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FORMULATION AND ACTIVITY TESTS OF NANOEMULSION OF TURMERIC RHIZOME (CURCUMA LONGA L) EXTRACT FOR *METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS* (MRSA) BACTERIA

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ABSTRACT
Turmeric rhizome *(Curcuma longa* L) contains the main active compound curcumin, which has antibacterial activity which inhibits *Methicillin-Resistant Staphylococcus aureus* (MRSA). Developing a nanoemulsion formula for turmeric rhizome extract can overcome bacterial resistance by protecting the active substance from degradation and blocking the efflux pump in bacteria. This research aims to create a turmeric rhizome extract nanoemulsion formulation and determine the activity of against MRSA bacteria in vitro. Method: Turmeric rhizomes were extracted using the maceration method using 70% ethanol solvent. The technique of nanoemulsion preparation was by using the spontaneous emulsification method with varying extract concentrations of 1.25% (F1), 2.50% (F2), and 3.75% (F3). Evaluation of the physical characteristics of nanoemulsions (particle size, polydispersity index, zeta potential), viscosity, and pH. In vitro activity test formula using the diffusion method against MRSA bacteria. The turmeric rhizome extraction process produces a yield value of 14.3%. The best formula is F1 with a viscosity value of 134.6 ± 21.3 Cps, pH value of 6.34, particle size value of 33.4 ± 12.8 nm, polydispersity index of 0.407 ± 0.01, zeta potential -14.2±2.9 mV. Turmeric extract nanoemulsion can inhibit the growth of Methicillin-Resistant Staphylococcus aureus bacteria with an inhibition zone of 11.00 mm at F3. Conclusion: Curcumin can be formulated in a nanoemulsion system without providing significant changes in organoleptic tests, viscosity, pH, nanoemulsion type tests, particle size, polydispersity index, and zeta potential. Turmeric extract nanoemulsion has an antibacterial effect against MRSA in vitro.

Keywords: Nanoemulsion; MRSA; Turmeric Rhizome

1. INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that is genetically resistant to the antibiotic methicillin as well as various forms of other β-lactam antibiotics such as flucloxacillin, cephalosporins and carbapenems (Wildana et al., 2018). Vancomycin is the current treatment option for MRSA infections. However, the effectiveness of therapy is slower in vitro when compared to β-lactam antibiotics (Salsabil et al., 2021). The nose, throat, and perineum are the primary sites of MRSA colonization (Anggraini et al., 2021), with hands being the most prevalent site at 32.50%, followed by the nose at 17.50%. Notably, the Asia-Pacific region bears a substantial burden of MRSA, with transmission within the hospitals contributing significantly to its prevalence of 23.5%, ranging from 0.7%-10.4% (Budiman et al., 2020; Wong et al., 2018).

Turmeric (*Curcuma longa* L) contains the compound curcumin, which has pharmacological activity in the form of antibacterial. Curcumin has an antibacterial mechanism like other bacterial phenolic compounds, explicitly inhibiting metabolism by damaging the cytoplasmic membrane and denaturing cell proteins (Ramadhani et al., 2018). Curcumin at a concentration of 250 µg/mL was able to kill MRSA. Additionally, turmeric rhizome extract, at concentrations of 5%, 10%, 20%, and 40% can produce inhibitory zone diameters of 11 mm, 13.5 mm, 14.5 mm, and 15 mm against Staphylococcus aureus, respectively (Pangemanan et al., 2016; Teow & Ali, 2015). However, curcumin's susceptibility to crystallization inacidic solutions (pH < 7) and rapid degradation at elevated temperatures of 70 and 90ºC pose significant challenges (Kharat et al., 2017).

The delivery of nanoparticles, particularly for topical preparations, offers a promising approach to overcome bacterial resistance by protecting the active substance from degradation and blocking the efflux pump in bacteria. In addition, nanoparticles can release active substances with a long half-life, so using small doses can provide maximum therapeutic effects (Alabdali et al., 2022; Chamundeeswari et al., 2019). Nanoemulsions, in particular, enhance the stability and effectiveness of the active substances and protect them from environmental factors such as high temperature, pH, and oxygen (O'Sullivan et al., 2017). Moreover, nanoemulsions can prevent particle aggregation compared to conventional emulsions (Saifullah et al., 2016). The mechanism of nanoemulsion in delivering active substances is by combining nanoemulsion globules with the lipid bilayer structure on the bacterial cell membrane so that it can release the active substance, and this combination also damages the lipid membrane of the bacteria (Jiang et al., 2020).

The ratio of oil, surfactant, and water plays a pivotal role in shaping physicochemical properties, stability, and therapeutic efficacy of nanoemulsions, offering a fertile ground for innovation. The loading of curcumin into nanoemulsions was explored using a combination of medium chain triglyceride (MCT), Tween 80, and lecithin as surfactants. The precise ratio of medium chain triglyceride (MCT), Tween 80, lecithin, and water used (10:6:4:80, respectively) produces a particle size of 113.93 nm with a polydispersity index of 0.23 and a zeta potential of -36.23 mV. Furthermore, the synergistic effects of mixed surfactants, exemplified by combinations of Tween 80 and Span 80, were highlighted by Chong et al. (2018) underscoring the importance of leveraging diverse surfactant combinations for enhanced emulsion stability. Non-ionic surfactants, such as Tween 80, serve as a co-surfactant can prevent aggregation and increase pH stability. Surfactant concentration also influences physicochemical stability (particle size, polydispersity index (PDI), surface charge, and curcumin concentration) under the influence of ionic strength, pH, and thermal (Chuacharoen et al., 2019). Additionally, the utilization of virgin coconut oil (VCO) as the oil base presents a novel approach. Its unique composition, featuring oleic acid and medium-chain fatty acids, facilitates a bond with Tween 80, mitigating Ostwald ripening and yielding nanoemulsions with droplets smaller than 100 nm thus opening avenues for further exploration in nanoemulsion formulation design.

2. METHODS

2.1. Tools

Beker glass (Pyrex®), Magnetic stirrer (Thermo Scientific®), Particle Size Analyzer (Malvren®), ultra-turrax (IKA T25 digital®), sonicator, pH meter (Ohauss®) and Viscometer (Brrokfield®).

2.2. Material

Turmeric rhizome simplicia (PT Palapa Muda Perkasa), Methicillin-Resistant Staphylococcus aureus bacteria, tween 80, span 80, methylparaben, glycerin, VCO, Mueller Hinton agar (Oxoid®), Mannitol Salt Agar, paper disk (Macherey Nagel®), Nacl 0 solution .9% (w/v) (Otsu-Ns®), and vancomycin antibiotic disc.

2.3. Extraction Process

Turmeric rhizome was extracted using the maceration method. 600 g of turmeric rhizome simplicia powder was macerated using 6 L of 70% ethanol (1:10) solvent. The maceration was carried out for two days while stirring thrice a day for 2-3 minutes. The macerate was collected and subjected to evaporation and concentration using a rotary evaporator at 70 °C to obtain turmeric rhizome filtrate. Subsequently, to eliminate residual ethanol and water content, the filtrate underwent further treatment in a water bath. The yield of the thick extract was calculated.

2.4. Preparation of Turmeric Rhizome Extract Nanoemulsion

Turmeric extract nanoemulsion will be formulated using a water-in-oil (W/O) system and formulas as outlined in **Table 1**. The oil phase was prepared by dissolving VCO and Span 80, followed byhomogenization using a magnetic stirrer for 15 minutes. Meanwhile, the water phase was made by mixing Tween 80, thick turmeric extract, glycerin, methylparaben, and distilled water. This mixture is homogenized using a magnetic stirrer for 15 minutes. Next, nanoemulsion commences by slowly and homogenously adding the water phase into the oil phase while stirring continuously using a magnetic stirrer for 1 hour. The emulsion particle size was then reduced using an Ultra Turax Homogenizer at a speed of 14,000 rpm within 15 minutes until a stable nanoemulsion was obtained. Subsequently, the nanoemulsions undergo sonication for 30 minutes.

Table 1. Nanoemulsion formula of rhizome tumeric extract

Ingridient	Concentration (%b/v)		
	F1	F2	F3
Rhizome Tumeric Extract	1.25 %	2.50 %	3.75 %
VCO *(Virgin Coconut oil)*	45%	45 %	45 %
Tween 80	1.5 %	2%	2.5 %
Span 80	10%	14.5 %	12.5 %
Glycerine	8%	8%	8%
Methylparaben	0.1 %	0.1 %	0.1 %
Aquadest	20 mL	20 mL	20 mL

2.5. Analysis of particle size, polydispersity index, and zeta potential

The nanoemulsion preparation that has been made was dispersed with aquabidest with a ratio of 1:1.000. Place 2 mL in the cuvette and measured it on the PSA tool to get the particle size, polydispersity index, and zeta potential results.

2.6. Physical Evaluation of Preparations

2.6.1. Organoleptic Test

Organoleptic testing was done visually by observing the preparation's shape, smell, and color.

2.6.2. Test pH

pH measurements were carried out using an electrode pH meter on a digital pH meter dipped in nanoemulsion until the pH meter showed a number at a temperature of 25°C ± 2.

2.6.3. Emulsion Type Test

This test was conducted by dropping the nanoemulsion preparation on a glass slide, then add one drop of methylene blue and observe under a microscope with 40x magnification.

2.6.4. Viscosity Test

A 20 ml sample was placed in a vial. A size 64 spindle with a rotation speed of 100 rpm is selected, then the tool was run. The viscosity value can be determined by observing the analysis results displayed on the Brookfield viscometer screen.

2.7. In Vitro Antibacterial Activity Test

2.7.1. Production of Mueller Hinton agar (MHA) media

Dissolve 19 grams of MHA in 500 ml of sterile distilled water, and heat the mixture on a hot plate until completely dissolved or homogeneous. Sterilize the media in an autoclave at 121 °C for 15 minutes. Pour the solution into a sterile petri dish; allow it to solidify, then invert and store the dish in the refrigerator (Sari et al., 2022).

2.7.2. Preparation of Mannitol Salt Agar (MSA) Agar Tilt Media

Dissolve 10.8 grams of MSA in 100 ml of sterile distilled water and heat it on a hot plate until completely dissolved or homogeneous. Sterilize the media in an autoclave at 121 °C for 15 minutes. Pour the solution into a sterile petri dish; after it hardens, then invert and store the dishin the refrigerator (Pasaribu, 2024).

2.7.3. Bacterial Rejuvenation

Aseptically streak one needle tube of MRSA bacteria onto the surface of the MSA medium in a zig-zag pattern. Incubate the mixture for 24 hours at 37 °C (Octaviani et al., 2022).

2.7.4. Making Bacterial Suspensions

MRSA bacteria rejuvenated with a sterile tube needle were then suspended in a tube containing 5 ml of 0.9% (w/v) NaCl until a turbidity that is the same as the standard turbidity solution of 0.5 Mc was obtained. Farland equals 1.5 x 108 (Colony Forming Unit) CFU/ml. Incubate the suspensions for 24 hours at 37 °C (Kurniawan et al., 2021).

2.7.5. Activity Test of Turmeric Extract Nanoemulsion Preparation

Antibacterial activity test utilizes the disc diffusion method On MHA plates previously inoculated with MRSA bacteria in 5 ml sterile NaCl were equated with MC Farland 108 CFU/ml/Mc Farland no.0.5. Lown's culture is made in MHA media by dipping a cotton swab in NaCl containing MRSA, then culture for 5 minutes to dry. Disc paper was dipped into each nanoemulsion preparation. The disc paper was drained until it did not drip, then left for 30 minutes to absorb the nanoemulsion into the disc. The paper discs were placed on MHA media inoculated with MRSA and then incubated at 37 °C for 18-24 hours. The inhibition zone or clear zone that forms was observed around the paper disc and then measured using a ruler and caliper to observe the diameter of the inhibition zone (Kurniawan et al., 2021).

2.8. Analysis of Results

Data analysis from research on nanoemulsion preparations of turmeric rhizome extract was carried out descriptively, where the data obtained were described and compared to the specification requirements that had been determined. The data obtained will be subjected to comparative analysis using the ANOVA analysis method to determine significant differences between the nanoemulsion preparation formulas. If the results obtained (p Value) are <0.05, it shows that there is a significant difference.

3. RESULTS AND DISCUSSION

3.1. Extraction Process

The development of the nanoemulsion formula for turmeric rhizome ethanol extract starts from extraction. One way to see the effectiveness of extraction is the % yield value. **Table 2** indicates a favorable yield, surpassing the threshold of 11% (Depkes RI, 2017), suggesting a robust extraction process. The greater the yield value of the extract produced, the more efficient the treatment determined and the better the active compound components extracted (Dewatisari et al., 2018).

Table 2. The yield value of turmeric rhizome ethanol extract

Dry Simplicia Weight (Grams)	Condensed Extract Weight (Grams)	% yield
600	86.18	14.3

3.2. Physical Characteristics of Turmeric Rhizome Extract Nanoemulsion

The organoleptic assessment of nanoemulsion formulation is presented in **Table 3**, complemented by visual representation in **Figure 1**. The development of a nanoemulsion formula based on the characteristics of turmeric extract contains non-polar polyphenolic compounds, which are relatively insoluble in water but higher solubility in oil (Subagia et al., 2019). The stability of the nanoemulsion system is based on the interaction of surfactant, cosurfactant, water phase, and oil phase. To ensure biocompatibility and safety, non-ionic surfactants are preferred over ionic ones due to their lower toxicity. There needs to be more than surfactants to reduce the interfacial tension between oil and water. Therefore, cosurfactants are needed to help reduce the interfacial tension. Non-ionic surfactants such as tween and span are widely used for drug delivery systems because of their low toxicity and ability to form stable emulsions on nanocarriers (Gupta et al., 2010; Hua et al., 2018).

VCO is one of the ingredients in the nanoemulsion formulation because it can prevent Ostwald ripening and produce preparations with a droplet size of <100 nm. VCO is an oil that contains 43-53% lauric acid, which is a medium-chain saturated fatty acid (MCFA). A more stable nanoemulsion can be produced using VCO by forming smaller droplet sizes than other MCT oils (Hartini, 2016). The potential for nanoemulsion formation can be done in two ways: spontaneous and non-spontaneous. Spontaneous formation occurs during the mixing process of water and oil phases aided by a magnetic stirrer. Conversely, non-spontaneousl formation necessitates external energy input, typically achieved through a sonicator.

Table 3. Organoleptic observation results

Visualization	F1	F2	F3
Color	Yellow	Yellow	Yellow
Odor	Turmeric	Turmeric	Turmeric

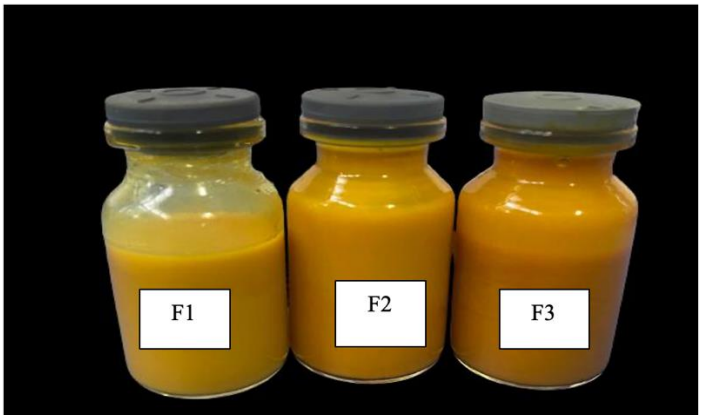

Figure 1. Visualization of Turmeric Rhizome Extract Nanoemulsion Preparations: F1: Nanoemulsion Formula Contains Turmeric Extract 1.25 %; F2: Nanoemulsion Formula Contains Turmeric Extract 2.50 %; F3: Nanoemulsion Formula Contains Turmeric Extract 3.75 %

Figure 2 illustrates the entire nanoemulsion formula, depicting a water-in-oil type emulsion, as evidenced by theblue coloration of water phase. The utilization of methylene blue, awater-soluble dye, aids confirming the emulsion type. After testing, the presence of blue granules throughout the entire nanoemulsion formula corrobates its classification as a sater-in-oil-emulsion. The choice of methylene blue as the dye stems from its intrinsic water solubility, facilitating its dipersion within water phase (Anief, 2019; Hartini, 2016).

3.3. pH and Viscosity Test

Table 4 shows the pH and viscosity values. The pH test determines the degree of acidity or alkalinity of the resulting emulsion. pH measurement is essential in determining the suitability

of topical preparations because the preparations will be applied directly to the skin. Topical preparation must align with the skin's pH to mitigate the risk of skin irritation and esure comfort during its application. Skin's pH divided into some of layer. The pH gradient of the stratum corneum (SC) is characterized by a steady decline, with values around pH 6.8–7.2 near the stratum granulosum (SG), which is the topmost layer of living keratinocytes located just below the SC. This gradient decreases towards the surface of the SC, where the pH ranges between 4.5 and 5.5 (Fukuda et al., 2024). After carrying out a pH test, each formulation has been confirmed to fall within the desired skin pH range, underscoring their safety and compatibility for topical use. Furthermore, the inclusion of excipients in the nanoemulsion preparations is carefully calibrated to maintain a pH that harmonizes the skin's natural pH. This strategic formulation approach ensures that when combined, the excipients contribute to a final pH that is conducive to safe and effective topical application.

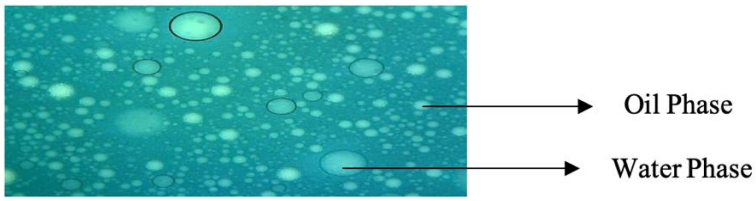

Figure 2. Emulsion type microscope observation (40x magnification)

Table 4. pH and viscosity

Formula	pH	Viscosity (cPs)	*P Value
F1	6.34±0.3	134.6 ± 21.3	
F2	6.36±0.03	154 ± 14.9	>0.05
F3	6.41±0.04	184.6 ± 11.4	

The viscosity test aims to determine the viscosity of the turmeric rhizome extract nanoemulsion. Numerous factors, such as changes in temperature, pH, manufacturing conditions, and the quality and concentration of raw materials, influence the viscosity value. A higher viscosity indicates improved product stability; however, they may also render the preparation challenging to apply and remove from its container. Among the formulations tested (F1, F2, and F3), F3 exhibits superior viscosity value compared to F1 and F2 (**Table 4**). The greater the extract concentration causes an increase in the viscosity of the nanoemulsion preparation. The increase in viscosity is directly proportional to the rise in surfactant because Span 80 surfactant has a dominant effect on increasing density due to the lipophilic chemical nature of Span 80, which tends to bind to the oil phase. It causes the nanoemulsion to be thicker and has a higher viscosity due to the water phase being wrapped in the oil. Nejadmansouri et al. (2016) show that Span 80 affects the viscosity of fish oil nanoemulsions because it interacts more with the oil phase, reducing the fluidity of the droplets and increasing the overall viscosity of the system.

3.4. Characteristics of Turmeric Rhizome Extract Nanoemulsion

Table 5 presents characteristics of turmeric rhizome extract nanoemulsion. The particle size in the nanoemulsion preparation will increase skin penetration (Kaur & Ajitha, 2019). An emulsion is categorized as a nanoemulsion if the particles have a size range of 10-1000 nm (Prakash et al., 2018). Based on **Table 5**, the droplet size of turmeric extract nanoemulsion is included in the nanoparticle category. Particle size influences absorption and release rates. The smaller the particle size formed, the greater the surface area, so the drug will be easily absorbed. However, variations in the extract concentration affect the need for the oil phase to coat the extract droplets. Higher concentrations of extract lead to increased droplet sizes as the oil layer expands to accommodate the extract. Moreover, surfactant concentration also influences physicochemical stability (particle size, polydispersity index (PDI) and surface charge) (Chuacharoen et al., 2019).

Table 5. Characteristics of turmeric rhizome extract nanoemulsion

Formula	Size of Particle (nm)	Polydispersity Index	Zeta Potential (mV)	**P Value*
F1	334.4 ± 12.8	0.407 ± 0.01	-14.4 ± 2.9	
F2	440.4 ± 6.8	0.573 ± 0.01	-7.2 ± 2	>0.05
F3	632.6 ± 24.5	0.305 ± 0.01	-19.1 ± 0.2	

The polydispersity index is a measurement of particles' dispersity quality, indicating whether the particles are monodispersed or polydispersed. A polydispersity index value of less than 0.5 is an ideal condition for nanoparticle formulation because it prevents aggregation (Kaur & Ajitha, 2019). **Table 5** shows nanoemulsion preparations F1 and F3 produce values < 0.5, but F2 has a polydispersity index value > 0.5. It can increase the occurrence of aggregation due to collisions between particles (Danaei et al., 2018).

The zeta potential value will determine the potential for flocculation to occur. It is done to predict the storage stability of the preparation. A high zeta potential will increase the prevention of flocculation because the electro is stable; preferably, a low zeta potential value tends to thicken and be unstable. The zeta potential value of a stable preparation is < -30 Mv and > +30 Mv (Prakash et al., 2018). The zeta potential value of the preparation of the turmeric extract nanoemulsion shows the stability of a system containing dispersed globules through repulsive forces between particles with the same charge when they are close together (**Table 5**).

3.5. Antibacterial Activity Test

The results of the in vitro test of turmeric rhizome extract nanoemulsion against MRSA bacteria suggest that the high concentration of turmeric extract will affect the antibacterial activity produced by the nanoemulsion preparation (**Table 6**).

Table 6. Antibacterial activity test

Group	Mean ±SD
F1	8,17±0,53
F2	9,64±0,81
F3	11,00±0,36

The antibacterial activity of turmeric extract nanoemulsion preparations was tested against MRSA bacteria using the disc diffusion method (Kirby-Bauer). This method is considered more efficient in work and has a lower risk of failure than other test methods. The sensitivity of MRSA bacteria to the turmeric extract nanoemulsion was assessed by measuring the size of the clear zone or inhibition zone formed. The test parameter observed was the clear zone, namely the clear area around the paper disk, as an indication of inhibited growth of microorganisms' excretion of antimicrobial substances by their competitors. The mechanism by which curcumin inhibits MRSA's growth is through binding curcumin to peptidoglycan found in the cell wall membrane. The results of this interaction cause the integrity of the MRSA cell wall to be damaged (Mun et al., 2013). As depicted in **Table 6**, the results highlight that nanoemulsion with a concentration of 3.75% (F3) is the most effective in inhibiting MRSA bacteria and has relatively strong antibacterial power.

4. CONCLUSION

Turmeric rhizome extract nanoemulsion emerges as a promising delivery system to combat MRSA resistance, particularly evident in F3 formulation. This formulation has good particle characteristics and a substantial inhibition zone. This condition is partly influenced by the comparison of the concentrations of span 80 and tween 80 which not only affects the characteristics of the nanoemulsion but also the effectiveness of the formula.

5. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

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ANTIBACTERIAL ACTIVITY OF PURIFIED EXTRACT OF AFRICAN LEAVES (*Vernonia amygdalina Delile*) AGAINST *Escherichia coli*

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ABSTRACT
The African leaf (*Vernonia amygdalina* Delile) is an Indonesian plant used by the community as a traditional medicine. *V.amygdalina* contains active compounds such as flavonoids, tannins, saponins, and steroids which have antibacterial properties. This study aims to determine the antibacterial activity and the most effective concentration of purified extracts of *V.amygdalina* against the Escherichia coli bacteria. *V.amygdalina* simplicia was extracted using the maceration method. Ethanolic extract *V.amygdalina* was tested for the quality of the extract parameters and purified. Then, the purified extract *V.amygdalina* was tested for phytochemical screening and antibacterial activity against Escherichia coli bacteria by well-diffusion with concentrations of 30%, 40%, and 50%. The result of the quality parameter test for ethanolic extract *V.amygdalina* meets the requirements of specific and non-specific parameters. Purified extract *V.amygdalina* positively contains flavonoids, tannins, saponins, and steroids. Antibacterial activity of 0.05% chloramphenicol and purified extract *V.amygdalina* concentrations of 30%, 40%, and 50% against Escherichia coli bacteria had inhibition of 23.42 mm, 8.40± 0.315 mm, 9.44± 0.543 mm, and 11.59 ± 1,640 mm. The produced most effective inhibition was at a concentration of 50%. The results showed that the purified extract of *V.amygdalina* has antibacterial activity in the moderate to strong category, and the most effective concentration against *Escherichia coli* bacteria is the concentration of 50%.

Keywords: African leaves; Purified extract; Antibacterial activity

1. INTRODUCTION

Plants in Indonesia have many uses in people's lives, one of which is used in traditional medicine. Traditional medicine is a concoction of natural ingredients or preparations that can come from plants, minerals, animals, or galenica preparations, or a mixture of ingredients that have been used for generations as a healing medium that can be applied according to the norms prevailing in that society. The advantage of the basic ingredients of a medicinal plant is that the side effects produced are relatively low (Isnawati et al., 2019). One of the plants that can be used in traditional medicine is African leaves. This leaf is better known as the leaf of a thousand diseases, namely a leaf that people believe can be efficacious in treating various diseases (M. Muhammad et al., 2023). African leaves have anti-diabetic activity (Yunitasari et al., 2022), antioxidant (Karlina et al., 2023), treat cholesterol, headaches, fever, throat infections, and cancer, treat malaria, heal wounds, and coughs, are antibacterial, and treat digestive problems (Hasibuan

et al., 2024; Oyeyemi et al., 2018). The various properties of various medicinal plants can be caused by the presence of secondary metabolite compounds (Yunitasari et al., 2022). Based on research, the secondary metabolites found in African leaf plants are flavonoids, alkaloids, terpenoids, glycosides, tannins, and saponins (Ogidi et al., 2019).

Indonesia has a tropical climate, which makes bacteria grow easily, one of which is a type of bacteria that is pathogenic (disease). One example of pathogenic bacteria is Escherichia coli bacteria. This bacterium belongs to the enterobacteria group of gram-negative bacteria which are often found in human digestion, animal digestion, soil, water, and air (Booth, 2018). Apart from that, Escherichia coli is also often found in the human digestive tract such as the large intestine as a microorganism or normal flora which can cause large amounts of salt and water to be lost from the body. Thus, pathogenic bacteria such as Escherichia coli can be the main cause of diarrhea (Riley, 2020). Research on antibacterial tests on African leaves showed that the ethanolic extract of African leaves on the proliferation of Escherichia coli bacteria could provide inhibitory power at an extract concentration of 50% (Habtom & Gebrehiwot, 2019).

The purified extract is an extract that is free from various components of ballast substances (impurity compounds contained in the sample) which can disrupt a matrix (marker compound) in natural materials, disrupting the resulting biological activity (Carolia & Noventi, 2016). The extract purification process aims to obtain various pure extract components that are free from other chemical components that are not needed in the extract such as wax, plasticizer, and fat (Ramadhani & Novema, 2022), in this research purification of the extract was carried out to maintain some of the chemical contents of the extract which have a synergistic effect and can maximize activity in the treatment process. Therefore, this study aims to determine the activity of purified extract of African leaves (*Vernonia amygdalina* Delile) against *Escherichia coli* bacteria.

2. METHODS

2.1. Plant Materials

African leaves obtained from Astana Village, Gunung Jati, Cirebon Regency were collected first. Then, plant determination is carried out to ensure the correctness of the plant parts and types of African plants that will be used in the research. The plant determination process was carried out at the Plant Taxonomy Laboratory, Biology Department, FMIPA, Padjadjaran University. The results of the African Leaf determination are based on the plant determination certificate Number 28/HB/04/2023.

2.2. Preparation of the Extracts

African Simplicia leaf powder is extracted using the maceration method. A total of 600 grams of simplicia powder was put into a closed glass vessel. Add 4.5 L (75 parts) filter liquid in the form of 96% ethanol, cover, and let sit for 3 days, stirring occasionally. After 3 days, the solution was filtered and squeezed, the dregs were washed with 1.5 L (25 parts) filter fluid until a filtrate was obtained. Then cover the container, and leave it in a cool place protected from light for 2 days. After that, concentrate using a rotary evaporator at 40 °C to one-third. Continue evaporation with an electric water bath until a thick extract is obtained (Karlina et al., 2023). Specific parameter testing of African leaf ethanolic extract includes organoleptic tests (odor, texture, and color), water-soluble extract, and ethanol-soluble extract (Kunaedi et al., 2023) while non-specific parameter tests include water content, total ash, acid-insoluble ash, and drying shrinkage test (Depkes RI, 2017). The ethanolic extract of African leaves was purified using n-hexane and distilled water (1:1) (Iryani et al., 2021).

2.3. Qualitative Analysis of Phytochemical Screening

Phytochemical screening includes tests for alkaloids, flavonoids, saponins, tannins, steroids, or triterpenoids. The purified extract of African leaves which had been diluted with distilled water was tested for flavonoids by adding 2 mg of magnesium powder and 3 drops of concentrated

hydrochloric acid. Positive results indicate the appearance of an orange color. Alkaloid test by adding 2-3 drops of Mayer and Dragendorf reagent (Putri et al., 2021). The result is positive if a yellowish-white precipitate forms with Mayer's reagent and a brownish-red precipitate forms with Dragendorf's reagent (Ladeska & Dingga, 2019). The saponin test is carried out by adding 10 mL of hot water, then cooling and shaking vigorously. The presence of saponin If a firm and stable foam forms for no less than 10 seconds and does not disappear when 1 drop of 2N hydrochloric acid is added. tannin test by adding 2-3 drops of 1% iron (III) chloride (Indriaty et al., 2023). The result is positive if a blackish-green color forms. In the steroid or Triterpenoid Test by adding 1 drop of glacial acetic acid and 2 drops of concentrated sulfuric acid, the results obtained are green, indicating the presence of steroids, if a purple color is formed, it indicates the presence of triterpenoids.

2.4. Preparation of Culture Media and Sterilization

2.4.1. Sterilization

The tools to be used are washed thoroughly using detergent and dried. After that, certain tools are covered on top with fatty cotton wrapped in gauze, then wrapped in parchment paper, and tied with mattress thread. For glass beakers, the mouth is only covered with parchment paper and tied with mattress thread, while Petri dishes are only wrapped with parchment. Sterilize using an autoclave at 121°C for 15 minutes.

2.4.2. Nutrient Agar Media

Media in Petri Dishes - Weigh the nutrients so that they are 1.6 grams. Dissolve 80 mL of distilled water in an Erlenmayer, then stir until dissolved. Heat the nutrient agar solution over low heat until clear and homogeneous. Cover with greased cotton. Then wrap it in parchment paper and tie it with mattress twine. Sterilize using an autoclave at 121 °C for 15 minutes.

The Media is Tilted - Weigh the nutrient so that it weighs 0.16 grams. Dissolve with 8 mL of distilled water in an Erlenmeyer flask, and stir until dissolved. Heat the nutrient agar solution over low heat until clear and homogeneous. Then pour the agar medium into a test tube. Sterilize by autoclaving at 121 °C for 15 minutes. After that, tilt it at 10° and let the media solidify (Balouiri et al., 2016).

Bacterial Rejuvenation - Take the ose needle in the flambir until the tip of the needle becomes incandescent, do this 3 times. Then take the Escherichia coli bacteria from the test tube, open the tube cap, and flammable the mouth of the tube. Take 1 dose of Escherichia coli bacteria, then flammable the mouth of the tube and close it again. Inoculate on agar media slanted in a zig-zag manner, cover, and incubate at 37 °C for 24 hours (Chavez-Esquivel et al., 2021).

Bacterial Suspension - Take 3 mL of 0.9% Sodium Chloride using a syringe, then put it in a test tube. Take 3 inocula of Escherichia coli bacteria, then put them in a test tube filled with 0.9% Sodium Chloride. Then the turbidity is seen by comparing the standard turbidity Mc. Farland (Balouiri et al., 2016).

2.5. Antimicrobial Activities

A total of 1 mL of bacterial suspension was inoculated into 80 mL of warm Nutrient Agar media, and shaken gently. The nutrient media is poured into 3 petri dishes of 20 mL each, then wait for 15 minutes (Indriaty et al., 2022). Before printing the holes, first mark the surface of the area at the back of the petri dish with a marker (A = positive control, B = negative control, C=30% concentration of purified extract of African leaves, D=40% and E=50%) (Héjja et al., 2024). After marking, print a hole using a perforator with a diameter of 6 mm which has previously been flammable. Then, the holes in the media were filled with purified extract of African leaves according to the predetermined concentration, positive control, and negative control 20 µl each using a micropipette. After that, leave it for 2 hours, then put it in an incubator at a temperature of 35-37 °C for 18-24 hours. The resulting clear zone around the hole is observed and measured using a caliper (Jarriyawattanachaikul et al., 2016; Karimi et al., 2018).

3. RESULTS AND DISCUSSION

Plant determination carried out at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University, Bandung Number 28/HB/04/2023 showed that the plant part used for research was correct, African leaves from the Asteraceae tribe with the species *Vernonia amygdalina* Delile. The organoleptic test results on African leaf Simplicia have a dark green color with a distinctive, aromatic, and quite pungent odor. The resulting texture is a dry, coarse powder. The organoleptic test results of the ethanolic extract of African leaves have a dark green color, a distinctive aromatic odor that is not too strong and the resulting texture is thick like a paste. Extract standardization testing is carried out to determine the quality parameters of the extract contained in an extract under study by established standards. The results of testing extract quality parameters are in the **Table 1**.

Table 1. Value of specific parameters and non-specific parameters of ethanolic extract

Parameters	Mean±SD %	Standard
Water Content	7.53±0.0055	<12.5%
Water Soluble Extract	17.20±0.0006	>12%
Ethanol Soluble Extract	31.49±0.0055	>8%
Total Ash	6.30±0.0281	<10.2%
Acid-insoluble ash	0.28±0.0015	<0.6%
Dry Shinkage	5.09±0.0052	<10%

The results of the phytochemical screening test for purified extracts of African leaves were positive for containing active compounds of flavonoids, saponins, tannins, and steroids. Based on the inhibition zone classification, the antibacterial activity test of the purified extract of African leaves has a clear zone in the moderate to strong category (Sanam et al., 2022). At concentrations of 30%, 40%, and 50%, it has antibacterial activity against Escherichia coli bacteria with the resulting inhibitory power being 8.40 ± 0.315 mm; 9.44 ± 0.543 mm; and 11.59 ± 1,640 mm. The most effective inhibitory power of the purified extract of African leaves is at a concentration of 50% with an average inhibitory power of 11.59 ± 1,640 mm. Antibacterial activity of purified extract of African leaves against Escherichia coli bacteria in **Figure 1**.

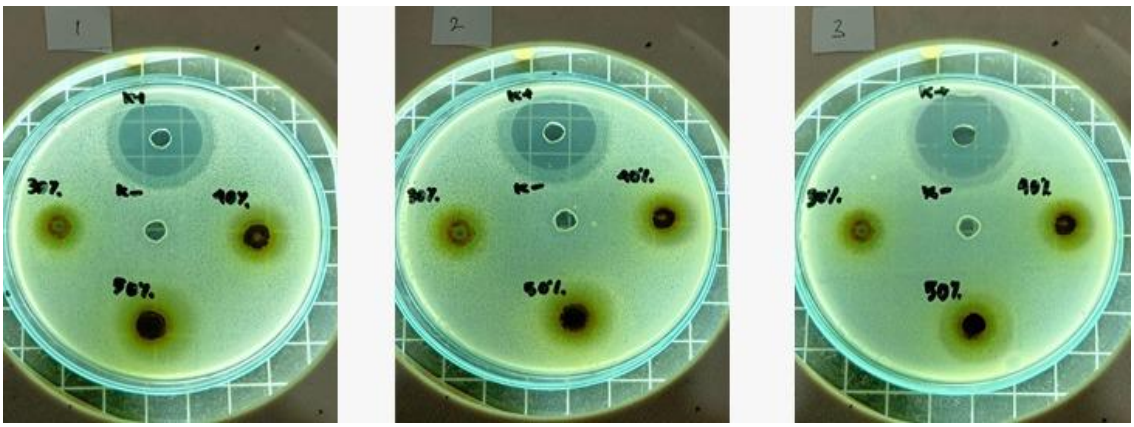

Figure 1. Antibacterial activity of purified extract of african leaves against *Escherichia coli*

Based on the data obtained, it shows that the ethanolic extract of African leaves has met various extract quality parameter requirements including water content, water-soluble essence content, ethanol-soluble essence content, total ash content, acid-insoluble ash content, and drying loss. Standardization of extract water content in African leaf extract was carried out using a tool in the form of MAB (Moisture Analyzer Balance). The water content results obtained from research that has been carried out are 7.53±0.0055%. The requirements for good extract quality parameters for African leaf extract according to the (Depkes RI, 2017) are no more than 12.5%.

This shows that the ethanolic extract of African leaves has water content that meets the requirements, the result of which is no more than 12.5%. The purpose of determining the water content in extracts is to provide a minimum limit or range for the amount of water content in a sample, where the higher the water content in an extract, the easier it is for fungi or mold to grow, thereby reducing the biological activity of the extract during the storage period.

Testing the levels of water-soluble essence and the levels of ethanol-soluble essence aims to determine the number of compounds dissolved in water (polar) and ethanol (semi-polar-non-polar in nature). In this study, the results of the water-soluble essence from African leaf extract were 17.20±0.0006%, while the ethanol-soluble essence was 31.49±0.0055%. The quality parameter requirements for water-soluble essence content and ethanol-soluble essence content are that the water-soluble essence content is not less than 12% and the ethanol-soluble essence content is not less than 8%. This shows that the extract quality parameters of African leaf extract are more soluble in ethanol than in water because African leaf extract has active compounds that are more likely to be easily dissolved in ethanol than water. After all, ethanol is a universal solvent so it can attract polar compounds and non-polar while water is only able to attract polar compounds.

The ash content testing carried out included total ash content with the results obtained in the research carried out being 6.30±0.0281%, while the acid-insoluble ash content was 0.28±0.0015%. According to the (Depkes RI, 2017), the requirement for a good total ash content for African leaf ethanolic extract is no more than 10.2%, while the acid-insoluble ash content is no more than 0.6%, so testing the ash content of the leaf ethanolic extract Africa has met the requirements. Determination of total ash content is carried out to determine the amount of internal and external mineral content remaining after the washing process which can come from the beginning to the end of the extract-making process. Meanwhile, determining the acid-insoluble ash content aims to evaluate the extract for contamination by materials containing silica such as soil and sand contained in the extract studied. Drying loss in testing the quality parameters of African leaf extract which was carried out was 5.09±0.0052%. The requirement for good extract drying shrinkage is <10%. Determination of drying loss is carried out to show how many compounds contained in the extract have been lost or easily evaporated during the drying process. Drying loss is a parameter for an extract to maintain quality to avoid fungal growth (Utami et al., 2022).

Based on research data from antibacterial activity tests, shows that all the wells in the samples containing purified extracts of African leaves have clear zones in the medium to strong category. The inhibitory power produced by the purified extract of African leaves is due to the secondary metabolite compounds contained in the purified extract of African leaves. These metabolite compounds include flavonoids, saponins, tannins, and steroids. Flavonoids act as antibacterials by denaturing bacterial cell proteins, damaging bacterial cell membranes, and inhibiting energy metabolism in bacteria so that bacterial growth will be hampered and disrupted (Gutiérrez-Venegas et al., 2019). Saponin has a role as an antibacterial with its mechanism of action, namely reacting with porins (trans-membrane proteins) on the outer membrane of bacterial cell walls, forming strong polymer bonds resulting in damage to porins which are the gates for the entry and exit of compounds which will reduce the permeability of bacterial cell membranes and result in cell Bacteria will experience a lack of nutrition, so that bacterial growth is hampered or dies (Alina et al., 2023). Then, tannins can also act as antibacterials by binding to the proteins of bacteria by contracting the cell walls so that permeability and cell wall formation will be hampered. Steroids can act as antibacterials whose mechanism of action is to interact with the phospholipid membrane of bacterial cells which are permeable to lipophilic compounds this causes the integrity of the cell membrane to decrease and the morphology of the cell membrane to change into cells that can easily become brittle and lyse (Othman et al., 2019).

4. CONCLUSION

A purified extract of African leaves (*Vernonia amygdalina* Delile) at concentrations of 30%, 40% and 50% has antibacterial activity against Eschericia coli bacteria with the resulting inhibitory power of 8.40 ± 0.315 mm; 9.44 ± 0.543 mm; and 11.59 ± 1.640 mm. The most effective inhibitory power of the purified extract of African leaves is at a concentration of 50% with an average inhibitory power of 11.59 ± 1.640 mm. The higher the concentration of purified extract of African leaves, the greater the inhibitory power of bacterial growth due to the increasing content of bioactive bacteria inhibitors. Further research is needed on African leaves using other extraction methods and bacteria.

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Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Competing Interests

The authors declare no competing interest.

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ANALYSIS OF POTENTIAL DRUG INTERACTIONS IN PNEUMONIA PATIENTS AT GUNUNG JATI REGIONAL HOSPITAL INSTALLATIONS, CIREBON

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ABSTRACT
Pneumonia patients with complex diseases require a variety of drugs so the possibility of drug interactions also increases. This study aims to determine patient characteristics, potential drug interactions, severity, description of the type of drug and number of interacting drugs, as well as the relationship between patient characteristics and drug interactions. This type of research is a retrospective analytical descriptive study. The research sample was the medical records of patients diagnosed with pneumonia who met the inclusion criteria. Determination of drug interactions using the Medscape application. The analysis uses a correlation test to determine the relationship between the number of drugs and comorbidities with potential drug interactions. The characteristics of patients based on age are 46-65 years (36%), the majority of gender is male (52%), the number of drugs most frequently used is ≥ 5 types of drugs (94%), the predominant comorbidity is asthma (19.3%). The number of potential drug interactions occurred in 60 patients (67%) out of a total of 90 pneumonia patients. The highest level of severity of potential drug interactions was 55.2%. The type and number of drugs that interacted most were dexamethasone with omeperazole (13.2%). The results of the Chi Square correlation test obtained a significance value of 0.00 where the significance result was <0.05, so it was concluded that the number of drugs used and comorbidities with drug interactions was correlated with an R value of 0.477, which means the correlation between the number of drugs used and comorbidities has a moderate level of correlation.

Keywords: Drug Interactions; Pneumonia; Medical records

1. INTRODUCTION

Pneumonia is a serious infection that can cause death if not properly treated. Pneumonia infection attacks the lungs at the end of the bronchioles and alveoli caused by invasion of pathogens such as bacteria, viruses, fungi and parasites. Pneumonia is prone to attack toddlers and the elderly, characterized by complaints such as fever, chills, cough with phlegm, or shortness of breath (Erviana, 2018).

The incidence of pneumonia is also high. According to data from the WHO (*World Health Organization*), 156 million cases of new pneumonia were found worldwide, and 61 million cases occurred in Southeast Asia. In 2015-2018, it was found that the incidence of pneumonia in children under five had increased by 3% from 94.12% to 97.30% (Azyenela et al., 2022). Based on data from the Cirebon District Health Service in 2020, pneumonia was found in 56.7% of children under five, namely 10,185 cases (Dinkes Cirebon, 2020). In children aged 29 days to 11 months, pneumonia is the main problem, resulting in 73.9% of deaths (Kemenkes RI, 2006).

The high morbidity and mortality rates of pneumonia in various countries have influenced the determination of patient care strategies. Correct treatment can determine the success of

pneumonia treatment. Antibiotics, which aim to kill bacteria, are the main therapy for treating pneumonia. Apart from antibiotic therapy, patients receive various other supportive treatments to achieve therapeutic success. Drug interactions occur due to the large number of treatments given to pneumonia patients (Azyenela et al., 2022).

Drug interactions are events in which changes in the pharmacokinetics and pharmacodynamics of a drug are caused by the influence of another drug. Patients with pneumonia have complex diseases that require a variety of medications. When a patient receives more drugs, the likelihood of drug interactions also increases (Erviana, 2018). According to Fatin and Pasha (2021), the potential for pneumonia drug interactions in one hospital in Bandung City was 480 interactions consisting of 5 minor interactions (1.04%), 214 moderate interactions (44.58%) and 261 major interactions (54.38%). According to Sari et al. (2019), his research found that there were 13 interactions (81.25%) between combinations of antibiotics and non-antibiotics in pneumonia patients at the Inpatient Hospital of Dr. Soedarso Pontianak with interaction severity levels major (25%), moderate (18.75%), and minor (37.50%). According to Farida (2022), of the 28 patients, there were 17 types of drugs that could cause drug interactions in pneumonia patients at Dr. Soedono Madiun. Previous studies have examined the relationship between drug interactions and the number of drugs used, but no research has been conducted regarding the correlation between drug interactions and comorbidities, especially pneumonia.

This study aimed to determine patient characteristics (gender, age, number of drugs, and comorbidities), description of the number of potential drug interactions, severity of drug interactions, description of the type and number of interacting drugs, and relationship between the number of drugs used and potential comorbidities. Drug interactions in pneumonia patients at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022.

2. METHODS

This was a non-experimental study with a retrospective data collection. This study was conducted from January to June 2023 at the Inpatient Installation at RSD Gunung Jati Cirebon. The population in this study comprised inpatients diagnosed with pneumonia at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022. Sampling was carried out by total sampling, taking all inpatients diagnosed with pneumonia at RSD Gunung Jati Cirebon in 2022 who met the inclusion criteria. The medical records used in this study included 90 patients. The inclusion criteria were as follows: medical records of patients with a primary diagnosis of pneumonia with or without comorbidities at the Inpatient Installation of RSD Gunung Jati Cirebon in 2022; medical records of pneumonia patients who received 2 or more types of drugs in the patient's treatment while inpatient; patients who have complete medical records, including: gender, age, diagnosis, name and number of drugs used, and comorbidities. The exclusion criteria in this study were patients' medical records that could not be read because they were damaged. The course of research: research permits and code of ethics, determination of sample size; data collection includes age and gender, diagnosis of disease, medicine name; processing and analysis of data. Data analysis in this study used descriptive analysis to examine patient characteristics, potential drug interactions, the severity of potential drug interactions (contraindicated, serious, monitor, or minor), and determine the description and types of drugs that interact with drug use in patients with pneumonia. The results of the analysis were in the form of a description of potential interactions in the form of percentages and tables. A Chi Square correlation test was also carried out to determine the relationship between the number of drugs and comorbidities and the potential for drug interactions in pneumonia patients at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022. This research received an Ethical Exemption certificate with No.014/LAIKETIK/KEPPKRSGJ/IV/2023 from RSD Gunung Jati Cirebon.

3. RESULTS AND DISCUSSION

The total sample in this study comprised 90 medical records of pneumonia patients at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022 who met the inclusion criteria. The data were collected in the form of patient characteristics such as age, gender, patient's main diagnosis, comorbidities, and data on medications received by the patient.

3.1. Patient Characteristics

Based on the results of this research, the characteristics of pneumonia patients at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022 are shown in **Table 1**.

Table 1. Characteristics of pneumonia patients in the RSD inpatient installation Gunung Jati Cirebon in 2022

Patient Characteristics	Group Variables	Number of Patients	Percentage (%)
Age	0-5 years	12	12%
	6-11 years	1	1%
	12-25 years	3	3%
	26-35 years old	4	4%
	36-45 years old	10	11%
	46-65 years old	36	40%
	>65 years	25	28%
	Total	**90**	**100%**
Gender	Man	47	52%
	Woman	43	48%
	Total	**90**	**100%**
Amount of medication used	2-4 drugs	5	6%
	≥ 5 drugs	85	94%
	Total	**90**	**100%**
Comorbidities	Patients with comorbidities	85	94%
	Patients without comorbidities	5	6%
	Total	**90**	**100%**

Based on **Table 1** of 90 patients with pneumonia, the results showed that patients in the age range of 46-65 years and those aged over 65 years were the largest group diagnosed with pneumonia. This is in accordance with the statement from Farida & Soleqah (2016) that in old age there are changes in anatomy and physiology and a decrease in body endurance. Anatomical and physiological changes resulting from the aging process have important consequences for the functional reserve of the lungs, ability to overcome decreased lung compliance, and increased airway resistance to infection. The next most frequent pneumonia patients were patients aged 0-5 years. This is caused by the body's immunity not being fully developed, the airways being rather narrow, and the high prevalence of pathogenic bacteria in the nose and throat area. In addition, malnourished children are 6.25 times more likely to get pneumonia than well-nourished children (Efliana et al., 2016).

52% more men were diagnosed with pneumonia than women. This is because the size of the respiratory tract of men is smaller than that of women, and the immune system of women is higher than that of men. According to Efliana et al. (2016) it was found that too much testosterone in men can reduce the immune response. Therefore, men are more susceptible to the disease than women are. In addition, pneumonia patients are dominated by male patients because pneumonia deaths are related to smoking. Long-term exposure to cigarette smoke in healthy adults can increase the risk of lung disease, bronchitis, and pneumonia (Farida & Soleqah, 2016).

Of the 90 patients diagnosed with pneumonia in 2022, treatment patterns were grouped into 2 based on the number of drugs received, namely 2-4 drugs and ≥5 drugs. From the research results, it is known that 6% of patients who receive 2-4 drugs and 94% of patients receive ≥ 5 drugs. When a patient receives many drugs (polypharmacy), the potential for drug interactions also increases (Reyaan et al., 2021).

The severity of pneumonia is influenced by comorbidities. The results of this study revealed that the patient had multiple diseases at the same time. Comorbidities in pneumonia patients cause a large number of drugs to be received by patients because, apart from prescribing drugs for the main disease, patients also receive drugs for comorbidities that may worsen the patient's health condition. As shown in **Table 2**, the dominant comorbidity was asthma, namely 28 patients (19.3%).

Table 2. Distribution of patients based on comorbidities

Comorbidities	Number of patients	Percentage (%)
Asthma	28	19.3%
Post Tuberculosis Obstruction Syndrome	17	11.7%
Dyspepsia	8	5.5%
Diabetes	7	4.8%
Pleura effusion	7	4.8%
Anemia	6	4.1%
Chronic Obstructive Pulmonary Disease (COPD)	5	3.4%
Chrinic Kidney Disease (CKD)	5	3.4%
Acute Respiratory Failure	5	3.4%
Hyponatremia	5	3.4%
Congestive Heart Failure (CHF)	5	3.4%
Malnutrition	3	2.1%
Hyperglycemia	3	2.1%
Hypocalemia	3	2.1%
Feeding difficulties	3	2.1%
Hypoalbumin	3	2.1%
Diarrhea	3	2.1%
Coronary Artery Disease (CAD)	2	1.4%
Stroke	2	1.4%
Hypertension	2	1.4%
Atrial fibrillation	2	1.4%
Human Herpes Virus (HHV)	2	1.4%
Gastroenteritis	2	1.4%
Hypertensive Heart disease (HHD)	2	1.4%
Radiculophaty Lumbal	1	0.7%
Generalized Anxiety Disorder (GAD)	1	0,7%
Hemophilia	1	0.7%
Hipoglycemia	1	0.7%
Hypercalemia	1	0.7%
Low intake	1	0.7%
Syndrom nefrotic	1	0.7%
Hepatic failure	1	0.7%
Bronchitis	1	0.7%
Coronary artery fistula	1	0.7%
Syok septic	1	0.7%
Hypotiroid	1	0.7%
Tumor Paru	1	0.7%
Parkinson	1	0.7%
Epilepsy	1	0.7%
Total	145	100%

3.2. Identify Potential Drug Interactions

Based on the results of research on pneumonia patients at the Inpatient Installation at Gunung Jati Hospital, Cirebon in 2022, of 90 patients, 60 (67%) experienced drug interactions,

while 30 (33%) did not experience drug interactions. Patients with pneumonia are at high risk of experiencing drug interactions because of the large number of drug therapies given. Because patients with pneumonia usually have accompanying or comorbid diseases, they usually receive 5 or more types of medication (polypharmacy). The results of this research are in accordance with the results of other studies (Fatin & Pasha, 2021), which stated that the number of patients with potential drug interactions in pneumonia patients in one of the Bandung City hospitals was 195 (48.51%) out of 402 patients analyzed. The number of potential drug interaction incidents among patients with pneumonia is shown in **Table 3**.

Table 3. Description of potential drug interaction events

Criteria	Number of Patients	Percentage (%)
Number of patients with potential drug interactions	60	67%
Number of patients who have no potential for drug interactions	30	33%
Total	**90**	**100%**

3.3. Description of the Severity Level of Potential Drug Interactions

Drug interactions can be classified according to their severity. The severity of drug interactions is divided into 4 categories: contraindicated, serious, monitored, and minor. Based on the results of research at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022, the results showed that the severity of potential drug interactions that occurred most frequently were drug interactions in the monitor category, with an incidence of 75 drug interactions (55.2%). Monitor interactions are drug interactions that have potential effects that may occur and require monitoring or medical treatment. The next highest level of severity of drug interactions was minor, with 46 interactions (33.8%). Drug interactions are included in the minor category if the effects are mild and do not cause a change in therapy (Reyaan et al., 2021). The severity is minor, and the effects are not disturbing or significantly visible; therefore, no special management is required (Agustina et al., 2023).

The level of severity of the interactions that occurred the least was the serious category of drug severity, with an incidence of 15 interactions (11%). Serious severity can potentially be life threatening or cause permanent damage (Agustin & Fitrianingsih, 2021). The results of this study did not identify any contraindication level drug interactions. Contraindication interactions have the highest level of drug interaction severity, which requires close monitoring of undesirable clinical effects if both drugs are given simultaneously (Fitriani et al., 2016). A description of the Severity Level of Potential Drug Interactions is shown in **Table 4**.

Table 4. Description of the severity level of potential drug interactions

Criteria	Number of Interactions	Percentage (%)
Contraindications	0	0%
Serious	15	11%
Monitors	75	55.2%
Minor	46	33.8%
Total	**136**	**100%**

The number of disease diagnoses given to patients will increase the number of drugs given for therapy. This increases the potential for drug interactions to occur during therapy. By administering three or more drugs, it is possible that potential drug interactions will arise during therapy (Bhagavathula et al., 2014). Based on **Table 4**, the most common severity of drug interactions is the monitor category with an incidence of 75 drug interactions (55.2%). Monitor interactions are drug interactions that have the potential to provide effects that may occur and

require monitoring or medical treatment. The next most common severity of drug interactions was minor interactions with 46 (33.8%). A drug interaction is categorized as minor if the effect is mild and does not cause a change in therapy (Reyaan et al., 2021).

The least severity of interaction was the serious category of drug severity with an incidence of 15 interactions (11%). Serious severity has effects that can be potentially life-threatening or can cause permanent damage (Agustin & Fitrianingsih, 2021). The results of the study did not find any contraindication level drug interactions. Contraindicated interactions are the highest severity level of drug interactions that require close monitoring of undesirable clinical effects when the two drugs are administered simultaneously (Fitriani et al., 2016).

3.4. Description of the Type and Amount of Interacting Drugs

It was found that there were 60 pneumonia patients (67%) who experienced drug interactions at the RSD Gunung Jati Cirebon Inpatient Installation in 2022, here are the 3 most frequent drug interactions at the Gunung Jati RSD Cirebon Inpatient Installation in 2022.

3.4.1. Dexamethasone and Omeprazole

The concomitant use of dexamethasone and omeprazole may decrease the levels or effects of omeprazole by affecting the hepatic/intestinal metabolism of CYP3A4 (Medscape). Based on the research results, the most frequent drug interactions occurred with the use of dexamethasone and omeprazole with 18 interactions (13.2%) with minor drug interaction severity.

3.4.2. Dexamethasone and Levofloxacin

The concomitant use of dexamethasone and levofloxacin increases the risk of tendon rupture (Medscape). Based on the research results, there were 12 drug interactions in the use of dexamethasone and levofloxacin (8.8%), with the severity of drug interactions monitored.

3.4.3. Aminophylline and Omeprazole

The concomitant use of aminophylline and omeprazole increases the toxicity of aminophylline and may decrease aminophylline levels or effects by affecting CYP1A2 liver enzyme metabolism (Medscape). There were 10 drug interactions in the use of aminophylline and omeprazole (7.4%), with minor drug interactions.

An overview of the types, numbers, and interactions of pneumonia patients at the Gunung Jati RSD Cirebon Inpatient Installation in 2022 is shown in **Table 5**.

3.5. Relationship Between Number of Drugs Used and Concomitant Diseases

Polypharmacy or the simultaneous use of more than or the same 5 types of drugs can increase the risk of interactions between drugs. Polypharmacy can cause drug interactions, side effects, and problems, thereby affecting patient clinical outcomes. Polypharmacy is classified into 2 categories: minor and major polypharmacy. Minor polypharmacy is a medication sheet containing 2-4 medications, while major polypharmacy is a medication sheet containing ≥ 5 medications (Reyaan et al., 2021). Polypharmacy can be detrimental to children because of their limited physiological conditions (Getachew et al., 2016). The greater the number of drugs used, the greater is the potential number of drug interactions. The relationship between the number of drugs used and drug interactions in patients with pneumonia at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022 is shown in **Table 6**.

The potential for drug interactions increases with the increase in comorbidities in patients diagnosed with pneumonia. Pneumonia with comorbidities has a high potential to cause drug interactions because if there are comorbidities in patients with pneumonia, additional drug therapy is required. If the more drugs given to a patient, the greater the possibility of drug interactions occurring. So, it can be concluded that the large number of drugs and the presence of comorbidities in pneumonia patients have an influence on the potential for drug interactions. The relationship between comorbidities and drug interactions in pneumonia patients at the Inpatient Installation at RSD Gunung Jati Cirebon in 2022 can be seen in **Table 7**.

Table 5. An overview of the types and numbers and interactions of pneumonia patients at the Gunung Jati RSD Cirebon inpatient installation in 2022

No.	Interacting Drugs		Severity level	Amount	Percentage
1.	Alprazolam	Codein	Monitor	1	0.7%
2.	Amikacin	Thiamine	Minor	1	0.7%
3.	Aminophylline	Dexamethasone	Monitor	7	5.1%
4.	Aminophylline	Metilprednisolon	Monitor	1	0.7%
5.	Aminophylline	Omeprazole	Minor	10	7.4%
6.	Atorvastatin	Azitromycin	Monitor	1	0.7%
7.	Atorvastatin	Metilprednisolon	Monitor	1	0.7%
8.	Azitromycin	Cetirizine	Monitor	1	0.7%
9.	Azitromycin	Bicnat	Monitor	1	0.7%
10.	Bicnat	Concor	Monitor	1	0.7%
11.	Candasertan	Concor	Monitor	1	0.7%
12.	Candasertan	Gentamicin	Monitor	1	0.7%
13.	Candasertan	Spironolactone	Monitor	1	0.7%
14.	Ceftriaxone	Furosemide	Minor	1	0.7%
15.	Clobazam	Cetirizin	Monitor	1	0.7%
16.	Clobazam	Codein	Monitor	1	0.7%
17.	Clobazam	Codein	Minor	1	0.7%
18.	Clopidogrel	Meloxicam	Monitor	1	0.7%
19.	Clopidogrel	Topamax	Monitor	1	0.7%
20.	Concor	Miniaspi	Monitor	1	0.7%
21.	Concor	Nicardipine	Monitor	1	0.7%
22.	Concor	Spironolactone	Monitor	1	0.7%
23.	Dexamethasone	Ciprofloxacin	Monitor	1	0.7%
24.	Dexamethasone	Furosemide	Minor	3	2.2%
25.	Dexamethasone	Levofloxacin	Monitor	12	8.8%
26.	Dexamethasone	Lansoprazole	Minor	1	0.7%
27.	Dexamethasone	Metronidazole	Monitor	1	0.7%
28.	Dexamethasone	Omeprazole	Minor	18	13.2%
29.	Dexamethasone	Ondansetrone	Monitor	9	6.6%
30.	Digoxin	Furosemide	Monitor	1	0.7%
31.	Digoxin	Ketorolac	Monitor	1	0.7%
32.	Digoxin	Lansoprazole	Monitor	1	0.7%
33.	Digoxin	Levofloxacin	Serius	1	0.7%
34.	Digoxin	Moxifloxacin	Monitor	1	0.7%
35.	Digoxin	Omeprazole	Serius	2	1.5%
36.	Digoxin	Omeprazole	Monitor	3	2.2%
37.	Digoxin	Sucralfate	Serius	2	1.5%
38.	Digoxin	V-Block	Monitor	1	0,7%
39.	Furosemide	Metilprednisolon	Minor	2	1.5%
40.	Furosemide	V-Block	Monitor	1	0.7%
41.	Isoniazid	Pyrazinamide	Minor	1	0.7%
42.	Ketorolac	Furosemide	Monitor	1	0.7%
43.	Ketorolac	Levofloxacin	Monitor	1	0.7%
44.	KCI	Vascon	Monitor	1	0.7%
45.	Lansoprazole	Metilprednisolon	Minor	2	1.5%
46.	Levofloxacin	Metilprednisolon	Monitor	1	0.7%
47.	Levofloxacin	Ondansetron	Serius	9	6.6%
48.	Miniaspi	Furosemide	Monitor	1	0.7%
49.	Miniaspi	Levemir	Monitor	2	1.5%
50..	Miniaspi	Metilprednisolon	Minor	1	0.7%
51.	Miniaspi	Ramipril	Serius	1	0.7%
52.	Miniaspi	Ramipril	Monitor	1	0.7%

No.	Interacting Drugs		Severity level	Amount	Percentage
53.	Miniaspi	V-Block	Monitor	1	0.7%
54.	Omeprazole	Neurobion	Minor	1	0.7%
55.	Omeprazole	Ciprofloxacin	Monitor	1	0.7%
56.	Ramipril	Furosemid	Minor	1	0.7%
57.	Ramipril	KSR	Monitor	1	0.7%
58.	Ramipril	Spironolacton	Monitor	2	1.5%
59.	Sentraline	Codein	Minor	1	0.7%
60.	Sentraline	Codein	Monitor	1	0.7%
61.	Sentraline	Loratadine	Minor	1	0.7%
62.	Simvastatin	Metilprednisolon	Serius	1	0.7%
63.	Simvastatin	Metilprednisolon	Monitor	2	1.5%
64.	Sucralfate	Lansoprazole	Minor	1	0.7%
66.	Sucralfate	Moxifloxacin	Monitor	1	0.7%
67.	Sucralfate	Vitamin D	Minor	1	0.7%
	Total			**136**	**100%**

Table 6. Relationship between the number of drugs used and drug interactions

Potential Event	Concomitant Diseases			
	There is		There isn't any	
	Amount	Percentage	Amount	Percentage
Interaction Occurs	57	67%	3	60%
No Interaction Occurs	28	33%	2	40%
Total	**85**	**100%**	**5**	**100%**

Table 7. Relationship between comorbidities and drug interactions

Potential Event	Concomitant Diseases			
	There is		There isn't any	
	Amount	Percentage	Amount	Percentage
Interaction Occurs	57	67%	3	60%
No Interaction Occurs	28	33%	2	40%
Total	**85**	**100%**	**5**	**100%**

The results of the Chi Square correlation test obtained a significance value of 0.00 where the significance result was < 0.05, so it was concluded that the number of drugs used and comorbidities with drug interactions was correlated with an R value of 0.477, which means the correlation between the number of drugs used and comorbidities has a moderate level of correlation. The relationship between the number of drugs used and comorbidities with drug interactions using the Chi Square correlation test can be seen in the **Table 8**.

Table 8. The relationship between the number of drugs used and comorbidities with drug interactions using the Chi Square Correlation Test

	Value	Df	Asymptotic Significance (2-sided)	R	Sig. F Change
Chi Square correlation test				.477	.000

Predictors: (Constant), Comorbidities, Number of drugs used

4. CONCLUSION

Based on the results of this study, from 90 patient medical records, the results of the most patient characteristics based on age were 46-65 years (36%), the most male gender (52%), the greatest number of drugs used was ≥ 5 types of drugs (94%), the most comorbidities were asthma (19.3%). The number of potential drug interactions occurred in 60 patients (67%). The highest severity of potential drug interactions was 55.2%. The type and number of drugs that interacted

the most was dexamethasone with omeperazole (13.2%). Chi Square correlation test results showed that there was a significant relationship between the number of drugs used, the type of comorbidities with drug interactions.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

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COMPARISON OF PHENOLIC CONTENT OF RED GINGER (*Zingiber officinale var. rubrum* Theilade) AT DIFFERENT GROWING LOCATIONS

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ABSTRACT
Red ginger (*Zingiber officinale* var. *rubrum* Theilade) as a traditional medicine can be used as anti-inflammatory, analgesic, antipyretic, lower cholesterol, prevent impotence, depression, and others. The rhizome of the red ginger plant has different phenolic levels in each region. The locations were chosen due to differences such as altitude, soil type, rainfall, temperature at the planting site, and harvest age of red ginger. To find out the comparison of the levels of these compounds, an examination was carried out with UV-Vis's spectrophotometry. The purpose of this research was to determine the comparison of phenolic content of red ginger from different growing locations, namely from Wonosobo Regency and Karanganyar Regency. This research used samples of red ginger extract (*Zingiber officinale* var. *rubrum* Theilade), as much as 500 grams of red ginger simplisia extracted using maceration method with 96% ethanol solvent in ratio of 1:5. Quantitative analysis phenolic content using UV-Vis's spectrophotometry methode. The results of quantitative tests using UV-Vis's spectrophotometry obtained a significant comparison of the total phenolic content of red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo Regency of 0.49% v/b and red ginger from Karanganyar Regency of 0.47% v/b.

Keywords: Red ginger; Phenolic; Maceration; UV-Vis Spectrophotometry

1. INTRODUCTION

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) is a Zingiberaceae family that has a role in various aspects of Indonesian society because it is used as traditional medicine. Red ginger rhizome can also be used to reduce fever, overcome indigestion, colds, as an analgesic, anti-inflammatory, lower cholesterol, prevent impotence, impotence, and others (Pakpahan, 2015). Red ginger (*Zingiber officinale* var. *rubrum* Theilade) contains many active phenolic components, besides that it also has a very sharp distinctive aroma with a very spicy taste (Wiendarlina & Sukaesih, 2019).

Phenolic compounds are known as antioxidants and antidotes to free radicals associated with oxidative damage. Phenolics are gaining traction at the moment because of their in vitro studies showing that they have various beneficial biological properties such as anti-inflammatory, anti-tumor, and anti-microbial activities. Studies have attributed that the antioxidant properties are due to the presence of phenols and flavonoids. The antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to the antioxidant (Nishanthini et al., 2012; Türkoğlu et al., 2007).

Phenolic compounds have important roles in human health. Red ginger contains phenolic compounds gingerol, shagaol, and zingeron. The role of phenolics in red ginger as a source of immunoregulators by affecting the regulation of pro-inflammatory cytokine synthesis, immune

cells, and gene expression (Luhurningtyas et al., 2021). Active components in red ginger contribute to its biological compounds. The levels of active components depend on several other factors such as the altitude of the growing location and the harvesting time of red ginger (Styawan et al., 2022). Climate, which includes rainfall, growing location, temperature and humidity, affects the development and production of ginger plants. Rainfall greatly influences the growth of ginger, requiring approximately 2500 to 4000 mm per year with a dry period of approximately 5 months per year. Sunlight is needed in the growth and planting of ginger to produce good rhizomes, especially at the age of 2.5 to 7 months of growth. The air temperature for ginger growth is between 25 and 35 °C. Temperatures of more than 35 °C will cause the leaves to burn and dry, while temperatures too low will cause the ginger to live longer. A good growing location is between 300 and 900 meters above sea level (Hapsoh et al., 2008).

Wonosobo Regency has an altitude of 200-2,250 meters above sea level and Karanganyar Regency has an altitude of 90-2,000 meters above sea level. Soil types in Wonosobo Regency are mostly regosols, which are soils resulting from volcanic eruptions that are gray, brown or yellowish brown in color. The average daily air temperature is between 14.3-26.5 °C (Pemkab Wonosobo, 2017). Soil types in Karanganyar Regency are mostly andosols, which are black or dark brown soils at the bottom. The average daily air temperature is between 18-31°C (Pemkab Karanganyar, 2013).

Determination of phenolic content using UV-Vis Spectophotometry method because phenol absorbs in the short UV region and can be detected with the best reagent, namely Folin-Ciocalteu to determine phenol with catechol or hydroquinone core which produces a blue spot color after spraying the reagent (Harborne, 1996). The Folin-Ciocalteu method has the principle that phenolic ions reduce phosphomolybdic acid-phosphotungstic acid under alkaline conditions into a blue molybdenum-tungsten complex compound. Phenolic ions are formed through proton dissociation under alkaline conditions obtained from an alkaline compound. The higher the content of phenol compounds, the greater the phenolic ions, so that more phenolic ions reduce phosphomolybdate-phosphotungstate, causing the blue color to form more intense (Andriani & Murtisiwi, 2018).

2. RESEARCH METHODS

2.1. Plant Determination

Determination of red ginger rhizome (*Zingiber officinale* var. *rubrum* Theilade) was conducted at the Plant Systematics Laboratory, Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Central Java.

2.2. Simplisa Manufacturing

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo was harvested at 10 months old and red ginger from Karanganyar was harvested at 12 months old. 5 kg of red ginger rhizomes were taken and washed thoroughly with running water. Red ginger rhizomes were chopped with a thickness of 2-3 mm and then heated at 60 °C. After the oven, it was pulverized with a blender and then sieved using a 40 mesh sieve (Luhurningtyas et al., 2021). Red ginger simplisia is carried out non-specific observations of the powder. As much as 2 grams of simplisia powder was placed on a cup and then dried in an oven for 30 minutes at 105°C. Cooling using a desiccator for 15 minutes then weighing the constant weight and calculating the water content. Water content is formulated as follow Eq. (1) (Handayani et al., 2019).

$$Water\ content = \frac{b-(c-a)}{b} x100\% \quad (1)$$

2.3. Extract Preparation

Extraction using the maceration method. The maceration method can avoid damage to compound components to heating, so this method was chosen because phenol compounds have

conjugated aromatic systems that are easily damaged at high temperatures. The solvent used is 96% ethanol because it can attract phenolic compounds optimally (Ramadhani et al., 2020). The purpose of maceration is to attract active compounds (Suhendar et al., 2020). Solvents used in extraction must have properties such as selectivity, ability to extract, not contain toxins, easy to evaporate, and affordable (Yunita & Khodijah, 2020). The solvent used in maceration extraction is 96% ethanol because it has a level of safety and ease when evaporated and its properties are able to dissolve almost all substances, both polar, semi-polar, and non-polar. This solvent can also attract phenolic compounds optimally (Ramadhani et al., 2020).

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) simplisia powder was taken as much as 500 grams each and soaked in 96% ethanol solvent in a ratio of 1:5, macerated with 96% ethanol as much as 1800 ml, covered with alumuniom foil, and allowed to stand for 1 x 24 hours at room temperature (20°C-25°C). In order for the active substance to be extracted perfectly, repeated shaking is carried out. After 24 hours the extract was filtered with flannel cloth, the pulp was macerated again using 700 ml of 96% ethanol solvent for 2 x 24 hours then filtered with flannel cloth. The filtrate obtained was collected and then concentrated using a water bath at 70°C to produce a thick extract. The thick extract obtained was then weighed to determine the weight of the extract and the calculation of the extract weight yield was carried out (Luhurningtyas et al., 2021). The calculation is formulated in Eq. (2) (Sani et al., 2013).

$$\%\ yield = \frac{weight\ of\ the\ extract\ produced}{initial\ weight} x100\% \quad (2)$$

2.4. Determination of Phenol Content

2.4.1. Gallic Acid Standard Solution

Gallic acid was weighed as 10 mg with 0.5 ml of ethanol pro analysis to make 1000 ppm gallic acid solution. Aquabides was added up to the limit mark and homogenized. The solution was pipetted as much as 1, 2, 3, 4, 5 ml and added ethanol pro analysis to 5 ml. The resulting solutions were 30, 40, 50, 60, 70, 80, and 100 ppm. Folin-Ciocalteu reagent was added as much as 1.5 ml at each concentration of 30, 40, 50, 60, 70, 80, and 100 ppm then shaken and allowed to stand for 3 minutes. Add with 7% Na_2CO_3 as much as 1.2 ml and shaken until homogeneous (Luhurningtyas et al., 2021).

2.4.2. Determination of Operating time

1000 ppm gallic acid standard solution was taken as much as 0.25 ml and put into a 5 ml volumetric flask and aquabidest was added to the limit. Gallic acid solution 0.3 ml was taken and added with Folin-Ciocalteu reagent as much as 1.5 ml. Sodium carbonate (Na_2CO_3) 7.5% was added as much as 1.2 ml. The absorbance of the solution was read every 5 minutes using a UV-Vis Spectophotometer with a maximum lamda of 747 nm for 60 minutes (Luhurningtyas et al., 2021).

2.4.3. Maximum Wavelength Determination

Gallic acid solution of 1000 ppm was taken as much as 0.25 ml and put into a 5 ml volumetric flask, aquabidest was added to the limit mark. Gallic acid solution was taken 0.3 ml and added with Folin-Ciocalteu reagent as much as 1.5 ml. Sodium carbonate (Na_2CO_3) 7.5% was added as much as 1.2 ml. Left for operating time then the wavelength in the range of 600-800 nm was read with 3 replicates (Luhurningtyas et al., 2021).

2.4.4. Phenolic Content of Extract

The extract solution was made by weighing the sample as much as 0.3 ml and adding Folin-Ciocalteu reagent as much as 1.5 ml then shaken and allowed to stand for 3 minutes. 1.2 ml of 7% Na_2CO_3 was added and shaken until homogeneous and incubated for 60 minutes at room temperature. The maximum wavelength absorption at 747 nm was measured with replication 3

times so that the phenol content was obtained as gallic acid equivalents per extract (Luhurningtyas et al., 2021).

3. RESULT AND DISCUSSION

3.1. Plant Determination

This study used the Red Ginger plant (*Zingiber officinale* var. *rubrum* Theilade). The determination results show that the plants used in this study are true red ginger plants (*Zingiber officinale* var. *rubrum* Theilade). Plant determination was carried out at the Plant Systematics Laboratory, Center for Research and Development of Medicinal Plants and Traditional Medicines, Tawangmangu, Central Java. Red ginger rhizome (*Zingiber officinale* var. *rubrum* Theilade) samples are presented in **Figure 1**.

Figure 1. Red ginger rhizomes (*Zingiber officinale* var. *rubrum Theilade*) from Wonosobo Regency and Karanganyar Regency

Plant determination was carried out to state that the plants used in this study were red ginger plants (*Zingiber officinale* var. *rubrum* Theilade). The determination results stated that the plants used in the study were true red ginger plants (*Zingiber officinale* var. *rubrum* Theilade).

3.2. Preparation of Red Ginger Rhizome Simplisia

The plant part utilized is the rhizome of red ginger. Wet sorting, washing, chopping, drying, and dry sorting were carried out. The chopped ginger rhizomes were then dried using an oven at 60°C for 2 days. Simplified dry sorted and pulverized with a blender then sieved using a 40 mesh sieve.

Simplisia was tested for moisture content using the gavimetric method. The water content of red ginger simplisia from Wonosobo Regency was 8% and red ginger simplisia from Karanganyar Regency was 7.5%. Test the moisture content of simplisia according to (Depkes RI, 2017) Indonesian Herbal Pharmacopoeia second edition 2017 that the moisture content of good ginger simplisia is < 10%.

3.3. Extraction of Red Ginger Rhizomes

Extraction using maceration method. Damage to compound components against heating can be avoided by maceration method, this method was chosen because phenol compounds have conjugated aromatic systems that are susceptible to damage at high temperatures. The solvent used is 96% ethanol because it can optimally attract phenolic compounds (Ramadhani et al., 2020). The maceration container uses a dark glass bottle to avoid direct sunlight, because it can affect the results of maceration and the solvent used does not evaporate at room temperature.

Simplisia of red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo Regency and Karanganyar Regency were each taken as much as 500 grams, macerated with 1800 ml of 96% ethanol for 1x24 hours then remacerated with a new 96% ethanol solvent as much as 700 ml. The resulting extract is brown in color and has a distinctive aroma of red ginger. The result of red ginger extract from Wonosobo Regency was 106.723 grams with a yield value of 21.3% b/w. The yield of red ginger extract from Karanganyar Regency was 92.662 grams with a

yield value of 18.5% b/w. The yield results are in accordance with (Depkes RI, 2017) Indonesian Herbal Pharmacopoeia edition II which states that the extract yield is not < 17%.

3.4. Total Phenolic Content Determination

Phenol is a compound that has an aromatic ring and one or two hydroxyl groups. Phenol compounds with more than two hydroxyl groups are called polyphenols (Hanani et al., 2014). Phenolic compounds have a role in antioxidant activity in the form of polyphenols or phenolic acids they contain (Haryoto & Ardiyani, 2021). The working principle of UV-Vis Spectrophotometer is when monochromatic light passes through a solution, some of the light is absorbed, reflected, and emitted (Yanlinastuti & Fatimah, 2016). Determination of phenolic content using the Folin-Ciocalteu method where the absorbance is measured at a wavelength of 747 nm. The standard solution used is gallic acid, because gallic acid is one of the stable and natural phenolic compounds. Gallic acid that reacts with Folin-Ciocalteu reagent produces a yellow color indicating the presence of phenol compounds. Na_2CO_3 solution is added as an alkaline atmosphere giver (Sari & Ayuchecaria, 2017).

Gallic acid standard solution was measured by UV-Vis Spectrophotometer method to determine the maximum wavelength. The maximum wavelength obtained was 747 nm with an absorbance of 0.802. The absorption reading of gallic acid solution was carried out to determine the operating time at a maximum wavelength of 747 nm. Determination of operating time in this study is 60 minutes.

Concentration and absorbance of phenolic standard curve solution using 9 concentration series. To find out the difference in absorbance, the concentration variation is carried out, where the higher the concentration, the greater the absorbance value. The concentrations used were 5,000 ppm; 15,000 ppm; 30,000 ppm; 40,000 ppm; 50,000 ppm; 60,000 ppm; 70,000 ppm; 80,000 ppm; 100,000 ppm.

The linear regression equation is used to determine the standard curve of the relationship between gallic acid content as the x-axis and Folin-Ciocalteu reagent with gallic acid absorbance as the y-axis. The relationship value (R^2) obtained from the determination of the standard curve of gallic acid is 0.9966 close to 1, it can be said that there is a linear relationship between the concentration of gallic acid and its absorbance results. The results of the standard curve graph are presented in **Figure 2**.

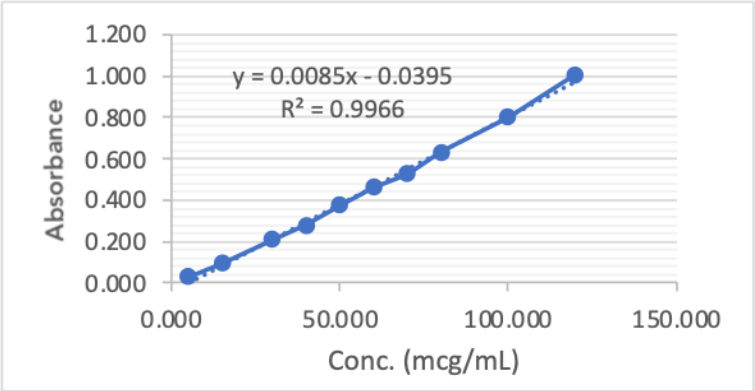

Figure 2. Standard curve graph

The results of the determination of total phenolic content of red ginger extract (*Zingiber officinale* var. *rubrum* Theilade) obtained from different growing locations using the UV-Vis Spectrophotometry method were replicated 3 times on each sample for accurate data acquisition. The results of the determination of total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) from wonosobo district are presented in **Table 1**.

Table 1. Determination of phenolic content of red ginger from Wonosobo Regency

Replication	Sample mass (mg)	Sample volume (mL)	ppm concentration	Abs	Content (%)
1	50.15	0.3	81.884	0.802	0.4898345
2	50.15	0.3	82.151	0.802	0.4914317
3	50.15	0.3	82.344	0.802	0.4925862
		$\bar{x}$ ± SD			0.4912841

Based on the results of this study, the total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) obtained from Wonosobo Regency was 0.49% b/b, and the results of the determination of total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) from Karanganyar district are presented in **Table 2**.

Table 2. Determination of phenolic content of red ginger from Karanganyar Regency

Replication	Sample mass (mg)	Sample volume (mL)	ppm concentration	Abs	*X*±SD
1	50.27	0.3	78.411	0.802	0.4703023
2	50.27	0.3	78.529	0.802	0.4710005
3	50.27	0.3	78.647	0.802	0.4717346
$\bar{X}$ SD					0.4710130

Based on the results of this study, the total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) obtained from Karanganyar Regency was 0.47% b/w. Phenolic levels obtained from each sample were then analyzed for normality with the Shapiro-Wilk Normality Test and continued using the Independent T-Test statistical analysis. Data analysis of the normality of phenolic levels tested with Shapiro-Wilk is presented in **Table 3**.

Table 3. Data analysis of normality of phenolic levels in red ginger extract of Wonosobo Regency and Karanganyar Regency with shapiro-wilk test

Sample	Normality
Wonosobo	0.991
Karanganyar	1.000

Based on **Table 3**, the normality value of the red ginger samples obtained from Wonosobo Regency and Karanganyar Regency is 0.991 and 1.000, indicating that both samples have normality> 0.05 so that the results are said to be normal and then conducted an *Independent T-Test.* Analysis of normality data with the *Independent T-Test* is presented in **Table 4**.

Table 4. Data analysis of normality of phenolic levels in red ginger extracts Wonosobo Regency and Karanganyar Regency with independent t-test and normality

Sample	P value/ sign
Wonosobo	0.000
Karanganyar	0.000

Based on **Table 4** the significant value (p) of red ginger samples obtained from Wonosobo Regency and Karanganyar Regency is 0.000 and 0.000 which both samples have a p value <0.05 so that H0 is rejected, then Ha is accepted. This means there is a significant difference between the phenolic content of red ginger obtained from Wonosobo Regency and Karanganyar Regency. Statistical data analysis of phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency is presented in **Table 5**.

The phenolic content of red ginger from Wonosobo Regency has a significant value (p) of 0.000 which means p < 0.05. This proves that there is a significant difference in phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency.

Table 5. Comparison of phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency with test independent t-test normality

Sample	N	Mean (%)	Standard Deviation	P value
Wonosobo	3	0.4912841	0.0013817725	0.000
Karanganyar	3	0.4710130	0.0007162250	

Phenolic content was determined using the Folin-Ciocalteu method because phenolic compounds can react with the Folin-Ciocalteu reagent to form a solution whose absorbance can be measured, where the absorbance is measured at a wavelength of 747 nm. Gallic acid is the standard solution used, because gallic acid includes stable and natural phenolic compounds. The yellow color that results from the gelling of gallic acid with Folin-Ciocalteu reagent indicates the presence of phenolic compounds, as a giver of alkaline atmosphere added with sodium carbonate. Hydroxyl groups in phenolic compounds react with Folin-Ciocalteau to form a blue molybdenum-tungsten complex that can be detected by spectrophotometer (Andriani & Murtisiwi, 2018).

Determination of the maximum wavelength aims to determine the wavelength required for gallic acid solution to reach maximum absorption. The maximum wavelength obtained was 747 nm with an absorbance value of 0.802. The difference in total phenolic content in red ginger from Wonosobo Regency and Karanganyar Regency in this study shows that the phenolic content of red ginger from Wonosobo Regency is higher. This difference is influenced by altitude, air temperature, soil, and rainfall which are different from each region.

The air temperature at the time of planting red ginger until harvesting can change due to the long planting period of ginger. Ginger cultivation lasts between 8 to 12 months. Air temperature affects the moisture content of red ginger rhizomes. The average temperature at planting time in Wonosobo Regency is 14.3-26.5 °C (Pemkab Wonosobo, 2017), while the average air temperature in Karanganyar Regency is 18-31 °C (Pemkab Karanganyar, 2013).

Harvesting of both red ginger samples was done in the morning. Harvesting in the morning is done because the ambient temperature is still low enough to increase harvesting efficiency and can reduce the effects of plant respiration. Air temperature has an important influence on the growth and development of ginger in physiological processes such as photosynthesis, transpiration, water and nutrient absorption, and flower primodia formation.

The soil used for red ginger growth in Wonosobo Regency is regosol soil while the soil in Karanganyar Regency is andosol soil. Regosol soils are soils resulting from volcanic eruptions that are gray, brown or yellowish brown in color. Andosol soil is brown or dark brown soil and is at the bottom. The types of soil commonly used for ginger planting media are brownish-red latosols, brownish-red latosol-andosol mixes, and soils in new forest clearings (Hapsoh et al., 2008).

The altitude of the red ginger growing location in Kalibawang District, Wonosobo Regency is 626 meters above sea level, while the red ginger growing location in Jatiyoso District, Karanganyar Regency is 875 meters above sea level. The altitude of the area in both locations is a good ginger growing location according to the Ministry of Agriculture (Hapsoh et al., 2008) that the best growing location for ginger is between 300-900 meters above sea level.

Rainfall in the process of planting red ginger is needed around 2500 to 4000 mm per year with dry months less than 5 months per year. Rainfall in Wonosobo Regency with high intensity in the first 3 months of planting with a dry month length of 5 months, while rainfall in Karanganyar Regency with high intensity in the first 5 months with a dry month length of 7 months.

According to Pakpahan (2015) Red ginger contains phenolics that are useful for analgesics, so it is necessary to develop research on the formulation of herbal medicinal preparations using red ginger taken from Wonosobo Regency. The herbal preparation in question is anti-pain herbal

syrup. The preparation of syrup is recommended because syrup is more quickly absorbed by the body and is already in dissolved form.

4. CONCLUSION

Red ginger extract from Wonosobo Regency has a phenolic content of 0.49% b/w and red ginger extract from Karanganyar Regency has a phenolic content of 0.47% b/w. There is a significant difference between phenolic content in red ginger of Wonosobo Regency and Karanganyar Regency with a significant value of 0.000 which means $p < 0.05$.

5. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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ANALYSIS OF LYCOPENE CONTENT IN RED GUAVA JUICE (PSIDIUM GUAJAVA L.) BY VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

Red guava (*Psidium guajava* L.) is a fruit that is rich in phytochemicals that are beneficial for health and is widely consumed in the form of juice. Lycopene belongs to the carotenoid group which has antioxidant potential and has an important role in the immune system to deal with homeostatic changes caused by oxidative stress. Lycopene is contained in a lot of red fruit. The purpose of this study was to analyze the lycopene content in red guava juice by visible spectrophotometry. Red guava juice extracted using n-hexane:acetone:methanol 1:2:1. Qualitative analysis was carried out by observing the spectrum, then measuring the maximum wavelength, which was supported by observing the spots by thin layer chromatography on silica gel 60 GF_{254} plate using petroleum ether:acetone 9:1. Quantitative analysis was carried out by measuring absorbance by visible spectrophotometry at 470 nm. The results of qualitative analysis by visible spectrophotometry obtained 3 typical peaks of the lycopene spectrum at 400-550 nm, with a typical maximum wavelength of lycopene at 470 nm. Qualitative analysis by thin layer chromatography obtained spot with Rf 0.64. The results of quantitative analysis obtained lycopene levels of 2.91 ± 0.465 mg/100 grams of juice.

Keywords: Lycopene; Red Guava Juice; Visible Spectrophotometry

1. INTRODUCTION

In general, people tend to be reluctant to consume red guava (*Psidium guajava* L.) directly because of the very high seed content in the fruit flesh. Red guava is more popular when consumed in the form of juice because it can present seedless fruit in a soft texture without reducing the taste and freshness of the fruit when consumed. In addition, fruit juice can be made in a very easy and fast way. Fruit juice is also one form of snack that is often found. One of the fruit juices that is in great demand by the public is red guava juice.

Various benefits of red guava juice have been studied. Consuming 100 grams of red guava juice per day can reduce tracheal damage due to exposure to cigarette smoke (Febrianti & Suryati, 2014). Giving 250 ml of red guava juice for 21 days significantly reduces the risk of heart disease (Anugrah et al., 2017). The effect of red guava juice on increasing immunity has also been studied by Aldi et al. (2012). These various benefits are related to the antioxidant content in red guava juice.

One of the very strong antioxidants in red guava juice is lycopene. Lycopene is a bright red pigment of the carotenoid group that is potentially contained in red-fleshed fruits, such as red guava. Lycopene is able to bind single oxygen 2 times more than β-carotene (Monica & Rollando, 2019) and ward off free radicals 100 times more effectively than vitamin E (Alfa et al., 2019; Fadilah, 2012; Hamsina et al., 2019). Supplementation with lycopene-rich intake for 1 week can increase serum lycopene levels, thereby suppressing lipid, protein, lipoprotein, and DNA oxidation. Thus, lycopene can eliminate or reduce oxidative stress which has a key role in

reducing the risk of various chronic and degenerative diseases (Kumar et al., 2017; Mehta et al., 2018).

Determination of lycopene levels has been carried out on various red-fleshed fruits using visible spectrophotometry (Arifulloh et al., 2016; Hamsina et al., 2019; Sima et al., 2019; Tristiyanti et al., 2018). Extraction of lycopene from watermelon juice (Hamsina et al., 2019) showed optimum results using n-hexane:acetone:ethanol 2:1:1 with a juice and solvent ratio of 1:2. Based on the research results Arifulloh et al. (2016) it is known that the optimal solvent for extracting lycopene from tomatoes is a mixture of n-hexane:acetone:methanol 1:2:1 with a material:solvent ratio of 1:5. While Maulida & Zulkarnaen (2010) states that the amount of lycopene in tomato juice can be up to 5 times more than fresh tomatoes. In the study Tristiyanti et al. (2018), red guava fruit lycopene was extracted by reflux method using n-hexane solvent. However, no research has been conducted to extract lycopene from red guava juice using liquid-liquid extraction method. Therefore, this study aims to analyze the lycopene content in red guava juice by visible spectrophotometry, which was extracted by liquid-liquid extraction method using n-hexane:acetone:methanol 1:2:1 at a juice:solvent ratio of 1:5.

2. METHODS

2.1. Research Materials and Tools

The materials used include: red guava (*Psidium guajava* L.) from 3 different supermarkets in Surakarta, silica gel GF254 (Merck), petroleum ether p.a (Merck), acetone p.a (Merck), n-hexane (Merck), acetone (Merck), methanol (Merck), distilled water (Brataco).

The tools used include: a set of UV-Vis spectrophotometers (Shimadzu UV-1280), cuvettes (Hellma Analytics 100.600-QG light path 10 mm), analytical balance (Ohaus Pioneer with a sensitivity of 0.0001 g), rotary evaporator (RV 10 basic), shaker (Rotator HSR-200), blender (Cosmos), and glassware commonly used in analytical chemistry.

2.2. Research Procedures

2.2.1. Making Red Guava Juice

Red guava from 3 supermarkets were washed and cleaned. The red guava whose skin surface was clean and dry was cut into several pieces, then homogenized and pureed with a blender until it became juice.

2.2.2. Lycopene Extraction

Red guava juice was weighed 200.0 mg, then added 50 ml of methanol and stirred for 5 minutes. Separate the seeds from the juice mixture by filtering. The juice mixture that had been freed from seeds was put into a closed Erlenmeyer flask and coated with carbon paper on the outside, then extracted using a mixture of 250 ml of n-hexane, 500 ml of acetone, and 250 ml of methanol. During the extraction process, it was shaken with a shaker at a speed of 150 rpm for 30 minutes. The mixture was put into a separating funnel, added with 10 ml of distilled water and shaken again, then left to stand until two phases were formed. The top layer was taken and then evaporated using a rotary evaporator at a temperature of 40 °C at a speed of 75 rpm. The concentrated extract obtained was then weighed and put into a brown glass bottle (Arifulloh et al., 2016; Monica & Rollando, 2019).

2.2.3. Preparation of sample solution

The concentrated extract was weighed 10.0 mg then dissolved in petroleum ether p.a to 10.0 ml (Monica & Rollando, 2019). The solution was pipetted 3.0 ml then diluted with petroleum ether p.a to 10.0 ml to obtain a concentration of 3 µg/ml, which was then used as a sample solution. Qualitative Analysis by Visible Spectrophotometry The sample solution was scanned at a wavelength of 400-550 nm. The spectrum shape and maximum wavelength produced by the sample solution were compared with standard lycopene in the reference (Arifulloh et al., 2016).

2.2.4. Qualitative Analysis by Thin Layer Chromatography

The sample solution was spotted on a silica gel 60 GF254 plate and then eluted with petroleum ether: acetone 9:1. The Retention Factor (Rf) value of the spots produced by the sample solution was compared with the standard lycopene in the reference (Harini & Sumathy, 2016).

2.2.5. Quantitative Analysis by Visible Spectrophotometry

The absorbance of the sample solution was measured at a maximum wavelength of 470 nm. The lycopene content was calculated using the equation C = A/($\sum_{cm}^{1\%} \times B$), with C the concentration of the sample solution (%), A the absorbance of the sample solution, $\sum_{cm}^{1\%}$ lycopene absorptivity, B the thickness of the solution in a 1 cm cuvette (Hamsina et al., 2019).

3. RESULTS AND DISCUSSION

3.1. Making Red Guava Juice

In this study, red guava (*Psidium guajava* L.) from supermarkets was used to ensure a more guaranteed level of fruit freshness, because the storage temperature in supermarkets is better maintained compared to traditional markets. The red guava used has the characteristics of ripe fruit with a texture that is not hard, yellowish skin, no defects, and fresh red flesh. The red guava was washed first to clean dirt from the surface of the skin. The clean red guava was cut into several pieces to facilitate the juice making process. Before being made into juice, all pieces of red guava from 3 supermarkets were homogenized to ensure sample homogeneity without comparing the origin of the sample.

3.2. Lycopene Extraction

At the start of extraction, red guava juice was first added with methanol and stirred for 5 minutes, as in Arifulloh et al. (2016). This aims to attract compounds in red guava juice that have polar properties so that in the extraction process more non-polar compounds will be extracted. After being filtered, the red guava juice residue is extracted using the liquid extraction method because lycopene is unstable at high temperatures (Fadilah, 2012) using n-hexane:acetone:methanol 1:2:1 with a juice:solvent ratio of 1:5. Arifulloh et al. (2016) stated that the use of n-hexane:acetone:methanol 1:2:1 was able to extract more lycopene than other solvent variations.

The extraction process is assisted by shaking using a shaker at a speed of 150 rpm for 30 minutes, then filtered to separate the filtrate from the sediment. During the extraction process, the solvent liquid will penetrate the cell wall and then enter the cell cavity containing the active substance. The solvent that has extracted the active substance will be pushed out due to the difference in concentration between the solution and the active substance in the cell and outside the cell. This event is repeated until there is a concentration equilibrium between the solution outside the cell and inside the cell. The results obtained are orange filtrate. The extraction process is continued by adding distilled water in a separating funnel to form two layers of polar and non-polar phases. The nhexane phase in the upper layer containing lycopene is taken and then concentrated with a rotary evaporator until a reddish concentrated extract is obtained.

3.3. Qualitative Analysis by Visible Spectrophotometry

The bright red color produced by lycopene is a result of the many double bonds in the chemical structure of lycopene, so that it is able to absorb electromagnetic radiation energy in the visible region (visible light) with a high wavelength (Maulida & Zulkarnaen, 2010). Compounds with more conjugated double bonds have higher maximum wavelength values. Based on the chemical structure presented in Figure 1, lycopene consists of 8 isoprene units with 11 unsaturated double bonds so that it will absorb at the highest wavelength compared to other carotenoids. This is what makes lycopene measurable by visible spectrophotometry.

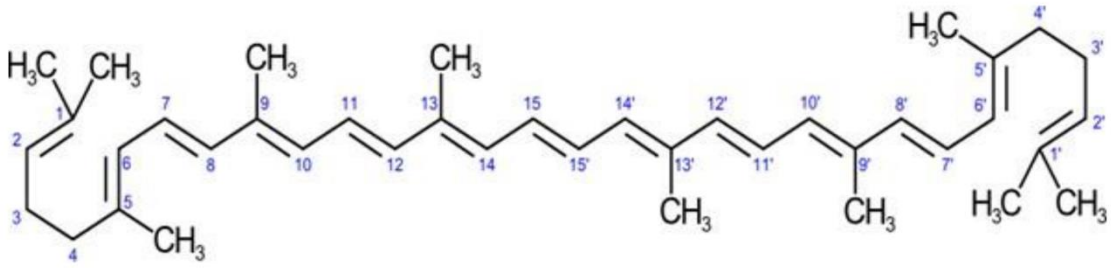

Figure 1. Chemical structure of lycopene (Monica & Rollando, 2019)

Identification of lycopene by visible spectrophotometry is done by comparing the spectrum shape and maximum wavelength of standard lycopene in the reference with the measurement results of lycopene in the sample solution. The absorption spectrum of lycopene is typical in the 400-550 nm region, with maximum absorption at 3 main wavelengths depicted in the form of 3 peaks around 444, 470, 502 nm as presented in Figure 2.

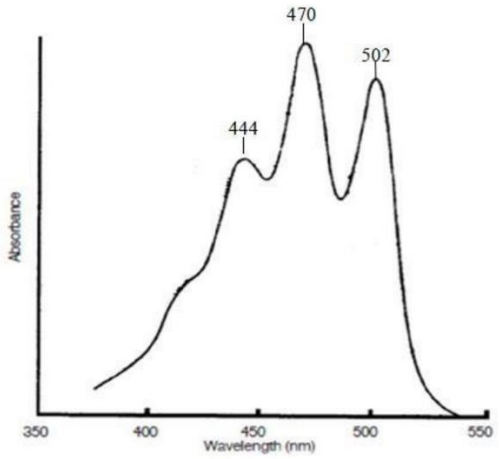

Figure 2. Standard spectrum of lycopene in petroleum ether (Arifulloh et al., 2016)

In the measurement of the absorbance of the sample solution, an absorption spectrum with 3 main peaks located between 400-550 nm was also produced, as shown in Figure 3. The absorbance spectrum of the sample solution indicates the presence of lycopene in red guava juice with the presence of three main wavelengths typical of lycopene, which are similar to the standard lycopene spectrum in the reference, with the results presented in Table 1. This is in accordance with the characteristics of unsaturated double bonds in the chemical structure of lycopene.

The conformity of the spectrum shape is strengthened by the conformity of the maximum wavelength value produced by the sample solution to the maximum wavelength of the standard lycopene in the reference. Based on the data in Figure 3 and Table 1, it is known that the highest absorbance of the sample solution was obtained at a wavelength of 470 nm. This is the same as the maximum wavelength of the standard lycopene in Figure 2, which is 470 nm. The maximum wavelength is determined by the chemical structure of the compound containing the chromophore (Gandjar & Rohman, 2018), namely the unsaturated group in the covalent bond which is responsible for the occurrence of electronic absorption (Dachriyanus, 2004). Therefore, the suitability of the maximum wavelength value of the analyte and standard is used as a parameter for identifying the analyte in the sample solution. Based on the suitability of the spectrum shape, the peak wavelength of the spectrum and the maximum wavelength produced by the absorbance of the sample solution to the standard lycopene in the reference, it proves that red guava juice contains lycopene.

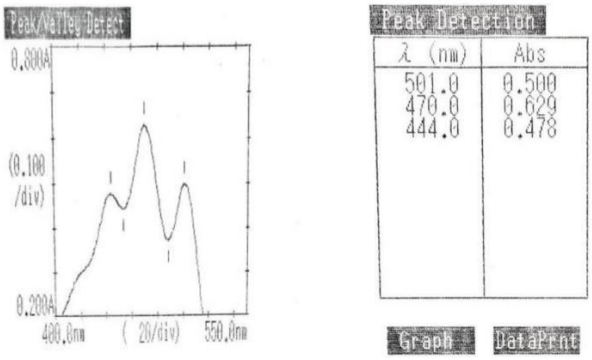

Figure 3. Lycopene spectrum of sample solution in petroleum ether

Table 1. Peak wavelength of the sample solution spectrum in petroleum ether

Sample Solution	Peak 1		Peak 2		Peak 3	
	λ (nm)	Abs	λ (nm)	Abs	λ (nm)	Abs
1	444.0	0.478	470.0	0.629	501.0	0.500
2	443.5	0.495	470.0	0.638	501.0	0.510
3	443.5	0.473	470.0	0.625	501.0	0.496

3.4. Qualitative Analysis by Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a method used to separate a compound based on the difference in distribution of two phases, namely the stationary phase and the mobile phase. Qualitative analysis using the TLC method aims to identify the presence of lycopene compounds in sample solutions by comparing the Rf value of the lycopene standard in reference to the Rf value of the spots produced by the sample solution. The elution process is carried out on a stationary phase of silica gel 60 GF254 with a mobile phase of petroleum ether: acetone 9:1 (Harini & Sumathy, 2016). The elution results as presented in Figure 4, are in the form of a single reddish orange spot with an Rf value of 0.64. These results are the same as the Rf value of standard lycopene in the research study Harini & Sumathy (2016). Thus, red guava juice is proven to contain lycopene.

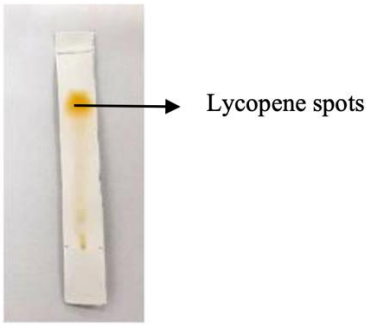

Figure 4. Results of Elution of Sample Solution on Silica Gel 60 GF254 with Petroleum Ether: Acetone 9:1 as Mobile Phase

3.5. Quantitative Analysis by Visible Spectrophotometry

Quantitative analysis was carried out by measuring the absorbance of the sample solution using visible spectrophotometry at 470 nm. Based on Sima et al. (2019), standard lycopene in the reference is used as a comparative standard in quantitative analysis, so that the calculation of lycopene levels is carried out using the Lambert-Beer equation with a value of $\sum_{cm}^{1\%}$ 3.450. Absorptivity expressed in $\sum_{cm}^{1\%}$ value describes the absorption power or ability of the analyte to absorb electromagnetic radiation at the measurement wavelength. Absorptivity does not depend on the concentration and intensity of radiation but is determined by the chemical structure of the compound, solvent, and wavelength of radiation. Thus, the $\sum_{cm}^{1\%}$ value becomes a setting or

constant that is specific to each compound molecule in a certain solvent and wavelength (Gandjar & Rohman, 2007), so that the $\sum_{cm}^{1\%}$ value is also called the specific extinction coefficient (Dachriyanus, 2004).

Table 2. Lycopene content in red guava juice

Sample	Content (mg/100 mg juice)
1	2.38
2	3.25
3	3.10
Average	2.91
Standard Deviation	0.465

Based on the data in Table 2, in this study, the lycopene content in red guava juice was obtained with an average of 2.91 ± 0.465 mg/100 grams of juice. These results were obtained using the liquid-liquid extraction method because lycopene is unstable at high temperatures (Fadilah, 2012) using n-hexane:acetone:methanol 1:2:1 with a juice:solvent ratio of 1:5 (Arifulloh et al., 2016). Acetone is soluble in both n-hexane and methanol, so acetone is expected to act as a polarity bridge in the mixed solvent. Therefore, in a ratio of 1:2:1, it is expected to optimize the withdrawal of lycopene from polar solvents (watermethanol) into non-polar solvents (n-hexane). Arifulloh et al. (2016) stated that the use of n-hexane:acetone:methanol 1:2:1 was able to extract more lycopene compared to other solvent variations, such as petroleum ether:acetone (3:1), n-hexane:acetone:methanol 2:1:1, and n-hexane:acetone:methanol 1:1:1. In the research study Tristiyanti et al. (2018), the lycopene content in guava fruit was obtained at 7.5 mg per 100 grams of fruit. The higher content in the study was thought to be due to the use of the reflux method with n-hexane as the sole solvent in the extraction process, so that it was better able to draw non-polar lycopene.

4. CONCLUSION

Red guava juice extracted by liquid-liquid extraction method using nhexane:acetone:methanol 1:2:1 at a juice:solvent ratio of 1:5, can obtain lycopene with an average content of 2.91 ± 0.465 mg/100 grams of juice. The use of n-hexane as a single solvent is recommended to obtain more lycopene.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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The authors declare no competing interest.

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ANALYSIS OF TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF GREEN TEA (*Camellia sinensis*) WITH VARIATIONS IN TYPE AND BREWING

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ABSTRACT
Free radicals can react to cause damage when they enter the human body. The use of antioxidants can neutralize free radicals in the body. This study aims to determine the total flavonoid levels and antioxidant activity in premium and original green tea with variations in brewing temperature. Green tea produced by Kulon Progo is brewed with aquadest at 75 ℃ and 100 ℃, and then the filtrate obtained is ground by freeze-drying. Identification of flavonoid compounds using the flavanoid-ammonia vapor color complex. The total flavonoid levels analysis using UV-vis spectrophotometry and antioxidant activity was tested by the DPPH (1,1-diphenyl-2-picrylhydrazil) method. Based on the test results, the highest to lowest total flavonoid levels were premium green tea at 100 ℃ original at 100 ℃, original at 75 ℃, and premium at 75 ℃ (155.095 ± 3.158; 153.333 ± 2.788; 151.7181 ± 4.944; and 148.634 ± 2,095 mgQE/gram extract). The highest to lowest potential antioxidant activity, respectively, is quercetin (flavonoid standard), premium green tea at 100 °C, premium at 75 °C, original at 100 °C, and original at 75 °C, with IC_{50} values of 3.0692 ± 1.064; 32.359 ± 7.346; 31.502 ± 6.746; 27.527 ± 7.868; and 27.167 ± 1.817 µg/mL. Analysis of the total flavonoids of green tea showed that the highest levels were in premium green tea at a temperature of 100 °C. An antioxidant activity test with the DPPH reagent produced the highest antioxidant potential in premium green tea at 100 °C. So premium green tea at a brewing temperature of 100 °C is more recommended to consumers.

Keywords: Brewing; *Camellia sinensis* L; Green tea; Flavonoid; Freeze drying

1. INTRODUCTION

Free radicals are unstable compounds and can harm health if they enter the human body (Wahdaningsih et al., 2011). Based on IQAir in 2022, Indonesia ranks 26th out of 131 countries with the highest air pollution and has the worst air pollution in Southeast Asia. Apart from that, the increase in health complaints in Indonesia in 2022 has increased to 29.94% from the previous 27.23% in 2022. Antioxidants are compounds that can neutralize and reduce the negative impact of free radicals on the body. Antioxidants are divided into two, namely enzyme and non-enzyme antioxidants. Enzyme antioxidants include catalase enzymes and glutathione peroxidase enzymes, while non-enzyme antioxidants such as vitamin C, anthocyanidins, and flavonoids (Zulaikhah, 2017). Antioxidants work by donating one electron to an oxidant compound so that the activity of the oxidant compound can be inhibited (Zulaikhah, 2017). Antioxidant compounds are easily oxidized, so the tea brewing procedure will affect the amount of antioxidant compounds extracted. The oxidation process that occurs due to the influence of the tea brewing procedure can affect the amount of antioxidant compounds produced. This is because the free radical scavenging group in the antioxidant compound is damaged.

Green tea (*Camellia sinensis* L.) is a plant that contains phenolic compounds, alkaloids, steroids, tannins, and flavonoids (Arya et al., 2019). In Indonesia, tea has long been known as a drink that is popular with Indonesian people (Tehubijuluw et al., 2019). Tea is divided into four based on processing methods: green tea, white tea, black tea, and oolong tea. of the four types of tea, green tea contains the highest antioxidant compound activity (Fajar et al., 2018). Tea is also rich in flavonoids (Sasmito, 2020). This content is thermolabile and volatile (Gloriana et al., 2023). So, the tea brewing temperature can affect the number of total flavonoids extracted (Sugiyanto et al., 2022).

The method that can be used to analyze total flavonoid levels is UV-Vis spectrophotometry. This is because flavonoids have conjugated aromatic groups and can show strong absorption bands in the UV-Vis region. Besides, flavonoids are polar, which can absorb radiation absorption lengths in the UV-Vis region (Winahyu et al., 2019). Based on this, research is needed to analyze the effect of brewing temperature on total flavonoid levels in premium and original green tea produced by Kulon Progo using the UV-Vis spectrophotometric method. Kulonprogo is one of the districts that has original tea products from the Special Region of Yogyakarta. This research used two variations, namely, brewing temperature and tea quality, with three replications for each variable. The difference between the two tea samples in this study is the premium class with original green tea. The difference between the two samples is based on the materials used. Premium green tea only uses young tea from the top of the plant, while original green tea uses a mixture of shoots and young leaves up to the fourth stalk. Based on the differences in the materials used, knowing their effect on the difference in total flavonoid content as antioxidant activity is necessary.

2. METHODS

2.1. Materials

The raw materials used are premium and original green tea leaf simplicia (*Camellia sinensis* L.), quercetin (Sigma), 2,2- *diphenyl-1-picrylhydrazyl* (DPPH) (Sigma), ethanol 96% (Brand), sodium acetate (Brand), $AlCl_3$ (Brand), and distilled water.

2.2. Sample Extraction

Each premium and original green tea sample was weighed at 10 g each, then brewed using distilled water in a dispenser at 75 °C and 100 mL of boiling water at 100 °C, ultrasonified for 10 minutes. The brewed water is filtered using filter paper until you get a clear filtrate (Chadijah et al., 2021). The clear filtrate is then dried using the freeze-dryer method.

2.3. Yield Test

The yield is obtained by comparing the final weight obtained (dry extract) with the initial weight of the sample (Salamah et al., 2017).

$$\%\, Yield = \frac{Initial\ weight\ of\ simplicia}{Weight\ of\ extract} \times 100\% \quad (1)$$

2.4. Flavanoid Content Test

Premium and original green tea that has been refined weighed 1 gram and dissolved in 10 mL of 96% ethanol. Two drops of green tea extract were dropped on filter paper, and then the filter paper was placed on the mouth of a beaker containing ammonia on a heater. A positive result is indicated by a change in the color of the filter paper to yellow or orange (Arnida et al., 2021).

2.5. Drying Shrinkage Test

Drying loss was carried out by weighing 2 grams of each simplicia sample, placing it on an aluminum foil plate (special), and then placing it in a halogen moisture analyzer at 105 °C for

15 minutes. Drying loss meets the requirements if the drying loss percentage value obtained is <10% (Silverman et al., 2023).

2.6. Ash Content Test

Determination of ash content is carried out using the gravimetric method. Each dry sample was weighed to 2 grams on a silicate crucible that had been tarred and ignited. The steam cup containing the sample is then heated in a furnace at a temperature of 600 °C until ash forms and the weight is constant. Ash content is calculated from the weight of the test material expressed in percent w/w (Silverman et al., 2023).

$$\%\ Ash\ content = \frac{w1 - w2}{w} \times 100\% \quad (2)$$

where, w: Sample weight before kilning (g); w_1: Weight of sample and cup after kiln (g); w_2: Weight of empty cup (g)

2.7. Determination of Total Flavonoids

Each tea sample (freeze-drying result) was weighed at 25 mg, and ethanol p.a was added to a 25 mL volumetric flask. Take 0.25 mL and put it in a 10 mL measuring flask. Add 3 mL of pa ethanol, 0.2 mL of 10% $AlCl_3$, 0.2 mL of 1 M sodium acetate, and distilled water to the mark. Leave it for operating time and measure the maximum absorption length (Silverman et al., 2023; Swandi et al., 2020).

2.8. Antioxidant Activity Test using the DPPH Method

The antioxidant activity test was carried out using the DPPH method and UV-Vis spectrophotometry. The sample (green tea) and standard (quercetin) were dissolved in 96% ethanol with a ratio of 0.15 mM DPPH: sample (2:1). The solution was incubated during OT at room temperature and covered with aluminum foil. The absorbance was measured at 517 nm lambda (corresponding to the DPPH lambda at that time). Absorption readings were carried out three times for each standard and sample. The IC_{50} (Inhibitory Concentration 50%) value, or 50% inhibitory concentration, can indicate antioxidant activity. The IC_{50} value is the antioxidant level that reduces DPPH by up to 50%. In addition, the IC_{50} value is one of the most commonly used parameters when using the DPPH method to test antioxidant activity. Please note that the greater the IC_{50} value, the smaller the antioxidant activity; vice versa; if the IC_{50} value is smaller, the antioxidant activity will be greater (Andriani & Murtisiwi, 2020). Antioxidant activity can be analyzed based on the percentage of antioxidant activity with the formula:

$$\%\ Antioxidant\ activity = \frac{(A\ negative\ control - A\ sample)}{A\ Negative\ control} \times 100\% \quad (3)$$

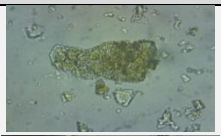
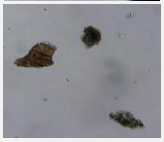
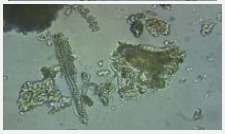
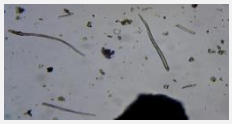
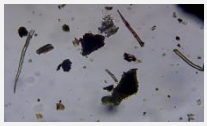
where, A is absorbance value

3. RESULTS AND DISCUSSION

The research began with sample collection; green tea samples were obtained from green tea producers in Padukuhan Suroloyo, Kulon Progo, Yogyakarta. Two types of quality green tea are used, namely premium quality and original green tea. Premium quality green tea only uses young leaves at the top of the leaf, whereas original quality green tea contains a mixture of stems and young green tea leaves. The green tea sample obtained was in the form of dried Simplicia, which was ready to be consumed, so to ensure that the sample taken was green tea, a microscopic test was carried out to look at the parts of the tea leaves in detail. The preparation is examined through a microscope using a water reagent in microscopic testing. Microscopic tests were carried out at the Laboratory of the Faculty of Applied Science and Technology (FAST), Faculty of Biology, Ahmad Dahlan University. They obtained microscopic test results for premium and

original green tea in parenkin tissue, vascular bundles, and trichomes, which can be seen in **Table 1**. This microscopic identification is very important to ensure that the sample used is truly the *Camelia sinensis* L. species because the sample used from the beginning was only a dry green tea Simplicia and not a whole plant. We use the microscopic results to identify the fragments of green tea leaves (Silverman et al., 2023).

Table 1. Microscopic test of premium and original green tea

No	Leaf parts according to the monograph	Sample	
		Premium	Original
1.	Parenkin		
2.	Vascular Files		
3.	Trichomata		

3.1. Sample Preparation

The samples were obtained from freeze drying; the freeze-drying of green tea brewing water obtained the weight of the dry extract, as shown in **Table 2** and **Figure 1**.

Table 2. Green tea yield

No.	Sample	Weight Simplisia (g)	Extract weight (g)	Yield (%)
1.	Premium Green Tea 100 °C	10.093	0.786	7.787
2.	Original Green Tea 100 °C	10.062	1.001	9.948
3.	Original Green Tea 75 °C	10.035	1.195	11.908
4.	Premium Green Tea 75 °C	10.078	1.394	13.832

Premium green tea extract 100 °C | Original green tea extract 100 °C | Original green tea extract 75 °C | Premium green tea extract 75 °C

Figure 1. Freeze drying green tea extract

Based on (Silverman et al., 2023), The required percent yield value of thick tea leaf extract (> 7.8%) means that the yield value of premium and original green tea produced by Kulon Progo is following the requirements.

3.2. Identification of Flavanoid Content

Flavonoid identification was carried out to ensure flavonoid compounds were in Kulon Progo Green Tea. The reagent is ammonia vapor, producing an intensive yellow color change on the filter paper if the sample contains flavonoids. The test results can be seen in **Table 3**. There is an OH- group in the flavonoid structure, which can easily release the H+ atom in the presence of an ammonia base and then combine with ammonia to make the O2- atom have additional free electrons. A quinoid structure is formed when these electrons are added, producing a yellow color. Because there is a large amount of water vapor (H2O) in the air, the H+ atom will be given to the

O2- atom, which has an excess of electrons, so that the color and reaction will quickly return to normal (reversible) (Nugraha et al., 2022).

Table 3. Flavonoid content test

Sample	Reactant	Before being evaporated by ammonia	After being evaporated by ammonia	Description
Quercetin standard	ammonia vapor			(+) There is an intense color change to yellow.
Premium green tea sample	ammonia vapor			(+) There is an intense color change to yellow.
Original green tea sample	ammonia vapor			(+) There is an intense color change to yellow.

3.3. Drying Shrinkage

Determination of drying losses is one of the requirements that must be fulfilled in the standardization of nutritious plants to provide a maximum limit (range) regarding the amounts of compounds lost in the drying process (Najib et al., 2017). The drying loss content of premium green tea was 6.999%, while original green tea was 6.6.45%. Based on (Silverman et al., 2023), the drying loss requirement for tea leaves is not more than 10%, so the drying loss value of premium and original green tea obtained follows the permitted requirements.

3.4. Ash Content Test

The ash content value aims to determine the mineral content in the simplicia or extract. The mineral content in a sample is divided into organic and inorganic salts. Apart from that, determining the ash content is useful for determining the high or low quality of the processing process, the type of material, and the nutritional value parameters of the material (Pine et al., 2015). Ash content is calculated from the weight of the test material expressed in % w/w. The research results showed that the percent ash content of premium green tea was 3.341% and original green tea was 4.403%. Based on the Indonesian herbal pharmacopeia, the limit value for the ash content of tea leaves is no more than 5.6%, so the percent ash content of premium and original green tea in this study is appropriate. Low ash content values indicate minimal mineral and inorganic contamination in the extract. Contamination can occur due to the growing location or an unclean extract manufacturing process. The lower the ash content, the higher the quality of the powder and extract.

3.5. Total Flavanoid Content

The standard quercetin solution was left for 30 minutes, and the absorbance was read at the maximum wavelength, as shown in **Figure 2**. The quercetin absorbance value obtained was then calculated to obtain the equation y = 0.0908x + 0.1406 with squared efficiency (R2) = 0.998, with y as the light absorption and x as the concentration of the quercetin solution. The quercetin standard curve graph can be seen in **Figure 3**. The content of flavonoid compounds in green tea samples is expressed in total quercetin equivalents per gram of green tea (mg EQ/gram extract).

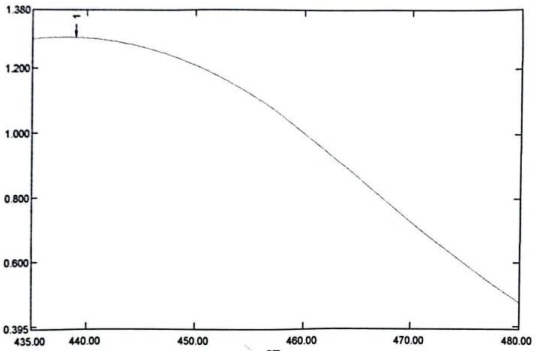


Figure 2. Graph of the maximum wavelength of quercetin 439 nm

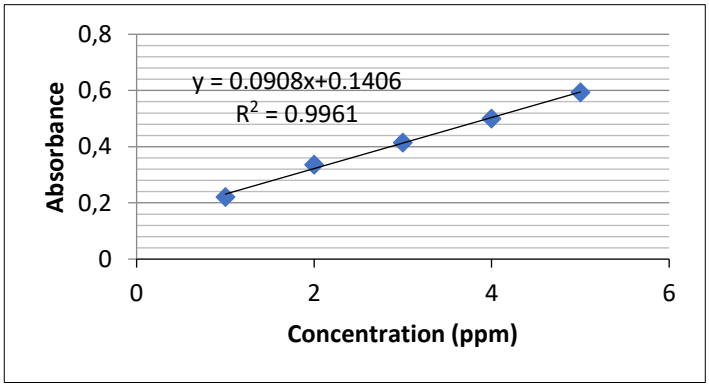


Figure 3. Quercetin standard curve obtained by UV-Vis spectrophotometry

Table 4. Total flavonoid content of green tea

No.	Name	Total Flavanois Levels (mg QE/g)
1.	Premium Green Tea 100 °C	155.095 ± 3.158
2.	Original Green Tea 100 °C	153.333 ± 2.788
3.	Original Green Tea 75 °C	151.718 ± 4.944
4.	Premium Green Tea 75 °C	148.634 ± 2.095

The total flavonoid content values from the research results can be seen in **Table 4**. Respectively, the total flavonoid content of green tea is premium green tea at 100 °C, original green tea at 100 °C, original green tea at 75 °C premium green tea temperature 75 °C. Based on brewing temperature, samples brewed using water at 100 °C produced higher levels of flavonoids than samples brewed in water at 75 °C. This is following research (Chatterjee et al., 2016), which states that the highest total levels of green tea flavonoids were obtained at a temperature of 100 °C with an extraction time of 15 minutes at various experimental temperatures of 60 °C, 80 °C and 100 °C. Meanwhile, regarding the differences in the quality of the samples used, based on the average total flavonoid content of the two samples, original quality green tea was superior to premium quality green tea. A graph of the total flavonoid content of premium and original green tea can be seen in **Figure 4**.

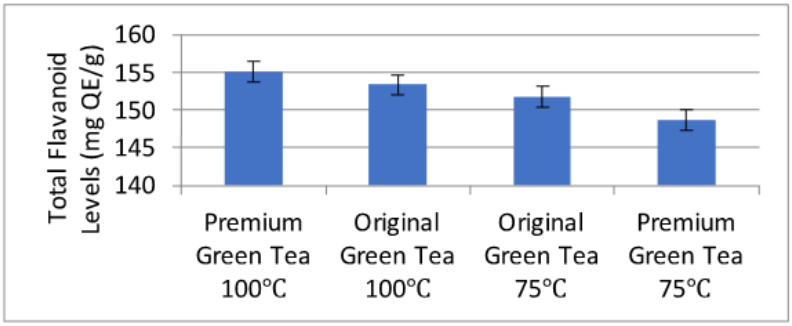


Figure 4. Graph of total flavonoid content of premium and original green tea

3.6. Preliminary Test for Antioxidant Activity

A preliminary test aims to find out whether the sample being tested has antioxidant activity. DPPH is a purple free radical. If DPPH reacts with antioxidant compounds, the intensity of the purple color is reduced. If free radical-reducing compounds react in large quantities or are strong antioxidants, DPPH can change color to yellow. In this test, react between DPPH at a concentration of 0.15 mM with original tea extract at a temperature of 100 ℃ and premium tea at a temperature of 100 ℃ and wait until the color is constant or does not change any more or at least 30 minutes. From this test, it is known that both green tea extracts contain antioxidants.

3.7. Measurement of Antioxidant Activity

The parameter for measuring antioxidant activity is the IC50 (Inhibitory Concentration) value (Purwanto et al., 2017). The percentage of free radical capture is the number of free radicals captured by standards and samples. The sample solution was left for 30 minutes at room temperature, and the DPPH free radical capture value was calculated at the maximum wavelength, as shown in **Figure 5**. The results from the percentage of free radical capture, the IC50 value, was calculated. Data on DPPH free radical capture and IC50 values for quercetin standards and samples can be seen in **Table 5**.

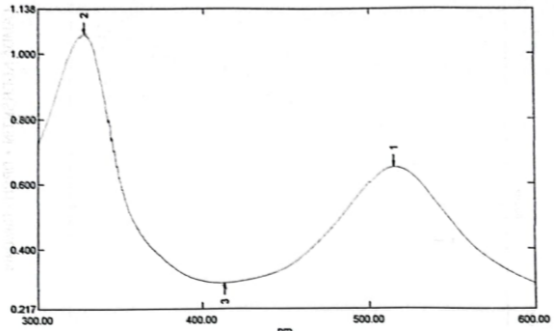


Figure 5. Graph of the maximum wavelength of a green tea sample, 515 nm.

Table 5. Data on % capture of DPPH free radicals and IC_{50} values of quercetin standards and samples

No.	Name	IC_{50}
1.	Standart Quercetin	3.069 ± 1.064
2.	Premium Green Tea 100 ℃	27.167 ± 1.817
3.	Original Green Tea 100 ℃	31.502 ± 6.746
4.	Original Green Tea 75 ℃	32.359 ± 7.346
5.	Premium Green Tea 75 ℃	27.527 ± 7.868

The results of the research show that green tea extract contains antioxidants. The IC_{50} value of standard quercetin is smaller than the IC_{50} value of the four samples, namely standard quercetin, original green tea at 75 ℃, original green tea at 100 ℃, premium green tea at 75 ℃, and premium green tea at 100 ℃. From the four tea extracts, it can be concluded that the smallest IC_{50} is in the premium tea extract at 100 ℃, which means that the premium tea extract at 100 ℃ has the best antioxidant activity. The IC_{50} value graph can be seen in **Figure 6**.

Analysis of the total flavonoid content of green tea with $AlCl_3$ using Uv-Vis spectrophotometry showed that the highest total flavonoid content value was premium green tea at a temperature of 100 ℃. Previous research was conducted by (Chatterjee et al., 2016), which stated that the highest total levels of green tea flavonoids were obtained at a temperature of 100 ℃ with an extraction time of 15 minutes at various experimental temperatures of 60 ℃, 80 ℃ and 100 ℃. The antioxidant activity test with DPPH reagent produced the strongest antioxidant activity with an IC_{50} value of 27.167 ± 1.817 µg/mL, namely premium green tea at 100 ℃. This follows research (Sasmito, 2020) in Sonneratia albapada green tea steeping water; it produces quite high antioxidant activity at a temperature of 100 °C for ten days with an IC_{50} value for DPPH

of 96.5 µg/mL. A compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 µg/ml, a strong antioxidant for an IC_{50} value of 50-100 µg/ml, and a moderate antioxidant if the IC_{50} value is 151-200 µg/ml (Andriani & Murtisiwi, 2020).

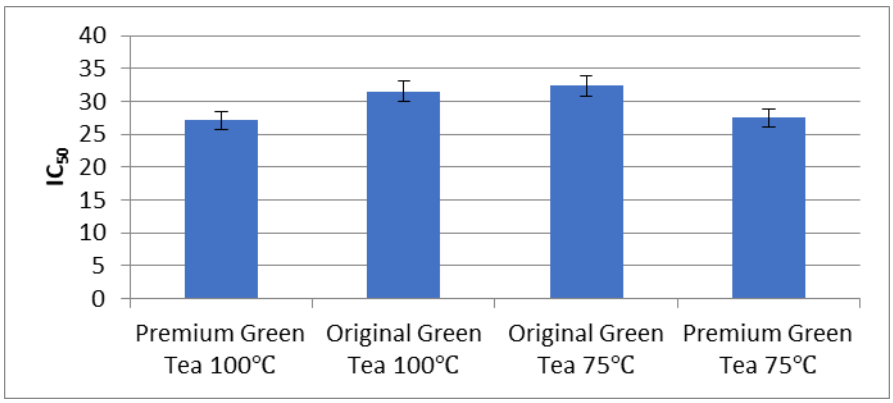


Figure 6. Graph of IC_{50} value for premium and original green tea

4. CONCLUSION

Analysis of the total flavonoids of green tea showed that the highest levels were in premium green tea at a temperature of 100 °C. The antioxidant activity test with DPPH reagent produced the highest antioxidant potential with an IC_{50} value of 27.167 ± 1.817 µg/mL, namely premium green tea at a temperature of 100 °C. So premium green tea at a brewing temperature of 100 is recommended to consumers. Premium quality green tea only uses young leaves at the top of the leaf.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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(2,2-Diphenyl-1-picrylhydrazyl) ASSAY of KENIKIR LEAVES (*C. caudatus* K.), BELUNTAS LEAVES (*Pluchea indica* L.), AND PURPLE CORN (*Zea mays*) AS A SOURCE OF ANTIOXIDANTS

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ABSTRACT
The purpose of this study is to assess and compare the antioxidant activity of the ethanol extract and ethyl acetate fraction of the leaves of Kenikir (*C. caudatus* K.), Beluntas (*Pluchea indica* L.), and purple corn seed (*Zea mays* L.) in an effort to find new sources of antioxidants. The samples were extracted with a 96% ethanol as the solvent. Afterward, the extract was separated using n-hexane and ethyl acetate to get ethyl acetate fraction. The antioxidant concentration was determined using the DPPH free radical technique using spectrophotometer UV-Vis. The results indicate that the purple corn's ethyl acetate fraction has the highest antioxidant activity as a free-radical scavenger, with an IC_{50} value of 10.47 µg/mL. The ethyl acetate fraction of *P. indica* leaves, the *P. indica* 96% ethanol extract, the *C. caudatus* leaves 96% ethanol extract, the purple corn's 96% ethanol extract, and the *P. indica* 96% ethanol extract are all listed after that. The purple corn's 96% ethanol extract has an IC_{50} value of 21.80 µg/mL. According to these findings, purple corn has the highest antioxidant activity and may be a new source of antioxidants.

Keywords: *C. caudatus* K.; *Pluchea indica* L.; *Zea mays* L.; Antioxidant; DPPH

1. INTRODUCTION

Degenerative disease is one of the world's most significant causes of death. The World Health Organization (WHO) identifies cardiovascular disease, cancer, and diabetes as the three most prevalent degenerative diseases. According to statistics, it kills 41 million people annually, or 71% of the global population. Oxidative stress and the excessive number of oxidation reactions occurring within the body produce degenerative disease (Werdhawati, 2014). Oxidative stress is typically used to describe an imbalance between the manifestation of free radicals in the systemic and the ability of cells to detoxify and negate the effects of damage to proteins, fats and DNA (Ahmadinejad et al., 2017). An antioxidant is needed to reduce the amount of excess free radicals and prevent the damaging effects of *oxidative stress* that can cause various diseases. The process of forming free radicals can occur through oxidation reactions. Antioxidants can inhibit oxidation reactions, even if only in small concentrations (Yadav et al., 2017). In order to stop the free radical reaction, antioxidants are chemical substances that can provide free radicals one or more electrons (electron donors). Due to its low molecular weight, this substance can inhibit the growth of oxidation reactions by inhibiting the production of radicals (De Araújo et al., 2014).

One such compound is a phenolic compound, a secondary metabolite that protects plant organs from oxidation. Therefore, phenolic compounds are referred to as natural antioxidants. In

addition to their activity as antioxidants, phenolic compounds in plants are known to have anticarcinogenic, antibacterial, antiallergic, antimutagenic, and anti-inflammatory properties (Sukweenadhi et al., 2020). Other phytochemicals that possess antioxidant activity are flavonoids. Flavonoids are a group of polyphenols and are classified by their chemical structure and biosynthesis. The basic structure of flavonoids consists of two aromatic groups combined by a carbon bridge (C6-C3-C6). The mechanism of flavonoids as antioxidants is divided into three, namely slowing down the formation of Reactive Oxygen Species (ROS), breaking down ROS and regulating / protecting with antioxidants. Flavonoids also stimulate internal antioxidant enzymes, suppress enzymes related to the formation of free radicals, and bind metals. Secondary metabolites of flavonoids derived from plants and some of them from *C. caudatus* leaves, *P. indica* leaves, and purple corn kernels since they have high level of flavonoids. Based on the study by (Mediani et al., 2013), the IC_{50} value of an ethanol extract of *C. caudatus* leaves at 80% concentration is 32 µg/mL (Werdhawati, 2014) revealed that the IC_{50} values for *P. indica* leaves ethanol extract and ethyl acetate, respectively, were 37.25 µg/mL and 3.33 µg/mL. The ethanol extract of purple corn kernels has an antioxidant activity with an IC_{50} value of 48.5 mg/mL. Because of this, the study's goal is to use the DPPH method to find out if the ethyl acetate and 96% ethanol extracts from these three samples have any antioxidant properties. The experiment selected three plants from highland areas in September, potentially offering a novel approach to this research.

2. METHODS

2.1. Materials

Plant materials: *C. caudatus* leaves were collected in Gunung Kidul, Yogyakarta, *P. indica* leaves were collected in UPT Materia Medica Batu, and purple corns were collected in Sukabumi, East Java. All of them were identified in Materia Medica, Batu, East Java, Indonesia. The specimen numbers are 074/555/102.7-A/2021.

Chemical materials: Ethanol p.a Merck (Germany), ethyl acetate p.a Merck (Germany), n-hexane p.a Merck (Germany), ascorbic acid Merck (Germany), *2-2-diphenyl-1-picrylhydrazyl*, Sigma-Aldrich (United States).

Instrument: UV-Vis Shimadzu 2700 Spectrophotometer (Tokyo, Japan).

2.2. Extract Preparation

Using 96 percent ethanol as the solvent, the dry powder of *C. caudatus*, *P. indica* leaves, and purple corn kernels was extracted with ratio 1:20 using ultrasound technology (Sonica® Ultrasonic Cleaners).

Three times within 10 minutes, the extraction was repeated. The extract was filtered through a membrane filtrate, and the resulting filtrate was evaporated at 40 °C in a rotary evaporator to get a 96 percent ethanol extract of *C. caudatus*, *P. indica* leaves, and purple corn kernels.

2.3. Fraction Preparation

The 96% ethanol extract of *C. caudatus*, *P. indica* leaves and purple corn kernels was first fractionated with n-hexane and aqueous with ratio 1:1. The aqueous phase was taken and fractionated with ethyl acetate until it turned colorless. The ethyl acetate phase was collected and evaporated at 40 °C in a rotary evaporator to obtain ethyl acetate fraction of *C. caudatus*, *P. indica* leaves, and purple corn kernels.

2.4. Flavonoid Test

The ethyl acetate fraction and 96% ethanol extract of *C. caudatus*, *P. indica* leaves, and purple corn seed were dissolved in 96% ethanol. A few drops of concentrated hydrochloride acid

and a pinch of magnesium powder were also added. The color of solution turning to reddish-orange shows that the test was successful (Suharyanto & Hayati, 2021).

2.5. Antioxidant Test (Damanik et al., 2014)

2.5.1. DPPH Stock Solution

A 100-ppm DPPH solution is prepared with 96% ethanol solvent and then incubated for 30 minutes at room temperature.

2.5.2. C.caudatus Extract and Fraction

Extract - Sample was prepared by weighing 100 mg of the extract and dissolved with 96% ethanol to 100 mL volumetric flask, then pipette 0.2 mL, 0.3 mL, 0.4 mL, and 0.6 mL into a 10.0 mL volumetric flask.

Fraction - A total of 25.0 mg of the ethyl acetate fraction sample was dissolved with 96% ethanol up to 25,0 mL. Then pipette 0.5 mL, 1.0 mL, 2.0 mL, and 3.0 mL into a 50.0 mL volumetric flask

Each extract and fraction sample were added a 0.5-mL solution of DPPH and then incubated for 30 minutes at room temperature.

2.5.3. Pluchea indica Extract and Fraction

Extract - Sample was prepared by weighing 25 mg of the extract and dissolved with 96% ethanol to 25 mL volumetric flask, then pipette 0.1 mL, 0.2 mL, 0.3 mL, and 0.4 mL into a 10.0 mL volumetric flask.

Fraction - A total of 25.0 mg of the ethyl acetate fraction sample was dissolved with 96% ethanol then transferred in a 25,0 mL volumetric flask. Then pipette 0.05 mL, 0.5 mL, 0.6 mL, and 0.7 mL into a 10.0 mL volumetric flask

Each extract and fraction sample were added a 0.5-mL solution of DPPH and then incubated for 30 minutes at room temperature.

2.5.4. Purple corn Extract and Fraction

Extract - Sample was prepared by weighing 25 mg of the extract and dissolved with 96% ethanol to 25 mL volumetric flask, then pipette 0.2 mL, 0.5 mL, 1.0 mL, and 2.0 mL into a 25.0 mL volumetric flask.

Fraction - A total of 0.07 g of the ethyl acetate fraction sample was dissolved with 96% ethanol up to 50.0 mL. Then pipette 0.1 mL, 0.3 mL, 0.5 mL, and 1.0 mL in to a 25.0 mL volumetric flask

Each extract and fraction sample were added a 0.5-mL solution of DPPH and then incubated for 30 minutes at room temperature.

2.6. Ascorbic Acid

Stock solution was prepared with concentration 1000 ppm and standard solution with range 10-50 ppm. Each solution was added DPPH solution 0.5 mL and incubated for 30 minutes.

2.7. Determination of Antioxidant Activity

The absorbance of the secondary solutions were collected by using spectrophotometer UV-Vis on the maximum wavelength. This process was repeated three times on each concentration. The percentage of inhibition was calculated from the following equation:

$$\%\ Inhibition = \frac{(A.Control - A.Sample)}{A.Sample} x100\% \quad (1)$$

The data of % inhibition was plotted with absorbance to obtain a linear equation. The equation was made to calculate Inhibition Concentration 50% (IC_{50}) to determine antioxidant activity.

2.8. Data Analysis

The data of the study were analyzed by a one-way ANOVA. The data were considered significant if P-value was less than 0.05.

3. RESULTS AND DISCUSSION

The imbalance between free radicals and antioxidants can lead in oxidative stress, which can develop to degenerative illnesses. Consequently, antioxidants are required to avoid degenerative illnesses. According to the classification based on source, antioxidants are classified into two types: endogenous, which are derived from the body's enzymes, and exogenous, which are derived from outside the body, such as plants. If the body's endogenous antioxidants are insufficient, exogenous antioxidants are required. According to this study, *C. caudatus*, *P. indica* and purple corn plants have sufficient antioxidant activity to meet demand.

3.1. Yield Value of Extract and Fraction

The result of extraction process using ultrasonic-assisted extraction method with 96% ethanol as the solvent was shown in **Table 1**. Pulling the desired compound was conducted by an extraction process using Ultrasonic Assisted Extraction (UAE) with 96% ethanol as a solvent. Extraction was carried out using UAE because it's a green extraction method, meaning it doesn't need a lot of solvents. It took an advantage of using ultrasonic wave to decrease the time of extraction without increasing the temperature. Using heat may resulting on degradation of some flavonoid. The 96% ethanol solvent was chosen because it is able to optimally attract nonpolar to polar flavonoid compounds. From the extraction process, the yield was 6.8% in *C. caudatus*, 14.7650% in *P. indica* and 5.37% in purple corn (**Table 1**). Based on the yield results, it was found that the yield obtained met the requirements with the respective yield value. *C. caudatus* yield value is the highest among the samples.

The fractionation process was carried out because the extraction results still consist of secondary metabolites with various levels of polarity. The extraction results were separated by the fractionation method using a solvent that can attract and optimally dissolve flavonoid compounds. The fractionation process was carried out using the stratified fractionation method. In the first stage, the process was conducted using n-hexane as solvent. The use of n-hexane solvent aims to separate non-polar compounds such as fatty acids with a carboxyl group because the carboxyl group inhibits the hydrogen atom donation reaction to neutralize free radicals. The next stage is fractionation using ethyl acetate solvent, intending to attract the flavonoid group compounds in the sample because the solvent is semi-polar. The process shows the principle of solubility, which means that compounds will be attracted to solvents with the same polarity level (Mamuaja, 2017).

Table 1. The 96% ethanol extract yield value of *C. caudatus, P. indica* leaves and purple corn

Name of the plant	Dry Powder Weight (g)	The obtained extraction (g)	Yield value
C. caudatus leaves	1099.96	82.45	7.50%
P. indica leaves	699.02	103.21	14.77%
purple corn	1649.67	58.14	5.37%

The result of fractionation process using liquid-liquid method with ethyl acetate as the solvent was shown in **Table 2**.

Table 2. The ethyl acetate fraction yield value of *C. caudatus, P. indica* leaves and purple corn.

Name of the plant	The obtained ethyl acetate fraction (g)	Yield value
C. caudatus leaves	12.16	0.88%
P. indica leaves	0.22	0.03 %
purple corn	0.08	0.005%

The *C. caudatus* secondary metabolite composition has been widely documented. Flavonoids and terpenoids are this plant's major secondary metabolites. Quercetin and catechin are some of the flavonoids that have been discovered (Widiyantoro & Harlia, 2020). *P.indica* leaves, which are rich in antioxidants, belong to the class of chemicals called flavonoids. It may be inferred from the Ultraviolet-Visible spectrum that the flavonoid chemicals found in beluntas leaves are of the flavonol type (Fitrya et al., 2023). Purple corn contains anthocyanins. Anthocyanins are naturally occurring pigments that are part of the flavonoid group. Their primary structure consists of two benzene aromatic rings (C6H6) joined by three carbon atoms joined by an oxygen atom (Rahmah et al., 2022).

Flavonoids include -OH groups that can establish hydrogen bonds; they are polar compounds. Nonetheless, a number of flavonoid types are less polar, including isoflavones, flavones, flavanones, aurones, chalcones, anthocyanins, and flavonols. Flavonoid extraction often uses a polar solvent, such as ethanol, due to the polar nature of most flavonoid molecules, including flavonoid glycosides and aglycones. However, to extract some less polar flavonoids, use ethyl acetate. Consequently, we use ethyl acetate solution to extract less polar flavonols (Widyawati et al., 2010). In this research, a yield percentage of 0,88% *C.caudatus* leaves, 0,03% *P.indica* leaves and 0,005% purple corn were obtained from the fractionation of 82,45 g of *C.caudatus* leaves ethanol extract, 103,21 g of *P.Indica* leaves's extract and 58,14 g of purple corn's extract.

3.2. Flavonoid Assay

The result of flavonoid assay on 96% ethanol extract and ethyl acetate fraction was shown in **Table 3**. Positive result was being indicated by the changing color of solution to reddish orange solution.Flavonoid assay was conducted to determine the presence or absence of antioxidant-functioning flavonoid compounds in three samples. The Wilstater method was used to conduct the test, and it was determined that the reddish orange-colored test results indicated the presence of flavonoid compounds in the three positive samples **(Table 3)**. This is due to the reduction of bonds caused by breaking glycoside bonds by flavonoids (Muthmainnah, 2019).

Table 3. The result of flavonoid test of *C. caudatus, P. indica* leaves and purple corn

Name of the plant	96% Ethanol Extract	Ethyl Acetate Fraction
C. caudatus leaves	(+)	(+)
P. indica leaves	(+)	(+)
purple corn	(+)	(+)

3.3. IC_{50} Value

IC_{50} value of *C. caudatus, P. indica leaves,* and purple corn by calculated the obtained linear equation of each sample was shown in **Table 4**.

Table 4. The obtained IC50 value of *C. caudatus, P. indica* leaves and purple corn.

Name of the plant	96% Ethanol Extract (µg/mL)	Ethyl Acetate Fraction (µg/mL)	P-value
C. caudatus leaves	93.98	33.90	
P. indica leaves	34.19	22.98	
purple corn	21.80	10.47	0.000
Ascorbic acid	4.792		

Antioxidant test was conducted using radical DPPH, colorimetric method based on the measurement of the scavenging capacity of antioxidants towards DPPH. DPPH is a free radical in the form of a stable organic nitrogen. it has pi electrons of the aromatic systems present in the molecule can compensate for the lack of an electron (Santos-Sánchez et al., 2019). A quick and accurate way to test for antioxidants is the DPPH method. The fundamental premise behind this approach is that antioxidants act as hydrogen donors. If the substance under test is an antioxidant, it will provide hydrogen to the radical DPPH and neutralize it (Tejaputri et al., 2019). In this

method, the 96% ethanol extract and ethyl acetate fraction of *C. caudatus, P. indica* leaves and purple corn were tested by adding DPPH solution. This study utilised a wavelength of 516.50 nm. The test results indicate the absorbance value of the test solution for each sample, after which the DPPH inhibition percentage is calculated. After obtaining the percent inhibition, linearity regression is calculated to determine the IC_{50} value. Purple corn ethyl acetate fraction had the highest IC_{50} value at 10.47 µg/mL.

This is followed by the purple corn's 96% ethanol extract, which has an IC_{50} value of 21.80 µg/mL., the *P. indica* leaves ethyl acetate fraction with an IC_{50} value of 22.98 µg/mL, the *P. indica* 96% ethanol extract with an IC50 value of 34.19 µg/mL., the *C. caudatus* leaves ethyl acetate fraction, which has an IC50 value of 33.90 µg/mL., and *C. caudatus* leaves 96% ethanol extract with an IC50 value of 93.98 µg/mL **(Table 4)**. The highest IC_{50} value is found in the ethyl acetate fraction of purple corn due to the presence of flavonoids from the anthocyanin group. Meanwhile, the compound which play as antioxidant agent of *C. caudatus* and *P. indica* are flavonoid as well, to be exact, catechin and quercetin. These flavonoids have conjugated double bonds in their structure, allowing them to be highly reactive and function as free radicals. Anthocyanins are capable of reacting with various free radicals derived from reactive oxygen, hydroxyl, and singlet oxygen (Priska et al., 2018). The mechanism for free radical inhibition is the termination of the chain of free radical propagation and the donation of electrons by all hydroxyl groups in ring B (Nurtiana, 2019). Catechins represent antioxidant activity by scavenging free radicals, chelating redox active transition-metal ions, inhibiting redox active transcription factors, inhibiting pro-oxidant enzymes, and inducing antioxidant enzymes (Zanwar et al., 2014). Quercetin works as antioxidant agent by maintaining oxidative balance. It regulates levels of glutathione (GSH) to neutralize free radical by donating a hydrogen (Xu et al., 2019).

Results indicate that temperature, pH, solvent system, solvent-to-solid ratio, and number of extractions are important factors that should be optimized for each sample to maximize extraction efficiency. The obtained IC_{50} in this study was analyzed using one-way ANOVA. The result show that there is a significant difference between the obtained IC_{50} because the p was less than 0.05. The limitation of this study was only analyzing antioxidant activity of ethanol extract and hexane fraction from *C. caudatus, P. indica leaves,* and purple corn. This study's limitation is that it didn't evaluate additional solvents, such n-hexane.

4. CONCLUSION

Purple corn's ethyl acetate fraction has the highest antioxidant activity as a free-radical scavenger with an IC_{50} value of 10.47 µg/mL., followed by its ethanol extract which has an IC_{50} value of 21.80 µg/mL. These three plants can be turned into preparations that act as antioxidants, according to the findings.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials
All data are available from the authors.

Competing Interests
The authors declare no competing interest.

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EFFECTIVENESS OF SEMBALUN ARABIC COFFEE (*Coffea arabica*) ACTIVE COMPOUNDS AS AN ALTERNATIVE FOR REDUCING OBESITY

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ABSTRACT
Obesity has become a global public health and nutrition problem, both in developed and developing countries. Based on the latest Riskesdas data in 2018, the prevalence of obesity in adults aged >18 years is still increasing, at 21.8%. Arabica coffee (*Coffea Arabica*) has active compounds such as caffeine, alkaloids, flavonoids, saponins, and tannins that can be used to reduce the risk of causing obesity. This study aims to determine the decrease in obesity index of wistar male white rats given ethanol extract of arabica coffee beans for 24 days in obese model rats induced with high fat diet for 50 days. Extraction was carried out on Arabica coffee beans using 96% ethanol solvent for 3x24 hours by maceration method. The treatment was carried out with extract doses of 200 (D1), 400 (D2), and 600 (D3) mg/kg BW for 24 days. Experimental animals were divided into 5 groups, namely negative control given 1% CMC, positive control given orlistat 30 mg/kg BW, control group given ethanol extract of Arabica coffee beans at a dose of 200 (D1) mg/kg BW, 400 (D2) mg/kg BW, and 600 (D3) mg/kg BW. Obesity parameters were measured by Lee index and fat index. Arabica coffee extract (ACE) at a dose of D1 mg/kg BW showed a decrease in body weight of 47.88%, higher than the dose of D2 by (23.49%) and D3 by (14.00%). Caffeine contained in Arabica coffee beans (*Coffea arabica*) shows potential as an obesity inhibiting agent. The effective dose of ethanol extract from Arabica coffee beans (*Coffea arabica*) which has the effect of lowering the obesity index is in the D2 group.

Keywords: Arabica coffee beans, *Index lee*, Obesity, Weight loss

1. INTRODUCTION

Obesity has become a health and nutrition problem for the world community, both in developed and developing countries. Based on the latest Riskesdas data in 2018, the prevalence of obesity in adults aged >18 years is still increasing, at 21.8%. Obesity is a condition of imbalance between height and weight due to an excessive amount of body fat tissue (Sinaga, 2020). One of the diseases caused by obesity is type II diabetes mellitus which is a chronic condition and is associated with complications such as neuropathy, nephropathy, and other diseases that are dangerous to health.

One way to reduce obesity is to consume 200 ml/day of caffeine, based on a simulation of human habits of drinking a cup of coffee. Arabica coffee (*Coffea arabica*) has active compounds such as caffeines, alkaloids, flavonoids, saponins, and tannins so that they can be

used as functional foods to reduce the risk of obesity. Previous research conducted by Ardiansyah *et al*., (2019) about the obesity index reduction activity test from ethanol extract of robusta green coffee beans *(Coffea canephora)* against male white rats of the Wistar strain. The difference between this study and previous research on the type of sample used, and the location of sampling. Sembalun Arabica coffee samples have never been the subject of research related to obesity. Josephine *et al*., (2022) conducted a test on Tea (*Camellia sinensis*) which can help reduce the risk of obesity due to the presence of bioactive compounds such as flavonoids, tannins, 1-deoxynojirimycin, caffeine, catechins, and theaflavins. In vitro and in vivo experiments have demonstrated caffeine's ability to induce browning of adipose tissue, increase UCP1 protein abundance, and enha hance mitochondrial biogenesis (Velickovic *et al.,* 2019). According to Trivana *et al*., (2023), based on the research of Matsumoto *et al.,* (1993), in vitro data show that green tea catechins can reduce glucose absorption by inhibiting digestive enzymes involved in nutrient digestion, specifically the activity of α-amylase and α-glucosidase. Green tea catechins may also interfere with fat digestion and absorption. From this background, there are similarities between *Coffea arabica* and *Camellia sinensis* due to their caffeine content, which can be used as an alternative to reduce obesity.

Arabica coffee has a distinctive and best taste compared to other types of coffee so Arabica coffee is more in demand in the world market (Arlius *et al*., 2017). One of the regions in Indonesia that cultivates coffee plants is Sajang Village, Sembalun District, East Lombok Regency, NTB. The Sajang area is an abundant coffee-producing area with the location of its plantations directly under the slopes of Mount Rinjani. The larger size of coffee compared to other types of Arabica makes it a superior product in the Sembalun area. Coffee cultivation in Sembalun is also directly carried out by coffee farmers and the community there. In addition, Lombok's local coffee also meets good coffee standards based on the standards of the *Specialty Coffee Association America* (SCAA) (Afifi *et al.,* 2022). This study was conducted to determine the activity of Arabica coffee beans as a decrease in the obesity index in male white rats Induced high-fat diet foods to obtain mice with an obese model.

2. METHOD

2.1. Tools and Materials

The tools used are evaporators, analytical scales, oral sondes, evaporation cups, glass utensils (pyrex), filter paper, Buchner funnels, and vacuums. The plant material used in this study is Arabica coffee beans from Sembalun District, East Lombok, West Nusa Tenggara. The materials used in this study were: CMC (Merck), Aquadest, Orlistat (Novell), beef fat, egg yolk, coconut oil, phokphand 551 standard feed, and chemicals used for phytochemical refining.

2.2. Symplisia Characterization and Phytochemical Filtration

Phytochemical filtration is carried out on simplicial and arabica coffee bean extract to determine the content of secondary metabolite compounds, the test is by MMI or Farnsworth which includes testing for alkaloids, flavonoids, tannins, phenolates, triterpenoids, steroids, monoterpenes, sesquiterpenes, and quinones (Ardiansyah *et al.,* 2019). The MMI or Farnsworth method is a method used to identify secondary metabolites in plant extracts. Various reagents are used to detect different classes of compounds, such as Mayer reagent for alkaloids, magnesium powder and HCl for flavonoids, and $FeCl_3$ for tannins and phenols (Handayani *et al*., 2017).

2.3. Extraction

Sembalun arabica coffee beans are obtained from Sembalun District, East Lombok, NTB. The plantation is located under the slopes of Mount Rinjani (-8.317242,116.482858) during the dry season in April 2024. The recommended spacing for coffee cultivation is 2,5x2,5 meters for arabica. This spacing is varied with a land elevation of 1,600 above sea level. The higher the land the moresparse and the lower, the tighter the spacing. Sembalun Arabica coffee harvest

period belongs to the winter season. Harvesting Coffee cultivation is done in stages gradually, the main harvest usually occurs in 4-5 months in April and May and this coffee harvest occurs twice a year 1 year 2 times with a picking time interval of every 10-14 days (Sarjan *et al*., 2021). A total of 1,500 kg of crushed arabica coffee bean powder was weighed and then macerated using 96% ethanol solvent as much as 1,000 ml for 3x24 hours. The resulting filtrate is accommodated and filtered and then concentrated using a rotary evaporator (Ardiansyah *et al*., 2019).

2.4. Preparation of Test Animals

The experimental animals were male white rats of the Wistar strain with a weight variation of 160-200 grams, with the age of 2-3 months given standard feed (Phokphand 551) and ad libitum drinking water. This experimental animal has obtained an *Ethical Clearance* (EC) from the University of Mataram. The experimental animals were divided into 5 experimental groups, namely the negative control was only given CMC 1%, the positive control was given orlistat 30 mg/kg BW, the control group was given ethanol extract of Arabica coffee beans with doses of 200 (D1) mg/kg BW, 400 (D2) mg/kg BW, and 600 (D3) mg/kg BW. The minimum number of groups based on Federer's formula is 5 mice (Ardiansyah *et al*., 2019).

2.5. Induction of Obesity in Experimental Animals

The experimental animals were induced with a high-fat diet for 50 days with a feed composition of 5% chicken egg yolk, 1% coconut oil, 10% beef fat, and 100% standard feed (phokphan 551) ad. Parameters to determine obese rats with lee index with the formula (rat weight (g)1/3/nasoanal length (cm)) x 103, rats that are said to be obese have a lee index value of >300 (Ardiansyah *et al*., 2019).

2.6. Obesity Index Testing

Experimental animals that were said to be obese were given a weight loss treatment for 24 days in each group given standard feed and drinking water. The mice that were said to be not obese had a Lee index value (<0.05), and continued *Analysis of Variant* (ANOVA) and continued the test *Post Hoc Least Significant Difference* (LSD). Statistical tests were conducted with a confidence level of 95% P (<0.05) (Ardiansyah *et al*., 2019).

3. RESULTS AND DISCUSSION

3.1. Results of Simplicia Characterization

The extraction method in this study is the maceration method using 96% ethanol solvent. The maceration method was chosen because of its simple equipment and workmanship. The main reason for the use of 96% ethanol as an extraction solvent is its ability to dissolve a wide variety of compounds, both polar, semipolar, and nonpolar, and its relatively low level of toxicity to living organisms (Vonna *et al*., 2021). The extraction process is carried out for 3x24 hours, this aims to maximize the process of taking chemical compounds contained in Arabica coffee. The thick extract obtained was 282.85 grams with a yield of 14.1425%. Yield is a comparison between the results of many extraction processes and the weight of the sample used. The yield is said to be good if the value is more than 10%, so the yield obtained can be declared good (Noviyanto *et al*., 2024).

3.2. Simplicia Filtering Results

Phytochemical filtration is a qualitative analysis carried out to determine the content of secondary metabolites contained in the simplicial ethanol extract of Arabica coffee beans (*Coffea arabica*). The results of phytochemical filtration of arabica coffee bean extract are shown in Table 1**.**

Table 1. Results of Phytochemical Filtration of Extract and Simplicia of Arabica Coffee Beans *(Coffea arabica)*

Testing	Reagents	Result	Simplisia	Extract
Alkaloids	Dragendoff	Brownish orange	+	+
Flavonoids	Mg + HCl concentrate	Deep black	-	-
Phenol	$FeCl_3$	Blackish green	+	+
Tannins	$FeCl_3$	Blackish green	+	+
Steroids	H_2SO_4	Blue	+	+
Saponins	Shuffled	No foam formed	-	-

From the results of the phytochemical filtration test above, it shows that the ethanol extract of Arabica coffee beans contains alkaloid compounds that are proven to change color and brown deposits, The purpose of adding HCl is because the alkaloid is alkaline so it is usually extracted with a solvent containing acid (Hadi & Permatasari, 2019). In the flavonoid test, it was declared negative which was marked with a deep black color with a concentrated Mg and HCl powder reagent. A positive result will show red, yellow, or orange when reduced with Mg and HCl. In this test, hydrogen gas bubbles (H_2) due to the reaction between Mg and HCl metals so that it can reduce the nucleotide (Mangiwa & Maryuni, 2019). The phenol test using $FeCl_3$ showed positive results which were characterized by a change in color to blackish-green. Phenol compounds form complex bonds with iron, causing color changes from purple to blackish-green (Fajriaty *et al.*, 2017). Tannin compounds are polar because there is an OH group, when $FeCl_3$ is added, there will be a color change to dark blue due to hydrolyzed tannins, or blackish green due to condensed tannins (Ningsih, 2017). The saponin test was negative when there was no foam after being shaken. Positive results were shown on foam 1-10 cm high in a few minutes because saponins are compounds that are easily detected through their ability to form foam (Ningsih, 2017).

3.3. Obesity Index Test Results

The weight gain test of the mice was carried out for 50 days by feeding a high-fat diet (MDTL), the goal of which was to obtain mice with an obese model. Rat test animals have obtained a permit *EC* with the protocol number UNRAM266123 from the University of Mataram. Weight gain parameters related to the level of obesity in rats expressed by *Index Lee*. Average results *Index Lee* can be seen in Table 2 and Figure 1. In Table 2 values *Index Lee* experienced an increase on the 0th to 50th day. This is related to the higher the value *Index Lee*, the greater the obesity rate (Ardiansyah *et al.*, 2019). The positive group on day 0 showed an average *Index Lee* The weight is quite high compared to other groups. This is due to the age factor where the positive group is older than the other groups. These results are by the research of Jensen *et al.*, (2021) that younger mice (3-5 weeks old at the start of the study) experienced a noticeable decrease in bone mass 12-16 weeks after following a high-fat diet compared to older mice (12-16 weeks at the time of the study). Therefore, age is one of the important factors in the change in bone mass due to obesity compared to the severity of obesity.

The administration of high-fat diet feed (MDTL) was able to increase the weight of rats in all dosage and control treatments because the intake of food entering the body was much greater than necessary so that nutrients and fat were stored automatically in muscle tissue and adipose tissue resulting in weight gain (Ardiansyah *et al.*, 2019). Weight gain was followed by an increase in fat involving viscera adipose regions such as parirenal, epididymal, and retroperitoneal compared to the normal group given standard feed. This means that high-fat diet feed affects fat formation (Istiqomah *et al.*, 2022). Here is the results *Index Lee* before induction on day 0 and after induction on day 50 are shown in Table 2 and Figure 1.

Table 2. Lee's *Index* Value of Weight Gain Induced by MDTL Day 0 and Day 50

Treatment Groups	Before Induction After Induction			
	Day 0 Index Lee	Day 50 Index Lee		
Positive Control	207.33	257.53	211.66	267.65
Negative Control	164.83	250.18	147.33	313.51
D1 mg/kg BW	118.83	262.99	155.33	334.09
D2 mg/kg BW	123.33	257.86	157.85	298.00
D3 mg/kg BW	138.16	278.02	160.66	316.18

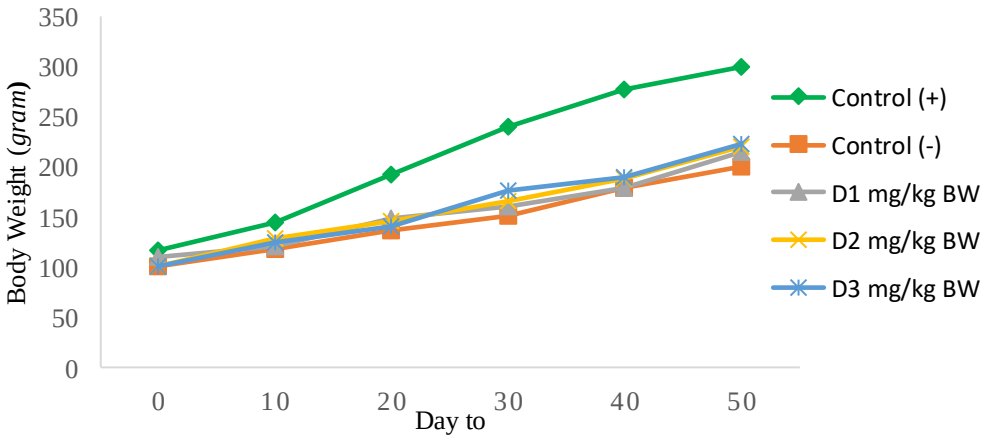

Figure 1. Weight Gain of Rats After MDTL Induction for 50 Days

3.4. Rat Weight Loss Results

Weight loss tests on rats were carried out for 24 days with standard feed and drinking water in each treatment group. Weight loss before the treatment, which is on the 50th day, is calculated as the 1st day, while the results after the treatment are calculated from the 51st day to the 74th day, as seen in Table 3.

Table 3. Average Weight Loss Before and After Arabica Coffee Bean Extract Treatment and Weight Loss Percentage Over 24 Days

Treatment Groups	Average Weight Loss (grams) in ±SD Rats		Percentage Decline Body Weight (%)
	Before Treatment	After Treatment	
Positive Control	211.67 ± 73.21[a]	140.83 ± 50.08	-33%
Negative Control	160.67 ± 48.38[b]	207.33 ± 33.00	29%
D1 mg/kg BW	157.67 ± 42.95[a]	118.33 ± 26.16	-24%
D2 mg/kg BW	155.33 ± 38.24[a]	143.5 ± 10.15	-9%
D3 mg/kg BW	147.33 ± 37.46[a]	138.16 ± 6.48	-6%

Remarks: a,b = if the letters are different, then significant (P<0.05)

Table 4. Post Hoc test results of arabica coffee bean extract treatment

Treatment Groups	Comparison Group	Mean Difference
Positive	Negative	64.333[b]
Negative	Positive	-64.333[b]
D1	D2	-10.333[a]
	D3	-13.333[a]
D2	D1	2.333[a]
	D3	-3.000[a]
D3	D1	5.333[a]
	D2	3.000[a]

Remarks: a,b = if the letters are different, then significant (P<0.05)

Caffeine functions as an adenosine receptor antagonist, which plays a role in increasing the release of the neurotransmitter dopamine. An increase in dopamine can improve mood and concentration, but can also cause side effects such as anxiety and restlessness at high doses.

Small doses of caffeine are generally anxiolytic (reducing anxiety), while high doses can be anxiogenic (causing anxiety), especially in susceptible individuals (Dewanti & Tadjudin, 2022). According to Dr. Erich Harnack (Tsatsakis *et al*., 2018), the minimum level of observable action that a drug induces is called the minimum effective dose. Therefore, the minimum dose of D1 is effective in reducing the body weight of rats. In the positive control group, Orlistat was given as an obesity reduction rate where the weight loss rate was (-33%), while the negative group was only given CMC 1% (29%), this means that CMC 1% does not affect weight loss. From the results of the *ANOVA* statistical analysis, there was no significant difference between the treatment groups, with a significance value of (P>0.05) which means that the results of the study did not show strong enough evidence to conclude that there was a difference between the treatment groups.

The Post Hoc test was carried out to determine the difference in the activity of arabica coffee bean ethanol extract at D1, D2, and D2, respectively, which showed significant or insignificant pharmacological effects on weight loss compared to the negative control group (CMC 1%) and positive control group (Orlistat 30 mg/kg BW). Based on the results of the Post Hoc test in the negative control group, there was a significant difference with the positive control group with a significance value (*P<0.05*). This showed that the positive control group (Orlistat 30 mg/kgBW) experienced greater weight loss than the negative control group (CMC 1%), so the positive group did not have the same effectiveness as the negative group or orlistat 30 mg/kgBW was more effective in losing rat weight than CMC 1%.

In the positive group, there was no significant difference in the significance value (*P>0.05*) with the D2 group so it is interpreted that D1 does not have the same effectiveness as the positive group (orlistat 30 mg/kg BW) in weight loss. Post Hoc test results between ethanol extract of Arabica coffee beans at D1, D2, and D3 showed no significant difference (*P>0.05*) (Table 4), this means that the dose group of Arabica coffee bean ethanol extract does not have the same effectiveness as orlistat 30 mg/kg BW which can cause weight loss. Orlistat works by inhibiting the absorption of fat and the pancreatic lipase enzyme, resulting in the excretion of fat from the body through feces (Shiyan *et al.,* 2017). Lipases are enzymes that catalyze the hydrolysis of fats and oils, breaking down triglycerides into free fatty acids and glycerol (Sholeha & Agustini, 2021). Therefore, the pancreas that does not produce enough lipase enzymes will result in impaired fat absorption.

Then, the weight gain parameter was related to the obesity rate in the mice which was expressed by *the lee index*. The average results of the decline *in the lee index* before and after the treatment as well as the percentage of decline in *the lee index* can be seen in Table 5 and Figure 2.

Table 5. Average Lee *Index Decline* Before and After Caffeine Extract Treatment and Percentage Decrease in *Lee Index* Over 24 Days

Treatment Groups	Average Lee Index Results in Rats		Percentage Decline *of Lee Index* (%)
	Dad1y 0	Day 24	
Positive Control	267.65	262.72	1.84%
Negative Control	313.51	308.12	1.71%
ACE 200 mg/kg BW Dose (D1)	334.09	281.66	15.69%
ACE 400 mg/kg BW Dose (D2)	298.00	284.45	4.54%
ACE 600 mg/kg BW Dose (D3)	316.18	278.85	10.03%

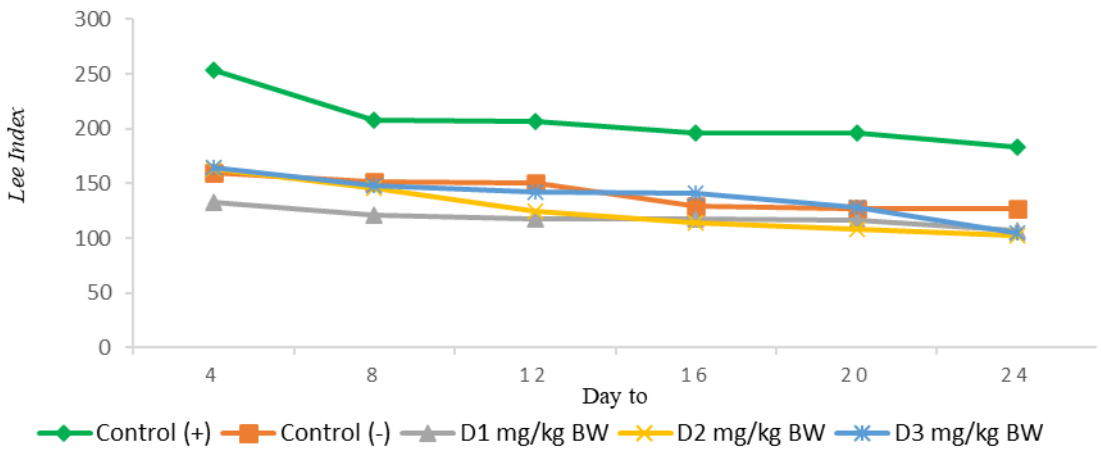

Figure 2. Caffeine Extract Rat Weight Loss for 24 Days

From Table 5 above, it is explained that there was a decrease in obesity in the treatment group of Arabica coffee bean ethanol extract with the highest D1 with a decrease in *Index Lee* (15.69%), when compared to D3 (10.03%), D2 (4.54%), positive control (1.84%), and negative control group with the lowest percentage (1.71%). Arabica coffee bean ethanol extract may contain various active compounds i.e. caffeine and chloronic acid with different mechanisms of action. At D1dose, this combination of compounds may work synergistically to boost metabolism and suppress appetite, thereby losing weight. On the other hand, in D2 and D3 Dose, the effects of either compound may be dominant, causing side effects. which inhibits weight loss. If you look at Table 4 above, the obesity rate after 24 days of extract treatment in the positive control group, the doses of D2, D2, and D3 extracts showed that they were not obese, while in the negative control group they were obese, this is because CMC 1% did not have an effect as an obesity reduction (Ardiansyah *et al*., 2019) So that the negative control group experienced obesity with a decrease in value *Index Lee* lowest (1,71%). Rats that were said to be not obese had an index value of Lee <300 (Ardiansyah *et al*., 2019). This means that weight loss and gain affect *Index Lee* as a parameter of the obesity index.

The average result of the increase in BW based on *the Lee index* for 50 days and the decrease in BW based on *the Lee index* for 24 days before and after the treatment of Ethanol Extract of Arabica coffee beans can be seen in Figure 3.

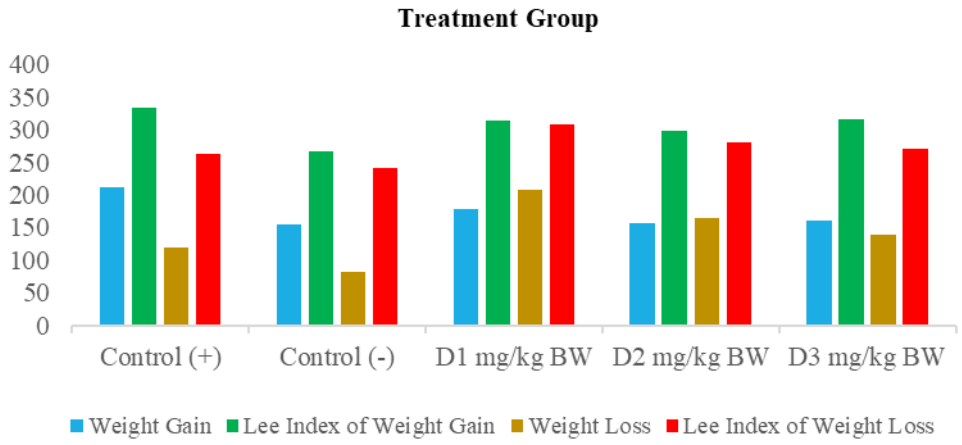

Figure 3. The average increase in BW based on the Lee index for 50 days and decrease in BW based on the Lee index for 24 days Before and After Ethanol Extract treatment of arabica coffee beans

From the figure above, it is explained that the average increase in BW and *Lee index* increased BW for the highest 50 days in the positive control group, while the increase in BW and *Lee index* increased the lowest BW in the negative control. The decrease in BW and *lee*

index of the highest 24-day decrease in BW was in the D1 group compared to the positive group and the negative group as well as D2 and D3. This shows that Arabica coffee bean ethanol extract has the potential as a weight loss and fat reduction agent. The most effective ethanol extract of arabica coffee beans is found at D1. Higher doses of D2 and D3 are less effective in promoting weight loss and fat reduction.

3.5. Fat Index and Organ Index

The fat index test is carried out by taking samples of abdominal fat tissue from the retroperitoneal area located behind the kidneys and liver. The following average results of testing fat index and organ index parameters over 74 days can be seen in Table 6. In Table 6, the fat index results at D1 were lower than all treatment groups, while the highest fat index was found at D2. The results of the analysis showed a significance value ($P<0.05$), meaning that there was a significant difference in the fat index between the treatment groups. The fat index can be interpreted as the body mass index (BMI), the higher the BMI, the higher the fat mass. This is by research by Susantini, (2021) which states that the relationship between BMI and body fat percentage with a positive coefficient means that there is a positive relationship between BMI value and fat mass percentage, the higher BMI will increase fat mass percentage. A high BMI is often associated with various health problems such as type 2 DM. Research by Masruroh, (2018), proves that there is a relationship between BMI and blood sugar levels of people with Type 2 *Diabetes Mellitus*.

Furthermore, the testing of organ index parameters aims to determine the fat deposits contained in the liver. Based on the analysis test, it showed a significance value ($P>0.05$) in the liver index, meaning that there was no significant difference in the treatment group and there was no fat accumulation in the liver.

Table 6. The average value of fat index and organ index parameter testing for 74 days

Treatment groups	Fat Index (%)	Organ Index (%)
Negative Control	5.91 ± 2.03	0.12 ± 0.18
Positive Control	6.90 ± 0.76	0.03 ± 0.01
ACE 200 mg/kg BW Dose (D1)	1.60 ± 0.55	0.05 ± 0.01
ACE 400 mg/kg BW Dose (D2)	7.64 ± 2.45	0.06 ± 0.02
ACE 600 mg/kg BW Dose (D3)	1.81 ±0.73	0.08 ± 0.04

4. CONCLUSION

Caffeine contained in Arabica coffee beans (*Coffea arabica*) shows potential as an obesity inhibiting agent. The effective dose of ethanol extract from Arabica coffee beans (*Coffea arabica*) which has the effect of lowering the obesity index is in the D2 group.

5. SUGGESTIONS

It is recommended to conduct pre-test and post-test tests to obtain more comprehensive data on changes in fat levels before and after obesity treatment.

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7. CONFLICTS OF INTEREST

The author states that there is no conflict of interest in this study.

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