Medical Laboratory Technology Journal
11(2), 2025, 197-207
Received 2025-09-08; Revised 2025-10-26; Accepted 2025-10-26
Available online at : http://ejurnal-analiskesehatan.web.id

Nutritional Content, Subchronic Toxicity, and Antidysmenorrhea Activity of Instant Herbal Medicine Mixture of Red Ginger and White Ginger

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Abstract: Dysmenorrhea is menstrual pain that occurs in 69.8% of women. Ginger (*Zingiber officinale*) contains various secondary metabolites with anti-inflammatory properties. This study aims to determine its characteristics, including nutritional value and stability, product safety through toxicity testing, and product efficacy. The research stages include research ethics testing and the production of instant powder from a mixture of red and white ginger. Nutritional content analysis included total fat, saturated fat, protein, and carbohydrates. Physical and chemical stability testing was carried out for three months. Subchronic toxicity testing used Wistar rats for 28 days by drawing blood before and after treatment, accompanied by liver delivery. Blood samples were analyzed for SGPT and SGOT levels. The test was conducted on 10 women aged 18-20, divided into two groups. The treatment group was given 20 grams of instant ginger in the morning and evening for 10 days, and compared with the control group without treatment. Pain was measured using a Visual Analog Scale (VAS) before and after the study. The study showed that instant ginger herbal medicine contained 0.83% total fat, 0.17% saturated fat, 3.73% total protein, and 35.42% total carbohydrates. The stability test results showed no changes in the physical and chemical parameters of the test. The results of the subchronic toxicity test showed that the body weight of mice, liver weight, SGOT levels, and SGPT levels of mice were normal. The activity test showed a decrease in pain from before to after administration of instant ginger, with an average VAS value of 7.8 to 4.2. This study concluded that instant ginger, a mixture of red and white ginger, has good nutritional content, is physically and chemically stable, is non-toxic, and is effective in reducing dysmenorrhea.
Keywords: Antidysmenorrhe; ginger; nutritional; toxicity.

INTRODUCTION

Dysmenorrhea is a painful menstrual cycle that occurs in women (Armour et al., 2019). Symptoms can occur before or during the menstrual cycle, including lower abdominal cramps, headaches, nausea, vomiting, excessive sweating, and diarrhea (Bahrami et al., 2017). The prevalence of dysmenorrhea in adolescents aged 12-15 years reaches 69.8%. Dysmenorrhea can affect quality of life, requiring adolescents to withdraw and rest from various routine activities (Esan et al., 2024). Dysmenorrhea is generally treated with non-steroidal anti-inflammatory drugs (NSAIDs). This class of drugs has bothersome side effects, including peptic ulcers and stomach pain. Reports of toxicity due to NSAIDs are standard in the United States (Adams & Wang, 2015). Herbal medicine can be a solution to this problem. Herbal remedies are known to relieve pain with minimal side effects.

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Natural products are widely available in Indonesia and have various uses, one of which is in traditional medicine. The use of natural medicines to prevent or treat disease has a prevalence of over 59.12% in Indonesia (Adiyasa & Meiyanti, 2021). Treatment using natural ingredients offers advantages including greater safety, minimal side effects, and readily available ingredients (Sumayyah & Salsabila, 2017). Ginger (*Zingiber officinale*) is a plant widely used by the public. Data from the Central Statistics Agency in South Kalimantan shows ginger is the most widely produced rhizome, reaching 1,967 tons (Badan Pusat Statistik, 2024). Farmers only sell the ginger as raw material, so its utilization is still not optimal. The final ginger product should undergo a series of scientific studies to be supported by scientific data.

Ginger rhizomes are the most widely used part of ginger. Ginger rhizomes contain secondary metabolites, including phenolics, alkaloids, flavonoids, terpenoids, and saponins. These secondary metabolites are potent substances that provide benefits to the body. Ginger has analgesic, anti-inflammatory, antioxidant, and anticancer properties (Ahnafani et al., 2024). Phenolics found in the plant can inhibit cyclooxygenase formation, thereby reducing inflammation and preventing uterine contractions in dysmenorrhea (Elsera et al., 2022). Flavonoids also can inhibit cyclooxygenase and have a relaxant effect, thus reducing contractions in painful or inflammatory conditions (Harianja & Septyani, 2021).

The use of ginger as a herbal medicine requires scientific data support. Characterization of raw materials, such as nutritional content, is essential (Bulgaru et al., 2025). Other characteristics include data on the physical and chemical stability of the product, which will describe its quality during storage (Saputro et al., 2021). Product safety data through toxicity testing is part of the preclinical testing that a product must undergo. This data provides scientific evidence of product safety for long-term use based on biochemical parameters in test animals (Rumaseuw et al., 2022). This study follows up on previous research. The previous study tested pain activity in mice, followed by an acute toxicity test. The results indicated that ginger can relieve pain. Ginger was proven safe based on acute toxicity tests (Dewi et al., 2024). In this study, testing was conducted on human subjects, and subchronic toxicity testing was conducted on test animals.

The novelty of this study is the use of a mixture of red ginger and white ginger from Kalimantan, as both have been shown to have a synergistic effect in relieving pain. The ginger was sourced from Kalimantan to increase the use of locally-based products. This ginger mixture was formulated as an instant powder, so testing was conducted on the final product. This study combined ingredient characteristics, product safety, and pharmacological activity testing. These three aspects comply with regulations for natural product ingredients, covering quality, safety, and efficacy. The objectives of this study were to determine the characteristics, including nutritional value and stability, product safety through toxicity testing, and product efficacy through testing on participants.

MATERIALS AND METHODS

Equipment

The equipment used is an oven (Labolytic), rotary evaporator (Buchi), refrigerator (Samsung), drying cabinet (Local), micropipette (Dragon Lab), analytical balance (Ohaus), spectrophotometer UV-Vis (PerkinElmer), thermometer (Joil), and vortex mixer (Buchi).

Materials

The materials used were red ginger, white ginger, albumin (Merck), glucose (Eralika), Wistar rats (local), sulfuric acid (Merck), sodium hydroxide (Himedia), Lieberman Burchard (Himedia), methanol (Merck), ethanol (Merck), gallic acid (Merck), Folin Cioceltau (Sigma Aldrich), gelatin, ferric chloride (Sigma Aldrich), lead acetate (Eralika), hydrochloric acid (Sigma Aldrich), and Mayer's reagent (Eralika).

Place and Time of The Study

The research was conducted from April to July 2025 at the Laboratory of the Department of Pharmacy, Lambung Mangkurat University, and the Pucuk Sirih Herbal Medicine Company in Banjarmasin.

Research Ethics Test

Before commencing this research, the Health Research Ethics Committee of Poltekkes Banjarmasin conducted an ethical review. The results were deemed ethically sound based on letter No. 210/KEPK-PKB/2025, dated April 17, 2025.

Preparation of Instant Ginger Powder

The red ginger (*Zingiber officinale var. rubrum*) and white ginger (*Zingiber officinale var. officinale*) used came from the Banjar Regency. The rhizomes were used, washed thoroughly with running water, and peeled. The ginger was cut into 1-centimeter pieces. One kilogram of red and white ginger was weighed and blended with 500 mL of distilled water for 1 minute. The ginger was then placed in a large container, and then, 4500 mL of distilled water was added, stirred for 15 minutes, and filtered using sterile gauze until a ginger solution was obtained. Next, the ginger solution was placed in a large pan, and 5 kilograms of granulated sugar were added. The mixture was heated over low heat, stirring until it reduced in size, resulting in instant ginger powder (Dewi et al., 2024).

Nutritional Content Analysis

Total Fat

Total fat content was determined using the Soxhletation method. The sample was wrapped in filter paper, then placed in a Soxhlet apparatus, and a condenser was installed. Reflux was performed for 5 hours until the fat completely dissolved in the collection flask. The solution was then evaporated using a rotary evaporator and a water bath to a constant weight.

Saturated Fat

One gram of the sample was weighed, and 5 mL of sodium hydroxide in methanol was added. The solution was heated in a water bath at 80°C for 20 minutes and then cooled. Borontrifluoride was added to the solution, heated again, and 1 mL of sodium chloride was added. 2 mL of hexane was added and vortexed to form two layers. Measurements were then performed using gas chromatography.

Total Protein

Total protein content was determined using Lowry's solution reagent with bovine serum albumin as a standard. 5 mL of Lowry B was added to 1 mL of the sample, homogenized, and 0.5 mL of Lowry B was added. Then, it was analyzed using a UV-Vis spectrophotometer at 600 nm.

Total Carbohydrates

Total carbohydrates were determined using glucose as a standard solution. 5% phenol was added to the sample solution, homogenized, and 5 mL of concentrated sulfuric acid was added. The solution container was immersed in water for 10 minutes. Analysis was performed using a UV-Vis spectrophotometer at 490 nm.

Physical and Chemical Stability Testing

The physical stability test was conducted by storing the instant herbal formula for three months, followed by analysis at month 0, month 1, month 2, and month 3 based on physical parameters. Physical parameters of the preparation included organoleptic tests, flow properties, homogeneity, and angle of repose of the powder. The chemical stability test was conducted by storing the instant herbal formula for three months, followed by analysis at month 0, month 1, month 2, and month 3. The chemical parameters of the preparation included the active compound content, namely total phenolics, which was analyzed using a UV-Vis spectrophotometer method (Nabilah & Mentari, 2023).

Subchronic Toxicity Testing

Subchronic toxicity testing was conducted for four weeks. In week 0, body weight was measured on all rats, and then five rats were sacrificed to determine liver weight, SGOT, and SGPT levels. Week 0 served as a control group without the instant ginger herbal medicine. The same procedure was performed in weeks 1, 2, 3, and 4, representing the treatment group (Adha, 2023). Forty Wistar rats were used as test subjects. Twenty adult males and twenty females were divided into four groups and given an instant ginger herbal medicine formula. The study was conducted for 28 days. Instant ginger herbal medicine of 100 mg/Kg/BW is given orally using an oral sonde for 28 days. After the 28-day treatment period, surgery was performed on all test animals, and blood and liver samples were taken. Blood samples of 1 mL were taken and then processed to obtain serum, and liver samples were processed into histological slides. SGPT and SGOT levels were measured spectrophotometrically at a wavelength of 340 nm (Dipayana et al., 2024).

Pain Testing Using Visual Analogue Scale (VAS)

Ten women aged 18-20 agreed to participate in the study by completing an informed consent form. These participants experienced mild to severe dysmenorrhea during menstruation. Prior to the treatment, participants were interviewed and asked to describe their dysmenorrhea pain using a Visual Analog Scale (VAS) (Andini & Rahmadiyah, 2022). Participants were divided into two groups of five. Next, two or three days before menstruation, each participant in the treatment group was asked to consume 20 grams of instant ginger in the morning and evening. The instant ginger was dissolved in 200 mL of mineral water. The instant ginger was consumed for 10 days. Patients were then asked to describe the pain they felt each time they experienced dysmenorrhea using a Visual Analog Scale (VAS). These parameters were then compared with those of participants who were not given any treatment.

RESULTS AND DISCUSSION

Results of Nutritional Content Analysis

The instant ginger herbal drink was based on the formulation used in the first year of research. It was then chemically analyzed to determine its nutritional value, including total fat, saturated fat, total protein, and total carbohydrate content. The nutritional analysis results for the instant ginger herbal drink are presented in Table 1.

Table 1. Results of Nutritional Value Analysis of Instant Ginger Herbal Medicine

No	Parameter	Replication 1 (%)	Replication 2 (%)	Replication 3 (%)	Average (%)
1	Total Fat	0,87	0,79	0,84	0,83
2	Saturated Fat	0,13	0,21	0,16	0,17
3	Total Protein	3,65	3,69	3,84	3,73

4	Total Carbohydrate	34,82	36,25	35,18	35,42

The nutritional analysis results in Table 1 show a total fat content of 0.83%. This is very low because ginger generally contains only minimal oil. The additional ingredients contain no fat, so they will not affect the total fat content. Other studies have shown that the fat content in ginger extract reaches 0.65-1.63%. Based on these results, the total fat content of instant ginger herbal drink is within the normal range (Chaerunnisa et al., 2024). Saturated fat content reaches 0.17%, which is very low for a product. For patients with fat or cholesterol problems, this drink will not be a problem. The low-fat intake also results in a low-calorie intake. Instant ginger drink is very different from other packaged drinks containing high-fat milk. This instant ginger drink would be a suitable choice for patients with hyperlipidemia.

Instant ginger herbal drink is known to contain 3.73% protein. Ginger is not a primary source of protein, so the protein content is not particularly high. This protein content is still far from that of other protein sources such as eggs, meat, and nuts. Another study found that ginger protein content ranges from 3.07 to 4.04% (Chaerunnisa et al., 2024). The results in this study fall within the appropriate protein content range. Protein content contributes to the body's nutritional needs. The protein referred to in these results is a general protein, consisting of various amino acids with various functions. Protein functions in growth, metabolism, tissue repair, and enzyme production.

Nutritional analysis showed a high carbohydrate content of 35.42%. This result is higher than other studies, which reported a carbohydrate content of 17.89% in ginger (Chaerunnisa et al., 2024). This difference is because in other studies, only the extract was analyzed, while in this study, instant ginger herbal medicine contains a high amount of sugar. Sugar as an additional ingredient in instant ginger herbal medicine increases the carbohydrate content (Mahbub et al., 2024). Sugar contains glucose, which is a source of carbohydrates. Patients with glucose-related problems such as diabetes mellitus should avoid this drink. Ginger generally contains carbohydrates because the rhizome is the plant's food reserve. Ginger is high in fiber, a form of cellulose that can be extracted during extraction. Carbohydrates serve as the primary energy source for humans.

Results of Physical and Chemical Stability Test

The formulated instant ginger powder was subjected to a three-month stability test and packaged in an airtight container. The results of the physical stability test for the instant herbal medicine are presented in Table 2. Based on organoleptic aspects, stability testing results for instant ginger herbal medicine showed stable physical characteristics after storage for up to three months. Instant ginger herbal medicine has a distinctive odor, sweet taste, yellowish color, and powder form during three months of storage. Organoleptic consistency is crucial because the product will be directly visible to consumers (Afdhaliah et al., 2024). Organoleptic changes over a specific period during storage will result in inconsistent product appearance, thus reducing consumer confidence in the product. The formulated instant ginger powder did not experience degradation of its aromatic components, allowing the ginger aroma to persist over time.

The flowability of a product is the ability of the powder to flow. Flowability is particularly beneficial during automated packaging (Achmadi, 2023). Accurately understanding the powder's flowability can predict the production process. The powder's flowability did not change significantly during storage for up to three months. Instant ginger herbal medicine consistently flowed for seven seconds. A flow time of

less than 10 seconds meets the flowability requirements for powders (Rahayu & Anisah, 2021). This will simplify the product handling and packaging process.

Table 2. Results of Physical Stability Testing of Instant Herbal Medicine

Month	Organoleptic	Flow Properties	Homogenity	Angle of Repose
0	Distinctive Smell Sweet Taste Yellowish Color Powder Form	7,4 second	Homogen	28°
1	Distinctive Smell Sweet Taste Yellowish Color Powder Form	7,6 second	Homogen	28°
2	Distinctive Smell Sweet Taste Yellowish Color Powder Form	7,5 second	Homogen	28°
3	Distinctive Smell Sweet Taste Yellowish Color Powder Form	7,8 second	Homogen	28°

Homogeneity is an indicator of the mixing of each substance in a preparation. Evenly mixed powder can be considered homogeneous (Saristiana et al., 2023). Mixing can be seen from the color and size distribution of the powder. After three months of storage, there was no difference in the homogeneity of the instant ginger herbal medicine. Homogeneity is crucial for a preparation because it indicates the even distribution of active ingredients in a product. The angle of repose is one of the physical parameters of powder preparations. Instant ginger powder consistently had an angle of repose of 28°. This indicates that the preparation is stable and meets the requirements. According to the literature, a good powder has an angle of repose of less than 30° (Manno & Setianto, 2022). Powder particles tend not to stick together, thus reducing the possibility of clumping and preventing the powder from obstructing its flow.

Instant ginger powder was tested for stability using chemical parameters. Testing was conducted based on the total phenolic content of the instant herbal medicine. The results of the chemical stability test of instant ginger herbal medicine are presented in Table 3.

Table 3. Results of Chemical Stability Testing of Instant Ginger Herbal Medicine

Month	Replication 1 (%)	Replication 2 (%)	Replication 2 (%)	Average Total Phenolic Content (%)
0	8,64	8,52	8,72	8,62
1	8,59	8,56	8,66	8,60
2	8,69	8,57	8,59	8,61
3	8,55	8,71	8,65	8,63

Chemical stability testing used total phenolic compounds due to the high phenolic content of ginger. Stability testing was conducted over three months, analyzing total phenolic levels at 0, 1, 2, and 3. The concentration was determined

using a spectrophotometer method with gallic acid as a comparator. The test results showed phenolic levels of 8.60-8.63%. This is a very narrow range for determining concentration, indicating the excellent chemical stability of the instant ginger powder. Chemical stability reflects consistent bioactive compounds, resulting in consistent pharmacological activity (Sadino, 2017).

Consistency is crucial for consumers to benefit from the production, distribution, and use processes. Chemical stability can be influenced by product formulation, storage conditions, contamination, and packaging (Qomara et al., 2023). Properly stored products prevent degradation of active compounds. Powdered preparations are more stable than liquid preparations. This is because the homogeneity of solids is easier to control than that of liquids, which can easily form precipitates. Powders also minimize water content, making them less susceptible to hydrolysis due to the presence of water, as liquids are.

Result of Subchronic Toxicity Test

Subchronic toxicity testing was conducted on instant ginger herbal medicine. The parameters used were rat body weight, liver weight, SGOT levels, and SGPT levels from rat. SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) are generally used as indicators of liver function problems (Widarti & Nurqaidah, 2019). The results of the subchronic toxicity testing on instant ginger herbal medicine are presented in Table 4.

Table 4. Results of Subchronic Toxicity Testing on Instant Ginger Herbal Medicine.

Week	Average Weight of Rats	Average Liver Weight	SGOT Levels (IU/L)	SGPT Level (IU/L)
0	246 g	26,5	76,4	37,3
1	253 g	26,8	78,6	38,2
2	256 g	26,7	78,2	39,4
3	264 g	26,9	79,5	37,2
4	267 g	26,8	78,3	38,6

The test results showed that the rats' weight increased each week from the first to the last week. This indicates no change in appetite in the rats, despite being given the instant ginger herbal medicine. Ginger has other properties, such as boosting immunity, which makes the rats healthy and have a good appetite.

The test results showed that the rats' liver weight consistently remained unchanged between groups. This indicates no liver problems occurred in the rats from the first to the fourth week of administration. The instant ginger herbal medicine did not cause liver problems. Liver problems can be seen in the enlargement of the liver's size and weight (Sijid et al., 2020), resulting from thickening of scar tissue in the liver. This problem did not occur in this study.

SGOT and SGPT levels tended to increase, but remained within normal limits. The parameters used to define normal SGOT and SGPT levels in rats are SGOT (45-100 IU/L) and SGPT (10-50 IU/L) (Prasuma et al., 2024). Another study by Rasyad et al. (2022) showed that administering a combination herbal preparation containing ginger at normal doses for a specific period did not cause toxicity. These findings suggest that long-term use of instant ginger herbal medicine (for 28 days) can be considered safe.

Result of Activity Test

The formulated instant ginger herbal drink, analyzed for nutritional value, stability, and subchronic toxicity, was then tested on adolescent girls. The testing on adolescent girls received approval from the research ethics committee. Prior to testing, patient consent was obtained through an informed consent form. The results of the instant ginger herbal drink testing on adolescents are presented in Table 5.

Table 5. Results of Testing Instant Ginger Herbal Medicine on Women

No	Group	Replication	Visual Analog Scale (VAS)			
			Before	Average	After	Average
1	Control Group	1	8	7,0	9	7,8
		2	7		8	
		3	8		8	
		4	6		7	
		5	6		7	
2	Treatment Group	1	7	7,8	5	4,2
		2	9		4	
		3	8		4	
		4	7		3	
		5	8		5	

Testing was conducted on 10 teenagers aged 18-20 years who experienced dysmenorrhea during each menstrual period. The test subjects were divided into two groups. The normal control group consisted of five adolescents who did not receive instant ginger herbal medicine. The treatment group consisted of five adolescents who received instant ginger herbal medicine. Pain was measured using a Visual Analog Scale (VAS), which is widely used in various studies (Putra et al., 2024). The results showed that in the normal control group, adolescents experienced pain before menstruation, with an average VAS score of 7.2 on a scale of 0-10. During menstruation, these adolescents experienced increased pain, with a VAS score of 7.8. This indicates that adolescents experienced pain during menstruation compared to the normal control group, who did not consume instant ginger herbal medicine.

In the treatment group, those who consumed ginger twice daily before menstruation experienced pain, with a VAS score of 7.8. After consuming instant ginger herbal medicine until the start of menstruation, pain was significantly reduced, with a VAS score of 4.8. Administering instant ginger herbal medicine from before menstruation until menstruation was known to reduce the incidence of dysmenorrhea. According to other research, ginger has analgesic or pain-relieving properties (Jayanti & Winarni, 2024) (Jayanti & Winarni, 2024), thus helping to reduce dysmenorrhea. Ginger has been shown to reduce dysmenorrhea in adolescents.

This study's limitations include the limited number of test subjects used. The researchers did not use a positive control group for comparison. The researchers were limited to a single dose in the subchronic toxicity testing.

CONCLUSION

This study concludes that the instant ginger herbal medicine contains 0.83% total fat, 0.17% saturated fat, 3.73% total protein, and 35.42% total carbohydrates. The stability test results showed no changes in physical and chemical parameters. The results of the subchronic toxicity test showed that the weight of the mice, liver weight, SGOT levels, and SGPT levels of the mice were normal during the test. The

activity test showed a decrease in pain from before to after administration of instant ginger, with an average VAS value of 7.8 to 4.8.

FUNDİNG

This research was supported by funding from the Higher Education Excellence Basic Research Program (PDUPT) of the Ministry of Health's Health Polytechnic. Banjarmasin, Fiscal Year 2025.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the results of this study.

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