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EDITORIAL WORDS

apt. Mohammad Rizki Fadhil Pratama, S.Farm., M.Si.

Editor in Chief
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Assalamu'alaikum Wr. Wb.

Alhamdulillahirabbil 'alamin. The next edition of **Borneo Journal of Pharmacy** (*Borneo J Pharm*), has been published at February 2022. This edition contains ten articles consisting of Pharmacognosy-Phytochemistry, Clinical-Community Pharmacy, Pharmaceutical, Microbiology Pharmacy, and Natural Product Development. This edition includes writings from three countries including Azerbaijan, Indonesia, and Ukraine. The authors come from several institutions, including Universitas Lambung Mangkurat, Universitas Pancasila, Bunda Mother and Child Hospital Jakarta, National University of Pharmacy, Kharkiv National University of Radio Electronics, Universitas Jenderal Achmad Yani, Institut Teknologi Bandung, Universitas Tanjungpura, Universitas Muhammadiyah Kalimantan Timur, Sekolah Tinggi Ilmu Farmasi Makassar, Universitas Gadjah Mada, Universitas Airlangga, and Azerbaijan Medical University.

Editorial boards are fully aware that there are still room for improvement in this edition, hence with all humility willing to accept constructive suggestions and feedback for improvements to the publication for the next editions. The editorial board would like to thank all editors and reviewers, and contributors of the scientific articles who have provided the repetoire in this issue. We hope that all parties, especially the contributors, could re-participate for the publication in the next edition on May 2022.

Wassalamu'alaikum Wr. Wb.

Palangka Raya, February 2022

Editor-in-Chief

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Author Guidelines

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1. Writing is the result of research or review of critical analysis study in the field but not limited to Pharmacology-Toxicology, Pharmacognosy-Phytochemistry, Pharmaceutical, Analytical Pharmacy-Medicinal Chemistry, Natural Product Development, Clinical-Community Pharmacy, Management Pharmacy, and other Pharmacy aspects which have never been published in other scientific publication media.
2. Manuscripts that have been published in the form of a preprint on several platforms such as arXiv, INA-RXiv, SSRN, and ResearchGate are welcome to publish.
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   e. Methodology, Research method consists of 2 sub-chapters including materials and methods. If data analysis is specific, or the way of analysis is novel, then data analysis subchapter could be added. The sub-chapters are written without numbering or bullet.
   f. Results and Discussion, at least contain three subjects (1) description of results, (2) interpretation or explanation of results, and (3) comparative of results with previous studies results. If the subchapters very length could be made sub-sub-chapters with Arabic numbering. Description of the results can be in the form of tables and figures with a serial number (Table uses a sequence of Roman numerals and placed at the top, while figures using Arabic numeric sequence and placed in the below).
   g. Conclusion, made in a single paragraph without the citation contains the final conclusion and suggestions for advanced research.
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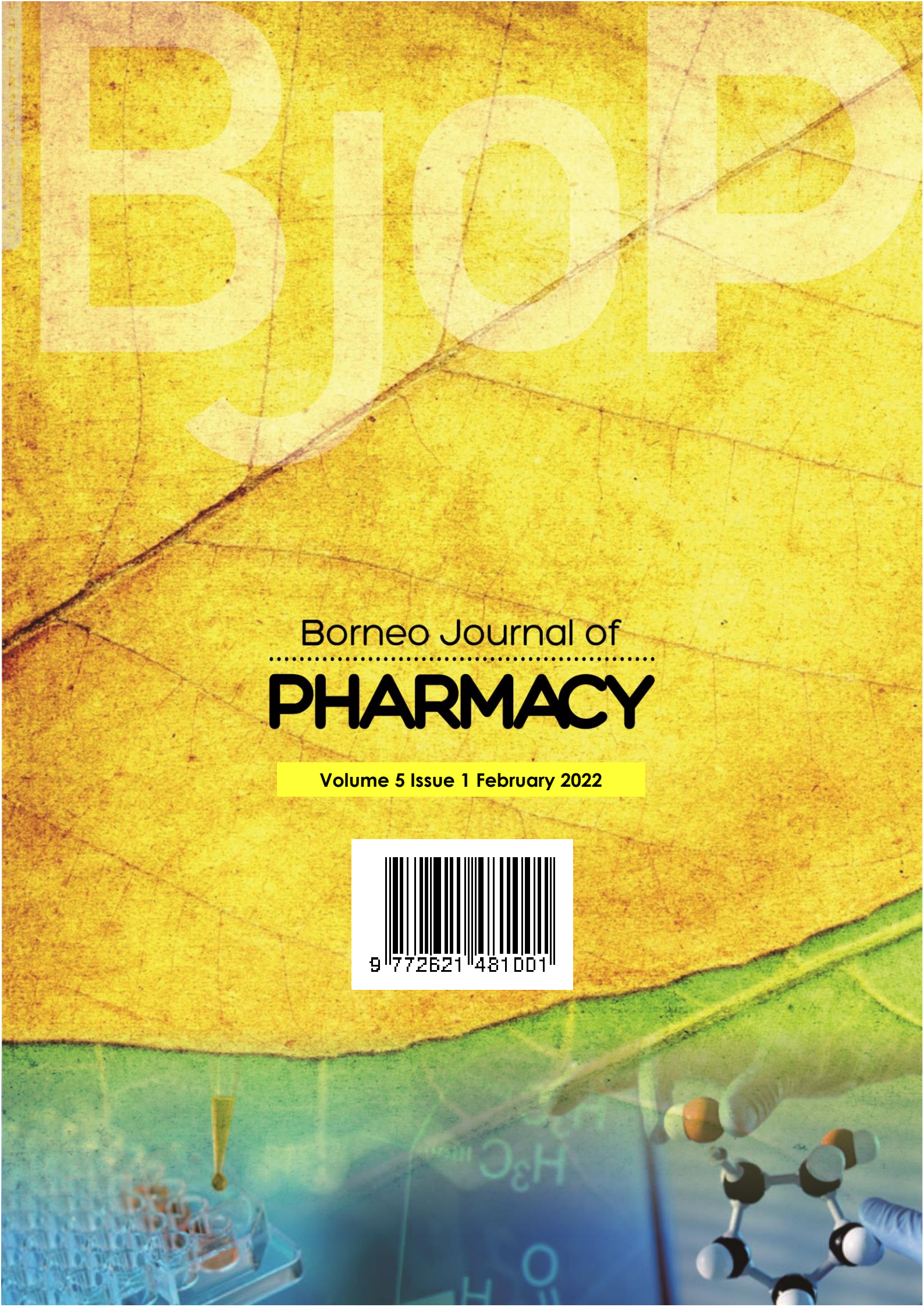
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Research Article

Ethnobotanical Study and Phytochemical Screening of Medicinal Plants Used by Local People in Belangian Village, South Kalimantan

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Keywords:
Belangian Village
Ethnobotany
Medicinal plants
Phytochemical screening

Abstract

The local people of Belangian Village are people of the Banjar ethnic group who utilize plants in the surrounding environment for medicinal purposes. This study aims to determine the number of plants, parts of plants that can be used, methods of application, methods of preparation, and phytochemical screening of plants with medicinal properties in Belangian Village. This study employed the descriptive-explorative method. The technique used for data collection was an interview with a semi-structured questionnaire. Respondents of this study were "*Pananamba*" who were selected by using the Purposive sampling technique. Phytochemical screening was carried out on plants with no previously known scientific names and which had never been tested before. The results showed 17 families and 31 species of plants with medicinal properties. Empirically, the plants were used as medicines for cough, bloody urine, diabetes, cancer, sprue, ulcer, hypertension, skin diseases, nausea, diarrhea, coronary heart disease, sinusitis, kidney stones, cleansing kidneys, menstrual pain, itchy eyes, paralysis, bone pain, increasing stamina, antibiotic, reducing body odor, wounds, bleeding, worm diseases, increasing appetite, boosting the immune system, and yellow fever. The most frequently used part of the plants were the leaves (45%), the most widely used method of preparation was boiling (52%), the most commonly used method of application was by drinking (81%). Phytochemical screening was conducted on three plants: Asam daun, Lukun, and Ulur-ulur. The results of phytochemical screening revealed the presence of quinones, saponins, and terpenoids in Asam daun, saponins in Lukun, and terpenoids in Ulur-ulur.

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INTRODUCTION

Indonesia is rich in diverse natural resources, resulting in various tribes who possess different knowledge, customs, and cultures, one of which is plants utilization[1]. An example of plants utilization is using plants as medicine. The knowledge of plants utilization as medicine is passed down from generation to generation. This knowledge is often passed down verbally, so it is necessary to collect information to assist the plants' utilization, development, and preservation[2]. Plants are utilized by ethnic groups or local people in an area as a life-support system, also known as ethnobotany. The ethnobotanical study focuses on studying how a particular ethnic group utilizes plants in its surrounding environment[3].

Borneo island is known for its forest, which possesses a high diversity of flora and fauna, some of which have medicinal properties. The Meratus Mountains is a mountain range that divides South Kalimantan into two parts. From a health aspect, the local people living around the Meratus Mountains area still use plants as medicine with the help of someone who is believed to understand and concoct[4]. Dayak and Banjar are the two largest ethnic groups that live around the Meratus

Mountains area. Belangian Village is located in Aranio district, Banjar Regency, South Kalimantan province, and is situated in Meratus Mountains[5]. The forest in Belangian village is still preserved, making it high in biodiversity. The population density of the Belangian village of 2 people/km^2 is relatively low compared to other villages in Aranio District. The population of Belangian Village is 90 families or 360 people. Generally, the villagers here work as field and vegetable farmers in addition to relying on garden produce in the form of local fruits. In addition to the remote location with transportation problems, other obstacles in this village are the unavailability of health facilities and amateur radio to communicate with the outside world[6].

The traditional healing process among the Banjar people is named *Batatamba*, and the person who gives treatment is called *pananamba*. *Batatamba* is conducted by using traditional concoctions and '*mantra*' or spells from a *pananamba*[7]. Limited access to health care services is one of the factors which causes people of Belangian village to use traditional medicine with the help of a *pananamba* more often. Looking at the potency of those medicinal plants, it is necessary to conduct a phytochemical screening test which is the initial stage to provide an overview of groups of chemical compounds found in plants[8]. This study aims to determine the number of plants, parts of plants that can be used, methods of application, methods of preparation, and phytochemical screening of plants with medicinal properties in Belangian Village.

MATERIALS AND METHODS

Materials

The materials used include the collected plant simplicia, Mg powder, HCl, Dragendorff's reagent, Mayer's reagent, NaOH, distilled water, chloroform, anhydrous acetic acid, and H_2SO_4.

Methods

Type of research

The type of research used was the descriptive-explorative method, and the data collection method was the interview with the aid of a semi-structured questionnaire (https://doi.org/10.5281/zenodo.5815108). Respondents were selected through the purposive sampling technique. This research was conducted from March to June 2020 in Belangian village, Aranio district, Banjar Regency, South Kalimantan.

Research location

The location of the plants could be reached in approximately an hour on foot. The zero point starts at house number 1 RT/RW 01/01 Belangian Village, walk to the north (forest) for 25 minutes, and find a river. A bamboo bridge crosses the river for about five minutes, then through the forest for 20 minutes until you meet the river again, cross the river on foot, then walk in the forest for up to 10 minutes. The sampling area is right around that area. Phytochemical screening was only conducted on plants that had never been tested before. The phytochemical screening included tests for flavonoids, alkaloids, quinones, terpenoids, and saponins.

Research respondents

The criteria for respondents were people of Banjar ethnic group in Belangian Village known as *pananamba*, descendants of father and mother from the Banjar ethnic group, residing in Belangian Village, and using plants to treat a disease. The number of *pananamba* who met the criteria were two people: Mrs. Hasriani and Mr. Ansorullah, which were unwilling to be photographed.

Retrieval procedure of research data

The search for information was carried out by interviewing *pananamba* using a semi-structured questionnaire. The information sought included the names of the plants, benefits of the plants, parts of the plants, methods of preparation, and methods of application of the medicinal plants. The plants obtained were photographed using a camera and measured using a measuring tool.

Preparation of herbarium

The sample was cleaned of dirt and then dried. The plant sample was evenly sprayed with 70% alcohol and then air-dried and pressed using a wooden board. After the plant sample was dried, parts of the plant were arranged on paper for identification.

Phytochemical screening

Identification of flavonoids was conducted by adding hot water to the sample and then filtering it. The filtrate was added with Mg powder, concentrated HCl, and then shaken vigorously. The red, yellow, or orange color formation indicated the test as positive[9-11]. Identification of alkaloids was conducted by adding Mayer's and Dragendorff's reagents. The formation of yellowish-white precipitate after adding Mayer's reagent and brick-red precipitate after adding Dragendorff's reagent indicated the test as positive[12]. Identification of quinones was conducted by adding NaOH 1 N into the sample solution; the formation of red color indicated the presence of quinones[13]. Identification of saponins was conducted by adding 10 mL aquadest to the sample shaken vigorously for 10 seconds. The test was positive when the foam was formed for 10 minutes with 1-10 cm height[14]. Identification of terpenes was conducted by dissolving the sample in 0.5 mL chloroform, adding 0.5 mL anhydrous acetic acid, and dropping 2 mL sulphuric acid through the test tube wall. The formation of a green ring indicated the test as positive.

Data analysis

The plants obtained were identified by their names, benefits, parts of the plants used, methods of preparation, and methods of application. Other data were presented in the form of a percentage diagram of parts of the plants used, preparation methods, and application methods. The percentage was calculated by using the formulas [**1**] to [**3**]:

$$\% \text{ part of the plants used} = \frac{\textit{the number of a specific part of the plants used}}{\textit{the number of all parts of the plants used}} \times 100 \quad \ldots [1]$$

$$\% \text{ methods of preparation} = \frac{\textit{the number of a specific method of preparation}}{\textit{the number of all methods of preparation}} \times 100 \quad \ldots [2]$$

$$\% \text{ methods of application} = \frac{\textit{the number of a specific method of application}}{\textit{the number of all methods of application}} \times 100 \quad \ldots [3]$$

RESULTS AND DISCUSSION

Research respondents

The respondents participating in this research were two people, Mr. Hasriani (53 years old) and Mr. Ansorullah (42 years old). Both were born in Aranio with a father and a mother from the Banjar ethnic group. Mr. Hasriani's last education was senior high school (SLTA), and Mr. Ansorullah's last education was an elementary school (SD). Mr. Hasriani worked as a teacher, and Mr. Ansorullah worked as a farmer. Both *pananamba* had practiced traditional medicine for more than ten years.

Ethnobotany of medicinal plants in Belangian Village

There were 31 species of plants used in traditional medicine. These plants were divided into 17 families, 28 species, and three plants with unknown scientific names, including asam daun, lukun, and ulur-ulur. Seventeen families reported were Graminae, Euphorbiaceae, Rhamnaceae, Convolvulaceae, Piperaceae, Fabaceae, Thymelaeaceae, Asteraceae, Zingiberaceae, Myrtaceae, Rubiaceae, Malvaceae, Verbenaceae, Lamiaceae, Campanu Laceae, Simaroubaceae, and Annonaceae, as shown in **Table I**.

Table I. Medicinal plants used by local people of the Banjar ethnic group in Belangian Village

No	Name (in Indonesian or Banjarese)	Familly	Species	Health Benefits	Parts of Plants Used	Method of Preparation	Method of Aplication
1	Insulin	Ateraceae	*Tithonia diversifolia*	Diabetes	Leaves	Boiling	by mouth
2	Sirsak	Annonaceae	*Annona muricata* L.	Stop bleeding after child birth	Leaves	Boiling	by mouth
3	Bilaran tapah	Convolvulaceae	*Merremia peltata*	Treat cough	Leaves	Boiling	by mouth

4	Lapak bumi/kitolod	Campanulaceae	*Lobelia deckenii*	Sores, itchy eyes	Flower	Soaking in water	Washed to the eyes
5	Mengkudu	Campanulaceae	*Laurentia longiflora*	Bone pain	Fruit	Steaming	by mouth
6	Betadine/jarak cina	Euphorbiaceae	*Jatropha multifida* Linn.	Treat wounds	Leaves	Taking the sap	Smeared
7	Keminting/kemiri	Euphorbiaceae	*Aleurites moluccana*	Antihypertension	Bark	Boiling	by mouth
8	Ubi kayu/singkong	Euphorbiaceae	*Manihot utilissima*	Ulcer	Bulb	Grating and then squeezing	by mouth
9	Carikan darah	Fabaceae	*Spatholobus sanguineus*	Open wounds	Root	Taking the sap	Smeared
10	Gulinggang/ketepeng cina	Fabaceae	*Senna alata*	Scabies ringworm itching of the skin	Leaves	pounded	Smeared
11	Bambu	Graminae	*Bambusa* Sp.	Bloody urine	Leaves	Grating and then squeezing	by mouth
12	Pelawan serai/sereh	Graminae	*Cymbopogon nardus* L. Rendle	Eliminate body odor, crush kidney stones	Leaves	Boiling	by mouth
13	Kumis kucing	Lamiaceae	*Orthosiphon aristatus*	Cleanse the kidneys	Flower	Boiling	by mouth
14	Jambu biji	Myrtaceae	*Psidium guajava*	Diarrhea	Leaves	Boiling	by mouth
15	Kapuk	Malvaceae	*Ceiba pentandra*	Sinusitis	Stem bark	Taking the sap	nose
16	Sinaguri/sidaguri	Malvaceae	*Sida rhombifolia* L	Wounds in patients with diabetes	Leaves	Pounding	Smeared
17	Pulantan putih/pelawan	Myrtaceae	*Tristaniopsis merguensis*	Antibiotic, deep wounds	Stem bark	Boiling	by mouth
18	Cambai/kemukus	Piperaceae	*Piper cubeba* L.	Sprue	Leaves	Boiling	by mouth
19	Bidara	Rhamnaceae	*Ziziphus mauritiana* Lam.	Diabetes, anticancer	Leaves	Boiling	by mouth
20	Kacapiring	Rubiaceae	*Gardenia jasminoides*	Internal heat	Leaves	Boiling	by mouth
21	Pasak bumi	Simaroubaceae	*Eurycoma longifolia*	Kidneys, stamina	Root	Boiling	by mouth
22	Gaharu	Thymelaeaceae	*Aquilaria malaccensis*	Ulcer, hypertension	Leaves	Boiling	by mouth
23	Kembang ulat/pecut	Verbenaceae	*Stachytarpheta jamaicensis*	Treat kidney stones	Leaves	Boiling	by mouth
24	Jahe	Zingiberaceae	*Zingiber officinale*	Reduce nausea	Rhizome	Grating and then squeezing	by mouth
25	Kencur	Zingiberaceae	*Kaempferia galanga*	Cough	Rhizome	Grating and then	by mouth
26	Kunyit	Zingiberaceae	*Curcuma longa*	Menstrual pain	Rhizome	Grating and then squeezing	by mouth
27	Temugiring	Zingiberaceae	*Curcuma heyneana*	Worm diseases	Rhizome	Grating and then squeezing	by mouth
28	Temulawak	Zingiberaceae	*Curcuma xanthorrhiza* L.	Increase appetite, boost immune system	Rhizome	Grating and then squeezing	by mouth
29	Asam daun	Unknown	Unknown	Treat cough	Lignum	Taken directly from the stem	by mouth
30	Lukun	Unknown	Unknown	Paralysis, bone pain	Lignum	Boiling	by mouth
31	Ulur-ulur	Unknown	Unknown	Hepatititis/liver	Root	Boiling	by mouth

The plant parts used were leaves with 45%, stem 7%, root 10%, rhizome 16%, stem bark 10%, flower 6%, fruit 3%, and bulb 3% (**Figure 1**). The most widely used preparation method was boiling with 52%, followed by pounding 6%, taking the water 3%, taking the sap 10%, soaking 3%, grating and then squeezing 23%, and steaming 3% (**Figure 2**). Methods of applying medicinal plants used by local people of Banjar ethnic group in Belangian Village were by mouth with 81%, smeared on the wound 13%, nose 3%, and washed to the eyes 3% (**Figure 3**).

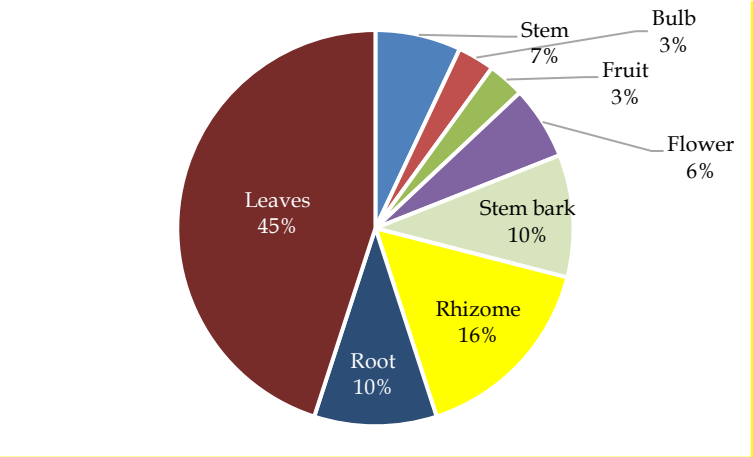

Figure 1. Percentage of plant parts used

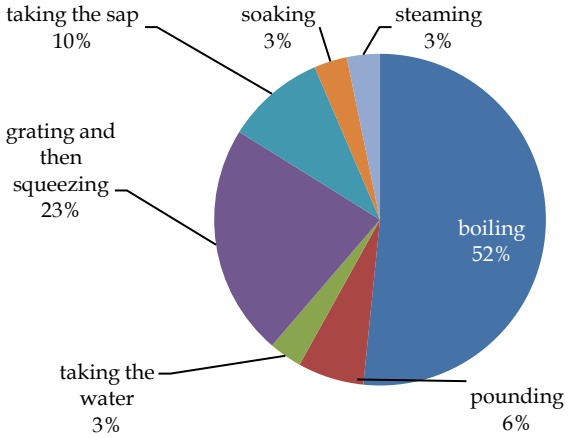

Figure 2. Percentage of plants preparation methods used

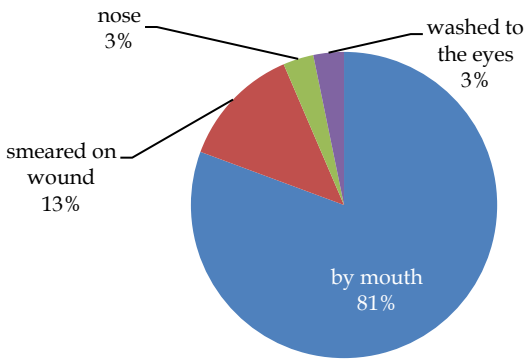

Figure 3. Percentage of plants application methods used

Phytochemical screening

A phytochemical screening test was carried out on unknown plants such as asam daun, lukun, and ulur-ulur. The compounds tested were quinones, saponins, terpenes, flavonoids, and alkaloids. Several compounds from the flavonoid group have shown antioxidant activity, anti-inflammatory, heart disease prevention, hepatoprotective, anticancer, and antiviral potential[15]. Alkaloid group compounds often have biological activities such as antimalarial, antiviral, antibacterial, cytotoxic, antitumor, antifungal, analgesic, and cholinesterase inhibition activities[16,17]. In general, saponin group compounds can break down erythrocytes, and their presence is developed through hemolytic tests. The hemolytic properties are generally attributed to the interaction between saponins and sterols of the erythrocyte membrane[18,19]. The results of phytochemical screening showed that asam daun contained quinones, saponins, and terpenoids, lukun contained saponins, and ulur-ulur contained terpenoids (**Table II**), while plant of asam daun, lukun, and ulur-ulur shown in **Figure 4**. The three plants were identified because the results of phytochemical tests had not been found.

Table II. Results of phytochemical screening

Phytochemical compounds	Procedure	Sample		
		Asam daun	Lukun	Ulur-ulur
Quinones	Sample + 2 drops of NaOH	+	-	-
Saponins	Sample + 10 mL distilled water, shaken vigorously for 10 seconds	+	+	-
Flavonoids	Sample + 3 drops of HCl + Mg powder	-	-	-
Alkaloids	Sample + 3 drops of Dragendorff's reagent	-	-	-
Terpenoids	Sample + 0.5 mL kloroform + 0.5 mL anhydrous acetic acid, + 2 drops of H_2SO_4 through the test tube wall	+	-	+

+: Present; -: absent

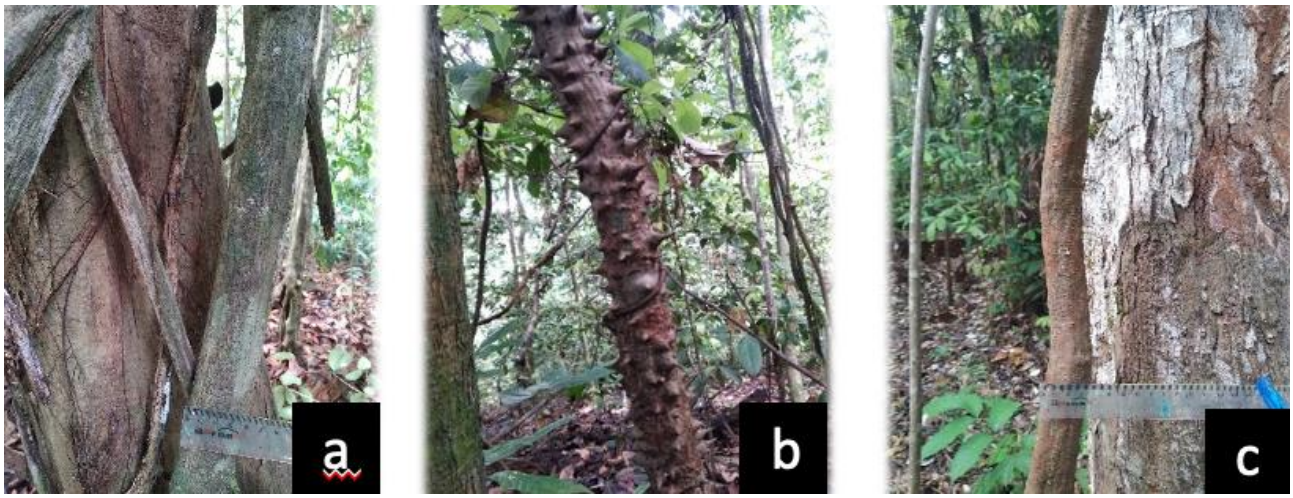

Figure 4. Plant of (**a**) asam daun, (**b**) lukun, and (**c**) ulur-ulur

Several researchers have also carried out research on ethnobotany. Syaifuddin *et al*.[20] reported that 24 types of medicinal plants had been used by the people of West Mandiangin Village, Banjar Regency, South Kalimantan. In addition, it was also reported that the Dayak tribe used several plants as medicinal ingredients in Haratai Village, Loksado. There are 110 medicinal plants with various families, including Poaceae, Lauraceae, Rutaceae, Zingiberaceae, Sapindaceae, Palmae, Graninae, Myrtaceae, Myristicaceae, and others[21]. Empirically the medicinal plants are used for rheumatism, gout, lumbago, jaundice, ulcers, postpartum care, bloody bowel movements, abdominal pain, diarrhea, knee weakness, crowds, chickenpox, bone pain, *Sawan*, anemia, relieving dandruff, strengthening the legs to be strong walking, and malaria. Moreover, the medicinal plants are used for herbal contraception, fever, headache, toothache, ringworm, immunity, flu, stopping bleeding, strong medicine, bloody cough, swollen gums, diabetes, ambient, and kidney abnormalities.

CONCLUSION

There were 31 medicinal plants used by local people of the Banjar ethnic group in Belangian Village South Kalimantan. The most frequently used part of the plants were the leaves (45%). The most widely used method of preparation was boiling (52%). The most widely used application method was by drinking (81%). The results of phytochemical screening showed that Asam daun contained quinones, saponins, and terpenoids; Lukun contained saponins; and Ulur-ulur contained terpenoid.

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Thank you to the Center for Natural Medicine Studies Universitas Lambung Mangkurat and local people of Belangian Village Banjar Regency South Kalimantan.

AUTHORS' CONTRIBUTION

Sutomo: conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, resources, supervision, validation, and writing - review & editing. **Vita Vitriana Awaliyah**: investigation, visualization, and writing - original draft. **Arnida**: data curation, formal analysis, methodology, supervision, validation, and writing - review & editing.

DATA AVAILABILITY

Semi-structured questionnaire deposited at https://doi.org/10.5281/zenodo.5815108.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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Research Article

Comparison of Pain Scale, Hemodynamics, and Side Effects of Percutaneous and Intravenous Fentanyl in Post Sectio Caesaria Patients at Bunda Hospital

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Abstract

This is novel research about comparison pain scale, hemodynamics, and side effects of percutaneous and intravenous fentanyl in post sectio cesarean patients. Sectio cesarean is a method of delivering a fetus through an incision in the abdominal wall (laparotomy) and the uterus wall. This method induces pain in the incision, so patients feel complicated or afraid to mobilize. Fentanyl is one of the opioid analgesics, which is the main choice in section caesarian surgery because safe for breastfeeding, is more potent than morphine, and acts as balanced anesthesia—comparing the use of percutaneous fentanyl with intravenous fentanyl with pain scale parameters, hemodynamics, and side effects in sectio caesarian patients at Bunda Mother and Child Hospital Jakarta. Before conducting this research, an observational study first makes an ethical approval. Data were taken prospectively and collected simultaneously to compare percutaneous and intravenous fentanyl performed on post sectio cesarean patients with the physical status of the American Society of Anesthesiologists (ASA) I–II at Bunda Mother and Child Hospital Jakarta from September to November 2020. Comparative data observed were pain scale parameters, hemodynamics, and side effects after percutaneous fentanyl therapy or intravenous fentanyl therapy. Data were processed using SPSS 22 version and Microsoft Excell 2016. In conclusion, intravenous fentanyl is more effective in reducing pain scale and has more minor side effects than percutaneous fentanyl. There is no significant difference in hemodynamic parameters (p-value >0.05).

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INTRODUCTION

Cesarean delivery is one method of delivering the fetus through an incision in the abdominal wall (laparotomy) and the uterus wall. Delivery by cesarean section impacts the mother because of the pain that appears in the incision after cesarean section so that the patient is difficult or afraid to mobilize[1]. Based on the results of *Riset Kesehatan Dasar* (Basic Health Research) 2018 of the Republic of Indonesia, there was an increase in the percentage of cesarean section delivery by 7.8%; in 2013, it was 9.8%, and in 2018 it was 17.6%[2].

Postoperative pain is a complex physiological reaction to tissue damage or a response to illness that the patient perceives as an unpleasant sensory and emotional experience and is still a significant problem faced by anesthetists. Inadequate pain management will cause physiological side effects, increasing morbidity and hindering the healing process[3]. Opioids are the analgesic of choice for moderate to severe pain. Opioid analgesics are drugs that act on opioid receptors in the central

nervous system (CNS). This drug is given to treat moderate to severe pain according to the strength of the pain that is felt and the strength of the drug[4]. This drug acts on the CNS selectively to affect consciousness and cause dependence if taken in the long term. The mechanism of this drug is to activate opioid receptors in the CNS to reduce pain. Activation of the drug is mediated by mu (μ) receptors which can produce analgesic effects in the CNS and peripheries[5]. Opioids are given to surgical patients, such as morphine, codeine, fentanyl, and pethidine[6]. Fentanyl is used in this study and is the main choice of an opioid analgesic in the cesarean section, consisting of[7,8]:

1. For consideration of the level of safety for breastfeeding mothers because fentanyl is not distributed in breast milk.
2. Fentanyl is 75 -125 times more potent than morphine due to its good analgesic properties with a fast onset of action, fewer cardiovascular depressants, and does not cause histamine release, which can trigger bradycardia.
3. Fentanyl as part of balanced anesthesia can help achieve good hemodynamic stability during anesthesia, both in response to surgery and reduced the need for inhalation anesthetics or other anesthetic drugs.

There are two fentanyl preparations given percutaneously and intravenously using the Patient Controlled Analgesia (PCA) method. Each form of fentanyl has several advantages and disadvantages. Intravenous fentanyl is lipophilic, so it quickly reaches μ receptors in the central nervous system so that fentanyl is suitable for labor analgesia[9]. Based on the research of Purnomo *et al.*[10], intravenous fentanyl given by PCA has a volume distribution of 4.0 L/kg, a clearance of 13.0 mL/min/kg, and an elimination half-life of 3.5 hours.

Fentanyl transdermal patch is an analgesic that has been approved for use in the United States and Europe for the management of moderate to severe postoperative acute pain approved by the Food and Drug Administration (FDA) which is easy to administer, designed for acute and chronic pain management, and can help postoperative analgesics for adult patients[11]. Apart from having advantages, intravenous and percutaneous fentanyl also has disadvantages. Percutaneous fentanyl has a slow effect that occurs only after 12 hours. This is due to the formation of fentanyl depots in the skin layer before the drug enters the systemic circulation. Percutaneous fentanyl distribution is characterized by a slow drug absorption rate and a sustained serum concentration after patch removal, making it unsuitable for acute pain management[12]. Patients with acute pain syndrome are not suitable candidates for percutaneous fentanyl. Time-limited acute pain syndromes are not compatible with the pharmacokinetics of the percutaneous fentanyl device. The FDA states that percutaneous fentanyl is contraindicated for postoperative pain control. However, some clinicians recommend percutaneous fentanyl for postoperative pain control but with caution and close clinical monitoring[13,14].

During the Covid-19 pandemic, delivery of sectio caesaria was still carried out at several hospitals in Jakarta, including at the Bunda Mother and Child Hospital Jakarta. Based on data from the Bunda Mother and Child Hospital Jakarta drug inventory, the average monthly use of percutaneous fentanyl was 78 patches; intravenous fentanyl was 228 ampoules; morphine 21 ampoules; and pethidine 13 ampoules. From these data, the use of fentanyl is 80% of other types of opioids that anesthetists have used in analgesic therapy for post-sectio caesaria patients. Based on the description above, there is no published data regarding the effectiveness of fentanyl in post sectio caesaria. So it is necessary to conduct a study to compare the effectiveness of two fentanyl forms at Bunda Mother and Child Hospital Jakarta in post sectio caesaria patients from September to November 2020.

MATERIALS AND METHODS

Materials

The materials and software used in this study include medical records, post sectio caesaria patient registration lists, informed consent, data collection forms, patient diaries, clinical pharmaceutical drug therapy monitoring sheets, clinical pharmacy checklists, SPSS 22 programs, and Microsoft Excel 2016.

Methods

Before conducting the research, the authors made ethical approval and dealt with patients using informed consent. Subject patients (inclusions) must be patient sectio caesaria with physical status ASA I-II, age more than 20, and used anesthesia medicines such as midazolam, lidocaine, and propofol with the same dosage. The patient who could not be subject (exclusions) is a patient with fentanyl allergy, using fentanyl for more than three months, or using inflammatory and steroid medicine within 24 hours before sectio caesaria. Data were taken prospectively, and data was collected at once to compare percutaneous and intravenous fentanyl use in post sectio caesaria patients from September to November 2020 at Bunda Mother and Child Hospital Jakarta. Comparative data observed were pain scale parameters, hemodynamics, and side effects after percutaneous fentanyl therapy or intravenous fentanyl therapy. The research scheme is presented in **Figure 1**.

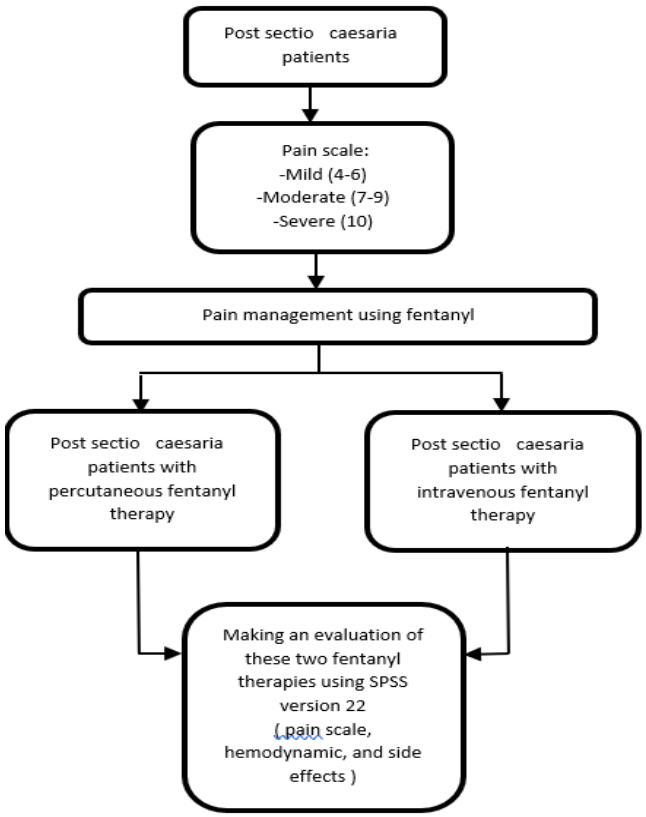


Figure 1. Research scheme

Research data were obtained from medical records, patient diaries, data collection forms, clinical pharmacy checklists, and drug therapy monitoring. The number of test subjects was 304 and divided into two test groups. The first group consisted of 152 patients receiving percutaneous fentanyl therapy and 152 patients receiving intravenous fentanyl therapy. The dose of percutaneous fentanyl was 25 µg/hour using transdermal preparations, and intravenous fentanyl was 25 µg/hour using PCA. The doses were recorded on the date and time of administration, and the progression of pain scale reduction, hemodynamics, and side effects was observed after three hours of administration.

Data were collected directly to the location of the study sample (the operating room) to determine the scale or degree of pain after surgery (post sectio caesaria). The doctor recorded the degree of pain in the patient's medical record or recorded by the nurse in the patient's diary. For observation of pain scale after intravenous or percutaneous administration of fentanyl after three hours of administration, it was carried out in an adult inpatient room. Drug therapy monitoring was carried out by identifying Drug Related Problems (DRP) and assessing the pain scale by interviewing post sectio caesaria patients and recording them on a clinical pharmacy checklist. The data obtained were carried out by statistical tests using SPSS 22.

RESULTS AND DISCUSSION

Data distribution tests were carried out to check the homogeneity of the research variables using the Levene test. After the homogeneity test was carried out for all research variables, the following results were obtained and presented in **Tables I** and **II**.

Table I. Homogeneity test of the characteristics of research subjects using the Levene test

No.	Characteristics of research subjects	N	Percutaneous fentanyl	Fentanyl intravenous	p-value
1.	Age (Years)	152	31 ± 3.38	30 ± 2.85	0.055
2.	Weight (Kg)	152	73 ± 5.28	70 ± 3.37	0.001
3.	Gestational age (GPAH)	152	37.8 ± 1.08	37.6 ± 1.29	0.294

Table II. Homogeneity test of the research variable parameters using the Levene test

No.	Parameter		N	Percutaneous fentanyl	Fentanyl intravenous	p-value
1.	Pain scale after drug administration		152	3.8 ± 0.68 n (2) = 4 n (3) = 37 n (4) = 90 n (5) = 21	3.3 ± 0.57 n (2) = 3 n (3) = 92 n (4) = 55 n (5) = 1 n (6) = 1	0.978
2.	Hemodynamics					
	a.	Temperature (°C)	152	36.3 ± 0.27	36.4 ± 0.23	0.054
	b.	Pulse (x/minute)	152	82.45 ± 3.89	82.35 ± 3.63	0.198
	c.	Systole (mmHg)	152	119.93 ± 8.79	114.9 ± 17.97	0.063
	d.	Diastole (mmHg)	152	77.38 ± 5.78	75.26 ± 5.58	0.309
	e.	Saturation (%)	152	98.9 ± 0.012	98.3 ± 0.0129	0.483
3.	Side effects					0.000
	a.	Nausea	152	0.532 ± 0.5 n (1) = 81	0.072 ± 0.25 n (1) = 11	
	b.	Throw up	152	0.335 ± 0.47 n (1) = 51	0.066 ± 0.081 n (1) = 1	
	c.	Headache	152	0.309 ± 0.46 n (1) = 47	0.065 ± 0.24 n (1) = 10	
	d.	Sleepy	152	0.914 ± 0.28 n (1) = 139	0.953 ± 0.21 n (1) = 145	

Identification of Research Subjects

The number of research subjects after going through the inclusion criteria was 304 patients. The subjects selected were patients with post-sectio caesaria physical status of ASA I-II at Bunda Mother and Child Hospital Jakarta from September to November 2020. Subjects were divided into two groups: the group that received percutaneous fentanyl therapy and intravenous fentanyl therapy. The characteristics of the research sample were presented in **Table III**. Data on general characteristics of research subjects showed that there was no significant difference ($p > 0.05$) on variables, age, weight, ASA physical status and pregnancy diagnosis. These results indicate that the samples taken for the research are homogeneous so that they are comparable.

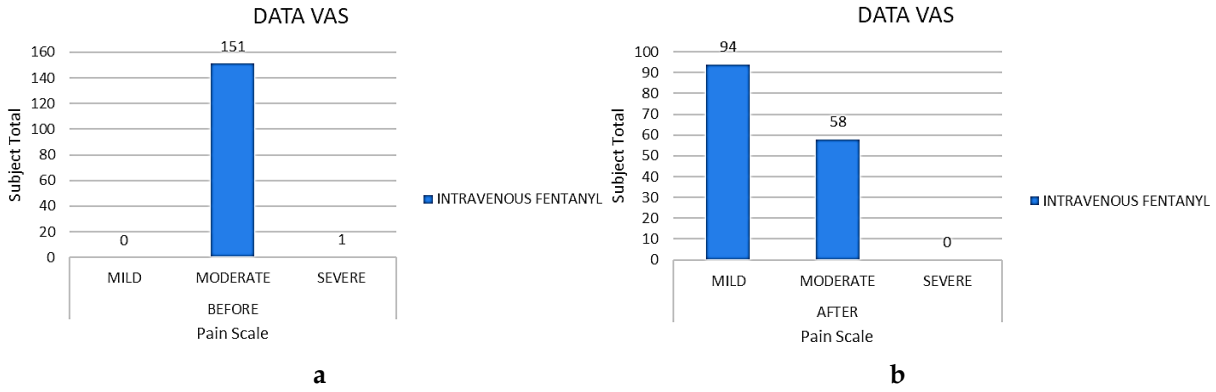
Table III. Characteristics of the research sample

Variable	Percutaneous fentanyl	Intravenous fentanyl	p-value
Age (years)	31.00 ± 3.39	30.56 ± 2.86	0.385
Weight (Kg)	73.04 ± 5.28	70.79 ± 3.38	0.001
Gestational age	37.79 ± 1.07	37.68 ± 1.30	0.966

Pain Effectiveness Analysis

To determine the effectiveness of pain, first a normality test was performed on the variable parameters of the pain scale on intravenous fentanyl and percutaneous fentanyl using the Saphiro-Wilk statistical method. From the results of the normality test of the pain scale parameter using the Shapiro-Wilk shows the p-value of the test is 0.000. The Sig value (p-value) of the two tests is above <0.05, which means that the data is not normally distributed. Then the test for the homogeneity of the variables was carried out using the Levene test obtained p value of 0.976. The Levene's test value is indicated by p-value

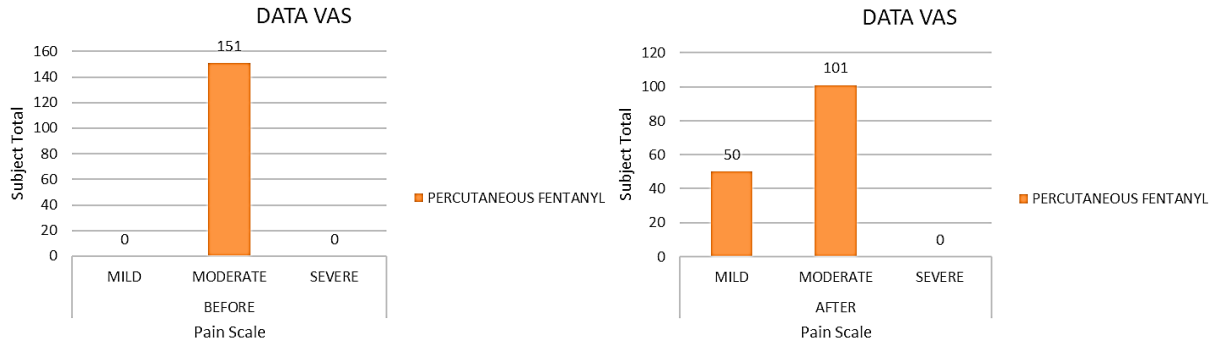
0.976 > 0.05, which means that the variance of the two groups is the same or what is called homogeneous. Thus, we will test different hypotheses using the Mann-Whitney test. To determine the effectiveness of pain in test subjects before and after intravenous and percutaneous administration of fentanyl, it can be seen from the **Figures 2** and **3**.



a **b**

Figure 2. Intravenous fentanyl VAS data before (**a**) and after (**b**) treatment

Based on the **Figure 2**, for the group of test subjects given intravenous fentanyl for three months on the basis of Lejus *et al.*[15], in the treatment before the administration of intravenous fentanyl, the visual analogue scale (VAS) results were 99.34% medium scale and 0.66% heavy scale. Observation of pain scale reduction was carried out after three hours of intravenous fentanyl administration, obtained a decrease in the VAS results from a severe pain scale to a moderate pain scale by 100% and a decrease in VAS from a moderate scale to a mild pain scale of 62.25%. However, there were still test subjects who experienced moderate pain of 38.16% after three hours of intravenous fentanyl administration.



a **b**

Figure 3. Percutaneous fentanyl VAS data before (**a**) and after (**b**) treatment

Based on the **Figure 3**, in the treatment before giving percutaneous fentanyl which was carried out for three months, 100% moderate-scale VAS results were obtained. Observation of pain scale reduction was carried out after three hours of percutaneous fentanyl administration, obtained a decrease in the VAS results from moderate pain scale to moderate to mild pain scale by 33.11%. But there were still test subjects who experienced moderate pain at 66.89% after three hours of percutaneous fentanyl administration.

In this study, anesthetists considered on the basis of giving fentanyl starting in the moderate category to prevent increased stress, anxiety and persistent chronic pain. This is in accordance with the basis for giving fentanyl according to WHO Three Analgesic Ladder on the assessment of the degree of pain based on the category of moderate (scale 4-6), severe (scale 7-9) and very painful (scale 10)[16]. To determine the effectiveness of pain in the two groups, different tests and hypotheses were carried out using the Mann Whitney test, as presented in **Table IV**.

Table IV. Mann Whitney test results on the effectiveness of intravenous and percutaneous fentanyl

Types of preparations	N	p-value
Intravenous fentanyl	152	0.005
Percutaneous fentanyl	152	

From the results of statistical tests for the effectiveness of pain, it was found that p-value <0.05, there was a significant difference between the two groups. Thus it can be said that there is a significant difference in the effect of the use of the two pain medications based on the observation of differences in the VAS, from the three hours VAS shows a significant difference in the three hours after analgesic administration (p-value <0.05). The distribution of fentanyl by the transdermal patch is characterized by a slow drug absorption rate and a sustained serum concentration after patch removal, making it unsuitable for acute pain management. Exogenous and endogenous factors such as the amount of subcutaneous fat, skin integrity, hair follicle structure and composition, possibly a dermal depot, body core temperature, skin thickness, first-pass skin biotransformation and environmental temperature cause strong differences in absorption of fentanyl into the bloodstream, this is a factor that influence change or decreas in pain scale when using percutaneous or transdermal fentanyl[17,18].

Percutaneous fentanyl has a slow effect, occurs only after 12 hours. This is due to the formation of fentanyl depots in the skin layer before the drug enters the systemic circulation. Patients with acute pain syndrome are not suitable candidates for percutaneous fentanyl. Time-limited acute pain syndromes are not compatible with the pharmacokinetics of the percutaneous fentanyl device. The FDA states that percutaneous fentanyl is contraindicated for postoperative pain control. However, some clinicians recommend percutaneous fentanyl for postoperative pain control but with caution and close clinical monitoring[12,13].

Intravenous administration of fentanyl does not use skin media for a longer period of time in the process of releasing and distributing fentanyl to its receptors, so this causes a reduction in acute pain, especially in post-cesarean patients, it is better at reducing the severe pain scale and moderate to mild pain scale[19]. Rayburn *et al*.[20] compared women who received intravenous fentanyl for labor pain with women who didn't receive analgesics or anesthetics during labor and determined that at low doses of intravenous fentanyl in women who delivered experience temporary analgesia and sedation without an immediate risk to mother and baby. When intravenous fentanyl was compared with intravenous meperidine for labor pain relief in a randomized, non-blind trial (N = 105 women with uncomplicated pregnancies during active labor), both fentanyl and meperidine produced similar reductions in pain scores; However, fentanyl was associated with less sedation, nausea, and vomiting and fewer newborns requiring naloxone therapy (1/49 vs 7/56, respectively; p <0.05). There was no difference in the rate of reduction in fetal heart rate variability, Apgar score, and neonatal neurologic and adaptive ability scores. Because fentanyl has fewer side effects in mothers and newborns, the investigators suggest that fentanyl may be preferred over meperidine for labor analgesia[21].

Hemodynamic Analysis

Opioids are drugs that act as receptor binding agonists μ. This drug has the effect of reducing pain and so is widely used as a regimen of postoperative analgesia[22]. Fentanyl is an opioid drug most widely used as a regimen for post sectio caesaria analgesia at Bunda Mother and Child Hospital Jakarta. The study was conducted on 304 patients who gave birth using the cesarean section method. These patients were divided into two test groups. The first group was given intravenous fentanyl therapy, and the second group was given percutaneous fentanyl therapy. Then, after three hours of intravenous administration of fentanyl and percutaneous fentanyl, the observed hemodynamic factors were tested, including temperature, blood pressure, pulse, and saturation. After the observations were made, the data obtained were subjected to statistical testing. The T-test results for hemodynamic parameters are presented in **Table V**.

The statistical test found that the administration of intravenous fentanyl and percutaneous fentanyl did not have a significant difference in the hemodynamic factors of temperature, pulse, blood pressure, and saturation. Intravenous fentanyl administration has a faster onset of action and a shorter duration of action because fentanyl is lipophilic. This reflects the large solubility of fentanyl in fat and can also cross the blood-brain barrier more quickly. The redistribution process of

fentanyl to inactive tissues such as fat and skeletal muscle is also accelerated. Administration of fentanyl by continuous infusion causes an increase in the saturation of the drug in the inactive tissue. As a result, the concentration of fentanyl in plasma does not drop rapidly, and respiratory depression can be prolonged. However, there was no change in saturation between the test group receiving intravenous and percutaneous fentanyl therapy[23].

Table V. Results of the T-test on intravenous and percutaneous administration of fentanyl

Variable	Percutaneous fentanyl	Intravenous fentanyl	p-value
Temperature (°C)	36.3 ± 0.27	36.4 ± 0.23	0.054
Pulse (x/minute)	82.45 ± 3.89	82.35 ± 3.63	0.198
Systole (mmHg)	119.93 ± 8.79	114.9 ± 17.97	0.063
Diastole (mmHg)	77.38 ± 5.78	75.26 ± 5.58	0.309
Saturation (%)	0.989 ± 0.012	0.983 ± 0.0129	0.483

The second hemodynamic factor to be monitored was blood pressure. Normal blood pressure is 120/80 mmHg, and blood pressure is declared high if the systolic pressure reaches more than 140 mmHg and diastolic blood pressure is more than 90 mmHg when sitting down[24]. From the study results, there were no significant differences in both systolic and diastolic blood pressure in patients who were given intravenous and percutaneous fentanyl. A drop in blood pressure can occur rapidly after anesthesia because of its vasodilating effect. A decrease in blood pressure is associated with decreased cardiac output, systemic vessel resistance, inhibition of the baroreceptor mechanism, depression of myocardial contractility, decreased sympathetic activity, and ionotropic effects[25]. The administration of fentanyl does not lead to depression of myocardial contractibility so that the decrease in blood pressure is not too large[26]. Myocardial depression and vasodilation effects occur depending on the dose of fentanyl given. Vasodilation occurs due to decreased sympathetic activity and the direct effect of calcium mobilization on intercellular smooth muscle[27]. Research conducted by Wickham *et al*.[28] said that giving fentanyl to induction of anesthesia with propofol can maintain hemodynamic stability of patients with a decrease in the mean arterial pressure (MAP) value <20% during induction of anesthesia. This occurs because fentanyl does not directly suppress sympathetic reflexes but maintains the patient's blood pressure. In this study, there was no hypotension because the administration of fentanyl served as hemodynamic stability. According to research conducted by Klamt *et al*.[29], the combination of fentanyl-midazolam assisted by isoflurane is effective and safe to provide long analgesic and hypnotic effects in pediatric patients undergoing cardiac surgery. Besides that, fentanyl also functions as hemodynamic stability, maintaining blood pressure and pulse within normal limits in pediatric heart surgery patients.

Laksono and Isngadi[30] said that shivers could occur after anesthesia. One way that is thought to be pharmacologically effective is to give fentanyl. The addition of intrathecal fentanyl can reduce the risk of shivering with minimal side effects of nausea and vomiting[28]. In patients with cesarean section, the incidence of shivering is more significant than in patients with other surgical methods. Because the method of spinal anesthesia performed on patients with sectio caesaria has more influence on temperature regulation. The effect of peripheral vasodilation on spinal anesthesia causes heat transfer from the central compartment to the peripheral compartment, causing hypothermia[30]. In addition, Techanivate *et al*.[31] concluded that adding 2 µg of fentanyl in 2.2 mL of 0.5% hyperbaric bupivacaine with 0.2 mL of morphine 0.2 mg intrathecally could reduce the incidence and the severity of intraoperative and postoperative shivering after spinal anesthesia in patients undergoing cesarean section without increasing the incidence of side effects. This study resulted in no significant change in the body temperature of the test sample. It is thought that the administration of fentanyl keeps the body temperature in normal condition or maintains thermoregulation to prevent hypothermia.

Fentanyl lowers the patient's shivering threshold so that even though there is hypothermia, it does not pass the shivering threshold that falls[30]. Another study conducted by Nugroho *et al*.[32] said that the labor process is a physical process that is thermogenic or causes heat due to increased oxygen consumption due to uterine and skeletal muscle contraction. The hypothalamus then triggers vasodilation, sweating, and hyperventilation to increase heat loss. Epidural analgesia is said to cause an imbalance between heat production and heat loss mechanisms. Generally, epidural analgesia causes a decrease in core temperature due to the redistribution of body heat from the core to the periphery, increasing heat dissipation by the

body. This effect is then offset by epidural analgesia, which lowers the threshold for shivering thermoregulation by blocking the intake of cold afferents from the anesthetic agent of the body. As a result, the patient will shiver to increase heat production[31]. Gleeson *et al*.[33] found that fever was twice as common in women who shivered than those who did not come after epidural insertion. Sweating is one of the body's responses to lower body temperature. Sympathectomy of epidural analgesia will prevent this from occurring. The epidural block alters the thermoregulatory response to heat generation by increasing the sweating threshold. In this case, the epidural is said to block sweat in the body segment that gets the epidural so that body temperature increases. Patients who do not receive analgesia during labor are also more likely to hyperventilate. This, together with the expulsion of sweating, will reduce the patient's body temperature. Providing adequate analgesia, such as epidural analgesia, reduces the degree of hyperventilation and heat loss, and body temperature will increase.

For pulse hemodynamic factors in this study, there were no significant differences in patients given intravenous or percutaneous fentanyl. Administration of fentanyl can cause bradycardia due to increased central vagal tone and depression of the SA and AV nodes. In hypovolemic patients, fentanyl causes a decrease in stroke volume, a decrease in heart rate, and cardiac output, causing the heart rate to decrease, but the heart rhythm does not change[34]. According to research conducted by Klamt *et al*.[29], infusion of a combination of midazolam and fentanyl can provide analgesic and hypnotic effects by maintaining hemodynamic stability such as heart rate in children undergoing heart surgery. Giving fentanyl is the right thing to do during surgery to maintain hemodynamic factors to remain stable, especially the pulse. Fentanyl is a suitable opioid that is suitable for use. Besides maintaining hemodynamic factors, it also has a fast onset and makes minimal hemodynamic changes even though it is given in large quantities.

Side Effects Analysis

From the results of statistical tests for the side effects of intravenous and percutaneous fentanyl administration (**Table VI**), it was found that the p-value critical limit was <0.05, so there was a significant difference between the two groups. Thus, there is a significant difference in the effect of using two pain medications from the side effect parameters. If you look at the mean value, where the intravenous fentanyl pain medication has a smaller mean, the resulting side effects are smaller. Thus, it can be concluded that intravenous fentanyl has more minor side effects than percutaneous fentanyl.

Fentanyl is an opioid with a μ agonist active against the μ receptor, where the μ receptor mediates analgesia, sedation, vomiting, respiratory depression, pruritus, euphoria, anorexia, decreased gastrointestinal motility, and urinary retention[35]. In transdermal preparations, fentanyl is sufficiently soluble in the lipid and water compartments of the skin to allow penetration. In its alkaloid (alkaline) form, fentanyl readily enters the stratum corneum keratin. This epidermal layer provides the most significant barrier for water movement both into and out of the body[17]. Only substances with sufficient fat solubility can dissolve and diffuse through this dermal layer's ceramides and other wax lipids. Subsequent drug movement from the lipid layer into the dermal water is required to allow systemic absorption. So the chemical must be lipid and water-soluble to be effectively internalized once it passes through the skin. The relationship between the lipid and water solubility of a chemical is indicated numerically by the octanol-water partition coefficient. It is expressed as the ratio of the concentration of a chemical in octanol and water when it is in equilibrium at a specific temperature. Fentanyl bases have an octanol-water partition coefficient of 860 (fentanyl citrate is 717 at pH 7.4), so they pass through the lipid portions of the epidermis with relative ease. Although fentanyl base and salt (citrate) are bioavailable, systemic base absorption appears to be slightly faster. In comparison, morphine is less lipophilic and has an octanol partition coefficient of 0.7, and predictably shows poor epidermal permeability. This is what chooses fentanyl in transdermal form compared to morphine.

Table VI. Mann-Whitney test results of side effects of intravenous fentanyl with percutaneous

Factor	N	Intravenous fentanyl	Percutaneous fentanyl	p-value
Nausea	81	0.072 ± 0.25	0.532 ± 0.5	0.000
Throw up	51	0.066 ± 0.081	0.335 ± 0.47	
Headache	47	0.065 ± 0.24	0.309 ± 0.46	
Sleepy	139	0.953 ± 0.21	0.914 ± 0.28	

The uptake of fentanyl in patch preparations depends on the transdermal site's exogenous and endogenous factors. The thickness and temperature of the skin can alter transdermal fentanyl bioavailability and blood flow to and from the patch site. Applying the patch to the damaged skin can cause an increase in blood fentanyl concentration, and an increase in skin temperature will also increase fentanyl absorption. The chest area is a site that can accept transdermal attachment and blood flow, and this is due to the minimal effect on systemic drug absorption under normal physiological conditions.

Pharmacokinetically, fentanyl patch (percutaneous) is detectable in serum 1-2 hours with onset for six hours after fentanyl action. Serum fentanyl concentration increased gradually at 12 hours and remained constant for 72 hours. This causes nausea, vomiting, and headache side effects found in this study because after the patch is removed from the skin media, fentanyl remains constant in the blood. The patient complained of being switched to TFP with other analgesics that did not cause the side effects of nausea, vomiting, and headaches.

The ideal general anesthesia can provide rapid and quiet induction, predictable loss of consciousness, stable intraoperative state, minimal side effects, rapid and smooth restoration of protective reflexes, and psychomotor function. In addition, the ideal physical and pharmacological properties of intravenous anesthetics should be soluble and stable in water, painless during injection, not releasing histamine or hypersensitivity reactions, rapid and gentle onset of hypnosis without causing excitatory activity, metabolism, rapid inactivation of drug metabolites, is related Steep dose and response to increase titration effectiveness and minimize tissue drug accumulation, minimal respiratory and cardiac depression, decrease cerebral metabolism and intracranial pressure, recovery of consciousness and cognition that is fast and gentle, and does not cause postoperative nausea and vomiting (PONV), amnesia, psychomimetic reactions, dizziness, headaches and prolonged sedation time (hangover effect)[36]. One of the disadvantages of fentanyl as a single anesthetic is that it requires a large initial dose with a large dose range ranging from 50-150 μg/kg BW or fentanyl concentration in plasma ranges from 20-30 μg/mL[37].

CONCLUSION

Based on the results, it can be concluded that intravenous fentanyl is more effective in reducing the pain scale in post sectio caesaria patients than percutaneous fentanyl. In hemodynamic parameters, p-value >0.05 showed no significant difference in hemodynamic factors (temperature, pulse, blood pressure, and oxygen saturation) in the administration of intravenous percutaneous fentanyl. Intravenous fentanyl has fewer side effects (nausea, vomiting, headache, and drowsiness) than percutaneous fentanyl.

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AUTHORS' CONTRIBUTION

Annisa``a Nurillah Moesthafa: conceptualization, funding acquisition, project administration, investigation, data curation, formal analysis, software, visualization, and writing - original draft. **Achmad Riviq Said**: conceptualization, resources, investigation, data curation, and validation. **Ros Sumarny**: resources, methodology, supervision, validation, and writing - review & editing. **Yati Sumiyati**: project administration, methodology, formal analysis, supervision, validation, visualization, and writing -review & editing.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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Short Communication

Control of the Nanoparticles Content in Cosmetic Medicines

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Determination of the concentration
Nanocosmetics

Abstract

The safety of nanoparticles used in medical cosmetology and dermatology raises significant concerns. One of the tasks of analyzing the concentration of nanoparticles that must be solved for the practical analysis of the quality of products with nanoparticles is the quantitative analysis of the content of nanoparticles. The research objectives were developing the simplified method for determining the quantitative content of magnetic nanoparticles in the sample and experimental testing the sensitivity of a previously developed acousto-magnetic method (AMM) for practical use for the samples prepared as a colloidal solution of cosmetic medicines with nanoparticles. The object of the study was a model colloidal solution containing a certain amount of magnetite nanoparticles. The possibility of using the simplified AMM method at a therapeutically permissible concentration of magnetic nanoparticles has been proven. Comparison of the used technique with other methods of measuring the concentration of magnetic nanoparticles showed several advantages of the AMM method. It is possible to create a device for practical use in the future.

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INTRODUCTION

The currently observed increased interest in metal nanoparticles (NPs) is caused by discovering their unique physical and chemical properties features of biological action, which often differ from the properties of this substance in a macrodispersed form. Good prospects are for metal NPs in medicine, particularly dermatology. A new direction of developing nano preparation is forming a complex between known drugs and NPs, which gives the possibility of a deeper penetration of such complex drugs into the pathological process. To maximize the benefits of nanoscale materials, accurate control of their concentration is a necessary condition. In particular, this is necessary regarding magnetic drug targeting to maximize the efficacy and minimize the toxicity of the nanomaterials. Towards this significant yet chronic problem, various strategies are currently under development[1].

Some of the concentration determination methods apply to the ensemble of physical properties of dispersions of NPs (for example, light absorption), while others, such as microscopy and sensors, directly count individual particles. The UV-Vis spectroscopy[2], turbidimetry[3], and dynamic light scattering (DLS)[4] are three optical methods that measure the intensity of light upon absorption or scattering by nanoparticles. The turbidimetry method measures a decrease in the intensity of the incident light caused by light scattering of nanoparticle suspensions. The values measured by these methods are ensemble properties of nanoparticle suspensions, which can reflect averaged concentrations with statistical significance. The

limitations of these three methods lie in the complexity of measuring extinction/scattering coefficient or employing a reference sample with a known concentration.

Laser-induced breakdown detection (LIBD) is another method measuring the plasma generation from nanoparticles irradiated by an intense, focused laser in a suspension. It has a wide application in various particles of different sizes, but a special laser system is required to ensure the breakdown of nanoparticles. Unlike measuring ensemble quantities of nanoparticle dispersions, several techniques enable counting individual nanoparticles under direct visualization[5].

Resistive-pulsed sensing, inductively coupled plasma mass spectrometry (ICPMS), and light scattering particle counter are three methods that can count particles. They provide concentration information based on the signal pulses from a sensor, and a standard reference sample is usually required for calibration. The ICPMS is a highly sensitive and rapid analytical technique for elemental analysis at ultra-low concentrations. The samples in traditional ICPMS are usually metal ions dissolved in solution, and the concentration of total metal can be calculated based on the averaged intensity of the ion peak over a measuring period[6].

Mössbauer spectroscopy is widely used to determine magnetic NPs (MNPs). The methods based on the analysis of X-ray diffraction data have a reasonably wide range of determined parameters (phase composition, structure, average size, and morphological characteristics of nanocrystals. However, using this method can lead to significant errors due to the influence of various factors on the effect of broadening of diffraction maxima and others. When the Mössbauer spectroscopy method is used for measurements at 4.2 K, the nanoparticles typically exhibit well-defined but complicated hyperfine spectra that may present some evaluation problems but eventually yield reliable results. The different situation was when nanoparticles of Fe_3O_4 (magnetite) and gamma-Fe_2O_3 (maghemite) had been studied by Mössbauer spectroscopy at room temperature when they are superparamagnetic. The magnetic hyperfine fields were averaged to zero, making Mössbauer spectroscopy useless for the characterization of superparamagnetic NPs[7].

Among the magnetic NPs suitable for use in medicine, particles of iron oxide Fe_3O_4 can be distinguished due to their biological compatibility with biological objects. These particles are superparamagnetic and cannot be studied by the Mössbauer spectroscopy method at room temperature. The characterization of magnetic iron oxide NPs is important for their use as contrast agents in magnetic resonance imaging, as carriers for magnetic drug targeting, for local hyperthermia. Magnetic resonance imaging (MRI) can be used for magnetic particles concentration determination[8].

Magnetic nanoparticles change the electromagnetic excitation spectra of organic molecules of the human body. Registration of these spectra by MRI provides information on the distribution of particles in space and, consequently, the concentration of drugs coupled with them. The special features of the MRI method are the high cost and the associated with it less availability and give information about NPs distribution. Analysis of existing methods shows no universal method for determining the concentration of NPs of various types[9]. Each of the listed methods has both certain advantages over others and limitations. In particular, developing a more straightforward method applicable to *in vivo* and *in vitro* use for superparamagnetic nanostructures at room temperature is important. This study aims to describes a simplified acoustomagnetic method (AMM) of detecting magnetic particles concentration[10] and experimental procedure for determining the concentration of MNPs in a colloidal solution that is the model of the cosmetic products with MNPs both *in vivo* and *in vitro* that is important in dermatology as the possibility of penetration through the skin, in particular through the sebaceous glands and hair follicles. Such carriers provide long-term drug release and protect it from degradation.

MATERIALS AND METHODS

Materials

The object of study was a colloidal solution of nanoparticles based on Fe_3O_4 in a mixture of oleic acid and kerosene, a model sample of a cosmetic product colloidal solution. The average particle size with magnetite was 50-150 nm, size of Fe_3O_4 was much less. Oleic acid prevents powder particles from sticking together in solution, and kerosene provides the necessary viscosity. For biophysical applications, it is recommended to use a solution with a concentration of nanoparticles not more

than 5%. In our experiments, the weight concentration of the actual magnetite was no more than 0.15%. The viscosity of the suspension was chosen close to the viscosity of the blood (5×10^{-3} poise).

Methods

The experimental verification of the applicability of the AMM method was carried out using the setup shown in **Figure 1**. The scheme consists of three component blocks (**A**, **B**, and **C**), allowing to determine the concentration of nanoparticles in a vessel with a studied medium. Block **A** is a conditional image of a plot of a model studied medium with nanoparticles affected by a constant magnetic field H_d and ultrasonic radiation (**US**). The result of these actions is the generation under the action of US on magnetic particles of an alternating magnetic field H_a, depending on the concentration of the nanoparticles in the indicated studied medium. Block **B** is a sensitive device for measuring the field H_a. Block **C** is a recorder of the value H_a. Ultrasonic radiation has induced excitation of vibrations of magnetic nanoparticles in the target area of the sample, located in the external uniform constant magnetic field H_d. Oscillations of the particle ensemble oriented (polarized) by H_d field caused the appearance in the surrounding space of the alternating magnetic field H_a with a frequency of **US**. The magnetic flux of this field depends on the concentration of nanoparticles in the studied region and can be measured by a sensitive detector located outside this region. As such a detector, it can be used a superconducting quantum magnetometer, which, as is known, has the highest sensitivity and dynamic measurement range among the known types of magnetometers[11]. In the experiments with a model of a cosmetic product due to the higher permissible content of magnetite nanoparticles, which themselves do not belong to highly toxic additives, a highly sensitive voltmeter was used. In addition, in practice, the possibility to use a sensor with a lower sensitivity compared to the superconducting quantum magnetometer will make the AMM technique available for practical use in the future.

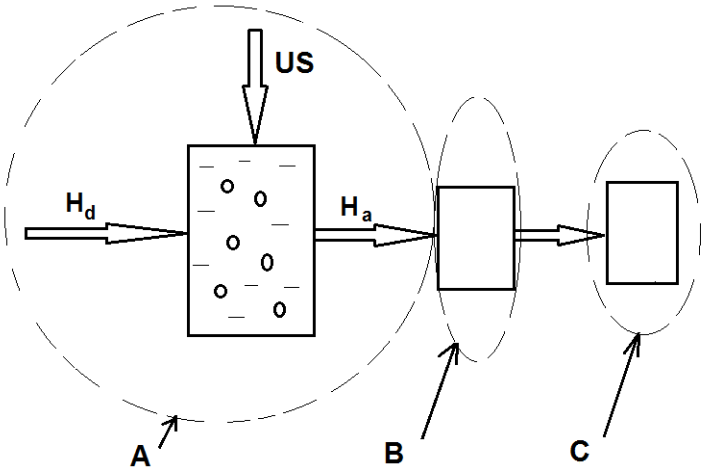

Figure 1. Block diagram of a measuring system

RESULTS AND DISCUSSION

Voltage (**U**) on the induction coil of unit **B** resulted from the excitation of oscillations of magnetic nanoparticles in the target area of the medium under the action of ultrasonic radiation. Accordingly to the Faraday's law on electromagnetic induction, the resulting **U** should be proportional to the magnitude of the nanoparticles' total magnetic field at the detector's location and their speed relative to the detector[12]. In turn, field H_a and **U** are proportional to the concentration (**K**) of the nanoparticles in the moving solution. In the specific case of the described experimental installation, the solution with the nanoparticles was moved using an ultrasonic wave, and the magnetic moments of the nanoparticles were oriented along the required direction by constant magnetic field H_d. In this case, the speed of movement of the nanoparticles is proportional to the power of the ultrasound. The magnitude of the **U** is proportional to the concentration of nanoparticles in the field of action of **US** and

magnetic field H_d and the **US** intensity (**I**). Dependence of the **U** at the induction coil on the **I** of ultrasound can be described by the **Equation [1]**:

$$U = k N I \qquad [1]$$

In which the parameter **k** characterizes the magnetic field properties in the coil area, the **N** number of the magnetic nanoparticles creates a magnetic field when all nanoparticles are oriented in the direction perpendicular to the plane of the coil by uniform constant magnetic field H_d. The value of **K** of the magnetic nanoparticles is equal to as described in **Equation [2]**:

$$K = (\mathbf{N\,V\,\rho/m})\ \mathbf{100}\% \qquad [2]$$

In which **V**, **ρ**, and **m** are the volume of one nanoparticle, its specific weight, and the total mass of the solution, respectively. The increase of the voltage on the induction coil proportionally to the **I** of the ultrasound confirmed the possibility of the magnetic particles registration using an acoustomagnetic method to measure the quantitative value of the concentration of the magnetic particles (**Figure 2**).

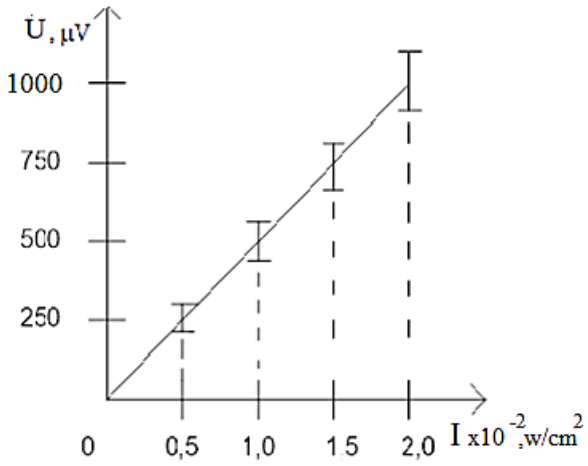

Figure 2. Dependence of the effective value of the alternating voltage U on the US intensity I

CONCLUSION

It is proposed to use a simplified method for determining the concentration of the cosmetic preparations when superconducting magnetometer usage is replaced by the highly sensitive voltmeter, which makes it possible to exclude the use of cryogenic liquid and makes the proposed method more accessible in practice for quality control of cosmetic preparations. Also, it has been experimentally shown that the sensitivity of detecting the response of an ensemble of MNPs using the selected measurement scheme is sufficient at the therapeutic dose with nanoparticles, which may be contained in a cosmetic product. The chosen method has several advantages over Mössbauer spectroscopy since it allows the concentration of superparamagnetic MNPs to be measured at room temperature, as Mössbauer spectroscopy is useless at this condition. Compared to MRI, the chosen technique is a direct method for determining the magnetic field's concentration and magnitude is significantly lower than it is used for MRI. Unlike most techniques, the chosen method allows its use not only for *in vitro* measurements but also for *in vivo* measurements at the penetration of MNPs through the skin in dermatology.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Research Article

Identification of Candesartan Cilexetil-L-Arginine Co-amorphous Formation and Its Solubility Test

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Co-amorphous
L-Arginine
Liquid-assisted grinding
Solubility

Abstract

The formation of co-amorphous is one alternative that can be attempted to enhance the solubility of drugs. The study aimed to identify the co-amorphous formation between candesartan cilexetil (CAN) and l-arginine (ARG) and to know its effect on the solubility and dissolution rate of candesartan cilexetil. Initial prediction of co-crystal formation was undertaken by observing differences in crystal morphology between the candesartan cilexetil-l-arginine (CAN-ARG) mixture and each of its initial components due to crystallization in ethanol. The CAN-ARG co-amorphous was produced by the liquid-assisted grinding (LAG) method with the same molar ratio of the CAN and ARG mixture using ethanol as solvent. The co-amorphous formation of CAN-ARG was identified by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) methods. The solubility and dissolution test was performed to know the impact of the co-amorphous CAN-ARG formation. The PXRD pattern of CAN-ARG of LAG result showed a very low peak intensity compared to pure CAN and ARG. The DSC thermogram of the CAN-ARG LAG result does not show any sharp endothermic peaks. The PXRD and DSC results reveal that CAN and ARG can form co-amorphous. The solubility and dissolution rate of candesartan cilexetil in co-amorphous CAN-ARG was better than that of pure CAN. It can be concluded that liquid-assisted grinding of CAN-ARG mixture is identified to form co-amorphous, which impacts increasing the solubility and dissolution rate of candesartan cilexetil.

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INTRODUCTION

Solubility is one of the essential parameters besides permeability that affects the bioavailability of active pharmaceutical ingredients (APIs), which impact pharmacological responses. Increasing the solubility of an API can be attempted by converting it into a solid form with a higher solubility than pure API. One approach that can be taken to overcome the problem of poor solubility of an API is to change crystalline solids to amorphous ones. The alteration of a crystalline solid to an amorphous one can be accomplished by combining the API with an excipient, either a polymer or a small molecule[1,2]. Combining API with a polymer to form an amorphous solid is known as a solid dispersion. However, solid dispersion has a disadvantage due to the hygroscopicity of the polymer in large quantities[3,4]. Modifying a crystalline solid from API with a small molecule often results in an amorphous solid known as co-amorphous. The co-amorphous formation is effective in increasing the solubility of some APIs. Co-amorphous is formed when a crystalline API and a crystalline excipient undergo intermolecular interactions, thereby preventing the rearrangement of their respective molecular arrangements into

separated crystal lattices and producing an amorphous material[5,6]. Co-amorphous has better physical stability than the single amorphous form[7].

Candesartan cilexetil (CAN) is an ester prodrug of candesartan that is widely used as an antihypertensive with its mechanism of action by blocking the angiotensin II receptor. This API has poor solubility in water, but its permeability is high, so it is classified into class II in the biopharmaceutics classification system. This poor solubility can be a problem causing low dissolution and bioavailability[8]. Several amino acids have been identified as being able to form co-amorphous with some APIs with the effect of increasing their solubility[9]. One of the amino acids often used as a co-former in the co-amorphous formation is L-arginine (ARG). Several drugs have been successfully increased their solubility through the co-amorphous formation with L-arginine, including indomethacin[10-12], ibuprofen[13], and hydrochlorothiazide[14].

Co-amorphous can be prepared by grinding or solvent-based techniques. Co-amorphous formation often occurs due to inhibition of the crystallization process during co-crystals or salts preparation. Some co-crystal or salt formers (co-formers) can prevent the molecule rearrangement of an API from forming crystal due to the API-coformer intermolecular interaction[2,15]. Co-crystal and salt formed from an API and a crystalline co-former do not recrystallize immediately after the manufacturing process by the liquid-assisted grinding (LAG) process. The grinding of two or more compounds using liquid-assisted grinding (wet grinding) can cause crystal breakdown that can induce intermolecular interactions to form new solid phases, such as co-crystal[16,17,] salt[18], and co-amorphous[19,20]. This study aimed to identify the co-amorphous formation of candesartan cilexetil-L-arginine (CAN-ARG) and to know its effect on the solubility and dissolution rate of CAN.

MATERIALS AND METHODS

Materials

Candesartan cilexetil and L-arginine were purchased from Afine Chemical Limited, Hangzhou, China, and Merck, Indonesia, respectively. Solvents and reagents such as ethanol, hydrochloric acid, sodium hydroxide, and potassium dihydrogen phosphate were purchased from Merck, Indonesia. Instruments used in this study include a polarizing microscope (Olympus BX-53), automatic mortar grinder (Retsch RM 200), powder X-ray diffractometer (PRXD; Panalytical Empyrean), differential scanning calorimeter (DSC; Shimadzu DSC-6 plus), orbital shaker (IKA KS-260), dissolution tester (ZRS6G), and ultraviolet spectrophotometer.

Methods

Observation of crystal morphology by polarizing microscope

Crystal morphology was observed using a polarizing microscope (Olympus BX-53) against CAN, ARG, and a mixture of CAN-ARG recrystallized in ethanol. The test was carried out by placing an amount of 1-3 mg of each CAN, ARG, and CAN-ARG on an object glass which was dropped with one drop of ethanol and allowed until the solvent evaporated. Observation of the crystal morphology of each sample was carried out using a polarizing microscope at a magnification of 200X.

Preparation of CAN-ARG co-amorphous by LAG

The co-amorphous preparation was carried out by the LAG method[21,22]. The co-amorphous preparation was carried out by grinding a mixture of 1.832 g (3 mmol) CAN and 0.522 g (3 mmol) ARG in an automatic mortar grinder (Retsch RM 200). The grinding was carried out for 10 minutes with the addition of five drops of ethanol until a soft and clear mass was formed. The soft mass was left in a desiccator to dry, powdered, and sieved through a 60 mesh.

Detection of CAN-ARG co-amorphous formation by PXRD

A total of 500 mg of LAG results from CAN-ARG that have been powdered are placed in a sample container and leveled. Scans were performed on a Panalytical Empyrean PXRD, using a Cu anode at a current of 30 mA and a voltage of 40 kV at a 2θ angle between 5 to 45°. The scanning under the same conditions was also performed on pure CAN and ARG as initial components.

Detection of CAN-ARG co-amorphous formation by DSC

About 3-5 mg of LAG powder from CAN-ARG was put in an aluminum crucible pan. The aluminum crucible pan containing the sample was positioned in the Shimadzu DSC-6 plus differential scanning calorimeter instrument and scanned at temperature intervals of 30-250°C at a scan rate of 10°/minute. The scanning under similar conditions was also executed on pure CAN and ARG as initial components.

Solubility test

The solubility tests were performed in the water at room temperature using the shaker method[23]. Each as much as 50 mg of CAN-ARG co-amorphous and pure CAN powder was placed into a vial. Five mL of water was put into the vial. The vial was placed in an orbital shaker at ambient temperature and shaken for two days at 250 rotations per minute (rpm). After shaking ends, the samples were filtered. The filtrate was measured using an ultraviolet spectrophotometer at 251 nm. Each test was repeated three times.

Dissolution test

The dissolution test was implemented as specified in the USP 40-NF 35 monograph of the CAN tablet[24]. The CAN-ARG co-amorphous powder was sieved through a 60-mesh sieve, and the equivalent of 32 mg of CAN was weighed for dissolution testing. Sampling was executed as much as 5 mL at 5, 10, 15, 20, 30, 45, and 60 minutes, and each sampling was replaced with the same medium and volume. Corrections to the calculations were made at each sampling point, and the amount of dissolved CAN was determined using an ultraviolet spectrophotometer. Dissolution tests with the same medium and conditions were also carried out on pure CAN. Each test was repeated six times.

RESULTS AND DISCUSSION

Crystal morphology

The crystals morphology of the CAN-ARG mixture, pure CAN, and ARG after recrystallization in ethanol were shown in **Figure 1**. Identification was carried out by comparing the morphology of the CAN-ARG mixture with the respective crystal morphology of pure CAN and ARG. The observations with a polarizing microscope at a magnification of 200 times showed that the morphology of the recrystallized CAN-ARG does not show any colors due to the interference of light from the crystal lattice, but the result recrystallization only looks black. This situation indicates that CAN-ARG forms an amorphous solid phase. This is different from the crystal morphology of pure CAN and pure ARG, both of which show crystalline morphology, characterized by light polarization that causes the crystals to be colored. This initial microscopic indication can be used as the basis for CAN-ARG co-amorphous preparation by a LAG method using ethanol to accelerate the co-amorphous formation.

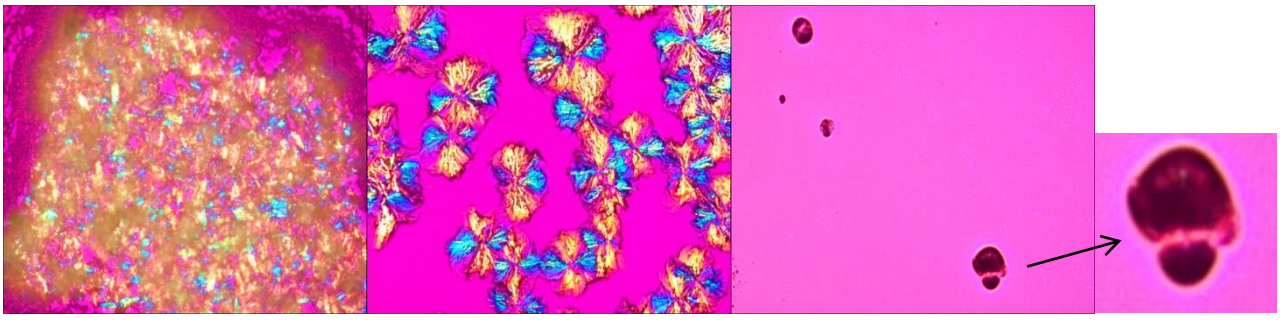

Figure 1. Morphology of (**a**) CAN, (**b**) ARG, (**c**) CAN-ARG, and (**d**) and further zoom of CAN-ARG mixture after recrystallized from ethanol compared its starting components observed by polarizing microscope at a magnification of 200x

Preparation of CAN-ARG co-amorphous by LAG

In the preparation of co-amorphous CAN-ARG, ethanol solvent was used because this solvent was able to dissolve both substances well. The addition of a solvent or solvent mixture in the wet grinding method helps accelerate the achievement of the amorphous state of each component, thereby increasing the movement of molecules that can accelerate the interaction[25]. In the preparation of CAN-ARG co-amorphous, ethanol was used as a solvent since this solvent could dissolve both substances well. Visually, the co-amorphous powder obtained after drying and grinding was white, the same as the powder of candesartan cilexetil starting material.

Powder X-ray diffraction

The X-ray diffractograms of the CAN-ARG LAG result and the two basic components were shown in **Figure 2**. The PXRD pattern of CAN showed the number of sharp peaks, which indicate that the CAN starting material was crystalline. The PXRD pattern of CAN powder corresponds to candesartan cilexetil form 1 reported by Matsunaga *et al*[26]. As with CAN, the PXRD pattern of ARG raw material was also crystalline. However, a different PXRD pattern was shown on the LAG result of CAN-ARG. The PXRD pattern of the LAG result of CAN-ARG showed a low peak intensity which indicates the formation of an amorphous phase. The high-intensity peaks previously present in the pure CAN and ARG disappeared. This situation indicates that CAN and ARG experienced intermolecular interactions during the LAG process, which prevented the CAN and ARG molecules from rearranging to form their respective crystal lattices and finally resulted in an amorphous material known as co-amorphous[27].

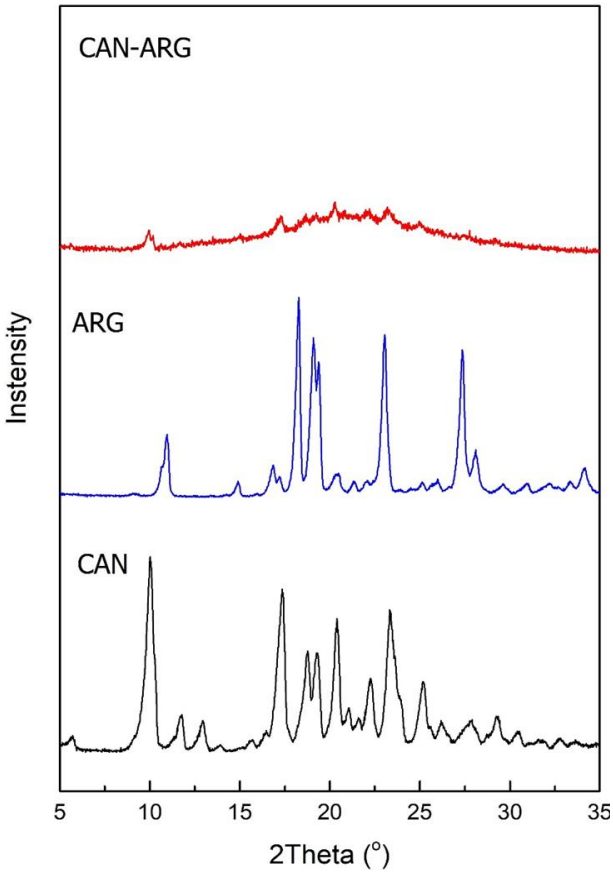

Figure 2. PXRD patterns of CAN-ARG LAG result, pure CAN, and pure ARG

DSC thermograms

DSC thermograms of CAN-ARG LAG result, pure CAN, and pure ARG were shown in **Figure 3**. The DSC thermogram showed that the thermal characteristics of the pure components of the starting material (CAN and ARG) were different from

those of the LAG of the CAN-ARG mixture. A sharp endothermic peak at 177.26°C in the DSC thermogram of CAN is due to the substance's melting, which corresponds to the melting point of the Form 1 polymorph[26,28]. The DSC thermogram of ARG showed two endothermic peaks, one sharp endothermic peak at 221.6°C corresponding to its melting point and another endothermic peak around 80-100°C due to the release of water molecules from the ARG raw material, which is slightly hygroscopic. DSC thermogram of the two starting components has sharp endothermic peaks that indicate both are crystalline. The DSC thermogram of the CAN-ARG grinding result did not show any sharp endothermic peaks indicating the co-amorphous formation. The formation of this co-amorphous was also confirmed by the presence of a glass transition (Tg) at 53.45°C, which is a characteristic of the amorphous form. The glass transition is the temperature at which an amorphous solid begins to change from a glassy to a liquid state when heated[29].

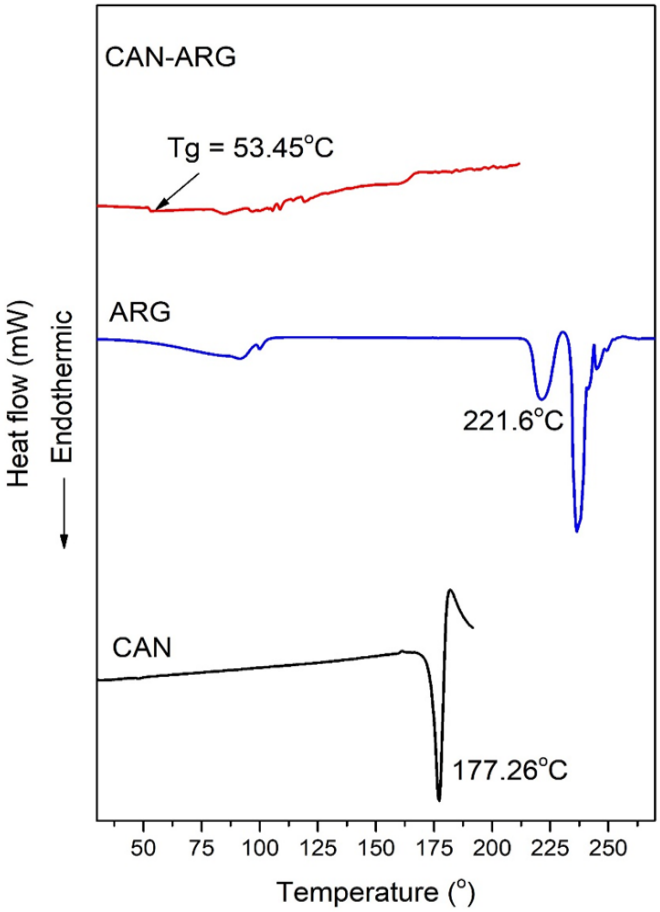

Figure 3. DSC thermograms of CAN-ARG LAG result, CAN, and ARG

Solubility

The solubility test aims to determine any changes in physicochemical properties that occurred due to the CAN-ARG co-amorphous formation. The solubility of CAN-ARG co-amorphous and pure CAN in water at room temperature were 2.837±0.080 and 0.009±0.001 mg/mL, respectively. The co-amorphous CAN-ARG showed 315-folds higher solubility than pure CAN. The increase in solubility through the formation of co-amorphous has been studied previously to increase the solubility of valsartan up to 1000-folds compared to the pure substance[30]. The possible reasons for this increase in solubility are that the co-amorphous form of CAN-ARG does not have a regular molecular arrangement, so the energy required to break the intermolecular bonds during the dissolution process is lower than that of CAN crystals. Candesartan is a weak acid, and the solubility of CAN in water depends on pH[31]. Therefore, the increase in solubility could also be due to the ionization of CAN in the presence of ARG (a weak base).

Dissolution

The dissolution rate profiles of CAN-ARG co-amorphous and pure CAN in 0.05 M phosphate buffer solution pH 6.5 containing 0.70% polysorbate 20 were shown in **Figure 4**. The dissolution profiles showed that the CAN released from the co-amorphous CAN-ARG had reached 100% in less than 20 minutes. In contrast, the CAN released from pure CAN was only 9.7% up to 45 minutes of testing. A significant increase in its solubility caused the increasing dissolution rate of CAN in the CAN-ARG co-amorphous after being co-amorphous.

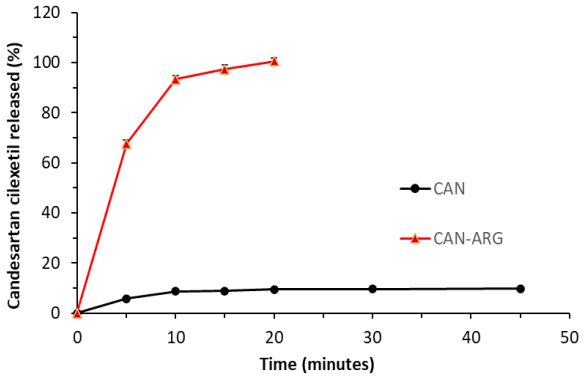

Figure 4. Dissolution profiles of CAN-ARG co-amorphous compared to pure CAN

CONCLUSION

The co-amorphous formation between CAN and ARG has been identified by the polarizing microscope, PXRD, and DSC of the LAG, which shows a CAN-ARG co-amorphous formation between CAN and ARG. The CAN-ARG co-amorphous led to a significant improvement in the solubility and dissolution rate of CAN.

ACKNOWLEDGMENT

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Research Article

Characterization of Onchidiid Slug (*Onchidium typhae*) West Kalimantan Waters as Antibacterials and Antifungal

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Microdilution
Onchidium typhae

Abstract

Onchidiid slug (*Onchidium typhae*) is a nudibranch that coastal communities in West Kalimantan have widely used as wounds. The study aims to characterize the West Kalimantan water *O. typhae* as antibacterial and antifungal. The study of *O. typhae* was carried out in several stages: preparation and optimization, extraction by Quinn method, characterization and identification of bioactive compounds, and antibacterial and antifungal assay using the microdilution method. The result of the proximate test showed that *O. typhae* powder contains high protein, namely 67.68%. Phytochemical screening results from methanol, ethyl acetate, and chloroform extracts contain alkaloids and amino acids. Methanol, chloroform, and ethyl acetate extract 1% of *O. typhae* showed inhibitory activity against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. The most significant inhibition value was indicated by chloroform extract 1%, where the inhibition value against *S. aureus*, *E. coli*, and *C. albicans* was 82±0.01%; 85.8±0.01%; 85±0.01%, respectively. From these results, *O. typhae* powder can be developed as a wound medicine through its antibacterial and antifungal activity.

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INTRODUCTION

West Kalimantan, part of a group of islands in Indonesia, is rich in animal and biological diversity. With the variety of its population, West Kalimantan will also be rich in traditional knowledge in using natural resources as medicine or healthy food preparations. The coastal communities of West Kalimantan use many natural ingredients, especially their aquatic products, as medicine. For example, the onchidiid slug (*Onchidium typhae*) has been used as a medicine for wounds/ulcers. *Onchidium typhae* is known to have the ability to produce secondary metabolites that are toxic to predators and some chemical compounds obtained from their food[1,2].

Onchidium genus (Onchidiidae family) is treated as a commodity with high economic value in waters along the Indo-Pacific coast due to its high nutritional and medicinal value. They are considered high-grade food due to their high protein and low-fat characteristics. This commodity has an aphrodisiac effect, digestive function, anticytotoxic activity *in vitro*, and antineoplastic *in vivo*[3-5]. According to a traditional Chinese medicine book, consuming fresh meat can maintain and improve as a cure for liver cirrhosis[6].

This research is essential because the wealth of marine biological resources and traditional knowledge that has been carried out empirically are abundant. However, exploration and exploitation are still minimal, even though marine natural resources have been proven to source various active ingredients with great potential as medicine[7]. Based on the literature

review, non-polar and polar active ingredients have promising pharmacological activities such as antioxidant activity[8]. In addition, reports of antibacterial activity from *O. typhae* have not been exploited much.

A wound is a form of tissue damage to the skin caused by physical contact (with a heat source), medical action, or changes in physiological conditions. The body naturally heals through sustainable bio-cellular and biochemical activities. The wound healing process is divided into five stages, including the stages of homeostasis, inflammation, migration, proliferation, and maturation[9-11]. Long-healed wounds are characterized by wounds that do not heal after 12 weeks. This condition is referred to as a chronic wound caused by infection. Infection can occur when bacteria get into an open wound. When an injury becomes infected, the body does more to fight off the infection than heal the wound. This condition can hinder wound healing[12,13]. *Staphylococcus aureus* and *Escherichia coli* are the bacteria that most often infect wounds. *Staphylococcus aureus* and *E. coli* are clinically relevant pathogens due to antibiotic resistance[14,15]. They are non-motile, non-sporing, facultatively anaerobic, catalase-positive, and oxidase-negative[16]. This study was conducted to explore the potential of *O. typhae*, commonly found on the coast of West Kalimantan, as an antibacterial. In addition, this study was also carried out to characterize the added value of *O. typhae* commodity.

MATERIALS AND METHODS

Materials

This study material used *O. typhae* with a length ranging from 4-6 cm in fresh conditions collected from the coast of Sambas, West Kalimantan (**Figure 1**). The sample was determined with specimen No. 023/A/LB/F.MIPA/UNTAN/2021 at the Biology laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. An antibacterial assay was performed using *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922), for the antifungal assay was performed using *Candida albicans* (ATCC 102310). Other materials were 1% DMSO, NaCl, 0.5 McFarland standard, sterile distilled water, Brain Heart Infusion (BHI) media, phosphate buffer saline (PBS) solution, and 1% crystals violet. The equipment used at the sample preparation stage was glassware for extraction, sieves, rotary evaporator, chopper, grinder, scales, refrigerator, vortex, and oven. The instrument used in the antibacterial assay was Laminar Air Flow (LAF), incubator (Moderna), micropipette (Socorex), multichannel micropipette (Socorex), microplate flat-bottom polystyrene 96 well (Iwaki), microtiter plate reader (Optic Ivymen System 2100-C, Spain), spectrophotometer UV Genesys 10 UV Scanning, 335903 (Thermo Scientific Spectronic, US), autoclave, and analytical balance (AB204-5, Switzerland).

Figure 1. *Onchidium typhae*

Methods

This research has passed the ethical clearance with No.700/UN22.9/PG/2022. It is critical to ensure that the research has complied with the principles of respect for the person, benefit and non-maleficence, and the principle of justice.

Extraction of Onchidium typhae

Extraction of active ingredients from *O. typhae* used was a multi-level extraction based on Quinn's method and was modified[17]. Modifications were made to the maceration time, 3 x 24 hours. The solvents used were chloroform (non-polar), ethyl acetate (semi-polar), and methanol (polar). Fifty grams of dry *O. typhae* powder and 100 mL of chloroform solvent were added until submerged, macerated at room temperature for 3x24 hours, then filtered (filtrate 1). The residue was added with ethyl acetate until submerged and macerated for 3 x 24 hours at room temperature, then filtered (filtrate 2). The remaining residue was added with methanol until submerged and macerated for 3 x 24 hours at room temperature, then filtered (filtrate 3). The filtrate 1, 2, and 3 were evaporated to obtain a crude extract. From the crude extract and then tested to identify *O. typhae* bioactive compounds. *Onchidium typhae* bioactive compounds were identified on alkaloids, steroids, saponins, carbohydrates, reducing sugars, peptide compounds, and free amino acids.

Proximate analysis of Onchidium typhae

A perfect *O. typhae* powder was obtained from the preparation under control, starting from fresh samples, cleaning from mud to the boiling process with constant stirring to ensure that the mucus was wholly removed. The drying of *O. typhae* samples was carried out at a temperature of 60°C for three days. The method provided a moisture content of <5%. Proximate analysis of *O. typhae* was carried out on powder samples. Proximate analysis was carried out using the Association of Official Analytical Chemists (AOAC) methods[18] in the Center Food and Nutrition Study laboratory, Universitas Gadjah Mada.

Bacterial strains

Staphylococcus aureus, *E. coli*, and *C. albicans* were grown within 24 hours at 37°C in BHI media. The optical density (OD) 600 of the microbial culture was adjusted to 0.1 (equivalent to the 0.5 McFarland standard - 1.5 x 10^8 CFU/mL) and then diluted in a new growth medium to 0.01 OD600[16].

Antibacterial and antifungal assay

The antibacterial and antifungal assay was carried out using the microdilution method. The assay was carried out with test compounds: 1; 0.5; 0.25; and 0.125% w/v on microplate 96 wells. The control for the antibacterial assay used was chloramphenicol 1% w/v, while the antifungal assay used fluconazole 1% w/v. The microplate was then incubated for 24 hours at 37°C — the percent inhibition was determined by observing the clarity of the solution[19,20]. Microplate absorbance reading process using a microplate reader at a wavelength of 595 nm[16,19].

RESULTS AND DISCUSSION

The sample preparation results obtained good *O. typhae* powder with less than 5% moisture content. *Onchidium typhae* powder was followed by proximate assay and extraction. The modified Quinn's method was chosen in the extraction process because it effectively extracts active compounds with different polarity levels. The crude extract obtained using chloroform, ethyl acetate, and methanol solvents were 8.7%, 0.25%, and 10%, respectively. Each extract obtained was then subjected to phytochemical screening. Phytochemical screening results can be seen in **Table I.**

Table I. Phytochemical screening of *Onchidium typhae*

Compound class test	Reagent	Result		
		Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	Wagner	+	-	+
	Mayer	-	-	-
	Dragendorff	+	-	+
Steroids & triterpenoids	Liebermann-Burchard	+	-	-
Saponins	Distilled water	+	-	-
Tannins	$FeCl_3$	-	-	-
Flavonoids	Mg and Cl ribbon	-	-	-
Reduction sugar	Benedict	-	-	-
Free amino acids	Ninhydrin	+	+	+

The test results showed that the chloroform extract contained alkaloids, steroids, saponins, and free amino acids, as shown in **Table I**. However, different results were shown in the methanol extract. It was shown that methanol extract does not contain saponins, steroids, and triterpenoids. These findings are in line with previous studies on the Onchidiidae family where polypropionate and its derivatives were found, amides and depsipeptides, terpenoids, and other types of compounds have been isolated from the dominant chemical constituents in the genus of Onchidium[3,4,6]. Previous research on the species *Onchidium sp*., a polypropionate compound with a pyrone ring main skeleton and several asymmetric centers like ilikonapyrone (**1**), is responsible for its biological activity. Ilikonapyrone is the first polypropionate secondary metabolite of this genus, followed by five ilikonapyrone-based derivatives (**2-6**), which were also isolated from the mixture of esters (**Figure 2**)[1,21].

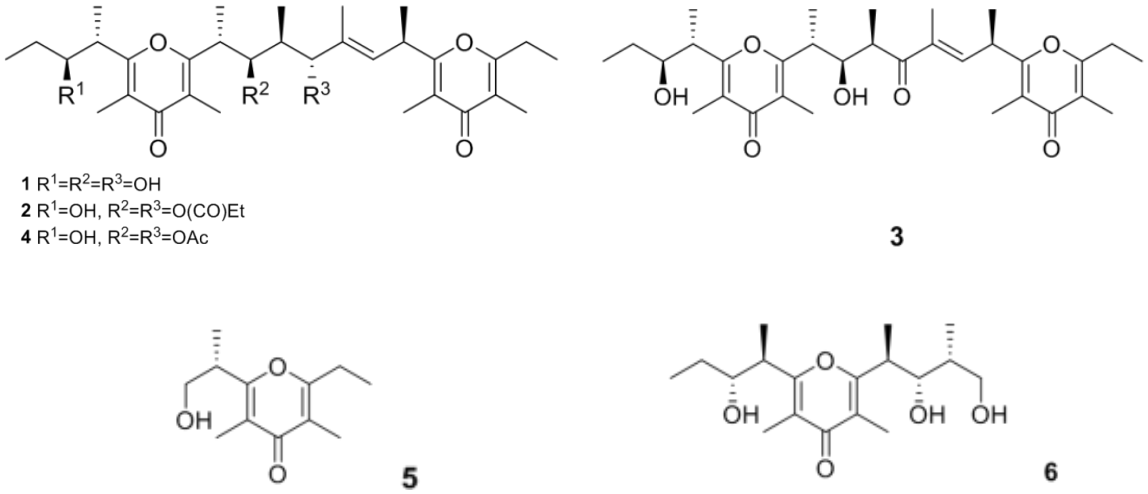

Figure 2. The structures polypropionate and its derivatives compounds[6]

Proximate analysis was carried out on *O. typhae* powder. Proximate analysis is carried out to determine the food's nutritional content, such as protein, carbohydrates, fat, and fiber. The results of the proximate analysis showed as shown in **Table II**. *Onchidium typhae* were rich in protein (67.88%) but a low number in fat (3.17%). The water, ash, carbohydrate, and crude fiber percentages were 4.37, 7.76, 14.55, and 0.65, respectively. Some literature explains that the high protein nutrient in food can be a supportive therapy in treating wounds, even postoperative wounds[22-24]. In other literature, steroid content such as squalene which is widely contained in Mollusca is known to have antioxidant activity[25].

Table II. Proximate analysis of *Onchidium typhae*

Test	Rate (%)
Water	4.37
Ash	7.76
Fat	3.17
Protein	67.88
Carbohydrate	14,55
Crude Fiber	0,65

Staphylococcus aureus, *E. coli*, and *C. albicans* were grown within 24 hours at 37°C in BHI media. Inhibition activity was carried out by observing the clarity of the solution—absorbance readings using a microplate reader at a wavelength of 595 nm. The antibacterial and antifungal activity assay results on 1% methanol, chloroform, and ethyl acetate extracts showed inhibitory activity against *S. aureus*, *E. coli*, and *C. albicans*. The most significant inhibition value was shown by 1% chloroform extract, where the inhibition value against *S. aureus*, *E. coli*, and *C. albicans* was 82±0.01%; 85.8±0.01%; 85±0.01%, respectively. The antibacterial and antifungal activity assay results are shown in **Table III**.

Table III. Antibacterial and antifungal result of *Onchidium typhae*

Crude extract	Percentage inhibitory (%)		
	S. aureus	*E. coli*	*C. albicans*
Chloroform 1%	82	85.5	84.9
Ethyl acetate 1%	77	85	86
Methanol 1%	73	67	74
Chloramphenicol 1%	85.2	86	-
Fluconazole 1%	-	-	85.3

Note: (-) the assay was not carried out

Recently, a new compound from the polyketides group known as penisclerotiorin A and penidepsidone A known to be responsible for antimicrobial activity (**Figure 3**)[26]. Onchidal isolated from another Onchidiid genus has shown the inhibition against *S. aureus*. The minimum inhibitory concentration was between 0.21 and 0.63 µg/mL, implying that *O. typhae* were as potent as antibacterial. These findings are in line with this study[27,28].

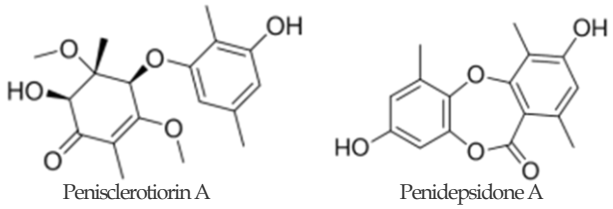

Figure 3. Structure of polyketides grup as antibacterial

CONCLUSION

Onchidium typhae extract has antibacterial and antifungal activity, especially in 1% chloroform extract against *S. aureus*, *E. coli*, and *C. albicans* with microdilution test.

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AUTHORS' CONTRIBUTION

Bambang Wijianto: research team leader and coordinator, validation, and article writing. **Hasyrul Hamzah**: antibacterial and antifungal assay, and article writing. **Annisa Larasati Nurhidayah**: sampling and phytochemical screening testing. **Guci Intan Kemuning**: extracting and determination of *O. typhae*. **Riyadh Aqilsya Amaryl Dyas**: extracting of *O. typhae*.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The author declares there is no conflict of interest and equivalent.

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Research Article

Peel-off Kefir Mask Arachi (*Arachis hypogaea* L.): Characterization and Antioxidant Activity

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Keywords:
Antioxidant activity
DPPH
Kefir
Peanut kefir
Peel-off mask

Abstract

This study aims to determine the best formulation for the peel-off mask Arachi or peanut (*Arachis hypogaea* L). *Arachis hypogaea* kefir as an active ingredient is added with variations in the concentration of F1 (0.5%) and F2 (2%) (w/v). Organoleptic tests, homogeneity, dry time, and pH were carried out on the peel-off mask that had been made. Antioxidant test (DPPH methods) was performed on masks F1 and F2. The results showed that the peel-off mask of *A. hypogaea* kefir had the best antioxidant activity at a concentration of 2% (F2) kefir with an IC_{50} value of 1.865 ppm and was very active. The characteristics of the peel-off mask have good physical stability, proven by not experiencing a change in color, odor, being homogeneous, having good dispersion power, and having a dry time ranging from 10-23 minutes. The pH value of the peel-off mask preparation is 4.52, and it is appropriate with SNI and the pH balance of normal human skin. The peel-off mask of *A. hypogaea* kefir can be produced because it has good physical stability and antioxidant activity.

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INTRODUCTION

Kefir fermented milk is produced from grains, a specific and complex mixture of bacteria and yeast. Kefir produces a sour taste from lactic and acetic acids[1,2]. It also contains CO_2, ethyl alcohol, and aromatic compounds that make it is unique organoleptic[3]. Kefir can be made from nuts milk, and it does not contain cholesterol[4].

Arachi or peanut (*Arachis hypogaea* L.) is one of the raw materials that can be processed into kefir milk[5]. *Arachis hypogaea* juice is proven to have higher nutrition than red bean and soybean extract and is a source of vitamin E and magnesium[6]. *Arachis hypogaea* were evaluated for total phenolic and flavonoid contents, antioxidants, and vitamins essential for optimum health[7]. *Arachis hypogaea* contain Vitamin E, known as α-tocopherol. Vitamin E has been in use for more than 50 years in dermatology and is an essential ingredient in many cosmetic products[8]. They are associated with obstructing the formation of free radicals by preventing oxidation and are believed to prevent damage to collagen and elastin fibers, increase skin cell regeneration, treat acne, and reduce the risk of decreased skin firmness and wrinkles[9].

The cosmetic preparation chosen in this study is in the form of a peel-off mask. This form was chosen because the peel-off mask is a popular facial treatment, does not cause dependence, and is easy to apply[10]. The peel-off masks can minimize pores and is valuable for recovering and treating facial skin[11]. Peel-off masks are usually made from polyvinyl alcohol (PVA) or hydroxypropyl methylcellulose (HPMC), developed in a hot aquadest of 80°C. This material has adhesive properties to form a film membrane that can be peeled off when dry[12].

The research on peanut kefir has been carried out, in which *A. hypogaea* kefir with a concentration starter lactic acid bacteria of 2% (w/v) with the fermentation of 48 hours was the best composition reported[13]. However, antioxidant activity and the

development of kefir in cosmetic product has not been carried out. This research will continue making formulation peel-off kefir mask with 2% yeast (w/v) and fermentation for 48 hours to have good physical stability and antioxidant activity.

MATERIALS AND METHODS

Materials

Arachis hypogaea was obtained and determined in Badan Penelitian dan Pengembangan Daerah Kebun Raya Banua (050/492-LIT/KRB) and the yeast "Fermipan" used in the study were obtained from the Banjarbaru market, South Kalimantan. Meanwhile, D-glucose was purchased in Prida Lab (Central Jakarta) and De Man, Rogosa and Sharpe (MRS) agar Merck was purchased in Nitrakimia (Yogyakarta). The antioxidant activity test was performed with UV-Vis Spectrophotometry (Genesys 10 UV-Vis).

Methods

Material preparation

Arachis hypogaea were peeled and washed with clean water. *Arachis hypogaea* were grounded using a blender with a ratio of water : beans (8 L : 1 kg of beans). The resulting *A. hypogaea* slurry was filtered and given the *A. hypogaea* filtrate. A total of 300 mL of *A. hypogaea* milk in Erlenmeyer was added with 12 g of D-glucose (4% w/v) and pasteurized at 80°C for 15 minutes. Then, 300 mL of *A. hypogaea* milk was added with 6 g of yeast (2% w/v) and fermented for 48 hours.

Preparation of peel-off mask

Nipagin was dissolved into CO_2-free water with a ratio of 1 : 30 at 80°C while stirring continuously with a magnetic stirrer. The nipagin solution was removed from the water bath and mixed with glycerin while continuing to stir. The HPMC powder was dissolved in CO_2-free water with a ratio of 1 : 15 at a temperature of 80°C, then left to stand until the HPMC expanded utterly. The two mixtures were stirred until homogeneous and added with the *A. hypogaea* extract kefir, then added with CO2-free aquadest until it reached 100% of the total weight. The formulations used are presented in **Table I**.

Table I. Peel-off mask formulation with *A. hypogaea* kefir

Materials (%w/w)	Function	Composition (%)	
		F1	F2
Arachis hypogaea Kefir	Active agent	0.5	2
HPMC	Gelling Agent	4	4
Glycerin	Humectant	12	12
Nipagin	Preservatives	0.2	0.2
CO_2-free water	Solvent	ad 100	ad 100

Physical evaluation of peel-off mask

Physical evaluation includes organoleptic test, homogeneity, spreadability test, and pH with the procedure as reported by Priani *et al*[14].

Antioxidant activity of peel-off mask

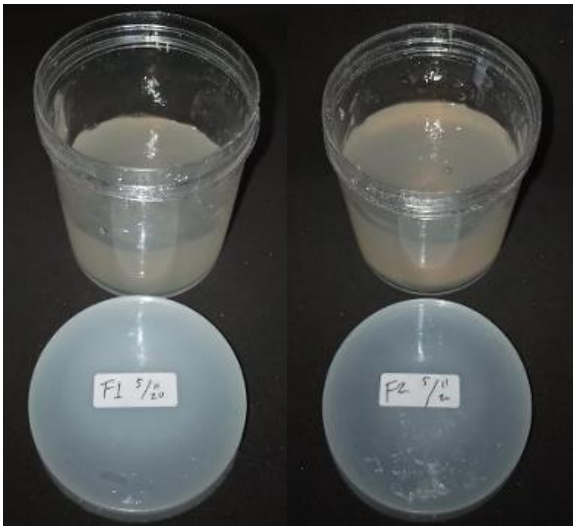
Antioxidant activity was tested using the DPPH method. *Arachis hypogaea* kefir was diluted and made at a concentration of 1000 ppm. Peel-off mask preparation with concentrations 0.5% and 2% each dissolved in 25 mL of ethanol. A series of solutions invariant peel–off mask concentrations were prepared (10, 15, 20, 25, and 30 ppm) until 10 mL volumetric flask. Furthermore, 1 mL of DPPH 0.4 mM was added to the solutions and incubated at room temperature and avoid light. The absorbance was measured at a maximum wavelength to calculate the inhibition percentage using the equation [**1**]. A_0 was the absorbance of the blank solution, and A_1 was the absorbance of the sample solution. The inhibition concentration of 50% (IC_{50}) was determined using the linear equation $y = bx + a$.

$$Inhibition\ percentage\ (\%) = \frac{A0-A1}{A0} \times 1 \qquad \ldots [1]$$

RESULTS AND DISCUSSION

Physical and chemical evaluation of A. hypogaea kefir and peel-off mask

Arachis hypogaea kefir was optimized with the addition of 2% yeast within 48 hours of fermentation and got pH of the kefir was 3.646. After the kefir was formed, the peel-off mask was formulated into two variance concentrations of *A. hypogaea* kefir as an active ingredient. Varian of kefir concentrations in the formula were 0.5% (F1), and 2% (F2) w/w. The various kefir concentrations were aimed to get the best formulation of mask and activity antioxidant. The physical evaluation of the peel-off mask was organoleptic, pH, drying time, and gel spread[15]. The peel-off masks in the two formulas were transparent yellow tended to be precise. For the two formulas, the distinctive aroma of *A. hypogaea* was produced. When applied to the skin layer, the gel was easily distributed and did not feel hot. Both F1 and F2 formulas had a good consistency. The physical appearance of the peel-off mask can be seen in **Figure 1.**



a **b**

Figure 1. Peel-off mask with (**a**) 0.5% and (**b**) 2% *A. hypogaea* kefir concentration

The organoleptic test was aimed to see the physical appearance by observing the color, smell, consistency, and homogeneity. The results of organoleptic observations can be seen in **Table II**. The homogeneity test was conducted to determine that the resulting peel-off mask preparation did not experience clumping when the active substance mixed with the base. Peel-off kefir mask, when applied to the skin layer, was homogeneous. There were no fibers and lumps or color differences.

Table II. Organoleptic observations of peel-off mask formulation with *A. hypogaea* kefir

No	Formula	Color	Odor	Consistency	Homogeneity
1.	F1	Clear	Characteristic *A. hypogaea* odor	Gel	Homogeneous
2.	F2	Clear	Characteristic *A. hypogaea* odor	Gel	Homogeneous

The spreadability test determines the ability to spread the gel on the skin layer. The peel-off mask is good if, when applied to the skin, the dispersibility of the gel has a standard diameter of 5-7 cm, and it can spread evenly that the effect can work optimally[16,17]. The peel-off mask spreadability test obtained for both formulas was 5.33 and 5.45 cm.

The dry time test in a peel-off mask preparation aims to determine the speed at which the mask forms a film when applied to the skin. The dry time of both peel-off mask formulas was 25 minutes. The drying time requirement for peel-off mask preparation is 15-30 minutes[18]. If the film forms faster, so active substances will be released so that consumers can immediately benefit from using these masks. The peel-off masks' pH was around 4.5-7.0, and it was appropriate with *Standar Nasional Indonesia* (SNI) and the pH balance of normal human skin[19]. The physical and chemical properties of the peel-off mask can be seen in **Table III**.

Table III. Physical and chemical properties of peel-off mask formulation with *A. hypogaea* kefir

Properties	F1	F2
Homogeneity	Homogenous	homogenous
Spreadability (cm)	5.33±0.061	5.45±0.133
Dry time (minutes)	25	25
pH	6.54±0.021	6.08±0.015

Antioxidant activity test of peel-off mask

The antioxidant activity test for the peel-off mask of *A. hypogaea* kefir was performed using the DPPH radical scavenging method at λ 519 nm. The proton radical scavenging activity with DPPH is quite reproducible and relatively simple[20]. The absorbance of the peel-off mask was linear with concentration according to the Lambert-Beer law (**Table IV**). The absorbance value was then used to calculate the % inhibition based on equation [**1**], and the results were presented in **Table V**. The IC_{50} value was then calculated based on a linear regression between concentration vs. %inhibition. The result shows that the peel-off mask F2 had better antioxidant activity than F1 with an IC_{50} value of 1.865 ppm and is included as a very strong antioxidant[21]. Meanwhile, F1 had an IC_{50} value of 6.950 ppm. This result shows that the concentration of *A. hypogaea* kefir added to the peel-off mask affects the antioxidant activity of the mask, with a higher concentration increasing its antioxidant activity. This result is in line with a study on *A. hypogaea* oil as an antiaging, in which 10% *A. hypogaea* oil has an antiaging effect. Another study reported that the antioxidant activity of 10% *A. hypogaea* shells was obtained IC_{50} of 380.18 µg/mL.

Table IV. Absorbance vs concentration of peel-off mask formulation with *A. hypogaea* kefir

Concentration (ppm)	Absorbance	
	F1	F2
10	0.650	0.721
15	0.737	0.716
20	0.938	0.742
25	1.002	0.835
30	1.031	0.912

Table V. %inhibition and IC_{50} of peel-off mask formulation with *A. hypogaea* kefir

Concentration (ppm)	% inhibition (%)	
	F1	F2
10	50.943	45.585
15	44.377	45.962
20	29.208	44
25	24.377	36.981
30	22.189	31.170
IC_{50} (ppm)	6.950	1.865

CONCLUSION

Based on the physical properties of the peel-off *A. hypogaea* kefir mask, including the organoleptic test, spreadability, dry time, and pH test, it shows that the F1 and F2 formulas had good physical properties of peel-off masks. The best antioxidant activity was obtained in F2 with the addition of 2% *A. hypogaea* kefir, indicating an IC_{50} value of 1.865 ppm.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The author declares there is no conflict of interest.

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Research Article

Cytotoxic Effect of the Paku Atai Merah (*Angiopteris ferox* Copel) Fraction on MCF-7 and HeLa Cells and its Compound Profile by GC-MS

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Abstract

Cancer is a condition of abnormal cell proliferation of tissue cells in the body that becomes malignant. It can attack other parts of the body and affect the normal function of the body organs. The sample used in this study was tubers of paku atai merah (*Angiopteris ferox* Copel), then extracted using 96% ethanol eluent to obtain a thick extract. The ethanolic extract of *A. ferox* was fractionated using column chromatography to get the active fraction to characterize the compound using thin-layer chromatography and gas chromatography-mass spectroscopy (GC-MS) and tested its cytotoxic effectiveness on MCF-7 and HeLa cancer cells. The results of this study were obtained from fractionation using the column chromatography method to get sub-fraction C and the results of compound characterization using GC-MS and obtained variations in the class of compounds contained in the sample: amino acids, glucosinolates, alkaloids, flavonoids, and terpenoids. Based on the cytotoxic effect test of sub-fraction C on MCF-7 cells, the results obtained moderate cytotoxic effects with an IC_{50} value of 61.027 µg/mL, and HeLa cells had an IC_{50} value of 521.03 µg/mL, which was categorized as having a weak cytotoxic effect. Based on the results obtained from this study, it can be concluded that sub-fraction C of *A. ferox* tubers has a cytotoxic effect on MCF-7 cells to be used as a reference for tracing pure compounds from *A. ferox* tuber.

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INTRODUCTION

Cancer is a condition of abnormal cell proliferation of tissue cells in the body that becomes malignant. It can attack other parts of the body and affect the normal function of body organs[1-3]. Cancer is a severe problem. As many as 8.2 million cases of death are caused by cancer. Breast cancer is the first most common sufferer in the Asian region, with 23% of breast cancer[2]. Breast cancer can be characterized by a disruption in the proliferation of abnormal mammary cells that turn into malignant cells through various pathways of cell mutagenesis. One of the mechanisms of breast cancer is signal transduction of estrogen receptors (ERα and ERβ) which is a factor in activating or suppressing the expression of target genes on ligand

binding[4,5]. The ERα has a significant role of about 75% in the pathogenesis of breast cancer by promoting the growth of breast tumor cells. The ERα reacted with cyclin D1, which can activate cyclin-dependent kinases (CDKs) to change the transition of cells from the G1 phase to the S phase into cancer cells[5,6].

Various technological and scientific developments for cancer treatment have been carried out, starting from surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, stem cell transplantation, and radiation therapy[7,8]. However, some of these therapies have various side effects: hair loss, decreased white blood cells, and decreased immune quality. The high cost of cancer treatment is not proportional to the success rate of therapy in cancer[7,9,10]. Therefore, to minimize excessive side effects on cancer treatment, several natural ingredients have been developed by looking at the cytotoxic effects of secondary metabolite compounds in plants that function as adjuvant anticancer therapy that have proliferative pro-apoptotic properties[11-14].

One of the plants with anticancer activity is paku atai merah or *Angiopteris ferox* Copel from the Marratiaceae family. The community has widely used *A. ferox*, especially in the Dayak area, Kalimantan, as a medicinal plant to treat various diseases. It is because *A. ferox* tubers contain a variety of compounds as reported in several studies by Nur *et al*[7,15,16]. Based on the results of phytochemical screening, the ethanolic extract of *A. ferox* tubers contains compounds such as flavonoids, tannins, saponins, steroids, terpenoids, phenolics, and angiopterosides. The various compounds in the *A. ferox* tubers also have antioxidant activity in reducing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals with a strong category and iron ions with a potent category. It also has strong categories for antioxidant activity using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitric oxide (NO), and lipid peroxidase methods[4]. Extracts and fractions of *A. ferox* tubers have also been reported to have anticancer activity on breast cancer cells (MCF-7 and T47D), colon cancer cells (WiDr), and epithelioid cancer cells (HeLa), indicating that the ethyl fraction acetate has an effect on each cancer cell with a toxic category[2,7]. Based on the activity background as an anticancer from *A. ferox* tubers, this study focuses on tracing the active compounds with anticancer activity by isolating the active compounds from *A. ferox* tubers and then characterizing the compounds using gas chromatography-mass spectroscopy (GC-MS) and testing the cytotoxic effect on MCF-7 and HeLa cells.

MATERIALS AND METHODS

Materials

The materials used were ethanol 70% (OneMed, Indonesia), ethanol 96% (JT-Baker), silica gel 60 GF 254 (Merck, Germany), thin-layer chromatography plate (TLC, Merck, Germany), acetonitrile (JT-Baker), methanol (Merck, Germany), ethyl acetate (Merck, Germany), $FeCl_3$ (Sigma Aldrich, Germany), H_2SO_4 (Merck, Germany), phosphate-buffered saline (PBS, Gibco), penicillin-streptomycin (Gibco), sodium dodecyl sulfate (SDS, Merck, Germany), trypsin EDTA 0.25% (Gibco), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and *A. ferox* Copel tuber simplicia obtained from West Kutai, East Kalimantan, Indonesia, and has been identified at the Anatomy and Science Laboratory of Universitas Mulawarman, Samarinda, Indonesia.

Methods

Sample preparation

The samples of *A. ferox* tubers collected were sorted by wet sorting, then washed under water to remove impurities still attached to the samples of *A. ferox* after the wet sorting was carried out. Then, the sample was chopped and dried by placing it in a simplicia oven at 40-60°C. Furthermore, after drying, the sample was done dry sorting and then pollinated for the extraction process.

Extraction

The extraction procedure was adopted from our previous research[2] under the same conditions. Dry simplicia as much as 1.5 kg was made into coarse powder by pounding. The coarse powder obtained was reduced in size by blending so that a

slightly coarse powder was obtained, as much as 1.2 kg. In general, simplicia powder with a larger surface area will improve the filtration because the surface of the simplicia powder in contact with the liquid filter is wider and breaks down the cell wall so that the filtered liquid can enter the cell. Simplicia powder as much as 1.2 kg was put into a tightly closed container and soaked with 96% ethanol. The simplicia was then allowed to stand for 24 x 3 hours, stirring occasionally for the first six hours, then allowed to stand for 24 x 3 hours. The filtrate was taken, the residue was re-macerated with 96%w/w ethanol. The filtrate was collected and evaporated. The viscous extract obtained was weighed, and the yield was calculated.

Thin-layer chromatography

The separation was carried out by TLC of the extract obtained to determine the eluent used in column chromatography. The extract was dissolved with the initial solvent and then spotted in the TLC and eluted with the appropriate eluent, after which it was put in a bucket and allowed to elude to the elution limit. The orientation of the eluent was carried out before separation by TLC using the ratio of methanol : ethyl acetate (9 : 1) and (8 : 2), and then one eluent was selected, which produced an excellent stain appearance with the ratio (8 : 2). Observations of the appearance of the stains were performed under UV lamps at λ of 254 and 366 nm[17].

Column chromatography

A set of column chromatography tools was prepared, then silica gel was inserted wet into the column tube. A total of 10 g of the extract was mixed using silica powder to obtain a dry powder extract. The mixture was then put into a column that already contained silica gel 60 and eluted using an eluent from non-polar to polar (*n*-hexane, ethyl acetate, ethanol with gradient concentration), starting from 100 mL *n*-hexane eluent, then further elution using ethyl acetate, and ethanol 96% with gradient concentration. The results of the obtained fractions were accommodated in a glass container. The incorporation of the fractions was carried out based on the color appearance of the solution and the stains on the TLC plate. Based on the similarity of the TLC profile, the combined fraction was then TLC to observe the spots at UV 254 nm and 366 nm. Eight fractions were obtained in the fractionation I process. The fractions were grouped according to their color and TLC profile. Fraction III (3.092 g) was then separated by column chromatography (polyamide, 60 cm x 5 cm column) using an eluent ratio of methanol : ethyl acetate (80 : 20 and 20 : 80) to obtain a sub-fraction of 7 (A-G). Sub-fraction C was characterized by compound profiles using GC-MS.

Fraction characterization

The characterization of the isolated fraction was carried out using a GC-MS to obtain the profile of the components in the fraction.

Cytotoxic assay of MCF-7 and HeLa cells

The active isolate fraction obtained was then subjected to cytotoxic testing to see the toxic effect of sub-fraction C on MCF-7 and HeLa cells using the MTT assay method following the test procedure from our previous research[4] with a slightly modified on serial concentration of sample test. The absorbance measurement of the sample using a microplate reader at a wavelength of 595 nm and the absorbance data obtained were then analyzed by looking at the percentage of cell viability and determining the IC_{50} value.

RESULTS AND DISCUSSION

Column chromatography

In this study, the sample used was *A. ferox* and then extracted using the maceration method. The maceration method was chosen because the extraction process is simple and avoids compound damage[18]. The extraction process using the maceration method uses 96% ethanol solvent to dissolve both non-polar and polar compounds so that the extraction process occurs entirely. Besides that, it avoids compound damage due to the growth of microorganisms during the process of making thick extracts of *A. ferox* tubers. The ethanol extract obtained was then fractionated by a silica chromatographic

column eluted using several solvents based on a concentration gradient. The results of column chromatography show that from the results of column chromatography, 42 fractions were obtained. The obtained fractions were combined based on the TLC color and stain profile eluted using methanol : ethyl acetate (8 : 2) in 10 mL. The merger results obtained eight fractions given each code (**Figure 1**). Fractions III were column chromatographed again with methanol : ethyl acetate (80 : 20 and 20 : 80) in 100 mL. The chromatography results obtained 35 fractions, which were then combined based on spot color and stain profile using an eluent ratio of methanol : acetone (8 : 2) in 10 mL to obtain seven fractions from the combined results. Sub-fraction C (7-11) was characterized using GC-MS to determine the profile of the compounds contained in the sub-fraction. Sub-fraction C was chosen for further characterization because the resulting spot pattern showed the presence of phenolic compounds after being sprayed using the $FeCl_3$ reagent, which formed a blue spot (**Figure 2A**).

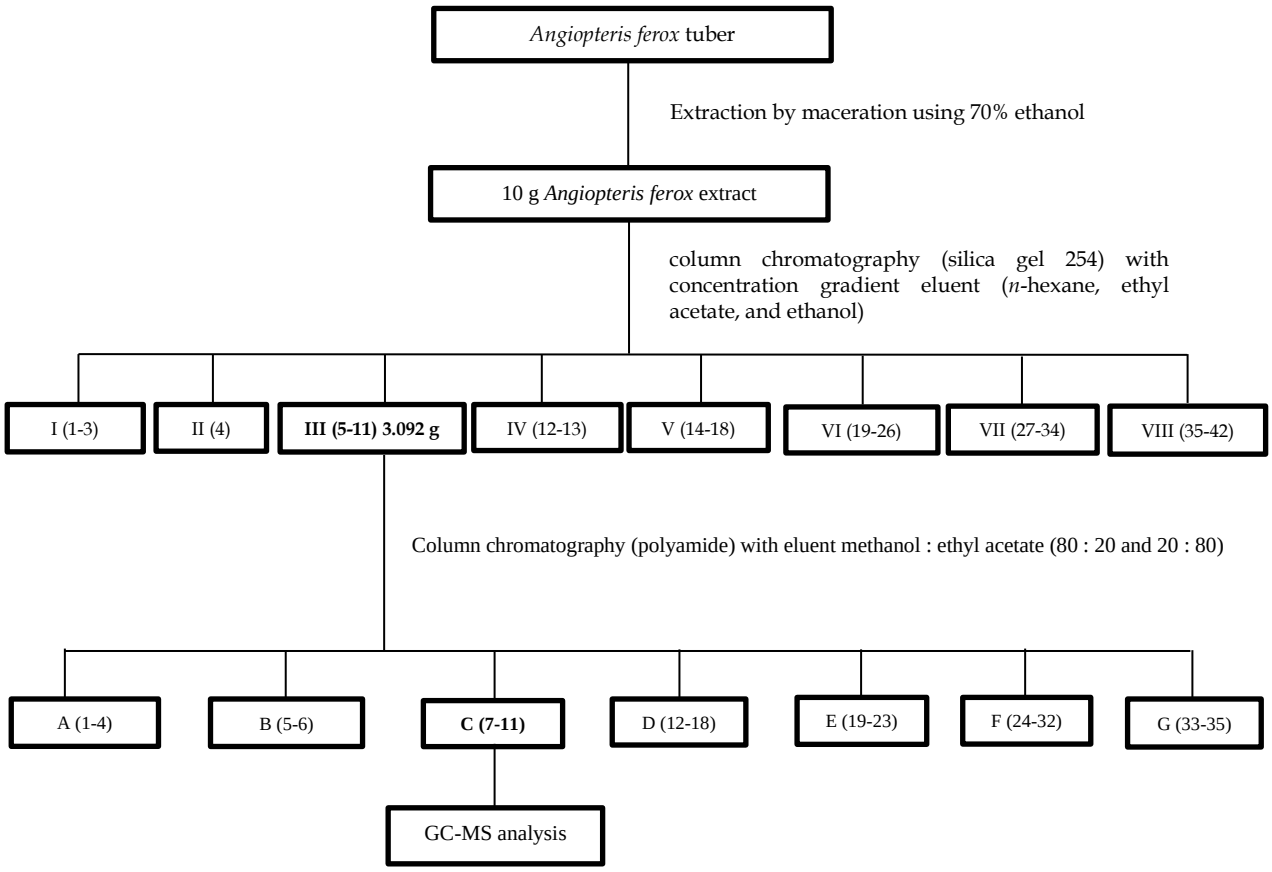
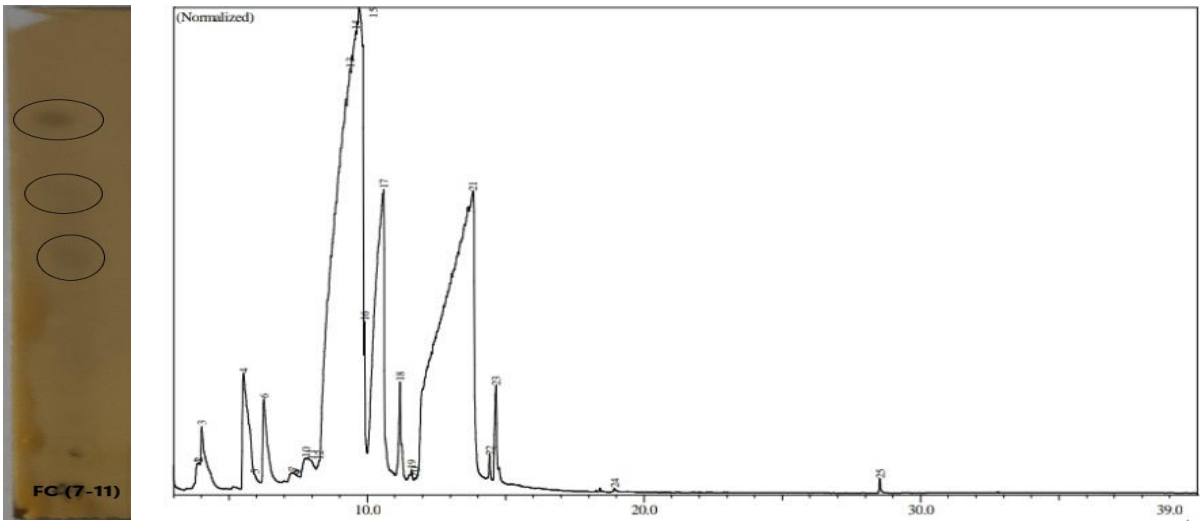


Figure 1. The process of compound fractions from the ethanol extract of *A. ferox*

Compounds characterizations

Compound characterization of sub-fraction C was carried out by looking at the profiles of the compounds found from sub-fraction using the GC-MS. The GC-MS data fragmentation (m/z) was processed using the *ReSpect for phytochemical* (http://spectra.psc.riken.jp/menta.cgi/respect/search/fragment) to see fragments that indicate the intensity of secondary metabolites contained in the isolates of *A. ferox* tubers (**Figure 2B**). Based on the GC–MS data obtained from identifying the *A. ferox* tuber isolates, it was shown that the sub-fraction C tested contained secondary metabolites, such as alkaloids, flavonoids, and terpenoids (**Table I**). Alkaloid compounds in the chromatograms obtained were indicated by peak numbers 4, 10, 14, 17, 18 and 19 in fragments 41, 42, 55, 57, 58, 68, 69, 73, 82, 84, 96, 97, 98, 101, 110, 113, 114, 129, 131 and 146. At the same time, the flavonoid compounds were shown by peaks 5, 6, 11, 22, 24 and 25 in fragments 41, 42, 57, 69, 70, 71, 73, 81, 84, 85 97, 103, 111, 129, 167, 199, 213, 256, 279 and 390. While the terpenoid compounds were shown by peaks 8, 12, 13, and 15 in fragments 55, 69, 97, and 115. The data show terpenoid compounds at peak 12-15, alkaloids at peak 17-19, and flavonoids at peak 22-25.



a **b**

Figure 2. Blue spot profile in TLC after spraying $FeCl_3$ reagent (**a**) and chromatogram of the sub-fraction C using the GC-MS (**b**)

Table I. Results of identification of compound groups using the GC-MS

No	Compounds detected	T_R (minute)	*m/z* prediction by ReSpect for phytochemicals
1	Amino acid	3.867	14 25 27 30 39 4453 55 58 84 86
2	Amino acid	3.908	14 25 27 30 39 4453 55 58 84 86
3	Glucosinolate	4.012	26 41 50 55 5766 69 7282 96 98
4	Alkaloids	5.529	18 26 31 43 54 55 61 68 82 108 110
5	Flavonoids	5.933	38 39 43 50 54 55 62 67 71 82 83 95 110 111
6	Flavanoids	6.264	28 39 41 54 68 82 110 111
7	Amino acid	7.302	39 43 55 57 85 86 128
8	Terpenoids	7.408	15 18 29 31 38 43 4655 61 69 85
9	ND	7.475	27 29 39 43 50 57 85 128
10	Alkaloids	7.790	29 41 45 54 56 63 69 73 84 85 95 98 113 131
11	Flavonoids	7.975	15 29 41 43 56 58 69 81 85 103 129 143 157
12	Terpenoids	8.258	29 39 45 55 58 69 86 87 97 115
13	Terpenoids	9.381	29 39 45 55 58 69 86 87 97 115
14	ND	9.587	27 41 42 51 58 6070 82 86 98 129
15	Terpenoids	9.702	29 39 45 55 58 69 86 8797 115
16	ND	9.905	39 45 57 85
17	Alkaloids	10.596	15 27 39 50 55 68 84 96 114
18	Alkaloids	11.183	15 29 41 44 57 69 74 86 97 146
19	Alkaloids	11.603	18 29 41 53 59 61 69 73 84 85 101 114
20	ND	11.725	28 30 41 44 56 66 78 84 88 99 115 143
21	ND	13.832	47 55 60 71 74 84 89100 118 160
22	Flavanoids	14.425	15 27 29 42 57 60 70 73 85 97
23	ND	14.652	29 33 42 4656 60 63 74 75 102 105 132
24	Flavonoids	18.939	29 43 57 73 85 98 111 129 143 157 171 185 199 213 227 239 256
25	Flavanoids	28.533	27 41 57 71 84 104 113 132 149 167 168 261 279 280 390

ND: compound not determined

Cytotoxic assay

Evaluation of the cytotoxic effects of the A. ferox tuber fraction using the MTT assay method on MCF-7 and HeLa cells was performed to evaluate the potential of the A. ferox tuber fraction in inhibiting cell proliferation with percent cell viability and toxic effect based on IC_{50} value. The IC_{50} value is a concentration value required for a sample to give a toxic effect of 50% on cells categorized as strong cytotoxic effect <50 µg/mL, moderate cytotoxic effect 50-200 µg/mL, weak cytotoxic effect 200-1,000 µg/mL and no cytotoxic effect >1,000 µg/mL. The cytotoxic effectiveness test of the *A. ferox* tuber fraction (**Figure 3**) showed that the *A. ferox* tuber fraction had a moderate cytotoxic effect on MFC-7 cells with an IC_{50} value of 61.027 µg/mL. Meanwhile, the *A. ferox* tuber fraction had a weak cytotoxic effect in HeLa cells with an IC_{50} value of >500 µg/mL.

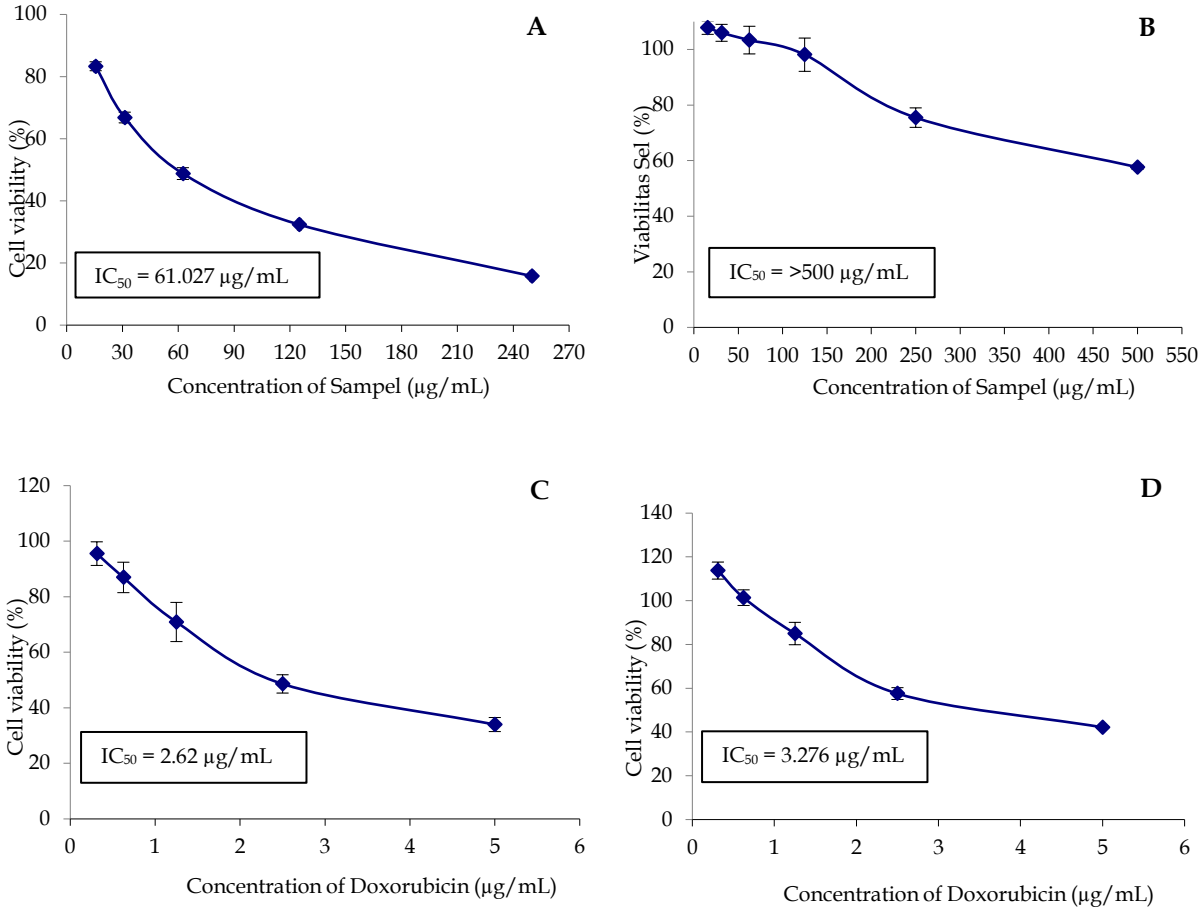


Figure 3. The graph of cytotoxic activity of sub-fraction C of *A. ferox* tuber toward MCF-7 (**A**) and HeLa cells (**B**) and doxorubicin as positive control toward MCF-7 (**C**) and HeLa cells (**D**). The data were observed in triplicate (n=3)

Meanwhile, the IC_{50} value of doxorubicin positive control against MCF-7 and HeLa cells obtained an IC_{50} value of 2.62 and 3.276 µg/mL, respectively, and included in the strong cytotoxic category. This study showed that sub-fraction C of *A. ferox* extract had a toxic effect on MCF-7 but not on HeLa cells. This mechanism is influenced by compounds' content in the sub-fraction of *A. ferox*, which could not cause apoptosis in HeLa cells. The sub-fraction C of *A. ferox* tubers has activity on MCF-7 cells based on the analysis of compound groups using GC-MS containing several compounds (**Table I**). According to previous research[13,19], phenolic compounds can inhibit the formation and growth of tumors by inducing cell cycle arrest and undergoing cell apoptosis. Phenolic compounds can induce cell cycle arrest with multiple cell cycles from G1-S-G2 so that they can downregulate cyclins and CDKs, and directly induce gene expression in p21, p27, and p53. According to other studies[20-22], flavonoid compounds have the potential as pro-oxidants so that they can suppress the proliferation of cancer cells by inhibiting the epidermal growth factor receptor or mitogen active protein kinase (EGFR/MAPK), phosphatidylinositide 3-kinases (PI3K), protein kinase B (Akt), and nuclear factor-kappa-β (NF-kB)[23].

CONCLUSION

Based on the results obtained from this study, it can be concluded that sub-fraction C of *A. ferox* tubers has anticancer activity, which was tested using an MTT assay on MCF-7 cells with an IC_{50} value of 61.027 µg/mL in the moderate toxic effect category. This result occurs because the sub-fraction C results from the compound groups' characterization using GC-MS. Several compounds are obtained, i.e., amino acids, glucosinolates, alkaloids, flavonoids, and terpenoids, to have a toxic effect on cancer cells.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The author declares there is no conflict of interest.

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Research Article

Anti-inflammatory Activity of Water Extract of *Luvunga sarmentosa* (BI.) Kurz Stem in the Animal Models

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Abstract

The study was aimed to determine the anti-inflammatory activity of water extract of the *Luvunga sarmentosa* stem in an animal model. Twenty-five Wistar rats were divided into five groups (n=5). Group 1 was administered 0.9% normal saline (negative control), group 2 was administered 150 mg/kg diclofenac sodium (positive control), and groups 3 to 5 were administered 50, 300, and 550 mg/kg BW of *L. sarmentosa* extract, respectively. Carrageenan was injected subcutaneously into each rat's subplantar region of the left hind paw. The paw volume was measured using a plethysmometer. The results showed that the water extract of *L. sarmentosa* stem (doses of 50, 300, and 550 mg/kg BW) significantly reduced the paw edema volume from the 4th to 5th hour compared to the negative control. The percent inhibition of edema at the 5th hour is 47.45; 46.95; 50.39%. The first phase of the edema (1st and 2nd hour) was not affected by the extract. Meanwhile, diclofenac sodium decreased paw edema volume from the 1st to 5th hour with a percent inhibition of 95.90% at the 5th hour. The histopathology result is relevant to the percentage inhibition of edema. Treatment with *L. sarmentosa* extract showed slight improvement, destruction of epidermal tissue, hyperkeratotic skin, and subepidermal edema. Meanwhile, positive control showed no inflammatory signs with normal keratin, subepidermal, and subcutaneous layers. The water extract of *L. sarmentosa* stem has anti-inflammatory activity. This extract effectively reduces the paw edema volume in the late phase with decreased neutrophil infiltration.

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INTRODUCTION

Inflammation is the body's normal response to wounds, injuries, microbial infections, allergies, and other harmful factors[1,2]. Symptoms of inflammation are pain, swelling, redness, heat, fever, and loss of body tissue function[3]. These symptoms are caused by inflammatory mediators and chemical agents such as prostaglandins (PG), serotonin, histamine, bradykinin, nitric oxide, and leukotrienes[4]. Inflammation plays a vital role in the physiological process. However, if the inflammatory process is prolonged and the offending agent persists, the intended protective process tends to be destructive that can damage the cell and cause various diseases[5-7].

Steroid and non-steroidal anti-inflammatory drugs (NSAIDs) are often used to treat pain and manage inflammatory conditions. The NSAIDs inhibit cyclooxygenase enzymes (COX-1 and COX-2), decreasing prostaglandin production[8,9]. The use of such drugs causes severe side effects, including severe gastrointestinal toxicities such as gastric ulcers and bleeding. Therefore, this instigates the development of effective, safe, and economic anti-inflammatory drugs[10].

Natural products from medicinal plants have been considered a potential alternative source of pharmacological substances with minimal adverse effects[11]. The plant represents a significant natural source of valuable compounds that might lead to novel drugs. World Health Organization (WHO) reported that about 70–80% of the world's population relies mainly on plant-based drugs. Its demand is increasing daily in developing countries[12-14]. Accordingly, there is a renewed interest in medicinal plant research to identify alternate agents that may be cheaper and have fewer adverse effects[15].

Luvunga sarmentosa (Bl.) Kurz, known as saluang belum in Uut Murung district, Central Kalimantan[16]. This plant is one of the endemic plants of Borneo Island, often used by local ethnic groups to increase male vitality[17]. The ethanolic extract of *L. sarmentosa* increased the number of spermatocytes and spermatid cells and showed aphrodisiac activity in male albino Wistar rats[18]. Several studies have reported compounds from *L. sarmentosa*. Flavonoids, steroids, and tannin have been isolated from the plants' roots[18]. Apotirucallane triterpenoids named luvungins A–G and 1a-acetoxyluvungin A (apotirucallane triterpenoids) were isolated from leaves[19].

The Dayak community uses a combination of *L. sarmentosa* and pasak bumi (*Eurycoma longifolia*) to increase stamina, sexual arousal, and male fertility by drinking root boiled water once a day. These plants are often used in a mix and prescribed root or stem, but the majority are used by the public, especially the root. Therefore, more attention is needed to avoid experiencing scarcity in nature, such as using stem parts instead of roots[16]. The use of mixed plants possibly aimed to obtain a synergism effect, in which *E. longifolia* was reported to have anti-inflammatory activity[20]. However, the effect of anti-inflammatory on *L. sarmentosa* has not been investigated. This study aims to determine the anti-inflammatory activity of water extract of *L. sarmentosa* (Bl.) Kurtz stem. This study's results could be used as supporting data on the utility of *L. sarmentosa* water extract in traditional medications.

MATERIALS AND METHODS

Materials

The stems of *L sarmentosa* was collected from traditional healers in the Pager, Rakumpit district, Palangka Raya City, Central Kalimantan, Indonesia on September 2019 (**Figure 1**). A licensed botanist made authentification and plant identification at Purwodadi Botanical Garden, East Java, Indonesia, with voucher specimen number No.1048/IPH.06/HM/IX/2019.

Figure 1. *Luvunga sarmentosa* stem simplicia

Methods

Plant extraction

The stem of *L. sarmentosa* was shade dried and powdered mechanically. The dried powdered (400 g) was extracted in water at 40-50°C for approximately 30 minutes. The extract was then filtered and concentrated with a vacuum evaporator and then dried with a freeze dryer to obtain a dry extract.

Experimental animal

Male Wistar rats (250-300 g) were obtained from the Laboratory Animal of the Department of Pharmacology, Faculty of Medicine, Universitas Airlangga. They were housed at a temperature of 25 ± 1°C, 12-hour light/dark cycles, and fed a standard rodent diet with water *ad libitum*. All the animals were acclimatized to the laboratory conditions before experimentation for seven days. Permission and approval for animal studies were obtained from the Faculty of Veterinary Medicine, Universitas Airlangga, with approval number KE.026.03.2021.

Anti-inflammatory activity by carrageenan induction

The carrageenan-induced paw edema model was used to evaluate the anti-inflammatory effect of *L. sarmentosa* extract (400 g). The initial paw volume was recorded using a plethysmometer (UGO Basile® 7140, Italy). Twenty-five male rats were selected and randomly divided into five groups (n=5). The negative control group was administered 0.9% normal saline (G1). The positive control group was administered 150 mg/kg of sodium diclofenac (G2), and the three test groups were administered 10, 40, and 80 g of simplicia *L. sarmentosa*, which is equal to extract doses of 50, 300, and 550 mg/kg BW, respectively (G3-G5). All drugs were administered an hour orally before the delivery of carrageenan injection. Carrageenan (0.1 mL of 1.5% w/v) was injected subcutaneously into the subplantar region of the left hind paw of each rat. The right hind paw was not treated and taken as a comparison. The paw volume was measured at 0, 30 minutes, 1, 2, 3, 4, and 5 hours following carrageenan injection using a plethysmometer[20-22]. The formula for calculating the percentage of inhibition was presented in equation [**1**], in which A was the mean paw volume for the test group and B was the mean paw volume for the control group.

$$Inhibition\ percentage = \frac{A-B}{B} \times 100\% \quad \ldots [1]$$

Histopathological analysis of paw tissue

The left hind paw of each rat was collected five hours after carrageenan was injected. The entire paw tissue sections (5 mm) were fixed by immersion in 10% formalin solution at room temperature. Paraffin-embedded paw tissue sections were stained with hematoxylin and eosin (H&E). Observation of structural abnormality and photographed under a light microscope (Olympus CKX41 microscope equipped with a digital camera). The observation was conducted at the Department of Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, to analyze the severity of paw tissue inflammation.

Data analysis

The results were presented in mean ± SEM, in which each value represents a minimum of five rats (n=5). The rise in paw volume data was tested for one-way analysis of variance (ANOVA) using GraphPad version 9.0 for Windows Software, followed by Dunnett's multiple comparison tests. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The extraction of the *L. sarmentosa* stem was carried out using water as a solvent at 40-50°C. The extraction yielded 5.5% w/w dry matter and was light brown. In this study, the water extract evaluated the anti-inflammatory activity induced by carrageenan. The carrageenan induction of rat paw edema is a suitable test for evaluating the anti-inflammatory activity of natural products[22,23]. Carrageenan-induced inflammation is acute, non-immune, well researched, and highly reproducible[24]. Carrageenan is used as a phlogistic agent, a substance that causes inflammation or edema[25].

The anti-inflammatory effect of water extract of *L. sarmentosa* stem on carrageenan-induced edema in rat's hind paws is presented in **Tables I** and **II**. Extract and sodium diclofenac significantly reduced the paw edema hours after carrageenan injection. For the control, swelling increased progressively to a maximum volume of 3.61±0.95 at five hours after carrageenan injection (**Figure 2**).

The first phase of the edema (1st and 2nd hour) was not affected by the water extract of *L. sarmentosa*. Administration of 50, 300, and 550 mg/kg extract significantly reduced the paw edema volume from the 4th to 5th hour compared to the negative control. Inhibition percentage of edema at the 5th hour of extract doses 50, 300, and 550 mg/kg showed no significant difference (47.45; 46.95; 50.39%), so we suggest using 50 mg/kg doses of the extract. This is to minimize the toxicity that may arise from the extract. On the other hand, 150 mg/kg of sodium diclofenac substantially decreased paw edema volume from the 1st to 5th hour compared to the negative control. Maximum percent inhibition of edema (95.90%) was estimated at the 5th hour after the carrageenan administration. This result confirms that sodium diclofenac has higher inhibition against inflammation than water extract of *L. sarmentosa*.

Table I. Average paw size of a rat in all groups after carrageenan injection

Groups	Dose (mg/kg)	Average paw size (mL)						
		0 minute	30 minutes	1 hour	2 hours	3 hours	4 hours	5 hours
Negatif control	-	3.67±0.29	4.83±0.27	5.40±0.30	5.92±0.53	6.40±0.90	6.93±1.08	7.28±1.09
Positive control	150	4.10±0.45	4.27±0.47	4.28±0.58*	4.20±0.37****	4.28±0.62****	4.33±0.53****	4.25±0.43****
Luvunga sarmentosa water extract	50	3.78±0.14	5.50±0.30	5.63±0.82	5.94±0.60	6.02±0.41	5.88±0.32*	5.68±0.25**
	300	4.05±0.40	5.90±0.37*	6.08±0.37	6.23±0.44	6.60±0.34	6.19±0.13	5.97±0.47*
	550	4.18±0.76	5.48±0.85	6.07±0.51	6.29±0.53	6.48±0.40	6.04±0.55	5.97±0.51*

Data were reported as mean±SD; n = 5. One-way ANOVA was carried out using Dunnett's multiple comparison test. Symbols represent statistically significant: *p <0.05 **p <0.01 ***p <0.001 ****p <0.0001

Table II. Percentage inhibition of inflammation in all groups after carrageenan injection

Groups	Dose (mg/kg)	Inhibition of edema (%)			
		2 hours	3 hours	4 hours	5 hours
Negative control	-	-	-	-	-
Positive control	150	95.47	93.56	92.87	95.90
Luvunga sarmentosa water extract	50	4.26	18.16	35.50	47.45
	300	3.29	6.88	34.40	46.95
	550	6.22	15.59	42.75	50.39

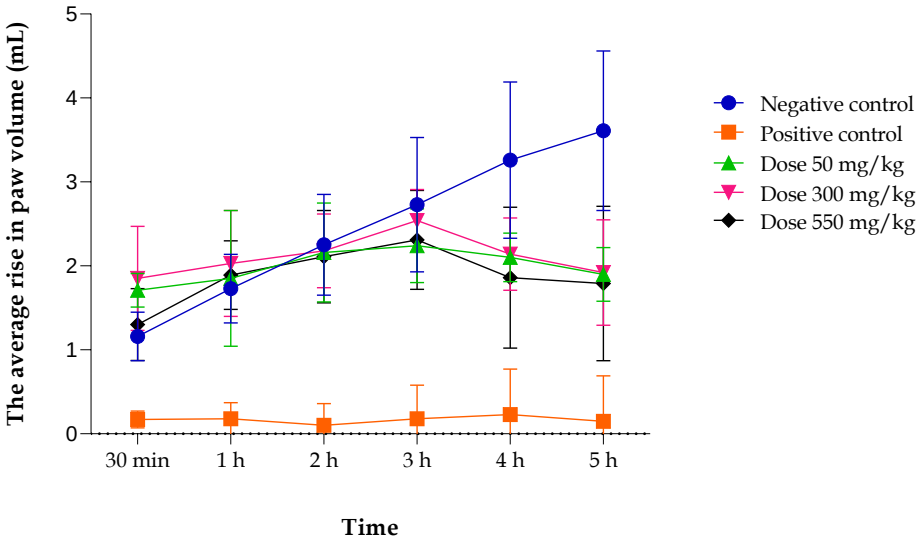

Figure 2. The average rise in paw volume in all groups after carrageenan injection (n=5)

Histopathology analysis of paw tissue showed a massive influx of inflammatory cell infiltration, proliferated collagen, keratinization was decreased dermis, and subepidermal edema in the negative control. Treatment with *L. sarmentosa* extract showed slight improvement, destruction of epidermal tissue, hyperkeratotic skin, and subepidermal edema. Meanwhile, positive control showed no inflammatory signs with normal keratin, subepidermal, and subcutaneous layer. The histopathology result was relevant to the inhibition percentage of edema (**Figure 3**).

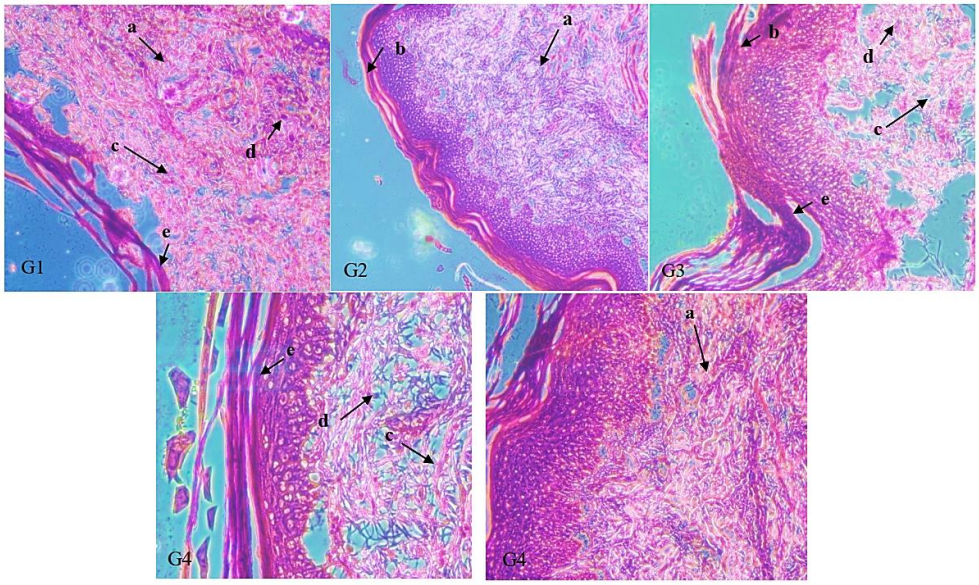

Figure 3. Histology of rat paw tissue after five hours injected with carrageenan. **G1**: negative control; **G2**: positive control; **G3**: dose 50 mg/kg BW; **G4**: dose 300 mg/kg BW; **G5**: dose 550 mg/kg BW of *L. sarmentosa* water extract with H&E staining and 400x magnification. **a**: dermis; **b**: epidermis; **c**: subepidermal edema; **d**: inflammatory cell infiltration (ICI); **e**: creatine

Carrageenan injection given subplantar will increase the rat paw's swelling, consisting of a relatively fast initial phase (up to 3 hours), followed by a late phase (3-5 hours)[2]. The initial phase was the release of histamine, serotonin, bradykinin, and a small number of prostaglandins produced by the COX enzyme. The late phase was associated with neutrophil infiltration, releasing free radicals, nitric oxide, pro-inflammatory cytokines, and continued prostaglandins[26]. We suggest that the administration of *L. sarmentosa* extract is effective in the late phase with decreased neutrophil infiltration.

CONCLUSION

The water extract of *L. sarmentosa* stem has anti-inflammatory activity, which effectively reduces the paw edema volume in the late phase.

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AUTHORS' CONTRIBUTION

Sabar Deyulita: Extraction, anti-inflammatory test, data analysis, and article writing. **Hilkatul Ilmi**: anti-inflammatory test, data analysis, and article writing. **Hanifah Khairun Nisa**: histopathological examination and data analysis. **Lidya Tumewu**: Extraction and article writing. **Aty Widyawaruyanti**: Supervision, conceptualization, validation of methods, writing review & editing. **Achmad Fuad Hafid**: Supervision, conceptualization, validation of methods, writing review & editing.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

Free Radical Scavenging and Analgesic Activities of 70% Ethanol Extract of *Luvunga sarmentosa* (BI.) Kurz from Central Kalimantan

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Abstract

Luvunga sarmentosa, commonly known as saluang belum, is widely used in Kalimantan to relieve pains, rheumatism, boost the immune system, and fever. The research on the free radical scavenging and analgesic effect of the *L. sarmentosa* stem extract has not been reported. This study aimed to evaluate the free radical scavenging and analgesic activity of the ethanol extract of *L. sarmentosa*. The *L. sarmentosa* stem was extracted using 70% ethanol and tested for free radical scavenging using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and analgesic activity, acetic acid-induced writhing test, and hot plate test in an animal model. The results showed that the 70% ethanol extract of the *L. sarmentosa* had an anti-free radical scavenging and analgesic activity. The extract has weak free radical scavenging with an IC_{50} value of 293.45 µg/mL. Analgesic activity using the writhing test indicated that the extract significantly reduced the writhes count after oral administration in a dose-dependent manner compared to the negative control. Extract at a dose of 550 mg/kg BW can reduce the writhing test by 67.60% compared to others. In contrast, the diclofenac sodium reduced the number of writhes by 74.74%. While in a hot plate, the extract at a dose of 550 mg/kg BW produced a maximum possible analgesia (MPA) of 17.64%, lower than the MPA of diclofenac sodium (51.01%). Analgesic activity of the extract has higher inhibition on the writhing test than on the hot plate. The extract could be responsible for the peripheral mechanism by inhibiting the prostaglandin biosynthesis.

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INTRODUCTION

Pain is a body's defense mechanism that reacts to stimuli to avoid further tissue damage[1]. Pain can also be defined as pathological conditions that arise due to free radicals and oxidative stress in body cells[2]. The body produces free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) in endogenous systems when exposed to various physiochemical or pathological conditions. Excessive ROS production causes oxidative stress, a process that can damage cell structures, including lipids, proteins, and DNA[3]. Pain is an unpleasant sensory and emotional experience associated

with actual or potential tissue damage[4]. However, pain is beneficial to the immune system. Nevertheless, it causes much suffering and discomfort to the victims, lowering the quality of life, disability, or mortality in several cases. Therefore, pain needs to be managed[5].

The onset of pain makes a person look for treatment to reduce pain. Attempts to reduce the pain are with analgesics drug[6]. Analgesics are substances that can reduce or dispel pain without losing consciousness. Evaluation of analgesic drugs in public hospitals in China from 2013 to 2018 showed an increase in analgesic drugs every year. From 2013 to 2018, NSAIDs' annual clinical drug dose increased by about 0.6 times[7]. However, prolonged use of these NSAIDs produces significant side effects and are toxic to the liver, kidney, gastrointestinal linings, and reduced auditory ability[8]. As such, research to discover other alternatives to treat pain is crucial.

Medicinal herbs have been used for centuries for therapeutic purposes. Many of these herbs with analgesic activity had been used without any side effects and at a lower cost[9]. World Health Organization (WHO) estimates that more than 80% of the world population relies on traditional medicines, and the market is rapidly growing. Saluang belum (*Luvunga sarmentosa* (BI.) Kurz) belongs to the family of Rutaceae, which develops and spreads in the tropical forests of Kalimantan[10]. Several studies have been carried out to identify the phytochemicals of *L. sarmentosa*. Its leaves contain apotirucallane triterpenoids named luvungins A-G, 1α-acetoxyluvungin A, coumarins ostruthin, and 8-geranyl-7-hydroxycoumarin, and triterpenes friedelin, flindissone, melianone, niloticin, and limonin have been isolated[11].

In Central Kalimantan, this plant was prescribed traditional medication to increase stamina and antioxidants[10]. The part of the stem, root, and leaves of *L. sarmentosa* is used in traditional herbal recipes. The stem and root were consumed three times a day by boiling or brewed with hot water and consumed once a day while warm[12,13]. The traditional healers prescribed the stem more to treat soreness, fatigue, or pains. Thus far, research on the analgesic activity of *L. sarmentosa* stem has not been widely reported. Therefore, this study was conducted to determine the analgesic and free radical scavenging activity of 70% ethanol extract of *L. sarmentosa* stem. The results study will provide scientific-based evidence on the use of stems in traditional medicine.

MATERIALS AND METHODS

Materials

The stems of *L. sarmentosa* was collected in September 2019 from traditional healers in Rakumpit District, Palangka Raya, Central Kalimantan, Indonesia (**Figure 1**). A licensed botanist at Purwodadi Botanical Garden, East Java, Indonesia, conducted authentification and identification of the plant with voucher specimen 1048/IPH.06/HM/IX/2019. Diclofenac sodium was used as a standard drug for analgesics and vitamin C for antioxidants. Both of them were obtained from PT. Kimia Farma Tbk, Indonesia. Other materials used were distilled water, acetic acid, carboxymethyl cellulose sodium (CMC-Na 0.5%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 70% ethanol.

a **b**

Figure 1. *Luvunga sarmentosa* at the forest (**a**) and the stem simplicia package from the traditional healer (**b**)

Methods

Plant extraction

The stem of *L. sarmentosa* was shade dried and powdered mechanically. The powdered (400 g) was macerated in ethanol 70% three times every 24 hours. The extract was then filtered and concentrated with a vacuum evaporator, then dried with a freeze dryer to obtain a dry extract.

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was tested by DPPH radical scavenging assay. The extract was diluted with methanol at 1000; 800; 600; 400; 200; 100; 50; 25; and 12.5 µg/mL. Meanwhile, vitamin C as standard was diluted at 100; 50; 25; 12.5; 6.25; 3.12; 1.56; 0.78; 0.39; 0.19; 0.095; and 0.0475 µg/mL. A solution of 0.25 mM DPPH in methanol was prepared, and 100 µL of this solution was mixed with 100 µL of extract/standard in methanol at different concentrations. The reaction mixture was incubated in the dark at room temperature (26°C) for 30 minutes. The experiment was carried out with three replications, and the absorbance was observed at a wavelength of 517 nm. DPPH radical scavenging activity was calculated using equation [**1**], in which A_0 was the absorbance of the control and A_1 was the absorbance of the extract/standard.

$$\%\ DPPH\ radical\ scavenging\ activity = \frac{A0-A1}{A0} \times 100\% \qquad \ldots [1]$$

Experimental animal

Male mice (Deutschland, Denken, and Yoken strains) weighing 25-30 g and aged 4-8 weeks were obtained from Farma Veterinary Center, Surabaya, Indonesia. All animals were maintained on a standard animal pellets diet and water ad libitum at the Animal Laboratory of the Institute of Tropical Disease, Universitas Airlangga, Surabaya. All the animals were acclimatized for seven days to the laboratory conditions before the experiment. All animal protocols were critically reviewed and approved by the Faculty of Veterinary Medicine, Universitas Airlangga, with approval number 2.KE.117.03.202.

Analgesic activity in animal model with acetic acid-induced writhing test

Thirty male mice were randomly divided into five groups, and each group consisted of six mice. Group 1 was treated with carboxymethyl cellulose (CMC-Na 0.5%) as a negative control, group 2 was treated with diclofenac sodium as a positive control at a 40 mg/kg BW, and groups 3, 4, and 5 were treated with ethanol extract of *L. sarmentosa* at a dose of 50, 300, and 550 mg/kg BW, equal to the dose of 10, 40, and 80 g of simplicia, respectively. All treatments were administered orally. The extract and the standard drug were treated 30 minutes before 1% acetic acid injection at a dose of 10 mL/kg BW intraperitoneally. After five minutes, each group of mice was observed for the number of writhes for 45 minutes. The mean value for each group was calculated and compared with the control. The percentage of analgesic activity was calculated using the equation [**2**], in which W is the number of writhing, c is the negative control, and t is the test[14,15].

$$\%\ Inhibition = \frac{Wc-wt}{Wc} \times 100\% \qquad \ldots [2]$$

Analgesic activity in animal model with hot plate test

The analgesic activity was also evaluated using the hot plate method[15-17]. Mice were given oral therapy according to groups. After 30 minutes of treatment, the experimental animals were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before treatment (0 minutes), then 30, 60, 90, and 120 minutes after administering the treatments. The maximum reaction time was fixed at 20 seconds to prevent any injury to the tissues of the paws. The maximum possible analgesia (MPA) was calculated using the equation [**3**][18].

$$\%\ MPA = \frac{test\ group\ mean - control\ group\ mean}{cut\ off\ time\ 20\ seconds - control\ group\ mean} \times 100\% \qquad \ldots [3]$$

Data analysis

The results of the study were presented in mean±SEM. Statistical analysis was used one-way ANOVA followed by post hoc Dunnett's test for multiple comparisons (GraphPad Prism 7.0, Co., Ltd., San Diego, US). The difference between groups was considered significant at a p-value <0.05.

RESULTS AND DISCUSSION

This study was conducted to determine the free radical scavenging and analgesic activities of *L. sarmentosa* stem ethanol extract. The *L. sarmentosa* was carried out using 70% ethanol as a solvent by the maceration method. The extraction yielded 3.9% w/w dry matter and was light brown.

DPPH radical scavenging assay

DPPH radical scavenging model is the widely used method to evaluate the anti-free radical activity of natural compounds and plant extracts. The results showed that extract had to scavenge the free radical, with an IC_{50} value of 293.45 µg/mL (**Figure 2**), while vitamin C had an IC_{50} value of 11.39 µg/mL (**Figure 3**). The extract was weak, and vitamin C was highly active as a free radical, based on Marjoni and Zulfisa[19]. The scavenging activity shows that extract and vitamin C could provide a hydrogen atom to the DPPH radical. The DPPH would oxidize and be decolorized. Stable free radical DPPH could accept an electron or hydrogen radical to become stable. Its solution appears a deep violet color. As this electron becomes paired off, the absorption vanishes, resulting in decolorization[20].

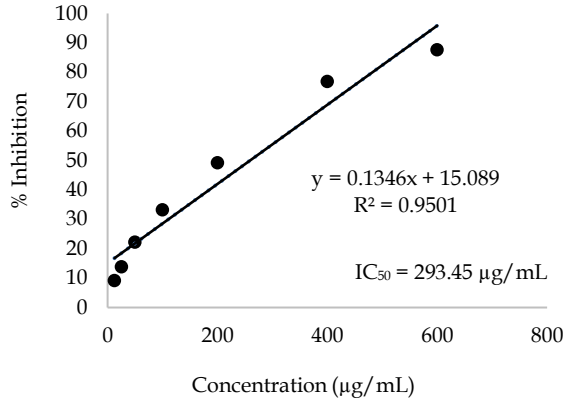

Figure 2. The IC_{50} value for radical scavenging activity of *L. sarmentosa* extract

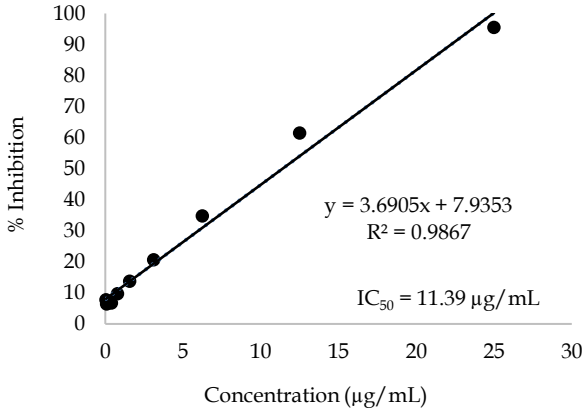

Figure 3. The IC_{50} value for radical scavenging activity of vitamin C

Analgesic activity with acetic acid-induced writhing test

The acetic acid-induced writhing test has widely been used for screening analgesic drugs[14]. The writhing test is used to assess peripheral acting analgesics. In the writhing test, the acetic acid injection causes pain by releasing serotonin, histamine, prostaglandins, and bradykinin from arachidonic acid through cyclooxygenase (COX) enzymes[1,14,16]. The synthesis of endogenous substances induces contraction of the abdominal muscles that touch the floor, pull the legs back, and stretch the body[21-23].

Analgesic activity in this study indicated that the 70% ethanol extract of *L. sarmentosa* stem significantly reduced the writhes count after oral administration in a dose-dependent manner compared to the negative control. After forty-five minutes of the test period, the extract at 550 mg/kg BW demonstrated the highest analgesic activity by reducing the number of writhes by 67.60%, while 300 mg/kg BW reduced the number of writhes by 49.30%. The 50 mg/kg BW dose reduced the writhes by 33.28%. However, diclofenac sodium reduced the writhes by 74.74% — the analgesic activity of 70% ethanol extract of *L. sarmentosa* stem presented in **Table I**.

Table I. Analgesic activity of 70% ethanol extract of *L. sarmentosa* stem in acetic acid-induced writhing test

Group	Dose (mg/kg)	Number of writhes in 45 minutes (mean ± SEM)	Inhibition (%)
Negative control	-	95.67 ± 5.70	-
Positive control	40	24.17 ± 1.58****	74.74
Luvunga sarmentosa extract	50	63.83 ± 1.68****	33.28
	300	48.50 ± 2.57****	49.30
	550	31.00 ± 2.72****	67.60

Data were reported as mean ± SEM and analyzed by ANOVA followed by Dunnett's multiple comparison test. * indicate a statistically significant value from negative control, ****p <0.0001.

Analgesic activity with hot plate test

The hot plate is a standard method for evaluating central analgesic activity in animal models that use thermal stimuli as pain inducers with temperature was maintained at 55°C. The principle of this method is a change in spinal cord level, which effectively describes the centrally mediated anti-nociceptive response[24]. The paw-licking or jumping are defined as pain reflex behavior[25-27]. The analgesic activity of the 70% ethanol extract of *L. sarmentosa* stem using the hot plate was presented in **Table II** and **Figure 4**.

The treatment of 70% ethanol extract of *L. sarmentosa* stem (50–550 mg/kg BW) and diclofenac resulted in a significant dose-dependent increase in the reaction time to thermal stimulation compared with the negative control. The MPA value of extract did not show any analgesic effect 60 minutes after treatment but increased at 90 minutes and declined after that. The highest increase in reaction time was observed with a 550 mg/kg BW dose at 90-minutes post-treatment (17.64%). Diclofenac sodium elicited significant analgesic activity within 30 minutes following administration, as evidenced by the gradual increase throughout the observation period. At the peak of activity (90 minutes), diclofenac sodium showed an MPA of 51.01%.

Table II. Analgesic activity of 70% ethanol extract of *L. sarmentosa* stem in hot plate test

Groups	Dose (mg/kg)	Reaction time (minutes)				
		0	30	60	90	120
Negative control	-	3.56±0.20	4.06±0.25	4.96±0.02	5.42±0.13	5.20±0.30
Positive control	40	5.13±0.52*	7.27±0.06****	9.26±1.29****	12.86±1.59****	11.54±1.29****
Luvunga sarmentosa extract	50	5.32±0.39**	6.25±0.41**	6.94±0.39**	7.40±0.47*	6.52±0.33
	300	5.49±0.21**	6.72±0.23***	7.34±0.30**	7.67±0.28*	7.16±0.26**
	550	5.43±0.52**	6.69±0.61***	7.36±0.56**	8.00±0.76**	7.43±0.55**

Data were reported as mean ± SEM; n=6. One-way ANOVA was carried out using Dunnett's multiple comparison test. *indicate a statistical significant: *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001

Writhing response induced by acetic acid and hot-plate latent pain response in mice are two common and important models for screening analgesics. These two models could cause pain by liberating PGs and many others that excite pain nerve endings. Both methods were used to study ethanol extract's peripheral and central analgesic activities. The results showed that ethanol extract has higher inhibition on the acetic acid test than the hot plate. This observation points out that extract possesses peripherally-mediated antinociceptive properties that may work via reducing the level of prostaglandin synthesis or other inflammatory mediators, which is much like diclofenac. Diclofenac sodium performs its action in peripheral acting by inhibiting the synthesis of prostaglandins (pain mediators) by inhibiting COX-1 and COX-2[15]. The analgesic activities of 70% ethanol extract of *L. sarmentosa* stem could be attributed to one or more phytochemical compounds present in the extract.

The phytochemical screening in this study showed that 70% ethanol extract of *L. sarmentosa* stem contained terpenoid and flavonoid compounds. Their compounds in the extract may contribute to the anti-free radical and analgesic activity. Flavonoids are polyphenolic compounds that can change or reduce free radicals[28]. Triterpenoids or steroids are compounds that have a role as antioxidants. The antioxidant mechanism of triterpenoids is by scavenging reactive species, such as superoxide and metal chelating[29]. Besides that, a flavonoid is also known to have analgetic activity[30]. Flavonoids can also reduce arachidonic acid production by inhibiting neutrophils' degranulation[31].

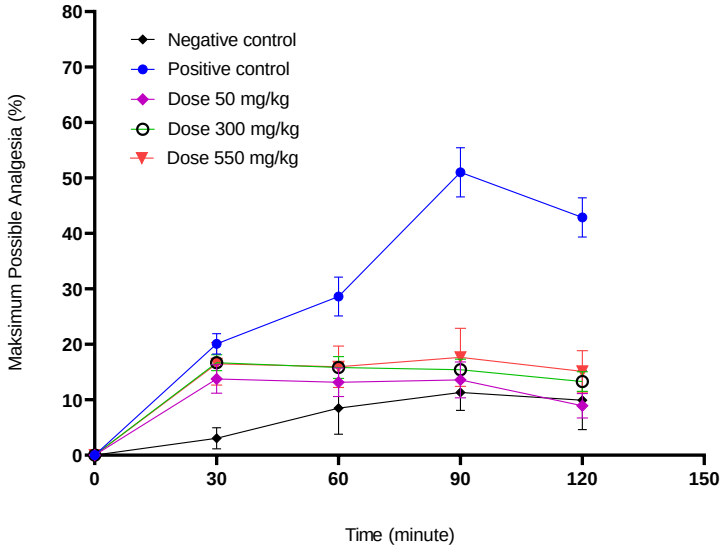

Figure 4. Maximum possible analgesia of *L. sarmentosa* stem extract compared to diclofenac sodium evaluated by hot plate test

CONCLUSION

The 70% ethanol extract of *L. sarmentosa* stems had weak anti-free radical activity. However, it exhibited significant analgesic activity, possibly by a peripheral pain mechanism inhibiting the prostaglandin pathway.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Mini Review

Cancer Statistics and Anticancer Potential of *Peganum harmala* Alkaloids: A Review

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Keywords:
Alkaloids
Cancer
Peganum harmala
Statistics

Abstract

Cancer is one of the most common diseases in the world. Although it develops in various organs and tissues, some species maintain a stable position in the ranking. Although the cancer causes are different, the specific grounds for each type are also noted. Sometimes the increase in incidents and mortality is associated with geographical reasons. Increases in statistics, expensive and chemotherapeutic methods focus on plant-based substances. One of such potential plants is *Peganum harmala*, which contains alkaloids such as harmine, harmaline, harmol, and harmalol. The effects of these compounds on many cancer cells have been tested, and positive results have been obtained. This fact reinforces the claim that more in-depth research on noted alkaloids is needed.

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INTRODUCTION

Cancer is one of the most dangerous diseases in the modern world. The reasons for its formation and the causing factors are different. Carcinogenic substances in the food we eat in modern life, the air we breathe, and the water we drink lead to this disease's greater spread. Chemical additives used in foods for long-term storage, especially nitrites in meat products; aflatoxins, pesticides that we come across as contaminants; toxic gases emitted from factories, machines; toxic wastes released into the water, heavy metals, water pollutants such as arsenic can be a typical example for our problem[1-3]. At the heart of the growing prevalence of this disease are also some of our addictions, such as smoking and alcohol[4]. Numerous studies have been conducted on the effects of smoking and alcohol consumption on cancer statistics, and it has been found that there is an increasing dependence graphic between these behaviors and cancer incidence and mortality rates[5,6].

The above are just some of the cancer causes. Even if we want to get rid of pollution around the world one day, even if we want to give up our addictions such as smoking and alcohol, reducing cancer would still take time. If we add genetic factors to these causes, we can see that we can not escape from this disease[7]. Therefore, besides eliminating the causes of the disease as much as possible, it seems to be the most logical way to find new therapeutical ways of fighting it. Many anti-cancer drugs are used today, and the exacerbation of the disease statistics leads to an increase in the substance choices used in its treatment and the search for new alternatives in this direction. The effects of many synthetic chemicals and plant-based compounds on various cancer cells have been studied, and this process continues to be relevant today[8]. There are specific drugs currently used in the body to slow down cancer. However, they are expensive and relatively difficult to reach[9]. For such an increasing rate of disease, more accessible sources are needed. In this case, the plants and plant-based compounds come to the fore. Some plants are especially noteworthy for their anti-cancer effects, making their usage potential closer to reality. One such plant is *Peganum harmala*[10].

Although its leaf extract is used in practice as an anti-cancer agent in Iran, the use of *P. harmala* is not widely spread around the world[11]. The effects of its most predominant compounds - alkaloids such as harmine, harmaline, harmol, and harmalol

on many cancer cell lines have been separately studied and obtained favorable results. Moreover, this plant is found on almost all continents, especially in Asia and Africa. Moreover, it does not require special care for growing and maturing; its primary habitat is arid and saline soils. Because it is so accessible to humans, its traditional use also has an extensive list[12]. However, despite all these properties, effectiveness, and availability, none of these plant alkaloids are used to prevent cancer. Thus, this article aims to stimulate more research on new natural alternatives, such as *P. harmala* alkaloids, and raise awareness of their therapeutic potential when the incidence and mortality of cancer are increasing.

CANCER STATISTICS

Lung cancer has been linked to tobacco usage in 90% of male and 79% of female patients. Smoking is thought to be responsible for 90% of lung cancer fatalities. Compared to non-smokers, lifelong smokers have a 20-40 times higher risk of developing lung cancer[13]. Men's mortality and incidence rates are nearly two times higher than women. Smoking is responsible for almost two-thirds of lung cancer deaths globally. It is known that men are more likely to drink alcohol, so the effects of alcohol on cancer are more intense. According to 2020 data, most alcohol-related types in men have been reported with esophageal, liver, and breast cancer[14].

It is a fact that there are social reasons besides just those related to the environment and our routines. For instance, in low- and lower-middle-income nations, cancer-causing diseases such as hepatitis and human papillomavirus (HPV) account for roughly 30% of cancer cases. Also, in these nations, late-stage presentation and lack of access to diagnosis and treatment are prevalent. According to reports, comprehensive therapy is available in more than 90% of high-income countries but fewer than 15% in low-income countries[15].

There are many types of cancer, depending on the organ and tissue in which they are located, but some differ significantly in terms of prevalence and mortality. Based on information for 36 cancers in 185 countries in 2020, the cancer types with the highest incidence and mortality are shown in **Table I**. When we compare the statistics of cancer types worldwide, we see that certain regions and countries are particularly conspicuous. The regions and countries with the highest prevalence and mortality rates are shown in **Table II**.

Table I. The most common cancer types in 2020[14]

Incidence		Mortality	
Cancer type	**Rate (%)**	**Cancer type**	**Rate (%)**
Female breast	11.7	Lung	18
Lung	11.4	Colorectal	9.4
Colorectal	10.0	Liver	8.3
Prostate	7.3	Stomach	7.7
Stomach	5.6	Female breast	6.9

Table II. Prevalence of cancer types by regions and countries in 2020[14]

Cancer type	Highest incidence		Highest mortality	
	Region	**Country/its region**	**Region**	**Country/its region**
Female breast	Australia/New Zealand	Belgium/Western Europe	Melanesia	Barbados/Caribbean
Lung	Micronesia/Polinesia	Turkey/Western Asia	Nothern America	Hungary/Eastern Europe
Colon	Southern Europe	Hungary/Eastern Europe	Australia/New Zealand	Norway/Northern Europe
Rectum	Eastern Europe	Portugal/Southern Europe	Eastern Europe	Latvia/Northern Europe
Prostate	Northern Europe	Ireland/Northern Europe	Caribbean	Zimbabwe/Eastern Africa
Stomach	Eastern Asia	Japan/Eastern Asia	Eastern Asia	Mongolia/Eastern Asia
Liver	Eastern Asia	Mongolia/Eastern Asia	Northern Africa	Mongolia/Eastern Asia
Oesophagus	Eastern Asia	Cape Verde/Western Africa	Eastern Asia	Malawi/Eastern Africa
Cervix uteri	Eastern Africa	Malawi/Eastern Africa	Eastern Africa	Malawi/Eastern Africa
Thyroid	Northern America	Cyprus/Southern Europe	Micronesia/Polinesia	Cyprus/Southern Europe
Bladder	Southern Europe	Greece/Southern Europe	Southern Europe; Western Europe	Hungary/Eastern Europe
Non-melanoma skin	Australia/New Zealand	Australia/ Australia/New Zealand	Australia/ Australia/New Zealand	Australia/New Zealand
Pancreas	Eastern Europe	Hungary/Eastern Europe	Western Europe	Hungary/Eastern Europe
Non-Hodgkin lymphoma	Australia/New Zealand	Israel/Western Asia	Australia/New Zealand; Northern America	Slovenia/Southern Europe
Corpus uteri	Northern America	Poland/Eastern Europe	Eastern Europe	Bahamas/Caribbean
Kaposi sarcoma	Southern Africa	Mozambique/Eastern Africa	Southern Africa	Zambia/Eastern Africa
Lip, oral cavity	Melanesia	Papua New Guinea/Melanesia	Melanesia	Papua New Guinea/Melanesia

Unfortunately, the cancer tumor, which has become so entrenched in human life today, will grow even bigger in 20 years. Forecasts show that in 2040, the highest increase in cancer rates will be in Africa (incidence +89.1%, mortality +92.9%), and the lowest increase will be in Europe (incidence +21.0%, mortality +29.2%) (**Table III**)[16]. According to the incidence data, the most common cancer types will be breast, melanoma, and lung; and in terms of mortality, lung, liver, intrahepatic bile duct, and colorectal cancer will take the first three places.

Table III. Predicted cancer growth rates in 2040[16]

Region	Incidence (%)			Mortality (%)		
	Both	Female	Male	Both	Female	Male
Africa	89.1	86.2	92.9	92.9	90.2	96.1
Latin America, Caribbean	65.6	59.0	72.5	77.3	72.7	81.8
Asia	59.2	52.6	65.1	69.7	68.0	70.9
Oceania	47.8	46.9	48.5	65.6	62.6	68.1
Nothern America	37.9	32.2	42.8	49.3	44.0	54.1
Europe	21.0	14.1	27.1	29.2	23.4	33.9

GEOGRAPHICAL CANCER REASONS

Some regions and countries in **Table III** differ significantly in the prevalence of specific types of cancer. For instance, stomach cancer in Eastern Asia; cervix uteri in Malawi/Eastern Africa; bladder in Europe; non-melanoma of skin in Australia/New Zealand; Kaposi sarcoma in Africa; lip and oral cavity in Papua New Guinea/Melanesia. Each of these similarities can be attributed to specific reasons. For example, Eastern Asia accounts for more than half of all stomach-gastric cancer cases[17], and it is related to high rates of infection with *Helicobacter pylori* and the increased consumption of salted and smoked foods[18]. The highest cervical cancer rates in Malawi/Eastern Africa are coordinated with a high prevalence of human immunodeficiency virus (HIV) with 10.6% and human papillomavirus (HPV) with 33.6%. Late diagnosis and limited cancer treatment options also increase the incidence of this disease[19].

Smoking is shown as the most critical cause of bladder cancer. This cancer type is most common in Europe[20]. Furthermore, given the high and growing smoking levels in Europe, we can say that this trend is expected. Even Greece has the highest smoking rate in Europe at 42%, and it is no coincidence that Greece ranks first in the world incidence and makes Southern Europa the world's largest region in this incidence[21].

The prevalence of skin non-melanoma in Australia/New Zealand is mainly due to the region's geographical location. Thus, it is considered that this cancer type in Australia/New Zealand is caused by exposure to UV radiation in sunlight. It should be noted that the incidence and mortality of this disease in this region differ sharply from other areas[22,23].

A virus called human herpesvirus, also known as Kaposi sarcoma-associated herpesvirus (KSHV), high-rated in Africa, is the cause of Kaposi sarcoma. Medical specialists believe that the virus is primarily transmitted from mother to kid through saliva. The malignancy develops in the context of a reduction in immune function, even if humans have carried the virus their entire lives[24]. Endemic Kaposi's sarcoma in Africa is also associated with geographical causes. The proximity of the regions where the disease is most prevalent to areas rich in volcanic clay minerals, the high incidence on the feet and legs, and the predominance of rural peasants and cultivators indicate the same etiology[25].

The most common oral cancer in Papua New Guinea is undoubtedly due to their traditional habit. This routine is associated with the Areca palm (*Areca catechu*) seed, called betel nut, and 80% of the country's population, even children, often chew this plant throughout the day. It is important to note that this plant has psychoactive properties, and the possibility of the population's dependence on it is a logical approach. For years, this ancient custom has ranked Papua New Guinea as the leading cause of oral cancer incidence and mortality[26,27].

PEGANUM HARMALA ALKALOIDS AGAINST CANCER CELLS

Most drugs used to treat cancer contain chemicals. However, given the current medical and social challenges in treatment and the predictions that cancer will be more prevalent in the future, there is a greater need for more readily available,

effective sources. In this case, attention is focused on plants[28]. One of such herbal substances used in modern practice is vincristine. It is derived from *Catharanthus roseus* and is used against cancer under the name Oncovin. Lymphoid blast crisis of chronic myeloid leukemia, acute lymphocytic leukemia, and Hodgkin and Non-Hodgkin lymphoma are the indications for vincristine approved by the US Food and Drug Administration (FDA)[29].

Peganum harmala (**Figure 1**) is one of the potential plants whose treatment area can be developed and expanded in the cancer problem. For example, Spinal-Z, medicament in the capsule form of methanolic extract of *P. harmala* seeds and *Dracocephalum kotschyi* leaves, is used for gastric cancer treatment in Iran[30]. According to the literature, this medicine can reduce the viability of cancer cell lines in mice[31].

Figure 1. *Peganum harmala* fruit and seeds[12]

Peganum harmala is a plant rich in amino acids[32], minerals[33], and lipids[34]. However, this plant is especially famous for its alkaloid content. The most frequently encountered alkaloids, quantitatively and qualitatively, are harmine, harmaline, harmol, and harmalol[35,36]. These compounds are in the researchers' focus with their anticancer effects. The antitumor properties of these alkaloids against various cancer cells have been studied, high results have been obtained, and research in this area is ongoing. The effects of Peganum alkaloids on many cancer cells have not been researched, meaning that some gaps and areas need to be investigated. **Table IV** shows this deficiency also cancer and cell types in which the effects of these alkaloids have been studied so far.

Table IV. Anticancer studies on *P. harmala* alkaloids

Types of cancer and cell lines	References			
	Harmine	**Harmaline**	**Harmol**	**Harmalol**
Breast; mammary gland				
MDA-MB-231	**11,37,38,39,40,41**	**11**	-	**42,43**
MCF-7	**11,37,41,44,45,46,47,48,49,50**	**11,48,50**	-	-
BT549	**51**	-	-	-
BCaP-37	-	**50**	-	-
4T1 (mouse)	**51**	-	-	-
Thyroid				
TPC-1	**52**	-	-	-
Large intestine; colon				
HCT116	**44,49**	-	-	-
SW480	**53**	-	-	-
SW620	**54**	-	-	-
LoVo	-	**50**	-	-
Stomach (gastric)				
SGC-7901	**55,56**	**57**	-	-
SGC-790	**53**	-	-	-
MGC-803	**56,58**	-	-	-
BGC-823	**53**	**50**	-	-
Brain				
U87	**59**	**59**	**59**	**59**
H4	**59**	**59**	**59**	**59**
U373	**60**	-	-	-
T98G	**60**	-	-	-
Hs683	**39,60**	-	-	-
GBM	**61**	-	-	-

U251MG	-	-	**39**	-
Oesophagus				
OE21	**60**	-	-	-
OE33	**60**	-	-	-
ESCC	-	**62**	-	-
Pancreas				
PANC-1	**63**	-	-	-
CFPAC-1	**63**	-	-	-
SW-1990	**63**	-	-	-
BxPC-3	**63**	-	-	-
Lung				
LLC (mouse)	**50,64**	-	-	-
CCD18LU (normal)	**53**	-	-	-
A549	-	**65**	**66**	**42,43**
H596	-	-	**67**	-
H1299	-	**65**	-	-
Liver				
HepG2	**46,50,64,68**	-	-	**42,43,50,69**
L02	**46**	-	-	-
Hep3B	**50**	**50**	-	-
WRL-68	-	-	-	**42**
SMMC-7721	**58**	-	-	-
HepA (mouse)	**64**	-	-	-
Hepa 1c1c7 (mouse)	-	-	-	**50**
Uterus; cervix				
HeLa	**50,53**	**50**	-	**42,43**
HEp-2 (HeLa derivative)	-	**50**	-	-
C-33A	**53**	-	-	-
Ovary				
OVCAR-3	**49**	-	-	-
Peripheral blood (leukemia)				
HL-60	**50,53,70**	**50,70**	-	-
Jurkat, Clone E6-1	**71**	-	-	-
Bone; marrow (leukemia)				
K562	**50,53**	**50**	-	-
Umbilical vein				
HUVEC	**57**	-	-	-
Urinary bladder				
RT112	**57**	-	-	-
RT4	**57**	-	-	-
SW780	**53,72**	-	-	-
BIU87	**72**	-	-	-
5637	**72**	-	-	-
Ureter; uroepithelium				
SV-HUC-1 (normal cell)	**72**	-	-	-
Skin				
SKMEL-28	**39**	-	-	-
UACC-62	**48,50**	**48,50**	-	-
B16F-10 (mouse)	**50,73**	-	-	-
L1210 (mouse)	-	**50**	-	-
Kidney				
TK10	**48,50**	**48,50**	-	-
Spleen				
Sp2/O-Ag14	**50,71**	-	-	-
Hemo-lymphocytic				
P388 (mouse)	-	**50**	-	-
B lymphocyte				
Raji	-	**50**	-	-
Muscle				
RD	-	**50**	-	-
Sarcoma				
S180	**50,64**	**50**	-	-
UCP-med (rat)	**71**	-	-	-
L2 reticulosarcoma (rat)	-	**71**	-	-
Carcinoma				
Med-mek (rat)	**71**	-	-	-
UCP-med (rat)	**71**	-	-	-

CONCLUSION

Given current cancer rates and future prognoses, there is a need for alternative compounds that are easier to find. In this case, the first thing that comes to mind is plants, and one of the most important plants in this area is *P. harmala*. In this article, the potential for cancer treatment through experiments with this plant and its essential alkaloids and scientific gaps have been shown.

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AUTHORS' CONTRIBUTION

Tohfa Nasibova performed the entire role of this review.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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