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Immunomodulatory activity of Pepolo (*Bischofia javanica Blume*) stem bark ethanolic extract in *Staphylococcus aureus*-stimulated macrophages and anticancer activity against MCF-7 cancer cells

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ABSTRACT

Pepolo (*Bischofia javanica* Blume) is an Indonesian native plant whose stem bark is widely used as a traditional medicine for various diseases. Therefore, this study aims to determine the chemical profile of the secondary metabolites of the Pepolo stem bark (PSB) extract and its effectiveness as a potential immunomodulatory and anticancer agent. The PSBs were extracted by a reflux technique with 96% v/v ethanol as solvent. The immunomodulatory activity of the PSB extract was evaluated based on its effect on the macrophages of BALB/C mice, while the cytotoxicity and anticancer potential were observed as the PSB extract response on brine shrimp lethality test and MCF-7 cancer cells, respectively. The chemical profiles of the secondary metabolites were analyzed using a Liquid Chromatography-Mass Spectrometry/Mass Spectrometry instrument. The result is that the PSB extract showed its potential as an immunomodulatory and anticancer agent. The toxicity evaluation showed that the PSB extract was classified as a toxic material. More importantly, the PSB extract showed an activity against MCF-7 cancer with lethal concentration 50% (LC_{50}) values of 68.46 µg/mL and an inhibition concentration 50% (IC_{50}) of 362.36 µg/mL, respectively. The chemical evaluation suggested that the terpenoid and polyphenolic compounds are responsible for the immunomodulatory and anticancer mechanisms. In summary, our work showed that the PSB extract is a potential alternative source for the treatment of breast cancer and supports the immune response.

INTRODUCTION

The immune system is responsible for protecting the body from unusual conditions. Of several certain unusual conditions mediated by the immune system, some may be difficult to treat, for example, autoimmune disorders, infectious diseases, cancer, and other chronic diseases (Childs *et al*., 2019). As an approach, a treatment by using an immunomodulator is often used to boost the immune system. Immunomodulators are substances that were used to restore the balance of the immune system, which has been disrupted by upregulation (immunostimulant) or downregulation (immunosuppression) mechanisms (Catanzaro *et al*., 2018; Krensky *et al*., 2012). Some synthetic drugs have been used as immunomodulatory agents. However, some of them have some inconveniences, including side effects as well as bioavailability and stability issues. For that reason, an immunomodulatory agent from natural sources is preferred (Jantan *et al*., 2015).

The Pepolo plant (*Bischofia javanica* Blume) is a type of tree in the Euphorbiaceae family widely used as a traditional medicine in Indonesia, specifically in Central Sulawesi. This plant can grow in the lowlands at altitudes of ± 1,500 m above sea level. Geographically, Pepolo is native to South Asia, Southeast Asia, Australia, and China, and it is widely distributed across Western India, Southern Japan, Eastern Australia, the Pacific,

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and the Indonesian Archipelago (Kundu et al., 2020). Several studies have demonstrated the pharmacological action of Pepolo as an anti-inflammatory (leaves), antioxidant (bark and leaves), antileukemic (leaves), antimicrobial, antiallergic, antitussive (leaves), anthelmintic (leaves), antidiarrheal (stem bark), antidiabetic, antinematode, and antimicrobial agent and as a hair growth stimulant (stem and leaf bark) an antiaging agent for the skin and for treating burns (bark) (Kundu *et al*., 2012; Lee *et al*., 2021; Lingadurai *et al*., 2011; Rajbongshi *et al* 2014).

The compounds responsible for those activities remain unclear. However, studies have shown the presence of alkaloid, flavonoid, phenolic, glycoside, tannin, and steroid/triterpenoid compounds which possibly having a relationship with pharmacological activities. More importantly, alkaloids, flavonoids, phenolics, and steroids/triterpenoids (Dhani *et al*., 2017; Jambak *et al*., 2019) have been shown to have an effect in supporting the immune response. Previous studies have proven that ethanol extract of pepolo stem bark (PSB) has a very strong antioxidant activity with an IC_{50} of 12.248 µg/mL (Jambak *et al*., 2019). There is a relationship between the antioxidants and immunomodulation, which is well known, so it takes extra antioxidants from outside the body in the form of supplement intake from herbals (Yarosz and Chang, 2018). For the aforementioned reason, we are interested in exploring the potency of the PSB as an alternative immunomodulatory agent.

Macrophages, which are found throughout the body, are the primary effector cells of innate immunity, engulfing microbes and secreting proinflammatory factors to provide the first line of defense against invading pathogens. Macrophages also play an important role in bridging the innate and adaptive systems. The presence of *Staphylococcus aureus* influences macrophage polarization and cytokine exudation toward either proinflammatory or anti-inflammatory activities. Macrophages possibly destroy *S. aureus* either intracellularly or extracellularly. Therefore, stimulating macrophages by infecting mice using *S. aureus* is of interest in this project.

In this present work, the potency of the PSB extract as an immunomodulatory and anticancer agent was evaluated by observing its phagocytic activity on *S. aureus*-stimulated macrophages and MCF-7 cells, respectively. Then, to investigate the toxicity potential of the PSB, a brine shrimp lethality test (BSLT) was performed. Additionally, the secondary metabolite profile of the PSB extract was also analyzed using a Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) instrument to predict the compounds responsible for those activities.

MATERIALS AND METHODS

Equipment and chemicals

The equipment and chemicals used in this work are a reflux extractor set, vacuum rotary evaporator (Buchi), autoclave, blender (Philips), oven (poL-HCD Aparathra), incubator, laminar air flow, electric microscope (Olympus), spectrophotometer 20 D, ACQUITY UPLC BEH C8 (1.7 µm, 2.1 × 100 mm), biosafety cabinet, centrifuge, CO_2 incubator (Thermo) and Multimode Reader, *S. aureus* ATCC 25293, Dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), Giemsa stain, cisplatin, PrestoBlue™ Cell Viability Reagent, Roswell Park Memorial Institute Medium, fetal bovine serum, Trypsin-Ethylenediaminetetraacetic Acid (EDTA), trypan blue, and Stimuno.

Plant material

The PSBs were collected from Sedoa Village, North Lore Subdistrict, Poso Regency in Central Sulawesi Province, Indonesia. The plant specimen was determined at the Laboratory of Plant Biosystematics, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Tadulako University of Palu City, Central Sulawesi Province, Indonesia, with specimen No. 484/UN28.128/BIO/2020.

Sample processing

The fresh PSBs were selected and then scraped to remove moss and other impurities. Subsequently, the PSBs were scratched from the top circle to the bottom, thoroughly washed, and drained. Then, the PSBs were cut into small pieces (2.5 × 3.5 cm) and dried in a drying cabinet at 40°C. The dried PSBs were then powdered and sieved by using a sieve with mesh No. 40. The PSB powder was stored in a tightly sealed container before extraction and evaluation. The reflux method was used for extraction with 96% ethanol as the solvent. Subsequently, PSB *Simplicia* was weighed and placed in a bottom flask with a 96% ethanol solvent, which was then refluxed for 3 × 4 hours before being allowed to stand and filtered. The filtrate was concentrated using a rotary evaporator, while the thick extract was stored in a tightly sealed container.

Phytochemical screening

Phytochemical screening was carried out using color reactions and/or precipitation with specific chemical reagents to identify alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids (Safitri *et al*., 2020; Tiwari *et al*., 2011).

Experimental animals

All experiments involving animals were carried out according to the protocols that had been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Tadulako University (Approval Tadulako University No. 1436/UN 28.1.30/Kl/2021). Healthy BALB/C male mice [body weight (BW), 20–30 g] were used in this investigation. The mice were fed, watered, and acclimatized for seven days prior to the experiment. During the experiment, the mice were divided into five groups of five animals each. Groups I–III received the PSB extract at doses of 100, 200, and 300 mg/kg BW, respectively. Group IV was a positive control and received a commercial meniran extract (Stimuno) at a dose of 19.5 mg/kg BW. Group V was the control and received 0.5% wt Na Carboxymethyl cellulose (CMC). All groups were treated once a day for seven consecutive days by gavage. Macrophages were prepared from the peritoneal fluids stimulated with *S. aureus*.

Immunomodulatory assay

Preparation of test bacteria

The *S. aureus* strain was grown overnight (24 hours) on a nutrient agar medium. The bacteria were then harvested and suspended in 0.9% NaCl. Before the infection procedure of macrophage phagocytic activity assay, the bacteria were adjusted to the desired inoculum concentration using McFarland 0.5 as a

standard (OD_{625} = 25% T for 1.5 × 10^8 CFU/ml) (Ibrahim *et al*., 2013).

Macrophage phagocytic activity assay

On day 8, the mice were infected intraperitoneally with 0.5 ml of *S. aureus* suspension and left for 1 hour. Then the mice were anesthetized by applying ether. The abdomen was dissected aseptically, and approximately 0.5 ml of peritoneal fluid containing macrophage cells (PFCMCs) from the abdomen was collected. The PFCMCs were then mixed with 1 ml of a PBS solution (pH 7.8) before being placed on the object glass. Next, the PFCMCs were fixed using methanol for 5 minutes and stained with 10% wt Giemsa stain. Before microscopic observation, the preparation was allowed to stand for 20 minutes and then rinsed with running water, and the excess water solution was drained by touching a blotting paper on one side of the slide. The microscopic observation of PFCMCs was conducted at 1,000× magnification (Yuliastri *et al*., 2021).

The immunomodulatory activity was determined by calculating the phagocytic activity of the PFCMCs using the ImageJ software. The phagocytic activity is expressed in percentage and calculated according to the following formula:

% phagocytic activity = (number of active macrophages) / (100 number of macrophages observed) ×100.

Then, the phagocytosis index was determined by % phagocytic activity positive control or treatment group / % phagocytic activity negative control.

When the phagocytosis index is less than 1, the activity is determined as an immunosuppressant. Meanwhile, if it is greater than 1, the activity is determined as an immunostimulant (Sagala and Murwanti, 2020).

The BSLT

To predict the toxicity of the PSB extract, the BSLT was applied. The PSB extract was dissolved in DMSO/water at varying concentrations and was incubated in vials containing brine shrimp larvae. About 10 brine shrimp larvae were placed in each vial. As a control, brine shrimp larvae were placed in a vial containing a mixture of seawater and DMSO only. After 24 hours, the nauplii were examined against a lighted background, and the average number of surviving larvae was determined. The percentage mortality was determined according to the following equation:

% mortality = (number of dead test larvae) / (total number of test larvae) × 100%.

Next, the percentage mortality was plotted against the concentrations, and the concentration killing 50% of the larvae [lethal concentration 50% (LC_{50})] was determined from the graph (Handayani *et al*., 2018).

Cytotoxic assay using MTT assay

For cytotoxic evaluation, the MCF-7 cancer cells were seeded at a concentration of 1.7 × 10^4 cells/well into a 96-well plate. The cells were incubated with the medium alone or with a twofold serial dilution of the PSB extract starting with the highest concentration at 1,000 µg/ml for 24 hours. Cisplatin was tested for 48 hours of incubation and served as the positive control. The Microtetrazolium (MTT) solution was added to each well and mixed. After 2 hours, the supernatant was removed and 100 µl of DMSO was added to each well to dissolve the precipitate. The cell viability percentage was calculated by measuring the absorbance at 570 nm using a multimode Enzyme-linked immunosorbent assay (ELISA) plate reader (Suzery *et al*., 2020).

Identification of active compound ethanol extract of PSB by LC-MS/MS

The PSB extract was then processed for the identification of bioactive secondary metabolites by LC-MS/MS analysis. The sample (1 mg) was dissolved in 1 ml methanol, and 100 µl was pipetted and made up to the 1.0 ml mark with methanol (LC-MS Chromasolv® grade). A 1 µl aliquot of the sample was injected into the column (ACQUITY UPLC BEH C8, 1.7 µm, 2.1 × 100 mm). A gradient elution method was used with 0.1% (v/v) water-formic acid as solvent A and 0.1% (v/v) acetonitrile-formic acid as solvent B and a flowing rate of 0.3 ml/minute with a 16 minutes gradient elution. It started with A:B in ratio 95:5 for the initial 1–8 minutes, 8 minutes 60:40, 11–13 minutes 0:100, and 16 minutes 95:5. Compounds were analyzed on a Water ACQUITY UPLC I-Class System with the XEVO G2-XS QTof Mass Spectrometer. The optimal conditions of analysis were as follows: column temperature, 30°C; sample temperature, 20°C; acquisition start time, 0.00–16.00 minutes; start mass, 50.00–1,200.00 m/z; scan time, 0.100 seconds; acquisition mode, ESI (+); capillary voltage, 2 kV; cone voltage, 30 V; collision energy, low CE, 10 eV and high CE, 40 eV; source temperature, 120°C; desolvation temperature, 500°C; cone gas flow, 50 l/hour; and desolvation gas flow, 1,000 l/hour. The LC-MS/MS data files were processed by the UNIFI software (version 1.8, Waters Corporation) with a screening solution workflow, which helped in automated data processing for reporting the positive identifications by comparison with a database.

Data analysis

A one-way analysis of variance and Duncan's test were used to determine the difference in the effect of the PSB extract at different doses on the phagocytic activity of the macrophage cells. The LC_{50} and IC_{50} were calculated using probit analysis to determine the cytotoxic potential. Lastly, probit analysis was performed using the SPSS 21 program, with a significance level of 0.05 (at a 95% confidence level).

RESULTS

The extraction of 1,678.16 g PSB using ethanol as solvent yielded a 2.84% wt concentrated extract. Then, it showed that the PSB extract contains alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids, as suggested by phytochemical screening evaluation. Those results were in line with previous work reported (Pangodian *et al*., 2020).

As shown in Figure 1(a), exposing macrophages to the PSB extract resulted in a significant phagocytic activity at nearly all PSB extract concentrations tested at a dose of 100–200 mg/kg BW. According to Figure 1(a), the highest phagocytosis activity was observed at a dose of 100 mg/kg BW, and the response was almost similar to the group which was given a meniran commercial extract (Stimuno). Stimuno contains a meniran extract (*Phyllanthus niruri* L.) which is one of the original Indonesian

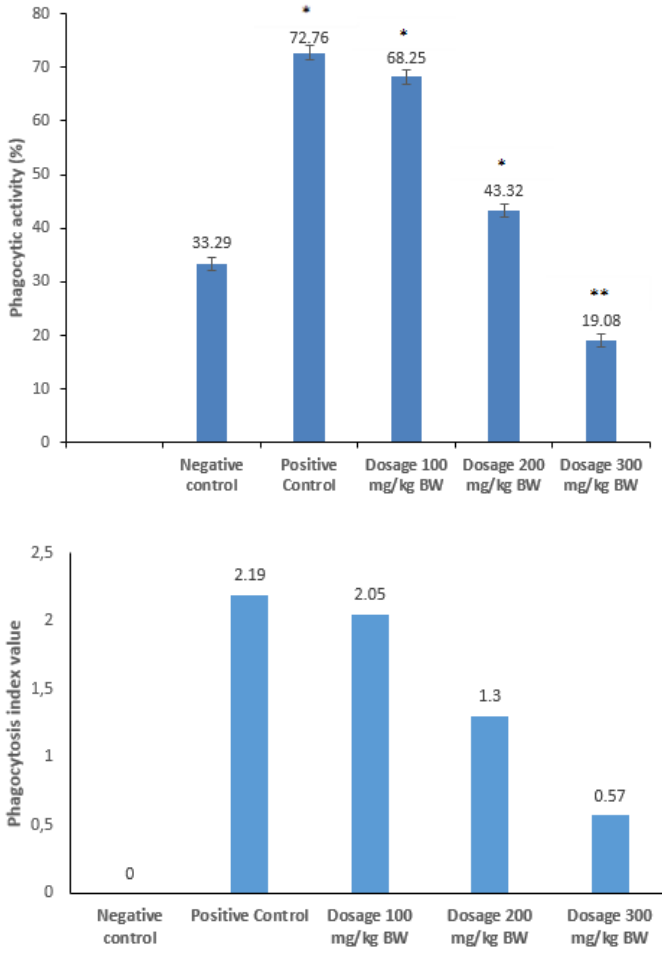

Figure 1. Phagocytic activity. (a) Phagocytosis index value and (b) data are expressed as means ± SD (n = 5). Asterisks indicate a significant difference compared to the negative control (*p < 0.01; ** p < 0.05).

immunomodulatory herbal phytopharmaca products and has been proven to be effective through clinical trials. The meniran extract can increase the activity of nonspecific and specific immune responses (Jantan *et al*., 2019; Tjandrawinata *et al*., 2005). The phagocytosis index as shown in Figure 1(b) suggested that, to provide an immunostimulant effect (>1), the concentration of the PSB extract should be below 200 mg/kg BW.

The toxicity of the herbal extract tested with the BSLT is expressed as the LC_{50} value. As tabulated in Table 1, it shows that, for the PSB extract at concentrations of 5–1,000 µg/ml, the % mortality varied in ranging of 6–100%. According to Meyer's toxicity index, extracts with LC_{50} < 1,000 µg/ml are considered as toxic, while extracts with LC_{50} > 1,000 µg/ml are considered as nontoxic. According to Clarkson's toxicity criterion, an extract with LC_{50} above 1,000 µg/ml is nontoxic, with LC_{50} of 500–1,000 µg/ml is low toxic, with LC_{50} of 100–500 µg/ml is medium toxic, while an extract with LC_{50} below 100 µg/ml is highly toxic. In accordance with the LC_{50} of the PSB extract and Meyer's criterion, the PSB extract is classified as a highly toxic extract (Hamidi *et al*., 2014).

Then, we evaluated the potency of the PSB extract for its potential in the prevention of breast cancer. The results of the MTT assay carried out with breast cancer (MCF-7) cells depicted

Table 1. Cytotoxicity profile of PSB extract by BSLT method.

Concentration (µg/ml)	Mortality (%)	LC_{50} (µg/ml)
5	6.7	LC_{50} = 68.46
10	13.33	
25	23.33	
50	53.33	
100	60	
150	70	
500	100	
1,000	100	

a certain degree of anticancer activity of the PSB extract; however, its anticancer potential varied in concentration ranges of 7.81–1,000 µg/ml (Table 2). The IC_{50} of the PSB extract was 362.36 µg/ml.

The secondary metabolite composition of the PSB extract was determined using an LC-MS/MS instrument. The compounds matched with the UNIFI software database are given in Table 3. The spectrum profile of LC-MS/MS of the PSB extract is shown in Figure 2. As presented, the PSB extract contained ambronal, asperulosidic acid, epigallocatechin(4β,8)-gallocatechin, stigmastan-3,6-dione, and unidentified compounds. The LC-MS/MS evaluation corroborated the phytochemical screening of the PSB extract.

DISCUSSION

The present investigation shows that the ethanol PSB extract exerted immunomodulatory potential as suggested by the phagocytic activity. Phagocytosis is an inherent function of macrophage cells, which is important for host protection and the initiation of innate and acquired immune responses (Harun *et al*., 2018). Therefore, peritoneal macrophages are often used in immunological studies to determine phagocytic activity (Pavlou *et al*., 2017). The percentage of phagocytic activity in peritoneal macrophage cells was calculated to determine how much the PSB extract increased the phagocytosis of invading foreign antigens. The results of the analysis showed that the administration of the PSB extract could significantly affect the phagocytic activity of peritoneal macrophages *in vitro*. Additionally, the phagocytic activity in the negative control indicates an innate (natural) immune response by macrophage cells, which protects the body from antigens that enter the body. The group that received PSB at doses of 100 and 200 mg/kg BW had higher macrophage activity than the negative control group. Furthermore, this shows that the phagocytosis of macrophages was triggered by the activation of the immune system by the stem bark extract, in addition to the natural immunity of macrophage cells. However, there was a decrease in the macrophage activity at higher doses (300 mg/kg BW) compared to other treatment groups, which shows that the administered dose can determine the immune response. If the minimum dose of an antigen is exceeded, there will be an increase in the immune response at higher doses and a decrease or elimination of the immune response at lower doses. This condition is referred to as immunogenic tolerance, which suggests that administering the extract at a higher dose may have an immunosuppressive effect (Horwitz *et al*., 2019). Additionally, this was supported

by the results of the cytotoxic test using the BSLT method and cancer cells. The prominent increase in the cytotoxic nature of the extract at higher concentrations led to a decrease in the viability of macrophage cells, which reduced the number of actively phagocytizing macrophage cells on foreign antigens. Furthermore, the effective dose of the PSB extract that increased the phagocytic activity was 100 mg/kg BW, which was due to the antioxidant-rich compounds found in PSB (Jambak *et al*., 2019). The presence of antioxidants has been shown to stabilize reactive oxidative species produced by cellular activity processes, such as the phagocytosis of macrophages, which protects the macrophage cells from free radical damage. The nonexcessive production of Reactive oxygen species (ROS) improves the phagocytosis of macrophages against foreign antigens (Nur *et al*., 2021).

The BSLT test was used to determine the toxic effect of the extract after 24 hours of treatment based on the LC_{50}. The *Artemia salina* Leach utilized in this study was 48 hours old in order to obtain the larvae at their most sensitive state with properly formed digestive tracts. Furthermore, this enabled the extract to trigger the desired effect since the cell walls of the larvae were still soft. Therefore, it could be inferred that the extract could be used as an anticancer agent if it exerted a high activity on the test animal, which was identical to cancer cells (Handayani *et al*., 2018). Preparation of two control media, including DMSO without samples and artificial seawater control, was conducted to determine the effect of DMSO and seawater on the cytotoxic test of the PSB extract. The BSLT method was reliable in determining the pharmacological activity of natural ingredients. If a plant extract is toxic based on the LC_{50} obtained from the BSLT, the plant has the potential to be used as an anticancer drug. However, if the plant is not toxic, it can be reexamined *in vivo* using other experimental animals larger than *A. salina* Leach larvae, such as mice and rats.

Table 1 shows an LC_{50} of 68.46 µg/ml (LC_{50} < 1,000 µg/ml), which implies that the PSB extract is toxic and can be used as an anticancer agent for further *in vitro* testing with MCF-7 breast cancer cells. Also, other studies using the BSLT method obtained similar results. Sinukaban *et al*. (2019), discovered that the crude methanol extract had an LC_{50} value of 56.92 ppm, while Manurung *et al*. (2020), proved that the crude ethanol extract had an LC_{50} value of 54,827 ppm.

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method with the PrestoBlue Cell Viability Reagent was used to test anticancer activity. The IC_{50} value of the extract against MCF-7 cells was 362.36 µg/ml. According to the classification of cytotoxic activity against cancer cells, the extract can be classified as "very active," "active," or "moderately active" if the IC_{50} value is < 10 µg/ml, between 10 and 100, or 100–500 µg/ml, respectively (Weerapreeyakul *et al*., 2012). Conversely, substances are said to have no cytotoxic activity if their IC50 value is >500 µg/ml (Machana *et al*., 2011). The PSB extract was classified as quite active in inhibiting the growth of MCF-7 cancer cells based on the IC_{50}. However, the cytotoxic activity of the bark extract is relatively low compared to conventional chemotherapeutic agents, such as cisplatin. The IC_{50} of the extract is quite promising for further development as a chemoprevention agent since the MCF-7 cells are known to be resistant to several chemotherapeutic agents. This development can be improved synergistically by the combination of the PSB extract with cisplatin on MCF-7 cells. Cisplatin is an anticancer drug in chemotherapy, which has a platinum complex form as an antiproliferative agent with side effects such as neurotoxicity, nephrotoxicity, and bone marrow suppression (Florea and Büsselberg, 2011). It was also reported that the use of cisplatin triggered resistance, which could be due to changes in cellular uptake, drug efflux, inhibition of apoptosis, and increased DNA repair. Cancer cell resistance and cisplatin side effects are triggered by high treatment doses. The use of combination chemotherapy is becoming increasingly popular. Furthermore, it involves the combination of nontoxic or less toxic chemopreventive compounds with chemotherapeutic agents to increase their efficacy and reduce their toxicity to normal tissues. One of the methods used to lower the dose of chemotherapeutic agents is through a combination with natural compounds, which produces a synergistic effect and also increases the sensitivity of the target cells to the chemotherapeutic agents (Achkar *et al*., 2018).

Table 2. Cytotoxicity profile of PSB extract against MCF-7 breast cancer cells.

Concentration (µg/ml)	Percentage cell viability (%)	IC_{50} (µg/ml)
7.81	98.48	IC_{50} = 362.36
15.63	97.38	
31.25	96.16	
62.50	95.39	
125	91.47	
250	67.21	
500	38.30	
1,000	15.22	

Table 3. LC-MS/MS profile of PSB extract.

No.	Component name	Observed m/z	Observed RT (minutes)	Mass error (mDA)	Class
1	Ambronal	439.3565 [M+H]	10.16	−0.6	Triterpenoid
2	Asperulosidic acid	455.1157 [M+Na]	1.51	−0.3	Monoterpenoid iridoid glycosides
3	Epigallocatechin(4β,8)-gallocatechin	611.1398 [M+H]	1.71	0.3	Polyphenolic
4	Stigmastan-3,6-dione	429.3718 [M+H]	11.18	−0.9	Steroid
5	Candidate Mass $C_{15}H_{18}O_{10}$	381.0787 [M+Na]	1.56	0.5	nd

Note: nd = not determined.

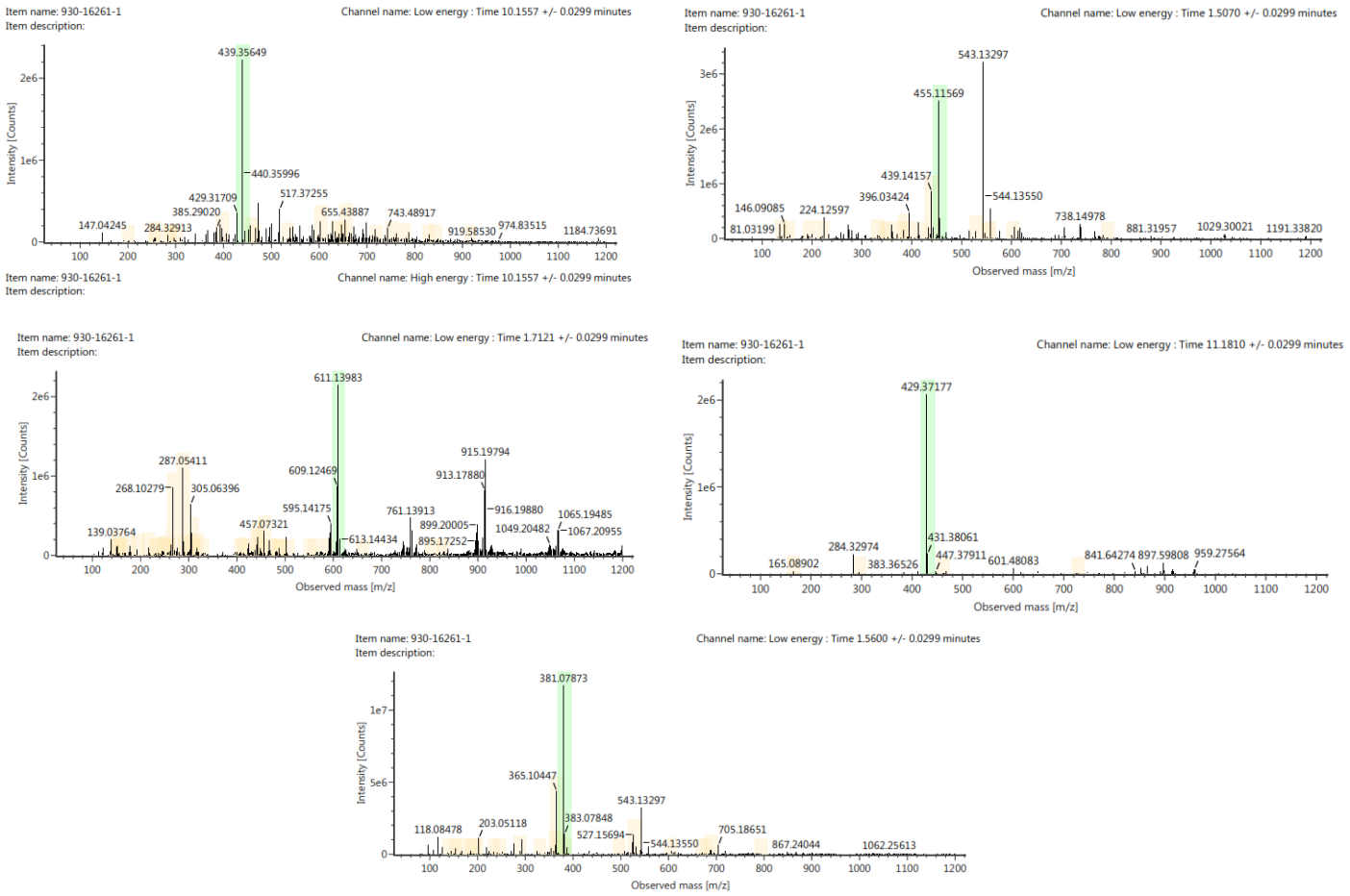

Figure 2. Spectrum profile of five compounds after LC-MS/MS analysis from PSB extract: (a) Ambronal [+H] : (44.5 PPM) 439.3565. (b) Asperulosidic acid [+Na] : (44.5 PPM) 455.1157. (c) Epigallocatechin(4β,8)-gallocatechin [+H] : (44.5 PPM) 611.1398. (d) Stigmastan-3,6-dione [+H] : (44.5 PPM) 429.3718. (e) Candidate $C_{15}H_{18}O_{10}$ [+Na] : (44.5 PPM) 381.0787.

The chemical profile of the extract was used as a reference to determine the mechanism of action of the extract. Several studies reported the presence of epi-fiedelanol acetate, friedelin (A), betulinate (B), and sitosterol in PSB. Meanwhile, this study found at least five compounds based on the results of LC-MS/MS analysis on the PSB extract, four of which could be identified in the literature, such as ambronal, asperulosidic acid, epigallocatechin(4,8)-gallocatechin, and stigmastan-3,6-dione. Additionally, this was due to the variation in the growing environment, which can affect the chemical content. The compounds identified in the extract were terpenoid and polyphenol compounds. The chemical content affects the mechanism of action as an immunostimulant, immunosuppressant, and anticancer agent. Epigallocatechin is a polyphenolic compound that serves as an antioxidant by inhibiting inflammatory signaling pathways, such as NF-B and AP-1, which are inducers and proinflammatory mediators. Furthermore, it has anticancer potential to prevent tumor cell angiogenesis and cell proliferation. Epigallocatechin is an immunostimulant and also an immunosuppressant by inhibiting cytokine transcription and weakening important immune system chains directly, specifically IL-2, which is required for lymphocyte multiplication and differentiation (Chourasia *et al*., 2021; Jantan *et al*., 2015; Kuo *et al*., 2014). The suppression of NF-B signaling pathways, mitogen-activated protein kinase, asperulosidic acid, and stigmastan are implicated in the anti-inflammatory action of the extract, which leads to the inhibition of inflammatory cytokines (TNF-, IL-6) and mediators Mitogen-activated protein kinase (MAPK). Meanwhile, the inhibition of AP-1 transactivation and cell transformation, as well as asperulosidic acid, has an antitumorigenic effect. Also, stigmastans can induce a T-helper cell response with immunomodulatory properties (Yuliastri *et al*., 2021). Ambronal inhibition of protein tyrosine kinase autophosphorylation might be the primary mechanism for the anticancer effect (Qian *et al*., 2020). The activity can result from the synergistic effect of the compounds. Therefore, the results of this study imply that the PSB extract can be investigated further to identify new resources for development as an immunomodulatory and anticancer agent. However, more studies are needed to isolate and purify these compounds with anticancer and immunomodulatory potential in *in vivo* studies.

CONCLUSION

The PSB extract can be used as a raw material for the development of anticancer and immunomodulatory drugs due to its high cytotoxicity, its ability to trigger phagocytosis, and its *in vitro* anticancer potential. Additionally, this study identifies compounds in the PSB extract which contribute to its pharmacological activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

There is no funding to report.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

ETHICAL APPROVAL

The Medical and Health Research Ethics Committee, Faculty of Medicine, Tadulako University (Approval Tadulako University No. 1436/ UN 28.1.30/Kl/2021).

AUSTHORS' CONTRIBUTION

YS (primary contributor) designed the study and contributed to data interpretation, data collection, data analysis, original manuscript writing, revision, and administration. S and R contributed to data acquisition, data analysis, and data collection. KK contributed to data analysis and interpretation. VS contributed to data interpretation and manuscript finalization. All authors drafted the article or revised it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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