of the presence of two singlet methyl signals at $\delta_H$ 0.68 (H-18) and 0.99 (H-19), indicating that they are

bound directly to the ring system. Three doublet methyls at $\delta_H$ 0.91 (H-21), 0.81 (H-26) and 0.79 (H-27), and one triplet methyl at $\delta_H$ 0.84 (H-29) are located at the side chain outside the rings of the main skeleton. A proton at $\delta_H$ 3.51 (H-3) is attached to the oxygen-binding carbon (C-3). The occurrence of three highly deshielded protons at $\delta_H$ 5.34 (H-6), 4.99 (H-22), and 5.14 (H-23) indicates the presence of two pairs of double bond. The typical signal for the olefinic H-6 of the steroidal skeleton was evident from a proton at $\delta_H$ 5.34 integrating for one proton. The similar coupling constants (J = 15.2 Hz) of the other olefinic protons, H-22 and H-23, reveal that they are neighbours and are in trans position. Based on the discussion above and data comparison with literature, 2 is identified as stigmasterol [18].

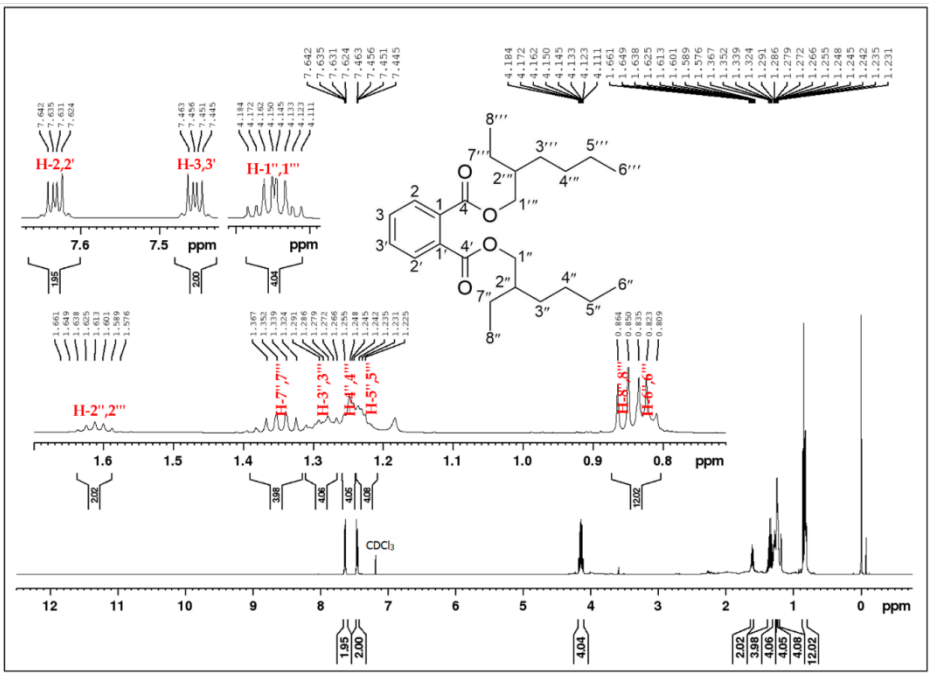



**Fig. 2: $^1$H-NMR in CDCl3 of Bis(2-ethylhexyl) phthalate (1)**

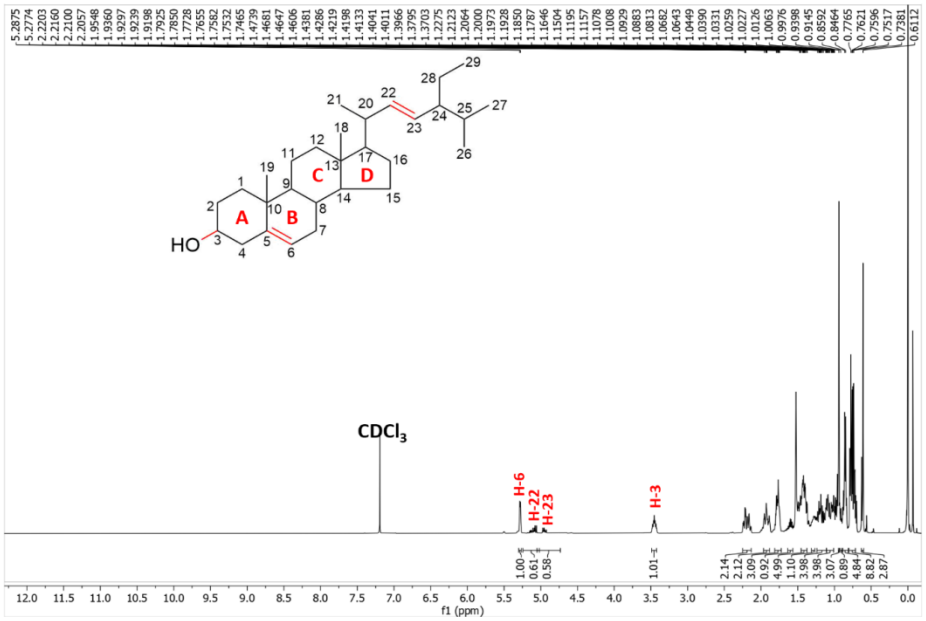



**Fig. 3: $^1$H-NMR in CDCl$_3$ of stigmasterol (2)**

**Immunostimulant assay**

The testing of two isolated compounds against immunostimulant effects revealed that stigmasterol had a greater impact on increasing the count of NK cells compared to pthalate. The immunostimulant effect for both isolates increased with the dosage administered. Based on the study results, it can be concluded that at a dose of 1 mg/kg bw, the average number of NK cells for stigmasterol was 5.31, higher than pthalate's count of 4.55. At a dose of 10 mg/kg bw, stigmasterol had a count of 6.45, while pthalate had 5.58. Finally, at a dose of 100 mg/kgbw, stigmasterol had a count of 6.44, and pthalate had 6.04. Therefore, it can be inferred that stigmasterol had a higher immunostimulant effect than pthalate at all administered dosages.

**Table 1: NK cell concentration (ng/ml) in various test conditions**

| Group | I | II | III | IV | V | Average | SD |
|---|---|---|---|---|---|---|---|
| Negative Control | 3.12 | 3.48 | 3.54 | 3.29 | 3.41 | 3.36 | 0.14 |
| Positive Control | 4.33 | 4.42 | 4.59 | 4.94 | 3.74 | 4.4 | 0.39 |
| A I | 5.03 | 5.73 | 5.03 | 5.38 | 5.38 | 5.31 | 0.26 |
| B I | 4.53 | 3.53 | 4.9 | 4.93 | 4.9 | 4.55 | 0.53 |
| A II | 5.79 | 6.76 | 6.69 | 6.28 | 6.73 | 6.45 | 0.37 |
| B II | 5.96 | 6.27 | 5.2 | 5.37 | 5.1 | 5.58 | 0.45 |
| A III | 6.51 | 6.98 | 5.64 | 6.75 | 6.31 | 6.44 | 0.46 |
| B III | 6.49 | 6.6 | 5.94 | 5.5 | 5.7 | 6.04 | 0.43 |
| S | 6.93 | 7.8 | 6.49 | 7.37 | 6.73 | 7.06 | 0.52 |

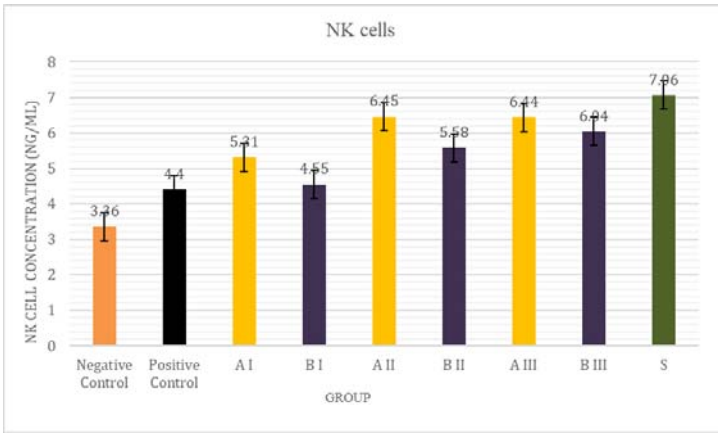



**Fig. 3: NK cells activity of stigmasterol (2) and Bis(2-ethylhexyl) phthalate (1), Information: Negative control: Na CMC 0,5%; Positive control: Moderna Covid vaccine 0,0013 ml; AI: Stigmasterol 1 mg/kg body weight; BI: Bis(2-ethylhexyl) phthalate 1 mg/kg body weight; AII: Stigmasterol 10 mg/kg body weight; BII: Bis(2-ethylhexyl) phthalate 10 mg/kg body weight; AIII: Stigmasterol 100 mg/kg body weight; BIII: Bis(2-ethylhexyl) phthalate 100 mg/kg body weight; S: Stimuno 6.5 mg/kg body weight**

## CONCLUSION

The structure elucidation results showed that the compounds obtained from the fractionation of sungkai leaf were isolated two known compounds, Bis(2-ethylhexyl) phthalate (1) and Stigmasterol (2). The administration of sungkai leaf isolate resulted in an increase in the level of Natural Killer cells in the body, with stigmasterol providing a better effect compared to Bis(2-ethylhexyl) phthalate. Based on the results of statistical tests, it was found that the increase in NK cell levels when stigmasterol at a dose of 100 mg/kg body weight was better than doses 1 mg/kg body weight and 10 mg/kg body weight. Duncan's test results showed a significant difference in the concentration of natural killer cells, $P<0.05$.

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Dwisari Dillasamola: Conceptualization, Supervision, Writing–Original Draft, Resources, Editing; Yufri Aldi: Writing–Original Draft, Writing–Review and Editing; Fatma Sri Wahyuni: Writing–Original Draft, Writing–Review and Editing, Rauza Sukma Rita: Supervision, Methodology; Yohannes Alen: Review; Dachriyanus: Review.

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All authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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