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Effect of guazuma ulmifolia lamk. extract on interleukin-1 and interleukin-2 levels as immunomodulatory agent candidates

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ABSTRACT

The incidence of infections that attack the body's immunity is a major problem in various Country. In 2019, Indonesia was shocked by the occurrence of Coronavirus infection that requires an immediate therapeutic approach. The infection attacks the body's immunity in biological and physiologically there are defense mechanisms that can protect body. Indonesia has abundant biodiversity and become an opportunity in the exploration of natural medicinal ingredients. *Guazuma ulmifolia* Lamk. is a type of plant and a parasite that is widely found in the highlands and is widely found in Janji Maria Village, North Tapanuli Regency Traditionally, it used for overweight, cholesterol, cancer therapy by boiling and drink it. This study purpose to analyze the activity of extract on IL-1 and IL-2 levels using male rats as test animals by the enzyme-linked immunosorbent assay. The method used is experimentally by measuring IL-1 and IL-2 levels in serum isolated from blood rats that have been given *Guazuma ulmifolia* Lamk. extract. *Guazuma ulmifolia* Lamk. extract at doses of 50, 100, 200, and 400 mg/kgBW and quercetin as a marker agent. The levels of IL-1 and IL-2 also increased following treatment with *Guazuma ulmifolia* Lamk. Extract and quercetin in both groups as compared to the negative control group. The extract at dose of 400 mg/kg BW and quercetin showed higher immunostimulatory activity than levamisole as a positive control. It was concluded that *Guazuma ulmifolia* Lamk. exhibit immunomodulatory activity, acting as immunostimulatory agent and has potential to be developed as a therapeutic agent.

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INTRODUCTION

The incidence of infections that attack the body's immunity is a problem in various countries. Developing countries with poor sanitation can increase the incidence of infection because the environment is a source of causative microbes infection (Gombart et al., 2020). Indonesia with a

tropical climate with humid air also increases risk of infection (Jayawardena et al., 2020)(Raeber et al., 2023). The body has an immune system that is able to provide a good immune response specifically and non-specific. The immune response provided is able to protect the body against the agent Causes of infection (Strzelec et al., 2023). However, there are times when the immune system weakens under certain conditions so that pathogenic microbes can damage the balance of immune system function and causing damage to various systems (Suardi et al., 2021). Physiological regulation of the immune system sometimes weakened in certain situations so that it requires a focused therapeutic approach for functional improvement. Macrophages are one of the cells that have big function in the immune response, functionally in the phagocytosis process and as an antigen presenting cells (APC) (Stopforth & Ward, 2020)(Zhao et al., 2021).

Cytokines in immune system regulation play a role in mediators and natural immunity regulators, namely Tumor Necrosis Factor (TNF) and IL-1 (Idrus & Budu, 2020)(Jarczak & Nierhaus, 2022). This is important in initial immune response when an infection occurs. IL-2 is a cytokine that is widely produced when an advanced immune response is needed in dealing with secondary infections (Sierawska et al., 2022). Infection *Staphylococcus aureus* is performed by administering 100 µL of this suspension injected into rat peritoneum. Quercetin as a marker compound is given a total of 25 mg/kg BB and administered concurrently with the extract for 14 days. IL-2 is an important cytokine which enhancing generation of memory T cells, which are capable of undergoing secondary expansion when found antigens (Yang & Lundqvist, 2020)(Inoue et al., 2022). This cytokine is said to be an inducer lymphocyte proliferation and B cell formation. B cells play a role in proliferation and differentiation into antibodies (Ghosh et al., 2021)(Harris et al., 2023). The inovating of natural drug candidates is needed in future treatment. Immunomodulators become agents or substances necessary for modulates the immune system. Traditional medicine is increasingly in demand by the community The availability is plentiful, easy to get and affordable. Indonesia is a country which is dominant in plants that are efficacious as traditional herbal (Damoiseaux, 2020)(Chopra & Dhingra, 2021).

Empirically, belief The community in using natural medicines is an indicator of renewal plants have medicinal potential. The chemical elements of a plant can be a new source of agentspotential immunomodulation. Assessment of immunological activity of phytochemicals can be based on on its specific effects on various components and functions of the immune system (Samec et al., 2020). *Guazuma ulmifolia* Lamk. can reduce fat and body weight levels, glucose and cholesterol in the blood. Empirically, the Batak Toba people are used by boiling it and drinking the stew. The community believes that coffee beans can be used as a antihypertensive, antidiabetic, antioxidant, and antidiarrheal. Secondary metabolite content of *Guazuma ulmifolia* Lamk, namely Flavonoids, Alkaloids, Steroids, Triterpenoids, and Phenol Glycoside Compounds. Urgency of research: This research is important because of the increasing incidence of infections increasing requires comprehensive therapy. *Guazuma ulmifolia* Lamk. is declared to have several pharmacological activities. Secondary metabolite content strengthens potential analysis pharmacological from the extract. Therefore, it is necessary to analyze the activity of coffee bean extract on IL-1 and IL-2 levels in their potential as immunomodulators.

RESEARCH METHOD

Explaining This research is experimental, using the experiment method. This research was conducted at the Pharmacognosy, Pharmacology laboratory, Fakultas Farmasi Institut Kesehatan Medistra Lubuk Pakam.

Tools and materials

Equipment: Silica gel 60 F254 (Merck, Germany), Micropipette (Eppendorf), power balance (Vibra AJ), waterbath (Memmert), centrifuge (Thermo Fisher), rotary evaporator (Heidolph), autoclave (Hirayama), blender (Philips), chamber (Camag), hot plate, microtube, balance

Veterinary (Presica Geniweigher GW-1500), Oral Sonde, Oven (Memmert), Injection Syringe (Disposable Syring), vortex (Shimadzhu), and 96-well ELISA microplate (Komabiotech, Korea). Materials: Benalu Coffee. Chemicals used 70% ethanol, sodium methyl cellulose carboxy (CMC-Na), chloroform, HCl2N, DMSO, chloroform pro-analysis, methanol, acetonitrile, CDCl3, quercetin, and levamisole®. The chemicals used are 0.25% Tripsin-EDTA (Gibco), MTT salt (Sigma), saline buffer phosphate (Irvine Scientific), sodium dodecyl sulfate (SDS) in HCl 0.1 N, IL-1 protein standard (Komabiotech), IL-2 protein standard (Komabiotech), streptavidin-HRP conjugate (Komabiotech), tetramethylbenzidine (TMB) (Komabiotech), stop solution (Komabiotech). This research test protocol has been approved by the Health Research Ethics Commission (KEPK) with No. 1.701/ VII /HRE/ 2024.

The extraction was made by maceration with 70% ethanol solvent. Ethanol solvent was used for extraction. The plant material was air-dried for 20 days at 25°C. 500g of the dried powder underwent maceration in 70% ethanol for 24 hours, with initial stirring for the first 6 hours, followed by resting for 18 hours. The filtrate was then collected and evaporated (Nurcahyo et al., 2020). The extract is dissolved in 1 ml of each solvent of the extract and put in the stationary phase. Next, the plate is inserted into the chamber that has been saturated with moving phase. After the development is complete, the plate is removed and dried, then the plate is sprayed with a spot viscerer and heated in the oven at 110^{o}C for 5 minutes and then the color change is observed. The test will use 4 dose variations, namely dose 50; 100; 200; and 400 mg/kg bb. Weigh 50 mg of extract and put it into the mortuary, then pour little by little 0.5% CMC-Na and pour into a 10 mL calibration flask.

The test animal used was a male rat with an average weight of 190-200 grams. The rats were divided 5 rats into each groups of. Before being used as a test animal, it is first acclimatized for 2 weeks with standard feeding and in a good cage. Groups II-VIII were given *Staphylococcus aureus* bacteria on day 4. The animals were grouped as follows: Negative Control, given CMC-Na 0.5% suspension, Active compound (Quersetin), positive control (Levamisole® dose 25 mg/kg bb), extract dose 50, 100, 200, and 400 mg/kgBW. On the 15th day, the rats were first anesthetized using ketamine, then dissected, blood was collected from the heart to isolated serum and plasma. Serum collection, blood is stored in a tube, centrifuged for 15 minutes at a speed of 3000-4000 rpm until it separates between the supernatant and the precipitate. The supernatant layer is taken using a syringe and housed in a microtube (Marbun et al., 2020).

Measurement of IL-1 and IL-2 levels

Prepare the tools and materials, wash 3 times using 300 µL wash buffer to each holes, then turn the plate over to remove the remaining solution, add 100 µL of standard, put in to each well, closed and incubated for 2 hours protected from light. Plate cleaned with wash buffer 4 times add 100 µL of detecting antibody to each well and incubate for 2 hours at 37^{o}C protected from light. Washed plate with wash buffer 4 times then add 100 µL of strettavidin-HRP solution and incubated for 30 minutes. Washed plate 4 times using TMB solution to each well and incubated for 15–30 minutes protected from light. Observed changes the color in the well will change and add 100 µL of SDS to each hole and A change in color to yellow is observed. Absorbed reading using microplate reader at 450 nm and the rate is calculated (Knight et al., 2020).

RESULTS AND DISCUSSIONS

Results of macroscopic examination and symplisia characterization

The results of the characterization of simplicia powder are qualified based on the requirements in the VI edition of Materia Medika Indonesia which lists the moisture content of no more than 10%, while the moisture content of simplicia obtained is 7.31%. Moisture content checks are used to setting the water content in simplicia which easily absorbs water and has the potential to rot due to high moisture content. High moisture content will promote microbial growth. Water-

soluble compounds are glycosides, sugars, gums, proteins, enzymes, dyestuffs, and organic acids. The water soluble juice content of simplicia was obtained at 14%. The soluble sage content of simplicia ethanol is 8.63%. The results of sifting 500 g of paitan leaf simplicia powder with ethanol solvent obtained a thick extract that was evaporated using a rotary evaporator and dried to obtain 13.45 grams of extract.

Phytochemical Screening of *Guazuma ulmifolia* Lamk. Extract Compound by Thin Layer Chromatography Method

The determination of chemical compounds of simplicia to obtain information on the secondary metabolite compounds. The determination showing on simplicia and extracts is contain alkaloid, flavonoids, tannins, saponins, glycosides and steroids/triterpenoids (Marbun et al., 2020).

Determination of IL-1 Levels

The examination of the effect of the extract on IL-2 levels was carried out using the ELISA assays which reads the absorbance with a microplate reader at 450 nm. The levels of IL-2 against the treatment of *Guazuma ulmifolia* Lamk. 50; 100; 200; 400 mg/kgBW were obtained by measuring the absorbance with addition of solutions of 62; 125; 250; 500; 1000; 2000; and 4000 pg/mL.

Table 1. Effect of *Guazuma ulmifolia* Lamk. Extract on IL-1 Levels

Group of treatment	Level of IL-1 (pg/mL) (Mean ± SEM)
Negatif Control	224,62±3,02 [b,c]
Levamisole®	831,02±3,1[a]
Quercetin	780,86±3,2[a]
EBK 50 mg/kgBW	400,25±3,6[a,b,c]
EBK 100 mg/ kgBW	515,30±3,02[a,b,c]
EBK 200 mg/ kgBW	620,21±4,02[a,b,c]
EBK 400 mg/ kgBW	736,10±6,2[a]

Description:
a. Sig (P)<0.05: significant difference with the negative control group
b. Sig (P)<0.05: significant difference with the levamisole® group
c. Sig (P)<0.05: significant difference with Quercetin

The effect of treatment on IL-1 levels in rats shows that there is a significant effect of treatment on changes in IL-1 levels. The effect of *Guazuma ulmifolia* Lamk. extract on IL-2 levels in rats has a significant effect on changes in IL-2 levels ($p < 0.05$). The results showed that the extracts 50; 100; 200 mg/kgBW were significantly different from the negative control, levamisole, and quercetin ($p < 0.05$). *Guazuma ulmifolia* Lamk. 400 mg/kgBW was significantly different from the negative control and not significantly different from levamisole and quercetin ($p > 0.05$). IL-1ß is a cytokine that is cleavable by ICE, and plays a role in cellular activities such as proliferation, differentiation and apoptosis. IL-1 acts as an immunocompetent cell that plays a major role in the inflammatory response and controls the production of other immune cells (Griffiths et al., 2021).

Determination of IL-2 Levels

Examination of the effect of extracts on IL-2 levels using a microplate reader at 450 nm. IL-2 levels for treatments of extracts of 50; 100; 200; 400 mg/kgBW were obtained by measuring absorbance with the addition of standard solutions of 62; 125; 250; 500; 1000; 2000; and 4000 pg/mL.

Table 2. Effect of *Guazuma ulmifolia* Lamk. Extract on IL-2 Levels

Group of treatment	Level of IL-2 (pg/mL) (Mean ± SEM)
Negatif Control	224,73±8,02[b,c]
Levamisole®	826,12±4,21[a]
Quercetin	851,76±5,24[a]
EBK 50 mg/kgBW	425,31±3,52[a,b,c]
EBK 100 mg/ kgBW	576,52±3,01 [a,b,c]
EBK 200 mg/ kgBW	643,11±4,02[a,b,c]
EBK 400 mg/ kgBW	786,01±4,24[a]

Description:
a. Sig (P)<0.05: significant difference with the negative control group
b. Sig (P)<0.05: significant difference with the levamisole® group
c. Sig (P)<0.05: significant difference with the Quercetin

The effect of treatment on IL-2 levels in rats showed that there was a significant effect of treatment on changes in IL-2 levels. The effect of treatment on IL-2 levels in mice had a significant effect on changes in IL-2 levels ($p < 0.05$). The results showed that the extract of 50; 100; 200 mg/kgBW was significantly different from the negative control, levamisole, and quercetin ($p < 0.05$). EBK 400 mg/kgBW was significantly different from the negative control and was not significantly different from levamisole and quercetin ($p > 0.05$).

The enhancing in IL-2 can be caused by the content of Quercetin. Quercetin is a flavonol compound derived from flavonoids that can increasing the immune regulating, namely by increasing IL-2 activity and enhancing lymphocyte proliferation (Sipayung, 2023)(Al-Qahtani et al., 2024). IL-2 is one of the cytokines that can regulating the immune response, functioning as a mitogen for T cells (Gurjar & Pal, 2021)(Oo et al., 2022).

CONCLUSION

The effect of *Guazuma ulmifolia* Lamk. on increasing IL-2 antibody levels in rats infected with *Staphylococcus aureus* differed significantly from negative controls ($p < 0.05$). EBK 100; 200; 400 mg/ KgBW but EBK 50 did not differ significantly ($p > 0.05$). EBK 200; 400 mg/KgBW did not differ significantly from levamisole, and quercetin ($p > 0.05$). The discovery of phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, and steroids/triterpenoids has also enriched the literature on the chemical composition of Guazuma ulmifolia and its biological benefits.

The use of natural extracts such as Guazuma ulmifolia in boosting immunity can reduce the risk of side effects often associated with synthetic drugs, thus potentially offering safer and more affordable health solutions for the wider community. This study also supports initiatives to involve more herbal products in medicine, which is relevant to the global trend towards phytotherapy and plant-based alternative medicine.

Future research is expected to conduct further tests to determine the long-term safety of using Guazuma ulmifolia extract, especially at high doses (400 mg/kgBW). More in-depth studies also need to be conducted in humans to see its efficacy and toxicity before wider clinical application.

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