PHYTOCHEMICAL SCREENING AND DETERMINATION OF SECONDARY METABOLITICAL CAPACITY OF BUTTERFLY LEAVES (Bauhinia Purpurea L) AS A GUIDE FOR WOUND HEALING IN WOMEN AFTER PREGNANCY

a Tri Tunggal, bYuniarti, cRusmilawaty, dNur Rohmah Prihatanti, eNortaila Sofia, fNoor Adha Aprilea, gAnwar Mallongi

ABSTRACT

Objective: The objectives of the research were to break down and analyze the secondary metabolite chemical compounds identified in Butterfly Leaves (Bauhinia purpurea L) and determine the amount present in the plant.

Theoretical framework: Proper perineal wound care will help the perineal wound healing process in a short time. One of the ways to treat wounds in postnatal women is to use a traditional butterfly flower herb as a means of accelerating wound healing. Proper perineal wound care will help the perineal wound healing process, so the wound can heal quickly. One way to treat perineal wounds that can be done is to use a butterfly flower plant (Bauhinia Purpurea L).

Methods: This research design is experimental, testing the levels of alkaloid compounds, flavonoids, saponins, steroids, tannins in butterfly leaves (Bauhinia Purpurea L). The population and sample were all butterfly leaves. This research was conducted at a herbal medicine factory in Banjarmasin.

Results and conclusions: The group of secondary metabolite compounds contained in the butterfly leaf (Bauhinia Purpurea L): Flavoid, tannin, phenol, alkaloid, safonim and steroid). Secondary metabolic levels in butterfly leaves and flowers: Flavonoids: 19.03 mg/g (1.90%), Tannins: 23.39 mg/g (2.33 %), Phenol: 37.84 mg/g (3.78 %), Alkoloids: 2.48 mg/g (2.48 %), Saponins: 11.26 mg/g (0.12 %), Steroids: 2.32 mg/g (2.32%). Proper perineal wound care will help the healing process of perineal wounds, so that the wound can heal in a short time. One way of treating perineal wounds that can be done is using butterfly flower plants (Bauhinia Purpurea L).

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Implications of the research: This research provides a scientific basis for understanding the chemical composition of butterfly leaves (Bauhinia purpurea L) and highlights their potential benefits in perineal wound care. The implications extend to herbal medicine practices, healthcare for postnatal women, the integration of traditional and modern medicine, and the promotion of further research and development in this field.

Keywords: butterfly leaf, phytochemical screening, perineal, rupture, natural medicine, childbirth.

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TRIAGEM FITOQUÍMICA E DETERMINAÇÃO DA CAPACIDADE METABOLÍTICA SECUNDÁRIA DE FOLHAS DE BORBOLETAS (Bauhinia Purpurea L) COMO GUIA PARA A CICATRIZAÇÃO DE FERIDAS EM MULHERES APÓS A GRAVIDEZ

RESUMO

Objetivo: Os objetivos da pesquisa foram quebrar e analisar os metabólitos químicos secundários identificados nas folhas de borboletas (Bauhinia purpurea L) e determinar a quantidade presente na planta.

Estrutura teórica: O cuidado adequado da ferida perineal ajudará o processo de cicatrização da ferida perineal em pouco tempo. Uma das formas de tratar feridas em mulheres pós-natais é usar uma erva de flor de borboleta tradicional como meio de acelerar a cicatrização de feridas. O cuidado adequado da ferida perineal ajudará o processo de cicatrização da ferida perineal, para que a ferida possa curar rapidamente. Uma maneira de tratar feridas perineais que pode ser feita é usar uma flor de borboleta (Bauhinia Purpurea L).

Métodos: Este projeto de pesquisa é experimental, testando os níveis de compostos alcaloides, flavonoides, saponinas, esteroides, taninos em folhas de borboleta (Bauhinia Purpurea L). A população e a amostra eram todas folhas de borboletas. Esta pesquisa foi realizada em uma fábrica de medicamentos à base de plantas em Banjarmasin.

Resultados e conclusões: Grupo de compostos metabólitos secundários contidos na folha da borboleta (Bauhinia Purpurea L.): Fleve, tanino, fenol, alcaloide, safonim e esteroide. Níveis metabólitos secundários em folhas e flores de borboletas: Flanoides: 19,03 mg/g (1,90%), Taninos: 23,39 mg/g (2,33%), Fenol: 37,84 mg/g (3,78%), Alcoóides: 2,48 mg/g (2,48%), Saponinas: 11,26 mg/g (0,12%), Esteroides: 2,32 mg/g (2,48%) 32 %. O cuidado adequado da ferida perineal ajudará o processo de cicatrização das feridas perineais, para que a ferida possa curar em um curto período de tempo. Uma forma de tratar feridas perineais que pode ser feita é usar flores de borboletas (Bauhinia Purpurea L).

Implicações da pesquisa: Esta pesquisa fornece uma base científica para a compreensão da composição química das folhas de borboletas (Bauhinia purpurea L) e destaca seus potenciais benefícios no tratamento de feridas perineais. As implicações estendem-se às práticas de fitoterapia, aos cuidados de saúde para as mulheres pós-natais, à integração da medicina tradicional e moderna e à promoção de mais investigação e desenvolvimento neste domínio.

Palavras-chave: folha de borboleta, triagem fitoquímica, perineal, ruptura, medicina natural, parto.
EXAMEN FITOQUÍMICO Y DETERMINACIÓN DE LA CAPACIDAD METABOLÍTICA SECUNDARIA DE LAS HOJAS DE MARIPOSA (Bauhinia Purpurea L.) COMO GUÍA PARA LA CICATRIZACIÓN DE HERIDAS EN MUJERES DESPUÉS DEL EMBARAZO

RESUMEN

Objetivo: Los objetivos de la investigación fueron descomponer y analizar los metabolitos químicos secundarios identificados en las hojas de la mariposa (Bauhinia purpurea L.) y determinar la cantidad presente en la planta.

Estructura teórica: El cuidado adecuado de la herida perineal ayudará al proceso de curación de la herida perineal en poco tiempo. Una de las formas de tratar las heridas en las mujeres posnatales es utilizar una hierba tradicional de la flor de la mariposa como un medio para acelerar la cicatrización de las heridas. El cuidado adecuado de la herida perineal ayudará al proceso de cicatrización de la herida perineal, de modo que la herida pueda cicatrizar rápidamente. Una forma de tratar las heridas perineales que se pueden hacer es usar una flor de mariposa (Bauhinia Purpurea L.).

Métodos: Este proyecto de investigación es experimental, probando los niveles de compuestos alcaloides, flavonoides, saponinas, esteroides, taninos en hojas de mariposa (Bauhinia Purpurea L.). La población y muestra fueron todas hojas de mariposa. Esta investigación se llevó a cabo en una fábrica de medicamentos a base de plantas en Banjarmasin.

Resultados y conclusiones: Grupo de compuestos metabólicos secundarios contenidos en la hoja de la mariposa (Bauhinia Purpurea L.): Luz, tanino, fenol, alcaloide, safonina y esteroide. Niveles metabólicos secundarios en hojas y flores de mariposa: Flanoides: 19,03 mg/g (1,90%), Taninos: 23,39 mg/g (2,33%), Fenol: 37,84 mg/g (3,78%), Alcoholes: 2,48 mg/g (2,48%), Saponinas: 11,26 mg/g (0,12%), Esteroides: 2,32 mg/g (2,48%) 32 ). El cuidado adecuado de la herida perineal ayudará al proceso de cicatrización de las heridas perineales, de modo que la herida pueda curar en un corto período de tiempo. Una forma de tratar las heridas perineales que se pueden hacer es usar flores de mariposa (Bauhinia Purpurea L.).

Implicaciones de la investigación: Esta investigación proporciona una base científica para entender la composición química de las hojas de mariposa (Bauhinia purpurea L.) y destaca sus beneficios potenciales en el tratamiento de heridas perineales. Las implicaciones se extienden a las prácticas de la medicina herbal, la atención sanitaria para las mujeres posnatales, la integración de la medicina tradicional y moderna y la promoción de nuevas investigaciones y desarrollo en este campo.

Palabras clave: hoja de mariposa, cribado fitoquímico, perineal, ruptura, medicina natural, parto.

1 INTRODUCTION

Childbirth is a complex process encompassing a multitude of factors, including the psychosocial experiences, family dynamics, socio-cultural norms, and the provision of maternity services (Olza et al., 2018). One of the paramount expectations during childbirth is the delivery of maternity care that prioritizes safety and humanization for both the mother, the newborn, and their families (Downe et al., 2018). Although labor is a
natural process, the potential for perineal rupture always looms, necessitating vigilant observation by healthcare providers, especially midwives, to ensure the well-being of the mother and her child throughout the delivery process (Dunn et al., 2015).

Perineal rupture, a common occurrence during childbirth, affects both primiparous and advanced laboring women and can result from either episiotomy or spontaneous tearing (Jansson et al., 2020). The World Health Organization (WHO) reported a staggering 2.7 million cases of perineal rupture in maternity mothers (Novelia et al., 2023). Research conducted at Muntilan Hospital in February 2015 revealed that grade 2 perineal rupture affected 24 individuals (58.4%), grade 3 perineal rupture affected 9 individuals (21.95%), and grade 1 perineal rupture affected 8 individuals (19.51%) (Prawitasari, 2015).

Perineal lacerations, which are incisions occurring in the perineum due to labor, are almost ubiquitous in first deliveries and often recur in subsequent ones (Dunn et al., 2015). These lacerations primarily manifest in the midline and may become extensive if the fetal head is delivered too quickly (Girsang and Elfira, 2023). The quality of perineal wound sutures is considered good when signs of infection, such as redness, swelling, heat, pain, and impaired function, are absent. Effective perineal wound care plays a pivotal role in expediting the healing process, which aims to prevent infection during tissue regeneration (Himalaya and Maryani, 2022; Novelia et al., 2021).

To facilitate this process, both conventional and alternative therapies are available. Alternative therapies, often referred to as traditional or herbal medicine, utilize natural ingredients with active secondary metabolites known for their pharmacological properties (Diksha Sharma et al., 2022). Indonesia, in particular, has a rich history of employing natural remedies for medicinal purposes (Yaman et al., 2014). Such natural medicines offer a safer alternative to chemical drugs, reducing the risk of side effects and harm to breastfeeding infants (Sharma et al., 2014). Secondary metabolites present in plants are key players in their pharmacological activities (Utami et al., 2022).

Natural compounds with diverse properties, including anti-infective, anti-inflammatory, and wound-healing capabilities, have been extensively studied for their potential in wound healing (Chaniad et al., 2020; Oluwole et al., 2022; Schilreff and Alexiev, 2022; Shukla et al., 2019; Trinh et al., 2022; Yang et al., 2020). Butterfly flower (Bauhinia purpurea L), a commonly used ornamental plant in public parks, possesses leaves with documented antioxidant, anti-inflammatory, anticancer, antibacterial, and
wound-healing properties (Negi and Sharma, 2012). While butterfly flower leaves are traditionally used by brewing them and drinking the infusion, this method lacks flexibility as it necessitates boiling before consumption.

For effective integration of butterfly flower leaves in wound healing for postpartum women, it is essential to ascertain the content of secondary metabolites through phytochemical screening tests (Asma’a Mohsen Al-Wajih et al., 2022; Hanafiah et al., 2017; Nurcholi S et al., 2023). Identifying the specific compounds and their levels is crucial for understanding their role in the observed pharmacological activities (Omar et al., 2021). Standardization parameters are required to guarantee the quality and safety of natural ingredients intended for use as traditional medicines (Martinez-Farina et al., 2019; Wang et al., 2023).

In conclusion, the utilization of natural products, such as butterfly flower (Bauhinia purpurea L), for perineal wound healing in postpartum women represents an attractive alternative to chemical drugs. This approach aligns with Indonesia's rich tradition of natural medicine and the growing concern for the safety of breastfeeding infants. A comprehensive research plan spanning three years is outlined, encompassing phytochemical screening and standardization, preclinical testing, and potential clinical trials, aimed at developing butterfly flower leaves as a standardized herbal remedy for postpartum wound healing.

This research holds great promise in enhancing maternal care, ensuring the well-being of both mothers and infants during the critical postpartum period, and contributing to the field of herbal medicine, aligned with Indonesia's medicinal tradition and the global interest in safer alternatives to conventional pharmaceuticals.

2 THEORETICAL FRAMEWORK

An essential component of postpartum recovery is the healing of wounds after pregnancy. Maternal wound healing during pregnancy may be aided by fetal microchimeric cells, which remain in mothers for decades after birth. According to studies, microchimeric fetal cells may express collagen and TGF-β3 in healed maternal scars and move to the site of harm in response to signals provided by maternal skin injury at cesarean section (CS) scars (Mahmood and O’Donoghue, 2014).

Finding out what women think and do about wound healing after cesarean sections
was the goal of a research. According to the survey, the majority of women thought that proper diet, antibiotic use before to surgery, and cleanliness of surgical instruments were all necessary for wound healing. In the past, women were also given multivitamins and a high-protein diet to help with wound healing. Additionally, they made sure the wound was kept out of the water and dressed it to prevent infection (Ghani, 2013).

In a research, postpartum moms' episiotomy wounds were examined to see how well normal saline treated them. According to the study, postpartum moms who use normal saline to their episiotomy wounds report great success with it. (Beula, 2014).

A another research examined how well infrared radiation treatment affected the way early postnatal women with episiotomies perceived pain and healed their wounds. According to the study's findings, infrared radiation treatment effectively reduced episiotomy discomfort and sped up the healing of wounds (Rani, 2018).

A study aimed to determine the secondary metabolites and antioxidant activities of shade-dried Bauhinia purpurea leaf aqueous extract. The study found that the flavonoid content was higher compared to phenolics, and the nitric oxide scavenging activity and reducing power activity were found to be higher compared to total antioxidant and metal chelating activity(Krishnaveni, 2014). Another study aimed to compare the antioxidant activity of different parts of Bauhinia purpurea L. The study found that all the extracts exhibited potent antioxidant activity in terms of DPPH and NO scavenging capacity(Urmi et al., 2013). Additionally, a study investigated the hepatoprotective activity of chloroform extract of B. purpurea leaves in paracetamol-induced liver injury (PILI) rats. The study found that the extract had a high antioxidant capacity when evaluated using the oxygen radical absorbance capacity assay(Zakaria et al., 2023). Nevertheless, there is no precise information regarding the constant metabolic capacity of butterfly leaves (Bauhinia Purpurea L) in the search results. To identify the precise secondary metabolites found in butterfly leaves and any possible medical benefits, more investigation would be required.

According to one study, Bauhinia purpurea leaf extract had more flavonoids than phenolics. It also showed greater levels of nitric oxide scavenging and reducing power activity than overall antioxidant and metal chelating activity (Krishnaveni, 2014). According to a separate investigation, every extract from every component of Bauhinia purpurea L showed strong antioxidant activity in terms of its ability to scavenge...
NO and DPPH (Urmi et al., 2013).

Additionally, a study investigated the hepatoprotective activity of chloroform extract of B. purpurea leaves in paracetamol-induced liver injury (PILI) rats. The study found that the extract had a high antioxidant capacity when evaluated using the oxygen radical absorbance capacity assay (Mahmood and O’Donoghue, 2014).

Based on the previous discussion, we proposed the following main hypothesis:

H: Phytochemical screening and determination of secondary metabolitical capacity of butterfly leaves (Bauhinia Purpurea) affect the wound healing in women after pregnancy.

3 METHODOLOGY

This research design is experimental. The first year tested the levels of alkaloid compounds, flavonoids, saponins, steroids, and tannins in butterfly leaves (Bauhinia purpurea L). The population and samples of first year were all butterfly leaves. This research was conducted at a herbal medicine factory in Banjarmasin from March to September 2021.
Data processing techniques are: 1) data checking (editing), namely the activity of checking data on variable phytochemical compounds of butterfly leaves (*Bauhinia purpurea* L); 2) coding, namely giving codes to variable compound levels coded 1 and activity coded 2; 3) data processing, namely entering data on phytochemical compound variables of butterfly leaves (*Bauhinia purpurea* L); 4) data cleaning, namely checking the variable phytochemical compounds of butterfly leaves. (*Bauhinia purpurea* L.) Data Analysis: Phytochemical screening data will be obtained in the form of qualitative data presented in tabular form. Level determination data was obtained from quantification results using a linear regression formula in Microsoft Excel. Linear regression is obtained from the results of making a standard curve using standard compounds in each test, then the samples are tested using a UV-vis spectrophotometer, and the absorbance data of the samples is integrated into the standard curve equation so that the levels are obtained.

The research flow was tested three times in replication; more details can be seen in the following table of butterflies (*Bauhinia purpurea* L).
3.1 PREPARATION OF BUTTERFLY FLOWER LEAF POWDER

Fresh butterfly flower leaves were selected according to specifications, namely perfect leaves. Leaves were washed thoroughly using sterile aqua pro injection three times to remove impurities. The leaves are dried using an oven at a controlled temperature of 60°C until dry. The dried leaves are refined to particle size with a special herbal simplisia grinder, then sieved using a mechanical sieve, the powder is sterilized with a UV light lamp.

3.2 PHYTOCHEMICAL SCREENING

The dried sample that has been obtained is taken as much as 10 grams, then dissolved into 500 ml of ethanol for analysis, then filtered with Whatman paper. The results of the search were carried out through phytochemical screening in the manner described below:

First is the alkoloid test. The solution was taken as much as 2 ml, evaporated using a porcelain cup, obtained residue, and then dissolved using 5 ml of HCL 2 N. The test tube is filled with a solution that has been made before. HCL 2N is dripped on the first test tube, which is used as a blank. Dragendorff reagent was dripped 3 drops in the second test tube, and Mayer reagent was dripped 3 drops in test tube 3. Identify the presence of alkaloids by the formation of orange precipitate in the second tube and yellow precipitate in the third tube.

The second is the flavonoid test. The solution was heated to 2 ml. HCL was added as much as 2 drops. Magnesium powder is added enough. Identify the presence of flavonoids by forming a red solution.

The third is the saponin test. A solution of 2 ml is dissolved in warm water and shaken vertically for 10 seconds. The solution is allowed to stand for 10 seconds. Identify the presence of saponins by forming a 1–10 cm-high foam that is stable for not less than 10 minutes. HCL 2 N added as much as 1 drop will not remove the foam.

Next is the steroid test. The solution is dissolved using chloroform in as much as 0.5 ml and acetic acid anhydride in as much as 0.5 ml. Sulfuric acid ditambahkan sebnayak 2 ml by dripping through the tube wall. Identify the presence of steroids by the formation of a bluish-green color.
Then there is the Tannin Test. 2 ml of solution was added to the gelatin solution. Identify tannin by the formation of a white precipitate at the bottom.

The last is the phenolic test. A solution of 2 ml was taken, then put into a test tube, and FeCl3 was added at 10%. Identify phenolic with the formation of a dark blue or greenish black color.

### 3.3 Determination of Secondary Metabolite Levels

**Phenolic.** The gallic acid in quantities of 10 mg was dissolved in 10 ml of ethanol at a rate constant until the limit mark, and then the flask was shaken. The concentrations used to create the serial levels were 30, 40, 50, 60, 70, and 80 ppm. Then, 2.5 ml of 5% Folin-Ciocalteu reagent was added to each quantity taken to obtain 0.5 ml in a test tube. A standard curve $y = bx + a$ connection between gallic acid content (g/mL) and absorbance was established by measuring the absorbance of each solution utilizing a UV-Vis spectrophotometer with a maximum wavelength of 736 nm. Every reagent employed in the production of ethanol solvent makes up the blank. In a 10 ml volumetric flask, a 10 mg sample was dissolved.

**Flavonoids.** 80% ethanol was utilized to extract a 6-gram sample before it was filtered using the Whatman No. 1 filter paper. The resulting filtrate was evaporated until a consistent weight for flavonoid content was attained.

**Terpenoids.** Petroleum ether was used to extract 20 mg of the sample powder, which was then concentrated. The value of terpenoids was determined using the calculated yield. **Saponins.** A volumetric flask containing sample powder weighing up to 15 grams was filled, and up to 20 ml of 20% ethanol was added. The mixture was cooked for four hours at 55°C before being filtered. 200 ml of the filtrate were extracted with 20% ethanol, and the remaining 40 ml were evaporated at 90°C. 20 ml of diethyl ether were added to the remaining filtrate, and the mixture was violently agitated. Shake vigorously after adding 60 cc of n-butanol to the water layer. Add up to 10 mL of 5% NaCl to the n-butanol part and shake vigorously.

**Alkaloids.** Thick solution of ethyl acetate fraction weighed as much as 20 mg was dissolved using 80% acetic acid (ethanol) as much as 200 ml. The mixture was allowed to stand for 4 hours, then filtered with Whatman paper. Put in a cup and tetesi with
ammonium hydroxide, allowed to settle. The filtrate was added with 1% ammonium hydroxide and filtered. The calculated precipitate was used as the alkaloid content.

3.4 EXTRACT PREPARATION

Running water was used to properly wash and then drain fresh butterfly leaves. The samples were then divided into smaller pieces and dried in an oven for three days at 60°C. After that, a grinder was used to crush the dry samples into powder.

The dry sample powder was weighed to a maximum of 1 kilogram, and each was then immersed in a solvent of 10 liters of ethanol for three days. Each day, the solvent was changed. Utilizing filter paper and a hydraulic press, the extract solution was filtered to separate the pulp from the active components. The extract solution was then reduced to 1/10 of its original volume by rotary evaporation. It was then once again evaporated over a water bath until a thick extract was produced.
4 RESULTS AND DISCUSSION

4.1 RESULT

4.1.1 Powder and Extract Preparation

Samples of butterfly flower leaves are wet-sorted to separate impurities or foreign materials from the leaves. The next process is washing with clean water to remove other impurities attached to the material and draining to reduce the amount of water still attached to the simplisia. Furthermore, kneading is carried out to expand the surface of the material so as to facilitate the drying process. Butterfly flower leaf simplisia is processed using the oven drying method. The oven used is equipped with a blower to speed up the drying process.

The cold extraction by maceration technique is used to remove the leaves of butterfly flowers. Due to its simplicity, ease of use, and suitability for chemicals that are not heat-resistant, the maceration technique is utilized (Purwanto et al., 2022). The Indonesian herbal pharmacopoeia recommends using an ethanol solvent since it is volatile, practically mixes with water in different situations, is non-toxic, and can dissolve at different degrees of polarity. Ethanol is commonly used as a solvent in the preparation of herbal extracts in Indonesia (Dewi and Kartini, 2023; Gurning and Sinaga, 2020; Johan Sukweenadhi et al., 2020; Rahmaniati M et al., 2018; Welly Ria Utami Lubis et al., 2023; Wijiyanto et al., 2016)

4.1.2 Phytochemical Screening

1. Results Of Phytochemical Screening Tests

To provide a general description of the class of chemicals present in the plant under research, a first step known as phytochemical screening is carried out. The phytochemical screening technique uses the reaction of color change or the creation of a precipitate with a particular perekasi (Khotimah, 2016). The test results for phytochemical screening are shown in Table 1 and the Appendix.
Table 1

Phytochemical Screening

<table>
<thead>
<tr>
<th>Test</th>
<th>Reagent</th>
<th>Description</th>
<th>Result</th>
<th>Documentati on</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. flavonoid</td>
<td>NaOH</td>
<td>Color of solution intense yellow</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2. Tannin</td>
<td>Gelatin Test</td>
<td>White precipitate formed</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3. Fenol</td>
<td>FeCl3</td>
<td>Formed blackish green precipitate</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4. Alkaloid</td>
<td>Meyer</td>
<td>There is a yellow precipitate</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5. Saponin</td>
<td>Aquades</td>
<td>Foam formed</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6. Steroid</td>
<td>Lieberman Burcard</td>
<td>Formed bluish green</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Source: Prepared By the Author(2021)(Singh et al., 2022)

2. Material Of Butterfly Flower Leaf Extract

According to the findings of phytochemical screening, butterfly flower leaf extract contains flavonoids, tannins, phenols, alkaloids, saponins, and steroids. Secondary metabolites have the potential to treat many different medical conditions. Secondary metabolites are the active ingredients in charge of pharmacological activity. Table 2 contains the material.
Table 2
Material Of Butterfly Flower Leaf Extract

<table>
<thead>
<tr>
<th>NO</th>
<th>Parameter</th>
<th>Content (mg/g sample)</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoid</td>
<td>19.03 mg/g</td>
<td>1.90%</td>
</tr>
<tr>
<td>2</td>
<td>Tanin</td>
<td>23.39 mg/g</td>
<td>2.33%</td>
</tr>
<tr>
<td>3</td>
<td>Fenol</td>
<td>37.84 mg/g</td>
<td>3.78%</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloid</td>
<td>2.48 mg/g</td>
<td>0.24%</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>1.26 mg/g</td>
<td>0.12%</td>
</tr>
<tr>
<td>6</td>
<td>Steroid</td>
<td>2.32 mg/g</td>
<td>0.23%</td>
</tr>
</tbody>
</table>

Source: Prepared By the Author(2021)(Flower, 2022)

The results showed flavonoid content of 1.90%, tannin content of 2.33%, phenol content of 3.78%, alkaloid content, 0.24%, saponin content of 0.12%, and stethoid content. The highest levels were dominated by phenolics, tannins, and flavonoids which generally have various pharmacological properties.

5 DISCUSSION

1. Secondary metabolite compounds contained in butterfly leaf (Bauhinia purpurea)
   a. Powder and Extract Preparation

      This method is used because it is more effective than sun drying and is suitable for drying butterfly flower leaf samples. The oven method has the advantages of even heating, avoiding contamination and adjustable temperature. Drying is carried out at a temperature of 60 °C which is the maximum temperature for drying simplisia, for three days(Anggarani et al., 2018). This drying also serves to reduce water content so that mold cannot grow (Krisyanella et al., 2012). Then dry sorting is carried out to separate impurities, foreign organic matter and simplisia that are damaged by the previous process(Pruteanu et al., 2023). The last process is pollination of dry simplisia until powder is obtained for extraction. The extraction results will obtain an extract solution which is then evaporated to remove organic solvents. The solvent evaporation process uses a rotary evaporator and oven. The extracts obtained were then collected to obtain dry extracts consisting of active compounds. The dry extract obtained is brownish in color, characteristic odor, bitter taste and dry powder form. These results are in accordance with the research which states that the extract from butterfly leaves is brown in color(Aryantini, 2021). The presence of tannin and alkaloid compounds in food can cause a bitter taste(Kam et al., 2022; Setiadi, 2020; Soares et al., 2020).

   b. Phytochemical Screening
If the solution noticeably changes color to yellow, brown, or red, the flavonoid test using NaOH is successful (Lisi et al., 2017). The experiment yielded a bright yellow precipitate that showed the sample had flavonoids in it. Flavonoid substances are reported to be present in butterfly leaf extract. When Mg powder and concentrated HCl are applied, the hue changes to yellow, indicating this (Aryantini, 2021). Compounds from the flavonoid group have anti-inflammatory and healing properties (Arora et al., 2020). Figure 2 depicts the reaction between flavonoids and NaOH.

**Figure 2**
*Reaction between flavonoids and NaOH*

![Reaction between flavonoids and NaOH](Lisi et al., 2017).

Tannin testing using gelatin shows a white precipitate. This process occurs because tannin compounds are able to agglomerate gelatin proteins. Tannin reacts with gelatin to form a copolymer that is insoluble in water. These results are in accordance with research which states that tannin compounds are contained in butterfly leaf extract (Arora et al., 2020). Research conducted by (Awasthi and Verma, 2020) states that Bauhinia purpurea which grows in India contains tannin group compounds. The reaction between tannin and gelatin can be seen in Figure 3.

**Figure 3**
*Reaction between tannin and gelatin*

![Reaction between tannin and gelatin](Sa’adah, 2010).
Testing phenolic compounds produces a blackish green color, this is due to the formation of complex bonds from Fe2+. These results are in accordance with other studies that tested butterfly leaf extract using FeCl3 reagent with positive results, forming a color change to black (Arora et al., 2020). Research by Kumar & Chandrashekar (2010) states that Bauhinia purpurea contains the main secondary metabolites, one of which is phenolic group (Kumar and Chandrashekar, 2010). The phenolic compound test reaction can be seen in Figure 4.

**Figure 4**
*Reaction between phenol and FeCl3*

\[
\text{OH} + \text{FeCl}_3 \rightarrow \text{Fe}^{2+} + \text{K}^{+} + \text{H}^{+} + \text{HCl}
\]

(Chang, 2005).

Findings that are positive for a white or yellow precipitate that is assumed to represent a potassium-alkaloid compound in the Mayer reagent. When mercury (II) chloride and potassium iodide are mixed together, a crimson precipitate of mercury (II) iodide results (Das et al., 1990). If too much potassium iodide is applied, potassium tetraiodomercurate (II) is produced. When alkaloids are tested using the Mayer reagent, the metal ions K+ from potassium tetraiodomercurate (II) will react with the nitrogen in alkaloids to generate a potassium-alkaloid complex that precipitates. The findings of this study are consistent with those of earlier studies on butterfly leaves carried out in India (Arora et al., 2020). In India, butterfly leaves are utilized for their anti-inflammatory, analgesic, anti-fungal, anti-diarrheal, and anti-cancer properties in traditional medicine (Kamala et al., 2018).
Because saponin compounds have physical features that are water soluble, saponin testing results in stable foam. The appearance of foam also suggests that there are glycosides that can create foam in water that hydrolyzes into glucose and other chemicals (Nugrahani et al., 2016). The findings of this study are consistent with earlier literature that claims that butterfly leaves contain saponins (Kumar and Chandrashekar, 2010). According to research, butterfly leaf extract has a high concentration of saponins. Figure 6 depicts the equation for the saponin reaction.

The results of steroid phytochemical screening using Lieberman-Burchard reagent through the tube wall if positive will form a color change to blue, purple, or green (Lisi et al., 2017). The results obtained in this study are in line with the results presented (Arora et al., 2020), that contained sterol group compounds which are a subgroup of steroids.
Steroids were also found in butterfly leaf extracts studied in India (Kumar and Chandrashekar, 2010). The steroid reaction equation can be seen in Figure 7.

Figure 7

*Steroid reaction equations*

(Harborne, 1987).

2. Level Determination

a. Determination of flavonoid levels

Total flavonoids serve to determine the total amount of flavonoids in the sample. The method used is spectroscopic with AlCl3 reagent in an acidic atmosphere. Determination of total flavonoid content is carried out by colorimetric method, namely measurement based on color formation. The principle of flavonoid determination by colorimetric method is the complex formation between AlCl3 with ortho dihydroxy groups and hydroxy ketone groups on flavonoid compounds (Harborne, 1987). The formation of AlCl3 complex with flavonoids can be seen in Figure 8.

Figure 8

*Complex reaction between flavonoids and AlCl3*

The total flavonoid content was calculated using quercetin as a reference. The spectroscopic approach was employed in this investigation to determine the levels. A 19.03 mg/g ethanol extract of butterfly flower leaves had 1.90% of the total flavonoid content. In comparison to studies done by Mishra et al. on Bauhinia variegate, a genus
with butterfly leaves, this study's findings are more valuable (Mishra et al., 2013). According to Mishra et al., the flavonoid content of Bauhinia variegata is 0.88%. The plant is from India, thus it may grow in Indonesia as well as other areas.

b. Determination of tannin levels

Determination of total tannin content using the vanillin method. Vanillin is one of the aldehyde group compounds that is widely used in determining tannin levels, especially in condensed tannins. The vanillin method is more widely used especially in natural material samples because of its sensitivity, specificity, and simplicity. Vanillin is protonated in acid which will form a carboxylation so that it undergoes a dehydration reaction and produces a red color in the compound (Malangngi et al., 2012). Tannin group compounds will react with vanillin in an acidic atmosphere to form derivatives or red end products (Mavrikou, 2012). The reaction between catechins and vanillin is shown in Figure 9.

**Figure 9**

*Reaction between catechins and vanillin*

![Reaction between catechins and vanillin](Mavrikou, 2012)

Catechins are one of the compounds of the tannin group. Catechins are condensed tannins that react when reacted with aldehydes, especially vanillin. The reaction between catechins and vanillin must be in an acidic atmosphere to accelerate the reaction. Sulfuric acid or hydrochloric acid can be used as a solvent to provide an acidic atmosphere (catalytic agent). Hydrochloric acid is able to provide high absorbance, so it can provide higher sensitivity (Sun et al., 1998). Determination of levels used in this study is the spectroscopic method. The results of determining the total tannin content of ethanol extract of butterfly flower leaves amounted to 23.39 mg / g with a percent content of 2.33%. The results obtained in this study are slightly lower than the research conducted...
by Aryantini which obtained a total tannin content of 3.3%. This difference can be caused by different growing places (Aryantini, 2021). Differences in growing places affect the levels of secondary metabolites found in plants (Adawiyah and Rizki, 2018). Differences that are not too far can still be tolerated provided that the manufacturing process carried out is in accordance with the rules of scientific research (Asare et al., 2023). The difference in levels is also due to differences in methods in determining total tannin levels between this study and Aryantini's research (Aryantini, 2021).

c. Determination of phenol levels

The Folin-Ciocelteau technique, which is based on the principles of reduction and oxidation, is used to determine the total phenol concentration (Tursiman, 2013). When phenol group chemicals react with the Folin-Ciocelteau reagent, a yellow hue results; when 1% NaOH is added, a blue color results (Alfieri et al., 2023). The proton dissociation of phenolic compounds into phenolic ions causes phenolic group compounds to react with the Folin-Ciocelteau reagent in an alkaline environment. The hydroxyl group on the phenolic molecule reacts with the Folin-Ciocelteau reagent to generate the blue hue. A spectrophotometer may be used to identify the blue molybdenum-tungsten complex that results from this reaction's oxidation of the hydroxy group of phenol group molecules (Alfian and Susanti, 2012).

Figure 10

*Reaction of phenol with Folin-Ciocelteau reagent*

Because gallic acid is one of the naturally occurring and stable phenolic groups, it is used as a standard solution to measure the total phenol level in samples (Paula et al., 2017). Hydroxybenzoic acid, which is categorized as a simple phenolic acid and is the source of gallic acid, is a phenolic chemical (Hussain and Reigosa, 2021). The
A spectroscopic approach was utilized to determine the levels used in this investigation. The total phenol concentration of the ethanol extract of butterfly flower leaves was calculated to be 37.84 mg/g, or 3.78% of the total. The results outperform those reported in the literature, which claims that butterfly leaf extract has a total phenolic content of 1.83% (Sofiah et al., 2022).

d. Determination of alkaloid levels

The determination of levels used in this study is the gravimetric method. The gravimetric method is one method of quantitative determination by measuring the weight of components in a pure state after going through a separation process. Determination of alkaloid compound levels in the extract is preceded by a precipitation process (Ahmad et al., 2017). Ammonia is added to free the alkaloid from its salt form. The addition of ammonia is carried out until the pH reaches ± 10 so that the alkaloid is freed from the salt form to form free alkaloids that precipitate (Ibnu Gholib and Rohman, 2007). Hydrochloric acid in the process is used to extract alkaloids that are alkaline (Astuti et al., 2023; Ernyasih et al., 2023; Mallongi et al., 2023; Robinson, 1995). The filtrate results are checked using a pH meter to obtain the desired pH, if the pH measurement results are not appropriate then ammoniac is added little by little until the desired pH is obtained.

Figure 11

*Reaction of Alkaloid Salts with Ammonia*

![Reaction of Alkaloid Salts with Ammonia](Rosyidah, 2016)

The results of the determination of total alkaloid levels in ethanol extracts of butterfly flower leaves amounted to 2.48 mg/g with a percent content of 0.24%. No research has been found that examines total alkaloid levels in butterfly leaf plants or plants of the same genus or family. Research on butterfly leaves is limited to the alkaloid compounds contained, namely Beta-Carboline. This compound is dominant in several genus of butterfly leaves (Murrukmihadi et al., 2015).

e. Determination of saponin levels
The determination of levels used in this study is the gravimetric method. One of the advantages of this method does not require a comparison substance (standard saponin) and is the simplest analysis compared to other methods of analysis, where in gravimetry the amount of substance is determined by weighing directly the mass of the substance separated from other substances. The reflux method is used to draw chemical compounds by heating, by heating the extract that has a rough texture will be more easily drawn. Petroleum ether is used to attract non-polar compounds. After cooling the petroleum ether solution to remove nonpolar compounds, the residue left behind is dissolved in ethyl acetate where the level of polarity is higher than petroleum ether. Ethyl acetate was used to remove semipolar compounds. The residue left behind was dissolved with N-butanol 3 times for 50 mL each (Robbika et al., 2022).

Because they include several hydroxyl and glycoside groups that are conveniently soluble in N-butanol solvent, saponins are polar molecules. To concentrate the obtained extract, the entire N-butanol solution was combined and then evaporated using a rotary evaporator. Where the degree of polarity is higher than N-butanol, the leftover evaporation was dissolved with methanol. Then, while stirring, this solution was slowly poured into 50 mL of ether. Since saponin is insoluble in ether, ether may precipitate saponin, and petroleum ether acts as a precipitating agent. To separate the saponin precipitate from other contaminants, the precipitate generated in the mixture was then placed onto filter paper. Prior to being weighed, the precipitate on the filter paper is first allowed to dry. The filter paper was next.

f. Determination of steroid levels

Steroids were detected qualitatively in butterfly leaves. Determination of total steroid levels in this study using spectroscopic methods. Stigmasterol is a precursor in the synthesis of progesterone and is involved in the biosynthesis of androgen hormones (aphrodisiac effects), estrogens, and corticoids (Tiwari et al., 2011). Steroid class compounds can increase serum levels of testosterone, FSH, and LH. In addition, steroids can also inhibit the enzyme phosphodiesterase-5 (PDE-5) which is responsible for sexual dysfunction disorders (Sharma et al., 2014).

The results of the determination of total steroid levels of ethanol extract of butterfly flower leaves amounted to 2.32 mg / g with a percent content of 0.23%. The results of this study are close to research data on Bauhinia purpurea conducted by Arain et al which states the steroid content of 0.25% (Arain et al., n.d.). Bauhinia purpurea plants
studied by Arain et al. came from Pakistan (Arain et al., n.d.). Acanthospermum hispidum plants that determine steroid levels as phytosterols are known to have levels of 0.14% (Araújo et al., 2013). Steroids in Bauhinia purpurea plants have the potential to function as vegetable oil because of the steroidal content that can be plant oil. The use of Bauhinia purpurea as a medicine in Pakistan is as an antipyretic, anti-inflammatory, analgesic, antifungal, anticancer, and antimicrobial (Arain et al., n.d.).

6 CONCLUSIONS

The group of secondary metabolite compounds contained in the butterfly leaf (Bauhinia Purpurea L.): Flavoid, Tannin, Phenolic, Alkaloid, Safronim and Steroid). Levels of secondary metabolites contained in butterfly leaves: Flavoids: 19.03 mg/g (1.90%), Tannins: 23.39 mg/g (2.33%), Phenol: 37.84 mg/g (3.78%), Alkoldi: 2.48 mg/g (2.48%), Saponins: 11.26 mg/g (0.12%), Steroids: 2.32 mg/g (2.32%)

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