Phytochemical Analysis of the Antioxidant Compounds of Baper Tea and Its Potential as an Immunomodulatory agent and Candidate for Standardized Herbal Medicine

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Abstract

The antioxidant content of Baper tea was determined using phytochemical testing, and the potential of these contents as immunomodulatory agents and candidates for standardized herbal medications was investigated. A randomized design was used in this experimental study. Phytochemical assays are used to determine the antioxidant content of Baper tea, whereas organoleptic studies are performed using hedonic tests. A literature review was used to assess the content's potential as an immunomodulatory agent and candidate for standardized herbal medicine. The study lasted four months at the Biology and National Food and Drug Agency Laboratory in Bali Province. Data analysis is based on descriptive methods. Baper tea products are prepared in a 2.1.1 ratio. According to the hedonic test results, 41 panelists (87.23 %) prefer Baper tea products based on their color assessment, flavor, aroma, community reception, and acceptability. The matrix and security test revealed a water content of 4.43 %, an IC₅₀ of 24.27 ppm, α-glucosidase inhibitors of 12.18 ppm, a total phenolic content of 28.00 %, and a total flavonoid content of 15.57 %. Baper tea raw materials contain components that have the potential to act as immunomodulatory agents and candidates for standardized herbal medications based on local wisdom.

Keywords: Baper tea, Covid-19, Immunomodulatory agents, Local wisdom, Standardized herbal medicine

Introduction

Corona Virus Disease (COVID-19) has spread to over 215 countries worldwide, including Indonesia, and has become a pandemic [1]. The COVID-19 etiology is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV-2). SARS-COV-2 targets the immune and human respiratory systems through droplet contamination, liquid sneezing, and coughing. [2,3]. The Coronavirus family has been known to mutate in several locations worldwide, including Indonesia [4]. According to a recent study, there are four distinct kinds of COVID-19: Alpha (B.1.17), Beta (B.1.351), Gamma (P.1/B.1.352), and Delta Coronavirus (B.1.617.2) [5,6]. Coronaviruses of the alpha and Beta kinds may infect both animals and people. On the other hand, Coronaviruses of the Delta and Gamma kinds can only infect animals [7]. However, recent advancements include the appearance of a new COVID-19 variation associated with the effects of a previous variant mutation. According to the Ministry of Health of the Republic of Indonesia, the new variety of COVID-19 was identified in Indonesia as Epsilon (B.1.42), Zeta (P.2), Eta (B.1.525), Kappa (B.1.617.1), and Lota (B.1.526) [6]. If the population is not disciplined in establishing a health protocol and maintaining body immunity, the growth in COVID-19 cases is predicted to increase.

The present issue is that the COVID-19 vaccine is in short supply, and its distribution is uneven throughout the area, particularly in Indonesia. The issue is that the COVID-19 vaccine is almost nonexistent. The community's reluctance to undertake immunization programs was exacerbated by the unpleasant effects of the COVID-19 vaccine and its effectiveness and efficacy. The government's efforts are focused on creating an Indonesian COVID-19 vaccine and urging people to take vitamins, herbal medicines, and immunomodulators to boost their immune systems throughout the pandemic. Chemical substances that regulate the immune system's function and activity to defend the body against viruses, infections and other external dangers are called immunomodulators. Each kind is classified as an immunostimulator (which promotes immune system activity), immunoregulator (which regulates immune system function), or immunosuppressor (which suppresses immune system function) [8]. Previous research
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has shown that plant compounds function as immunostimulators, overcoming immunodeficiency and improving immune system performance [9,10].

The Indonesian people have relied on traditional medicine to prevent and treat illnesses for decades. Traditional medicine includes traditional medicine, conventional medicine, and phytopharmaceuticals [11]. Many individuals are turning to conventional therapies to strengthen their bodies’ immunity due to the rising incidence of COVID-19 [12]. However, the use of combinations of plant materials as immunomodulatory agents packaged in standardized herbal medicines and phytopharmaceuticals is still limited, particularly in Indonesia, so preliminary studies are required to obtain plant materials that are thought to have benefits as traditional body healers and can later be used as candidates for herbal medicines in Indonesia. In this work, we created shallot skin (Allium cepa L.), morel berry leaves (Physalis angulata), and small gooseberry leaves (Phyllanthus urinaria) to be evaluated for antioxidant activity using phytochemical assays. We studied their potential as immunomodulatory agents. Based on past research and candidates for standardized herbal medicines.

The material was chosen because it is simple, inexpensive, has several advantages, and is healthy. The shallot skin, morel berry leaves, and small gooseberry leaves have an alkaloid, flavonoids (quercetin and epigallocatechin gallate), tannins, kumarin, polyphenols, saponins, allicine, hydrogen sulfide, filantin, hipofilantin, kalium, triterpenoid, taurine, glycosides and antrococyinon [13]. Flavonoid chemicals can boost IL-2 synthesis during T-cell activation and proliferation [14]. Filantin can increase macrophage cell phagocytosis, and hepatoprotective and EGCG play a role in stimulating the production of IL-1α, IL-1β, and TNF-α and increasing IL-12 secretion, IFN-γ and lymphocyte proliferation [15,16]. Based on previous findings, we sought to perform preliminary research on the antioxidant content of Baper tea, which was analyzed phytochemically and evaluated for its potential as an immunomodulatory agent and candidate for standardized herbal medicine during the COVID-19 pandemic. Based on the research of safe, inexpensive, and effective natural antioxidants, the findings of this study enable the development of formulations of prospective immunomodulatory agents. Because it is the outcome of herbal tea manufacturing based on local understanding, Baper tea has the potential to be utilized as a candidate alternative to standardized herbal medicine in Indonesia.

Materials and methods

Materials in research

Thermos, sterile handscones, haircups, masks, measuring cups, digital scales, tweezers, mercury thermometer, drops pipettes, measuring flasks, filters, 1cc and 3cc, iron spoon, gawai, and stationery were utilized in this investigation. The following items were employed in this study: 500 g shallot skin, 250 g morel berry leaves, 250 g small gooseberry leaves, tea bags, granulated sugar, boiling water, and a pouch for tea packaging.

Research design

The study was set up as an experimental study with a completely randomized design. The study involved conducting organoleptic testing on 47 panelists using the hedonics test to determine the quality of life-based on the color, taste, aroma, and public acceptance of Baper tea products using the assessment sheet, as well as content testing and security of Baper tea to determine the value of water content, IC50, α-glucoside inhibitors, total phenolics, and total flavonoids in 1 package [17] and WHO guidelines on good herbal processing practices for herbal medicines [18].

Ethical appropriate

The health research ethics committee of the Denpasar Health Polytechnic has accepted the use of 47 persons for organoleptic testing, with registration number LB/02.03/EA/KEPK/0133/2021.

The location and time of the research

This study lasted four months, from January to April of 2021. The Food and Food Supervisory Agency of Bali Province conducted research at the Department of Biology, Hindu University of Indonesia, for organoleptic testing. It tested the content and security of Baper tea.
Research implementation

Material preparation

The Shallot skin was collected clean after the older onions were stripped, while the Morel Berry and Small Gooseberry leaves were collected moist and cleansed. Additionally, all raw materials were dried during transportation to achieve a water content of \( \frac{1}{4} \) leaf weight. Additionally, Morel Berry Leaves and Small Gooseberry leaves are dried for 25 min at a temperature of 45 °C using a Rotary Dryer. The dried leaves were then dried at room temperature for three h.

Baper tea formulation and deciding

The early test findings sped up the development of the formulation in this investigation. In a 2.1.1 ratio, 500 g shallot skin, 250 g tiny gooseberry leaves, and 250 g morel berry leaves make up the Baper tea Formulation. In addition, a smooth blender is used for cooking the ready-to-use raw material. The fine powder is then packaged and placed in a tea bag weighing 1 g each bag.

Organoleptic Assays of Baper tea

Each panelist was given one glass of tea that had been trapped, and they were asked to rate the tea's color, flavor, fragrance, acceptability, and overall comparison to Baper tea. Each panelist completed the evaluation form that was provided.

Matrix Assays and security for Baper tea

Baper tea matrix testing and security are carried out by the National Food and Drug Agency (BPOM) of Bali Province to determine the content of DPPH free radical scavengers (IC\(_{50}\)), \( \alpha \)-glucosidase inhibitors, total phenolic, total flavonoids, and water content using spectrophotometric and thermogravimetric methods following Indonesia National Standard 3836:2013 guidelines for dry tea in the pack.

Data collection

An organoleptic assessment form, as well as the findings of the content laboratory and the security of Baper tea, were used to collect data for this study. The study subject is chosen through purposeful sampling. In this study, the Slovin method was utilized to calculate the sample size and a review based on panelist interviews [19].

Data analysis

The findings of hedonic tests, Baper tea content, and security tests were used in this study. Tables, graphs, and narratives are used to show research findings. Previous research publications, Indonesian national standard references, the National Food and Drug Administration and GMP Herbal Medicine supported data analysis using descriptive methods.

Results and discussion

The data from the distribution of organoleptic Baper tea utilizing the hedonic test technique for 47 panelists based on color evaluation, fragrance, flavor, community receipts, and overall were not trained with a repeat of the treatment once displayed in Figure 1. The findings of the hedonic test (Figure 1), which involved 47 untrained panelists, show that Baper tea is acceptable to the general public. Color analysis was carried out. Thirty-seven individuals said they were extremely similar, 35 were very similar, and 34 were pretty similar, and the public’s approval was measured. It was described as “quite similar” by 38 people and “very similar” by 41 others. This shows that Baper tea met the SNI 3836: 2013 standard requirement that organoleptic testing is practicable if the panelist’s value for the product supplied is more than 50 % and that Baper tea has the potential to be produced as a beverage.
Figure 1 Hedonic test result for Baper tea.

Testing the quality of Baper tea with an assessment tool following SNI 3836: 2013 yielded the following results: Baper tea has a bitter flavor, according to the reviewers. This comes within the “normal” category. Catechin compounds produced the feeling of drinking barrel tea; the compound was in the form of tannin, which did not correspond to the nature of the premise and smoldered the protein, resulting in a bitter taste [20]. The scent of Baper tea was judged to be as huge as it was fragrant, indicating that the gallate acid was dried on the raw material of oxidized tea, resulting in the production of thearubigin (TR) compound, which was responsible for the fragrant aroma generated [8]. Color analysis reveals that the color of the seduction generated by Baper tea is brilliant brown and yellowish-red brown, indicating the presence of a combination of filantin, quercetin, and theaflavin that alters the color of the seduction produced [21].

Baper tea has passed the content and security test at the Bali Province Food and Drug Supervisory Agency following SNI 3836: 2013, presented in Table 1. The water content of Baper tea fulfills the norm of < 8.0 %, according to the findings of evaluating its content and security. The DPPH Free Radical Scavengers (IC₅₀) in Baper tea have a very high antioxidant intensity, specifically IC₅₀ < 50 g/mL, according to the findings of phytochemical screening. Baper tea has a high potential as a candidate for immunomodulatory agents in preventing and healing cell damage caused by pathogenic illnesses such as viruses and free radical exposure. According to the Baper tea phytochemical screening results, tea’s components can be developed as candidates for standardized herbal medicines in Indonesia based on local wisdom [15].

Table 1 The results of testing the matrix and safety of Baper tea.

<table>
<thead>
<tr>
<th>No</th>
<th>Type</th>
<th>Result</th>
<th>Unit</th>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water content</td>
<td>4.43</td>
<td>%</td>
<td>Thermogravimetry</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>DPPH Free radical Scavengers (IC₅₀)</td>
<td>24.27</td>
<td>ppm</td>
<td>Spectrophotometry</td>
<td>Very active</td>
</tr>
<tr>
<td>3</td>
<td>α-glucosidase inhibitor</td>
<td>12.18</td>
<td>ppm</td>
<td>Spectrophotometry</td>
<td>Very active</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic total</td>
<td>28.00</td>
<td>%</td>
<td>Spectrophotometry</td>
<td>Equivalent floroglucinol</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoid total</td>
<td>15.57</td>
<td>%</td>
<td>Spectrophotometry</td>
<td>Equivalent quercetin</td>
</tr>
</tbody>
</table>

The higher the inhibitory action on free radicals in the body, the stronger the antioxidant activity on particular materials. According to Wangiyana’s research [14], the lower the IC50 value, the stronger the antioxidant activity. The findings of α-glucosidase inhibitors, total phenolic, and total flavonoids indicate that there is bioactive material with immunity booster properties and an increase in the number of free radicals and pathogens that are prevented. According to test results, Baper tea complies with SNI standard
Baper tea, made from shallot skin (Allium Cepa L.), morel berry leaves (Physalis angulata), and small gooseberry leaves (Phyllanthus urinaria), has the potential to be developed as an immunomodulatory agent candidate and deserves to be developed as it has thought safer for health, improve body health, and is extremely economical. The development of Baper tea as a candidate immunomodulatory agent presented in standardized herbal medicines is thought to be capable of naturally restoring immunological responses, fighting pathogens, anti-inflammatory by reducing cytokine activity, and protecting against pathogenic infections by activating various components and mechanisms in the body [22].

Antioxidant-rich compounds include flavonoids, polyphenols, filantine, hipofilantin, alkaloids, quercetin, allicin, terpenoid, phenol, and phytosterol are found in Baper tea raw material in the form of shallot skin, Morel Berry leaves, and tiny gooseberry leaves. Baper tea has the potential to be used in herbal tea preparations as a candidate for standardized herbal medicine. The flavonoid component in Baper tea, which is thought to enhance the body’s immune system, can trigger Th1 to generate IFN-γ. IFN-γ is an important mediator in the formation of T cells [15]. A suitably substantial correlation coefficient between IFN- and natural killer cells (NK cells) and CD8+ reveals this connection. Activated NK cells produce a variety of cytokines that control other immune systems. IFN-γ and TNF-α, which are powerful immunoregulator cytokines, can be produced by NK cells. Increased IFN-γ production enhances the immune surveillance component, namely NK cell activity as the primary defensive system and cytotoxic T cell function (CD8+) [23].

Flavonoids (flavonol, quercetin, and luteolin), bioactive components in Baper tea, can enhance the proliferation of T lymphocyte cells, which are indications of increased body immune systems [24,25]. Flavonoid content is a natural antioxidant that boosts immune response and fights lymphokine generated by T-cytotoxic cells, encouraging phagocytic cells to respond to phagocytosis. Putra et al. [26] study indicated that the flavonoid molecule has immunostimulant potential since it can enhance IL-2 synthesis, which is involved in T cell activation and proliferation. Filantine and literary compounds found in Shallot skin have been shown to activate Murine Macrophage Cell Lines and alter the immune system [27,28]. Research by Marefati et al. [16] stated that the Shallot skin has a very strong antioxidant that has immunomodulatory effects such as t-cell proliferation and macrophage activation, reducing IL-12 production, TNFα, IL-1α, IL -6, IL-8, IFN-γ, IL-2 and enhance IL-10 and increase the level of plasma no and IFN-α [29].

The ability of lectin compounds to regulate the immune system by enhancing macrophage phagocytosis has been demonstrated [30]. Toll-Like Receptors (TLR-1 and TLR-2) bind to lectin molecules, responding to the body's innate viral immunity. TLR-1 and TLR-2 can produce antipathogen cytokines, chemokines, and peptides in the natural world [31]. Baper tea’s polyphenol concentration is linked to its action and promise as an anti-inflammatory, antiviral, and immune-boosting agent. The polyphenol component in Baper tea is epigallocatechin gallate (EGCG), which has the potential to play a role in stimulating the production of IL-1α, IL-1β, and TNF- α. EGCG helps the phagocytosis process, increases lymphocyte resistance, proliferation, and secretion of IL-12 macrophages, increases IFN-γ, and inhibits the bond between FCSri and IgE, hindering histamine expenditure. FcCRI serves as an IgE receptor attached to the surface of the mast cell and plays a role in inhibiting IL-2 originating from T cells [32].

Research by Octarina et al. [33] and Rahayu et al. [34] the alkaloid chemicals phenol, tannin, lignin, saponin, flavonoids, phytosterol, essential oil, and anthraquinone found in tiny gooseberry and morel berry leaves have immunity-boosting properties. Research Febryantoro et al. [35] Anti-inflammatory and immunostimulant properties of phyllanthin and hypophyllanthin chemicals in tiny gooseberry leaves were discovered, along with inhibitory mechanisms and phagocytosis against infections. Research Octarina et al. [33] Morel berry leaves (Physalis angulata) contain saponin, tannin, and polyphenol, which act as an immunostimulator by increasing immunoglobulin M (IgM) activity. According to the description, Shallot Skin, Morel Berry Leaves, and Small Gooseberry Leave packed in Baper tea have very strong antioxidant activity and have the potential to be used as immunomodulatory agents in standardized herbal medical formulations, particularly in Indonesia. Figure 2 shows the composition of the Baper tea compound created with Chemdraw version 12.0.

The mechanism of action of Baper tea starch as an immunity booster in the preparation of standard herbal medicines, namely the content of Baper tea starch consists of secondary metabolite compounds in the form of flavonoids, tannins, quercetin, luteolin, polyphenols, saponins, alkaloids, and epigallocatechin-gallate, all of which have antioxidant properties and work to ward off free radicals [36]. Secondary metabolite chemicals, especially antioxidants, function by causing Th1 to produce more IFN-γ. IFN-γ has an antiviral impact by suppressing protein synthesis in the COVID-19 virus’s structure via a double-
stranded RNA inhibition mechanism, an antiviral replication product. The enzyme 2’-5’oligoadenylate synthetase is induced and activated by IFN-γ. Activated enzymes will polarize ATP into linked oligomers, activating the cell RNA, destroying single-stranded RNA, and limiting protein synthesis in the cell. Increased IFN-γ activity causes an increase in immune surveillance activation in the human body, which can boost the job of the Natural Killer Cell (NK cell) in melisis, the virus-infected cells. IFN-γ boosts the production of MHC I molecules, which CD8+ cells need to detect viral antigens, while also boosting MHC II. As a result, the body activates cytotoxic cells to generate and excite T cells, allowing antiviral activity to be carried out through both a cellular and a humoral immune response, which characterizes as an immunity booster in the body [37]. The potential of antioxidant chemicals as immune boosters and their action against free radicals that enter the body is also linked to an increase in T cell activity [38,39].

Figure 2 The content of the Baper tea compound as a natural immunomodulatory description: (a) epigallocatechin gallate; (b) Quercetin; (c) Anthraquinone; (d) Theaflavin; (e) Allicin; (f) Phenol; (g) Luteolin; (h) α-sitosterol; (i) Phenyl α-glucopopanoside.

Conclusions

Baper tea is made of shallot skin (Allium Cepa L.), morel berry leaves (Physalis angulata), and small gooseberry leaves (Phyllanthus urinaria), have significant antioxidant content and have the potential to be utilized as a candidate for immunomodulatory agents and standardized herbal medicines in Indonesia. Further study is expected to be able to examine the efficacy of the chemicals included in Baper tea against the effects of antiviral and immunomodulatory medications, as well as evaluate the results in experimental animals when ingested.

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