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Research Article

Evaluation of the mucolytic activity of ethanol extract of Temu Ireng (*Curcuma aeruginosa* Roxb.)

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ABSTRACT

Curcuma aeruginosa Roxb., known as Temu Ireng, is a species within the Zingiberaceae family that has been empirically noted for its potential as a cough remedy. Temu Ireng contains alkaloids, flavonoids, and saponins, which play crucial roles in mucus liquefaction. This study aims to investigate the mucolytic activity of the ethanol extract of *Curcuma aeruginosa* Roxb. In this study, we used an experimental method. The extract was obtained via maceration and analyzed in vitro for its ability to reduce the viscosity of bovine intestinal mucus using a digital viscometer. Bovine intestinal mucus was utilized due to its compositional similarity to human mucus. Test sample concentrations of the Temu Ireng extract were 1.0%, 1.5%, and 2.0%, with acetylcysteine serving as the positive control. Mucolytic activity was indicated by a decrease in mucus solution viscosity. The data were analyzed using one-way ANOVA. The results demonstrated a decrease in mucus viscosity for the Temu Ireng extract test solutions, with statistical analysis indicating a significant difference between the negative control and both the positive control and the Temu Ireng extract samples at concentrations of 1.5% and 2.0%, with p-values of 0.032 and 0.030 respectively ($p<0.05$). The study concludes that the ethanol extract of *Curcuma aeruginosa* Roxb. exhibits mucolytic activity at concentrations of 1.0%, 1.5%, and 2.0%.

1. Introduction

Coughing is a defensive reflex of the body to clear secretions and particles in the respiratory tract, as well as to protect against aspiration or inhalation of foreign materials, pathogens, inflammation, and

post-nasal drip (Wibowo, 2021). Symptomatic treatment using cough medicines is often employed to relieve cough symptoms (Clara et al., 2022). Mucolytics are a type of medication that works by liquefying respiratory secretions by breaking down or destroying mucoproteins and mucopolysaccharide filaments in mucus or phlegm (Ali, 2019). This mucolytic effect can also be found in various herbal plants that are widespread throughout Indonesia. Many of these plants have been traditionally used in Indonesian medicine for generations, harnessing their natural properties to treat respiratory conditions. The extensive biodiversity in Indonesia includes a multitude of species known for their medicinal benefits. Furthermore, Indonesia boasts an abundant biodiversity, with numerous herbal plants that can be utilized. Any type of plant containing one or more active components beneficial for treatment can be termed a medicinal plant or defined as any plant believed and known to have medicinal properties (Nurhazizah, 2021). The use of herbal concoctions for treatment can be a good choice, especially if you are looking for a natural alternative with a lower risk of side effects. One of the herbal plants that is empirically used as a medicine is black ginger. The use of herbal concoctions for treatment can be a good choice, especially if you are looking for a natural alternative with a lower risk of side effects. One of the herbal plants that is empirically used as a medicine is Temu ireng (*Curcuma aeruginosa* Roxb.).

Temu ireng (*Curcuma aeruginosa* Roxb.) is one of the Zingiberaceae species known to Indonesians as a medicinal herb (Setiadi et al., 2017). This plant is recognized for its distinctive dark purple rhizomes and is widely used in traditional medicine across Indonesia. Empirically, the community utilizes Temu ireng (*Curcuma aeruginosa* Roxb.) for various health benefits, including supporting skin health, treating asthma, serving as a remedy for coughs, stimulating appetite, and acting as an anthelmintic to expel parasitic worms (Andesmora et al., 2022). Further, according to Dwi Susiloningrum (2022), Temu ireng contains a rich array of bioactive compounds, including flavonoids, steroids, saponins, terpenoids, and alkaloids (Susiloningrum et al., 2022). These components are known for their diverse pharmacological effects. Flavonoids, for instance, possess antioxidant and anti-inflammatory properties, which can help reduce inflammation in the respiratory tract and support overall respiratory health (Wu et al., 2024). Steroids and terpenoids have been studied for their anti-inflammatory and immune-modulating effects, which may contribute to alleviating symptoms of asthma and other inflammatory conditions. Saponins are known for their expectorant properties, which can help in thinning and expelling mucus, thereby providing relief from coughs (Lalitha et al., 2024). Alkaloids, on the other hand, have been recognized for their therapeutic potential in various treatments due to their diverse bioactivities (Gonfa et al., 2024).

Collectively, these bioactive compounds in Temu ireng can provide a mucolytic effect, which helps break down mucus, making it less viscous and easier to expel from the respiratory tract. This action is particularly beneficial in managing coughs and respiratory infections where mucus build-up is a common issue. The traditional use of Temu ireng in Indonesian medicine highlights its importance and potential as a natural remedy for respiratory ailments, supporting its role in both historical and contemporary healthcare practices.

2. Materials and Methods

This study was an experimental research. It was conducted in vitro using bovine intestinal mucus to determine the mucolytic activity of Temu ireng (*Curcuma aeruginosa* Roxb.) extract in treating productive cough. The extract of Temu ireng (*Curcuma aeruginosa* Roxb.) was obtained using the maceration method and analyzed in vitro to reduce the viscosity of bovine intestinal mucus using a digital viscometer. Bovine intestinal mucus was used due to its composition being nearly identical to human mucus. Acetylcysteine 0.1% was used as a positive control, with test sample extract concentrations of Temu ireng at 1.0%, 1.5%,

and 2.0%. The mucolytic activity was indicated by the reduction in the viscosity value of the mucus solution. The obtained data were analyzed using one-way ANOVA.

Sample preparation

The extraction of Temu Ireng (*Curcuma aeruginosa* Roxb.) was performed using the maceration method with 96% ethanol as the solvent. Specifically, 500 g of powdered simplicia of Temu Ireng was dissolved in 96% ethanol at a solvent-to-powder ratio of 1:5 and left to macerate for 5 x 24 hours, with stirring once daily for 60 minutes. After five days, the solution was filtered, and the residue was re-macerated with 96% ethanol (1:5) for 2 x 24 hours. The combined filtrates were evaporated using a rotary evaporator at 40°C and 60 rpm until no solvent remained, followed by further evaporation in a water bath at 45°C until a thick, constant weight extract was obtained.

Phytochemical Identification

Alkaloid Identification. To identify alkaloids, 0.2 grams of concentrated Temu Ireng rhizome extract was dissolved in 5 ml of 2N HCl in a test tube. The solution was evaporated for 2 minutes, allowed to cool, and then 3 drops of Mayer's reagent were added. The formation of a red or reddish-brown precipitate indicates the presence of alkaloids (Oktavia et al., 2021).

Flavonoid Identification. To identify flavonoids, 2 ml of Temu Ireng rhizome extract was placed in a test tube, followed by the addition of a few milligrams of magnesium powder and 1 ml of concentrated HCl solution. A color change from orange-red to purple-red indicates the presence of flavonoids (Doloking et al., 2022).

Saponin Identification. To identify saponins, the Temu Ireng extract was vigorously shaken with 10 ml of distilled water in a test tube for 10 seconds and then allowed to stand for 10 seconds. The formation of a foam layer 1-10 cm high that persists for at least 10 minutes, even after adding one drop of 2N HCl, indicates the presence of saponins (Inalegwu et al., 2015).

Phenol Identification. To identify phenolic compounds, 1 ml of 10% FeCl3 solution was added to 1 ml of Temu Ireng extract. The formation of a dark blue or greenish-black color indicates the presence of phenolic compounds (Sukandiarsyah et al., 2023).

Tannin Identification. To identify tannins, 0.2 grams of Temu Ireng rhizome extract was placed in a test tube and 2 drops of 1% FeCl3 solution were added. The appearance of a dark blue or greenish-black color indicates the presence of tannins (Oktavia et al., 2021).

Mucolytic Activity Testing

Preparation of Negative Control Solution. For the negative control solution, 0.5 grams of Tween 80 was added to a phosphate buffer mucus solution up to a total volume of 100 ml, followed by homogenization.

Preparation of Positive Control Solution. For the positive control solution, 0.1% acetylcysteine and 0.5 grams of Tween 80 were added to a phosphate buffer mucus solution up to a total volume of 100 ml, followed by homogenization.

Preparation of Test Solutions. Test solutions with concentrations of 1.0%, 1.5%, and 2.0% Temu Ireng extract were prepared. For each concentration, 0.5 grams of Tween 80 was added, and the phosphate buffer mucus solution was added up to a total volume of 100 ml, followed by homogenization.

Mucolytic Activity Test. The viscosity of each test solution was measured using a digital viscometer with rotor number 2 at a speed of 60 rpm. Measurements were taken at time intervals of 0 seconds, 30 seconds, 1 minute, 3 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, and 35 minutes.

Data analysis

After the data results were obtained, the next step was to determine the significant differences between positive control, negative control, concentrations of 1% 1,5% and 2% using the One way Anova statistical test (α = 0.05) with a confidence level of 99%.

3. Results and Discussions

Extraction

The extraction method used in this study is maceration. According to Widyaningrum (2020), maceration is the simplest cold extraction method, wherein the solvent penetrates the plant cell walls and enters the cell cavities containing the active compounds. This process causes the concentrated active compounds to be expelled from the cells due to the concentration gradient between the interior and exterior of the cells. The maceration method was chosen because the equipment is very simple and easy to use. However, the drawback of this method is that it requires a long extraction time and the results may not be perfect. The primary goal of the maceration method is to produce an extract rich in active compounds without significantly damaging or altering them (Mugiyanto et al., 2018).

The obtained thick extract is dark brown with a distinctive odor, weighing 113 grams, with a yield of 22.6% and a moisture content of 0.25%. These results are higher compared to the study by (Sukandiarsyah et al., 2023), where the black turmeric extract weighed 65.44 grams using the same extraction method. According to the Indonesian Herbal Pharmacopoeia 2017, the standard yield for black turmeric extract is more than 13.19% and the moisture content is less than 10%. These results indicate that the obtained extract not only meets but also exceeds the set standards, demonstrating the effectiveness of the extraction method used. The yield results of the extract can be seen in **Tabel 1.**

Table 1. Table of yield of Temu ireng extract

Weight of simplicia powder (g)	Extract weight (g)	Extract yield (%)
500	113	22,6

Phytochemical Screening

Phytochemical screening is the preliminary stage in phytochemical research, aimed at providing an overview of the groups of compounds contained in the Temu Ireng rhizome. The phytochemical screening method involves observing color change reactions using specific reagents (Rubianti et al., 2022). The results obtained are presented in **Table 2**.

Table 2. Results of the black meeting screening test

No	Secondary Metabolite	Result
1.	Alkaloid :	
	Wagner	++
	Wragedrof	++
2.	Flavonoid	+++
3.	Tanin	++
4.	Saponin	+
5.	Fenol	++

+: The compound contains a small amount of secondary metabolites
++: The compound contains secondary metabolites
+++: The compound contains a large amount of secondary metabolites
-: The compound does not contain secondary metabolites

The qualitative test for alkaloids showed positive results with the formation of precipitates upon the addition of Bouchardat's reagent, Dragendorff's reagent, and Mayer's reagent. The principle of this analytical method is the precipitation reaction that occurs through ligand substitution. The nitrogen atom in alkaloids, which has a lone pair of electrons, can replace the iodine ion in the alkaloid reagents. Further, the positive alkaloid test result with Wagner's reagent is indicated by the formation of a brown precipitate. Mayer's reagent, a mixture of mercuric chloride ($HgCl_2$) and potassium iodide (KI), forms mercuric iodide (HgI_2). After the addition of excess KI solution, a complex potassium tetraiodomercurate (II), $K_2[HgI_4]$, is formed. In Mayer's test, the nitrogen atom with a lone pair of electrons in alkaloids reacts with potassium tetraiodomercurate (II) to form an alkaloid-potassium complex precipitate (Wahyuni et al., 2020).

The positive alkaloid result in the Dragendorff test for Temu Ireng extract is marked by the formation of a brownish-black precipitate. The precipitate formed is an alkaloid-potassium complex. In the preparation of Dragendorff's reagent, bismuth nitrate is dissolved in HCl to prevent hydrolysis reactions, as bismuth salts easily hydrolyze to form bismuth oxide ions (BiO^+). To maintain Bi^{3+} ions in solution, acid is added, shifting the equilibrium to the left. Additionally, Bi^{3+} ions from bismuth nitrate react with potassium iodide to form a black precipitate of bismuth (III) iodide, which then dissolves in excess potassium iodide to form potassium tetraiodobismutate (Rowe et al., 2021).

Saponins are surface-active compounds easily recognized by their foaming properties. The glycosidic bond component in saponins makes these compounds polar. The presence of saponins is confirmed if the test sample produces bubbles 1–10 cm high that persist for approximately 10 minutes. The presence of bubbles in the Forth test indicates the presence of glycosides that can form bubbles in hydrolyzed water, subsequently converting into glucose and other compounds. The foam produced in this study was slightly unstable.

A compound is considered to contain flavonoids if, during testing, it changes color to reddish-black, yellow, or orange. The flavonoid test using Wilstatter's reagent involves adding magnesium powder and concentrated HCl to the ethanol extract of Temu Ireng rhizomes. The addition of magnesium powder and concentrated HCl aims to reduce the benzopyrone core present in flavonoid structures, resulting in red or orange flavylium salts. For the Temu Ireng rhizome extract sample, the result was positive, as the sample turned orange.

Phenolic compounds are identified using $FeCl_3$ solution. Fe^{3+} ions react with phenolic groups in the sample, forming green, blue, or black colors, indicating the presence of phenolic compounds. Additionally, the phytochemical test for tannins involves adding ethanol extract of Temu Ireng rhizomes to $FeCl_3$

solution. The color change to dark blue, black-blue, or green-black indicates the presence of tannins due to the formation of a complex between tannins and $FeCl_3$. Phytochemical tests with $FeCl_3$ may indicate the presence of phenolic groups, but tannins might also be present, as tannins are polyphenolic compounds (Sommer et al., 2023). For the Temu Ireng extract sample, the results indicated the presence of tannins, as evidenced by the color change to greenish-black.

Mucolytic Activity Result

Mucolytics function by reducing the viscosity of mucus, particularly from the lower respiratory tract. This changes the physicochemical properties of the mucus, making it less viscous and easier to expel. The mucolytic activity test using bovine intestinal mucus was conducted because bovine mucus has a composition similar to human respiratory mucosa. Bovine intestinal mucus was obtained by carefully scraping the intestinal layer to avoid contaminating the mucus with blood or fat, which could interfere with the analysis process. The mucus obtained from the bovine intestine was brownish and viscous.

Mucolytic activity analysis is a test or research aimed at measuring the ability of a substance or compound to soften or reduce the viscosity of mucus formed in the respiratory tract. The mucolytic activity test needs to be statistically analyzed to ensure the validity and significance of the results. The results of the mucolytic analysis will indicate the extent to which the tested substance or compound is effective in softening mucus. The greater the reduction in viscosity or the change in mucus weight after treatment, the better the mucolytic activity. The test results for Temu Ireng extract (*Curcuma aeruginosa* Roxb.) are illustrated in **Figure 1**.

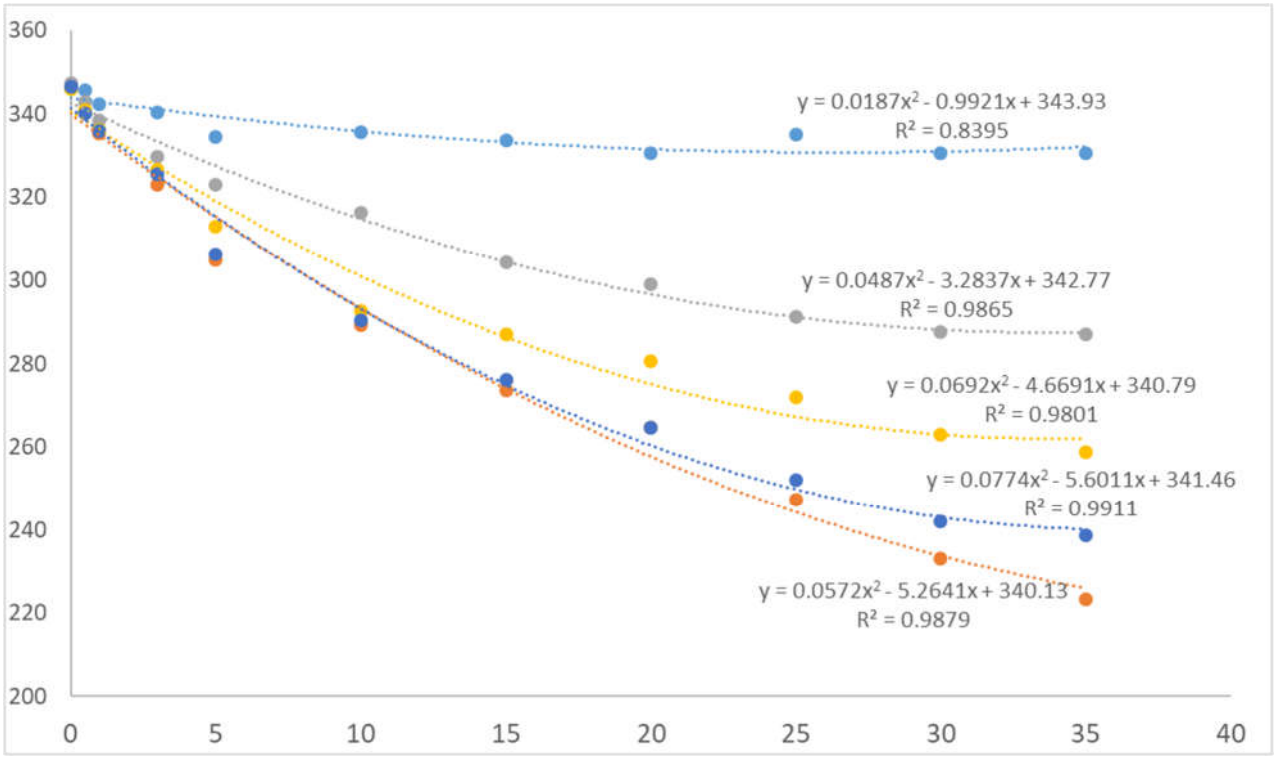

Figure 1. Viscosity value graph. Pale blue: negative control, grey: 1%, yellow: 1.5%, blue: 2%, and orange: positive control.

From **Figure 1**, it is evident that all concentrations of Temu Ireng extract and the positive control (acetylcysteine) experienced a decrease in viscosity over time. The graph shows that higher concentrations of Temu Ireng extract correspond to lower viscosity values, with the results for the 2% extract concentration closely matching those of the positive control (acetylcysteine). The data indicate that higher concentrations of ethanol extract of Temu Ireng rhizome (*Curcuma aeruginosa* Roxb.) are associated with enhanced mucolytic activity.

The presence of secondary metabolites likely plays a significant role in this mucolytic activity (Xu et al., 2016). Alkaloids found in the ethanol extract of Temu Ireng rhizome (*Curcuma aeruginosa* Roxb.) act as expectorants by releasing lysosomal enzymes that dissolve monosaccharides in bovine intestinal mucus, thereby reducing its viscosity. Flavonoids exhibit activity that can cleave mucoprotein and mucopolysaccharide chains in the mucus. Additionally, flavonoids possess antibacterial effects and can serve as anti-inflammatory agents in the respiratory tract by enhancing respiratory movement (Cushnie et al., 2016). Tannins have an astringent effect and can reduce mucus production in the intestines. Saponins exhibit activity by degrading mucoprotein and mucopolysaccharide chains in the mucus. Furthermore, saponins stimulate the secretion of bronchial mucus by increasing ciliary activity in cells, thus promoting efficient mucus excretion. Phenolic compounds present in Temu Ireng (*Curcuma aeruginosa* Roxb.) inhibit the growth of microorganisms. This inhibition is due to the disruption of bacterial cell membrane structural components (Caruana et al., 2012) Phenols are biologically active compounds that inhibit the growth of pathogenic microorganisms harmful to humans.

Statistical Analysis Result

The data analysis was conducted using SPSS version 16. The tests performed included the Normality Test, Homogeneity Test, and One-Way ANOVA Test. The first step was the Normality Test, which aims to determine whether the data used in the statistical analysis follow a normal distribution, The results of the statistical analysis of the normality test were 0.200 with the condition that the P value > 0.05. So this value shows that the viscosity test data of the ethanol extract of temu ireng (*Curcuma aeruginosa* Roxb.) is normally distributed.

Homogeneity is a critical assumption in statistical analysis, ensuring that data within each group are similar or not significantly different. This test also serves as a parameter to determine the most appropriate method for further analysis, based on the results obtained from the homogeneity test of mucus viscosity data for ethanol extract of Temu Ireng (*Curcuma aeruginosa* Roxb.).

The significance value obtained in the homogeneity test is 0.000, indicating that the data does not meet the criteria for homogeneity. This result suggests that the viscosity values for each sample varied significantly over time intervals, leading to the use of the Games-Howell post-hoc test for comparative analysis between groups.

One-Way ANOVA, or analysis of variance, is a statistical method that compares the means of three or more different groups or treatments to determine if there is a significant difference between the group means. This test compares the variation between groups with the variation within groups. The goal is to determine if the mean values of one group differ significantly from those of another group. One-Way ANOVA uses a 95% confidence level. The results obtained from the One-Way ANOVA are listed in **Table 5.**

Table 5. Anova Test

ANOVA *One-Way*	Value requirements	interpretasi
0,007	<0,05	Different meanings

The Games-Howell test is a post-hoc method used following an analysis of variance (ANOVA) to compare means between groups. This test is particularly useful when the assumption of homogeneity of variances is not met and when sample sizes vary across groups. Due to its ability to handle data with different sample sizes for each group, it provides greater flexibility compared to several other methods.

The Games-Howell test can validly identify specific differences between groups in their data, even when homogeneity is not met and sample sizes vary. The results obtained from the Games-Howell test are presented in **Table 6.**

Table 6. Comparison results with the Games-Howell Test

Treatment groups	Condition	Sig. (p)	Interpretation
Negative Control : Positive Control	p<0,005	0.032	Different Meanings
Negative Control : 1% Concentration		0.063	Meaningless
Negative Control: Concentration 1.5%		0.030	Different Meanings
Negative Control: Concentration 2%		0.030	Different Meanings
Positive Control: Concentration 1%		0.445	Meaningless
Positive Control: Concentration 1,5%		0.943	Meaningless
Positive Control: Concentration 2%		1.000	Meaningless
Concentration 1% : Concentration 1,5%		0.780	Meaningless
Concentration 1% : Concentration 2%		0.511	Meaningless
Concentration 1.5% : Concentration 2%		0.977	Meaningless

Table 6 shows significant differences between the positive control group and the negative control group with a p-value of 0.032, between the negative control group and the 1.5% concentration group with a p-value of 0.030, and between the negative control group and the 2% concentration group with a p-value of 0.030. The significance threshold for the Games-Howell test is $p < 0.005$. These significant p-values indicate that the positive control group, the 1.5% ethanol extract of *Curcuma aeruginosa* rhizome group, and the 2% ethanol extract of *Curcuma aeruginosa* rhizome group significantly differ from the negative control group. This supports the hypothesis that the mucus solutions treated with the ethanol extract of *Curcuma aeruginosa* rhizome and the active substance Acetylcysteine exhibit a significantly different reduction in viscosity compared to the negative control.

The groups with 1%, 1.5%, and 2% concentrations of ethanol extract of temu ireng *(Curcuma aeruginosa* Roxb.) have p-values that are not significant when compared to the positive control (Acetylcysteine), indicating that the viscosity of the mucus solution after treatment does not differ significantly from the mucus solution of the positive control (Acetylcysteine). This demonstrates that the ethanol extract of *Curcuma aeruginosa* rhizome possesses mucolytic activity comparable to that of the positive control (Acetylcysteine).

4. Conclusions

Based on this study, it can be concluded that the ethanol extract of *Curcuma aeruginosa* Roxb. rhizome has been proven in vitro to possess mucolytic effectiveness comparable to the drug acetylcysteine. The data from this research indicate that, in terms of the percentage of mucolytic activity,

the ethanol extract of *Curcuma aeruginosa* rhizome at a concentration of 2% demonstrates a percentage value nearly equivalent to that of 0.1% acetylcysteine, functioning effectively as an expectorant.

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