BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 2, October 2024 | Pages: 541-547 | DOI: 10.14421/biomedich.2024.132.541-547 ISSN 2540-9328 (online)

In Vitro Mucolytic Activity of Cardamom Fruit (*Amomum compactum*) Decoction on Duck Egg Albumens

Desy Aryanti Pardilla Vitri[1,*], Siti Rahmatul Aini[2], Legis Ocktaviana Saputri[3]
[1]Department of Pharmacy, Faculty of Medicine and Health Science, Universitas Mataram
[2]Department of Pharmacist Professional Education, Faculty of Medicine and Health Science, Universitas Mataram
[3]Department of Pharmacology, Faculty of Medicine and Health Science, Universitas Mataram
Jl. Majapahit no. 62, Mataram 83125, West Nusa Tenggara, Indonesia.

Corresponding author*
desyaryantipardillavitri15@gmail.com

Manuscript received: 26 Agustus, 2024. Revision accepted: 10 October, 2024. Published: 31 October, 2024.

Abstract

Cardamom (*Amomum compactum*) is empirically used as a cough medicine by using its seeds and fruits. Cardamom seeds have been shown to have mucolytic activity at a concentration of 0.8%, while cardamom fruit has not been studied. This study aimed to determine the mucolytic activity of cardamom fruit decoction at variation concentrations. Mucolytic activity tests were carried out *in vitro* on duck egg albumens at 3%, 6%, and 12% variation concentrations of cardamom fruit decoction. N-acetylcysteine 0.1% was positive control, while phosphate buffer and Tween 80 were negative controls. The mucolytic activity was determined based on the viscosity value measured by flow time using an Ostwald viscometer and density measurement using a pycnometer. Data from each group were analyzed using SPSS with parametric One-Way ANOVA and Post Hoc tests. The results showed that cardamom fruit decoction at concentrations of 3%, 6%, and 12% had values that were not significantly different from N-acetylcysteine ($p>0.05$) and there was no significant difference ($p>0.05$) between each concentration group. Accordingly, the research concludes that cardamom fruit decoction at concentrations of 3%, 6%, and 12% has mucolytic activity comparable to N-acetylcysteine.

Keywords: Cardamom Fruit; Decoction; Duck Egg Albumens; *In Vitro*; Mucolytic.

Abbreviations: Acute Respiratory Infections (ARIs); N-acetylcysteine (NAC).

INTRODUCTION

Acute respiratory infections (ARIs) are infectious diseases that cause the highest mortality rates worldwide. Each year, nearly 4,000,000 people die from ARIs, with the highest mortality rates observed among infants, children, and the elderly, particularly in developing countries, necessitating special attention (WHO, 2020). According to the National Basic Health Research Report 2018, the incidence of ARIs in Indonesia reached 1,017,290 cases (Riskesdas, 2018). One of the primary diagnoses in ARI patients is airway hypersecretion, characterized by coughing and increased sputum production (Susiami & Mubin, 2022; Fretes et al., 2020).

Coughing is a defense mechanism to expel harmful antigens from the respiratory tract (Baratawihaya, 2010; Pakadang et al., 2020). Antigens entering the respiratory tract get trapped and increase mucus production, leading to thicker mucus (Utama, 2018; Kurniawati et al., 2020). Mucus can obstruct airflow and cause complications of other diseases (Saminan, 2016). The viscosity of mucus can be reduced by mucolytic drugs, which can break down mucus, making it less viscous and more accessible to expel through coughing (Wahyuningtyas et al., 2016).

One such mucolytic drug is N-acetylcysteine (NAC), known for its strong ability to dissolve mucus (Liu et al., 2020). NAC has a rapid onset, reaching maximum plasma concentration within half an hour (Dawson et al., 1989 in Mahmoudi et al., 2015; Ferdinan et al., 2023). However, conventional drugs like NAC have side effects, including nausea, vomiting, diarrhea, epigastric pain, constipation, headaches, urticaria, and skin rashes (Adil et al., 2018). The most common side effects are nausea and vomiting, experienced by 23% of 145 patients taking NAC (Bebarta et al., 2010 in Tenório et al., 2021). Consequently, people are turning to traditional medicine, considered safer (Sari et al., 2019).

Cardamom (*Amomum compactum*) is a plant commonly used as a spice but also used empirically by people as traditional medicine. Cardamom contains essential oils about 2-4% (with active compounds like cineole, borneol, limonene, and alpha-terpineol acetate) (Ernawati et al., 2022). According to Nofriyaldi et al. (2023), ethanol extract of cardamom fruit contains

secondary metabolites such as tannins, alkaloids, saponins, and flavonoids. Empirically, cardamom is used as a traditional medicine for coughing (Hariana, 2013).

People use cardamom for cough medicine using its seeds and fruits. Cardamom seeds are used empirically for cough medicine by chewing and swallowing the juice (Latief, 2009 in Syaputra et al., 2021). Additionally, the seeds have been proven to has mucolytic activity *in vitro*. According to Syaputra et al. (2021), ethanol extract syrup from cardamom seeds have mucolytic activity comparable to acetylcysteine at a concentration of 0.8%. Cardamom fruit is used empirically for cough medicine by boiling 6 grams in 200 mL of water for 15 minutes (Hariana, 2013). However, using cardamom fruit as a cough medicine remedy has yet to be studied, necessitating further research into its mucolytic activity.

The mucolytic activity of cardamom fruit decoction can be tested *in vitro* using duck egg albumens. Duck egg albumens were chosen for the test method because they contain mucoproteins that can be broken down by compounds with mucolytic activity, thereby reducing their viscosity (Bragg & Haough, 1961 in Desiyana et al., 2021). Additionally, *in vitro* tests using duck egg albumens are simpler than bovine intestines (Desiyana et al., 2021). Obtaining duck egg albumens involves slowly separating the white from the yolk, whereas bovine intestines need to be cleaned of adhering waste, split longitudinally, and scraped to obliterate mucus (Sari et al., 2019). Therefore, this study aimed to determine the mucolytic activity of cardamom fruit (*Amomum compactum*) decoction *in vitro* on duck egg albumens.

MATERIALS AND METHODS

Materials

The materials used include aquadest, cardamom fruits, di-Sodium hydrogen phosphate dehydrate 0.1 N, $FeCl_3$ 1%, concentrated HCl, HCl 2 N, magnesium (Mg) powder, Wagner's reagent, Dragendorff's reagent, Mayer's reagent, N-acetylcysteine capsule, duck egg, sodium dihydrogen phosphate monohydrate 0.1 N, Tween 80. The equipment used includes laboratory glassware, stir bar, bulb pipette, blue tip, food dehydrator (Freeze Dryer), hot plate, filter paper, volumetric flask, micropipette, oven, dropper pipette, wooden clamp, ruler, pycnometer, pH meter, tube rack, stopwatch, spatula, analytical balance, Ostwald viscometer, water bath, yellow tip.

Procedures

Sample Preparation

Separated the cardamom fruits from the bunches and washed with running water until cleaned and drained. The clean cardamom fruits were dried in a food dehydrator at 40℃ for 12 hours. The dried cardamom fruits were sorted and blended. We weighed six grams of dried cardamom fruits, then boiled in 200 mL of distilled water at a temperature of 90℃ for 15 minutes (Hariana, 2013).

Figure 1. The cardamom plants (*Amomum compactum*) on Sapit (Documented by the author).

Phytochemical Screening

Flavonoid Test

Five mL of the sample was heated in a water bath for 5 minutes, then 2-4 drops of concentrated HCl were added, and 0.2 g of magnesium (Mg) powder was added (Fadilah et al., 2018). Flavonoid positivity is characterized by a color change to black, reddish, yellow, or orange (Rumagit et al., 2015).

Alkaloid Test

Nine mL of the sample was added with 1 mL of 2 N HCl in a test tube, and then the sample was heated over a water bath for 2 minutes. The solution is then allowed to cool. The sample solution was divided into 3 test tubes, each added with Mayer, Wagner, and Dragendorff reagents. Positive results are indicated by the formation of a white precipitate in the sample treated with Mayer's reagent, a reddish brown precipitate in the sample treated with Wagner's reagent, and the formation of a yellow precipitate in the sample treated with Dragendorff's reagent (Depkes RI, 1980; Raaman, 2006).

Saponin Test

One mL of the sample was added to 10 mL of distilled water, then heated over a water bath for 2 minutes, cooled, and shaken vigorously for 10 seconds. If the foam is formed that lasts for 10 minutes with a height of 1-10 cm and if one drop of 2 N HCl is added, the foam does not disappear; then the sample is positive for containing saponin (Depkes, 1989).

Tannin Test
Two mL of the sample was added with 2-4 drops of 1% $FeCl_3$. Positive tannin is characterized by a change in color to dark blue, blackish blue, or greenish black (Fadilah et al., 2018).

Preparation of pH 7 Phosphate Buffer
4.449 grams of di-Sodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$) 0.1 N was dissolved in 100 mL of distilled water. The solution was put into a 250 mL measuring flask, and distilled water was added to the mark. A total of 3.449 grams of 0.1 N sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) was dissolved in 100 mL of distilled water. The solution was put into a 250 mL measuring flask, and distilled water was added to the mark. A total of 244.4 mL of 0.1 N $Na_2HPO_4.2H_2O$ was added with 155.6 mL of 0.1 N $NaH_2PO_4.H_2O$, then stirred until homogeneous and measured with a pH meter (Mulyono, 2009).

Preparation of Duck Egg Albumens 20%w/w
Duck eggs were taken in Panjisari Village, Praya District, Central Lombok Regency. The duck eggs used are three days old. Mucus is obtained by carefully separating the egg albumens and egg yolk. A total of 20 grams of mucus (the albumens part of a duck egg) was added with phosphate buffer pH 7 until the weight reached 100 g to obtain a concentration of 20% w/w, then stirred until homogeneous (Sutoyo et al., 2020).

Preparation of Control and Test Solutions
- *Negative Control*

To reach a weight of 30 g, 0.15 g of tween 80 was added with 20% w/w duck egg albumens. The mixture was then stirred until homogeneous (Sutoyo et al., 2020).

- *Positive Control*

0.1 g of N-acetylcysteine was weighed, then dissolved in 100 mL of distilled water and stirred until homogeneous to obtain a concentration of 0.1%. 0.03 g of N-acetylcysteine 0.1% was added to 0.15 g of Tween 80, and duck egg albumens 20% w/w to 30 g. The mixture was stirred until it was homogeneous (Nerdy & Manurung, 2018).

- *Test Solution*

Twelve grams of cardamom fruit was boiled in 100 mL of distilled water at a temperature of 90°C for 15 minutes to obtain a concentration of 12%w/v. Boiled cardamom fruit with a concentration of 12% was then diluted to a concentration of 6% w/v and 3% w/v. A total of 0.15 g of each test concentration was added with 0.15 g of Tween 80 and 20% w/w duck egg albumens until it reached a weight of 30 g and stirred until homogeneous (Sulistanti et al., 2022; Sutoyo et al., 2020)

- *Mucolytic Activity Assay*

Each group (negative control, positive control, and test solution) was incubated for 30 minutes at 37°C. The sample flow time was measured using an Ostwald viscometer, and the sample density was measured using a pycnometer. Each measurement was carried out three times with new samples, and the mucolytic activity test was replicated two times. The results obtained were then processed to obtain the viscosity value, which was then averaged.

$$Sample\ viscosity = \frac{Sample\ density\ x\ sample\ flow\ time}{Water\ density\ x\ water\ flow\ time} x\ 0{,}89\ (cP)$$

Data analysis
Viscosity data were analyzed using the SPSS 26 version. Data normality was tested with Shapiro-Wilk, and homogeneity was tested using Levene Statistics. Parametric analysis was continued with one-way ANOVA ($p < 0.05$), followed by post-hoc analysis using LSD to determine the significance between treatments.

RESULTS AND DISCUSSION

Cardamom Fruit (*Amomum compactum*) Decoction
The yield of cardamom fruit simplicia obtained was 27.83%. Cardamom fruit simplicia was extracted using the boiling method. This method was chosen because people commonly consume medicines derived from plants or tubers. (Anggraini et al., 2020). Besides that, boiling is a method suitable for the empirical use of cardamom fruit as a cough medicine. Based on empirical use, 6 grams of cardamom fruit was boiled in 200 mL of distilled water for 15 minutes (Hariana, 2013). The results of boiling cardamom fruit are shown in **Figure 2.**

Figure 2. Results of cardamom fruit (*Amomum compactum*) decoction (Documented by the author).

Phytochemical Screening
The qualitative phytochemical screening test results showed that the cardamom fruit decoction contained flavonoids and tannins, as shown in **Table 1**. The decoction of cardamom fruit contained damaging alkaloid compounds because alkaline alkaloids generally

dissolve in relatively non-polar organic solvents and are difficult to dissolve in water, whereas the extraction process of cardamom fruit decoction uses distilled water solvent, which has very polar properties (Endarini, 2016; Fitriyanti et al., 2022). Nofriyaldi et al. (2022) also showed negative results of phytochemical screening for alkaloid compounds in simplicia and 96% ethanol extract of cardamom fruit. Decoction of cardamom fruit contains negative saponins because saponins are heat resistant up to a temperature of 70℃, whereas the process of boiling cardamom fruit uses a temperature of 90℃ (Kemenkes, 2017; Wahyuni et al., 2018). Therefore, it causes the saponins to be degraded.

Table 1. Phytochemical Screening Results of Cardamom Fruit (*Amomum compactum*) Decoction.

Test Compounds	Results	Interpretation of Results	Documentation
Flavonoids	The solution turns orange	+	
Alkaloids	No sediment	-	
	No sediment	-	
	No sediment	-	
Saponins	The foam formed does not reach a height of 1-10 cm	-	
Tannins	The solution turns to a greenish-black color	+	

Mucolytic Activity Test

Cardamom fruit (*Amomum compactum*) decoction was tested for mucolytic activity *in vitro* on duck egg albumens. Duck egg albumens was chosen as the medium because it contains a mucolytic agent similar to human mucus, namely ovomucin (glycoprotein) (Chaiyasit et al., 2019). When the test was carried out, duck egg albumens were mixed with phosphate buffer pH 7, which aimed to adapt to human mucus conditions (Henke & Ratjen, 2007 in Ladeska et al., 2020). The next stage was the addition of Tween 80, which functions as a wetting agent to reduce the surface tension between the phosphate buffer and egg albumens so that they can mix (Rowe et al., 2009). Before the test, the sample was incubated for 30 minutes at 37℃ to create conditions that resemble human physiological conditions (Windriyati et al., 2019).

The mucolytic activity test was carried out with an Ostwald viscometer because duck egg albumens, used as the test medium, had a water content of around 86.19% ± 0.10% (Chaiyasit et al., 2019). Ostwald viscometers are generally used to measure the viscosity of Newtonian fluids such as water (Martin et al., 2016; Spurk and Aksel, 2008 in Tiwow, 2015). In this study, N-acetylcysteine (NAC) was a positive control because its mechanism of action can break disulfide bonds in cross-linked mucus glycoproteins (mucin) so that the viscosity can decrease (Raghu et al., 2020).

Based on the viscosity data shown in **Table 2**, the viscosity value of the positive control and cardamom fruit decoction at variation concentrations of 3%, 6%,

and 12% decreased compared to the negative control. The negative control has the highest viscosity value. When viewed from the average viscosity value between test solutions, the higher the concentration of cardamom fruit decoction, the lower the viscosity value.

Table 2. Results of Viscosity Values.

Sample	Trial	Viscosity Value		
		I	II	III
Negative Control	1	1,4375	1,4101	1,3949
	2	1,4644	1,3838	1,3559
	3	1,5001	1,3559	1,4115
	Average ± SD	1,4673 ± 0,0314	1,3833 ± 0,0271	1,3874 ± 0,0285
Positive Control	1	1,0505	1,0666	1,1498
	2	1,0114	1,0457	1,0496
	3	1,1336	1,0847	1,0677
	Average ± SD	1,0652 ± 0,0624	1,0657 ± 0,0195	1,0890 ± 0,0534
Decoction 3%	1	1,1437	1,1570	1,1875
	2	1,1198	1,0635	1,1219
	3	1,1336	1,1209	1,1581
	Average ± SD	1,1452 ± 0,0262	1,1138 ± 0,0472	1,1559 ± 0,0329
Decoction 6%	1	1,0884	1,0847	1,1687
	2	1,1028	1,0989	1,0677
	3	1,1732	1,0858	1,1219
	Average ± SD	1,1214 ± 0,0454	1,0898 ± 0,0079	1,1194 ± 0,0506
Decoction 12%	1	1,0321	1,0847	1,1121
	2	1,0837	1,0635	1,0857
	3	1,1347	1,0666	1,1219
	Average ± SD	1,0835 ± 0,0513	1,0716 ± 0,0115	1,1066 ± 0,0187

Viscosity data was analyzed using the SPSS 26 version. The normality of the data was tested with Shapiro-Wilk and the significance results ($p>0.05$) showed that the viscosity data was normally distributed. Homogeneity was tested using Levene Statistics, with significant results ($p>0.05$) indicating that the data was homogeneous. After the normality and homogeneity requirements were met, the analysis was continued with the One-way ANOVA parametric test, which produced a significant value ($p<0.05$) (Dahlan, 2010). Significant differences between each sample group were analyzed using Post-Hoc (LSD) with a 95% confidence level, as shown in **Table 3**.

Table 3. Results of Post-Hoc analysis (LSD) of test group viscosity values.

Comparison Groups	Significant Value		
	1	2	3
NC-PC	0,000	0,000	0,000
NC-D3%	0,000	0,000	0,000
NC-D6%	0,000	0,000	0,000
NC-D12%	0,000	0,000	0,000
PC-D3%	0,056*)	0,051*)	0,063*)
PC-D6%	0,159*)	0,292*)	0,364*)
PC-D12%	0,631*)	0,790*)	0,595*)
D3%-D6%	0,536*)	0,295*)	0,281*)
D3%-D12%	0,126*)	0,080*)	0,154*)
D6%-D12%	0,329*)	0,421*)	0,696*)

Note: *) not significantly different ($p>0.05$); NC (Negative Control); PC (Positive Control); D3% (Decoction 3%); D6% (Decoction 6%); D12% (Decoction 12%).

Based on the Post-Hoc (LSD) results in **Table 3**, the positive control and negative control have a significance value ($p<0.05$), which indicates the viscosity values between the positive control and negative control were significantly different. That means the positive control shows mucolytic activity while the negative control does not. Cardamom fruit decoction at concentrations of 3%, 6%, and 12% had a significance value ($p>0.05$) when compared with the positive control but a significance value ($p<0.05$) when compared with the negative control. The results show that the cardamom fruit decoction with concentrations of 3%, 6%, and 12% has mucolytic activity equivalent to the positive control. Fruit decoction had a significant value ($p>0.05$) between variation concentrations. These results indicate no significant difference between each variation of concentrations of cardamom fruit decoction or that the mucolytic activity was comparable.

The results of this research can attest to the empirical use of cardamom fruit decoction. Flavonoids, alkaloids, saponins, and tannins are secondary metabolite compounds in plants with mucolytic effects (Wati, 2017; Kurniawati et al., 2020). However, this study's phytochemical screening of cardamom fruit decoction was positive for flavonoids and tannins. Flavonoids have mucolytic activity by breaking down mucoprotein and mucopolysaccharide fibers in mucus. Wahyuningtyas et al. (2016) showed that matteucinol was a flavonoid isolate with mucolytic activity. Meanwhile, as astringents, tannins have mucolytic activity, which can

shrink the mucous membrane (mucus) in the intestines (Wati, 2017). Humans have two mucus: bound mucus (intestines) and secreted mucus (respiratory tract). Both mucus have the same characteristics, but most of the mucus secreted was much higher than those bound to the membrane (Abrami et al., 2024). As for the connection with tannins, which can shrink the mucous membrane (mucus) in the intestines, it could have the same effect on mucus in the respiratory tract because both have the same characteristics. Thus, the mucolytic activity produced by the cardamom fruit decoction comes from these secondary metabolite compounds. N-acetylcysteine was a mucolytic substance with a mechanism of action comparable to flavonoids, which breaks the mucoprotein threads of the mucosa. (Estuningtyas et al., 2008 in Rambe et al., 2021).

CONCLUSIONS

The cardamom fruit (*Amomum compactum*) decoction has mucolytic activity comparable to N-acetylcysteine, and there is no significant difference between 3%, 6%, and 12 % concentrations.

Acknowledgements: The authors are grateful to the supervisors who provided suggestions, direction, and support in preparing this journal and to the laboratory staff who have provided convenience during research.

Authors' Contributions: The authors whose names are included in this journal contributed to this research.

Competing Interests: The authors claim that there are no conflicts of interests.

Funding: The authors claim that the research was self-funded.

REFERENCES

Abrami, M., Biasin, A., Tescione, F., Tierno, D., Dapas, B., Carbone, A., Grassi, G., Conese, M., Di Gioia, S., Larobina, D., & Grassi, M. (2024). Mucus Structure, Viscoelastic Properties, and Composition in Chronic Respiratory Diseases. *International Journal of Molecular Sciences*, *25*(3). https://doi.org/10.3390/ijms25031933

Adil, M., Amim, S., & Mohtashin, M. (2018). N-acetylcysteine in Dermatology. *Indian Journal of Dermatology, Venereology and Leprology*, *84*(1), 6–15. https://doi.org/10.4103/ijdvl.IJDVL

Anggraini, I. A. K. D., Darmayanti, L. P. T., & Sugitha, I. M. (2020). Pengaruh Lama Perebusan pada Pembuatan Minuman Herbal Daun Sawo (*Manilkara zapota*) Terhadap karakteristik dan Daya Hambat Pertumbuhan *Escherichia coli*. *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, *9*(3), 272. https://doi.org/10.24843/itepa.2020.v09.i03.p03

Chaiyasit, W., Brannan, R., Chareonsuk, D., & Chanasattru, W. (2019). Comparison of Physicochemical and Functional Properties of Chicken and Duck Egg Albumens. *Brazilian Journal of Poultry Science*, *21*(1), 1–9.

Dahlan, S. M. (2010). *Statistik untuk Kedokteran dan Kesehatan*. Jakarta: Salemba Medika.

Depkes. (1989). *Materia Medika Indonesia* (Jilid V). Jakarta: Departemen Kesehatan Republik Indonesia.

Depkes RI. (1980). *Materia Medika Edisi VI*. Jakarta: Departemen Kesehatan Republik Indonesia.

Desiyana, L. S., Vonna, A., Hafsyari, R., Illian, D. N., & Koresponden, P. (2021). Uji Aktivitas Mukolitik Daun Kersen (*Muntingia calabura* L.) secara In Vitro. *Indonesia. Jurnal Bioleuser*, *5*(3), 18–21. https://doi.org/https://doi.org/10.24815/j.%20bioleuser.v5i1.22977

Endarini, L. H. (2016). *Farmakognosi dan Fitokimia*. Jakarta: Kementrian Kesehatan Republik Indonesia.

Ernawati, Suharjon, & Fika, W. (2022). *Budidaya Kapulaga (Amomum cardamomum)*. Kementerian Pertanian.

Fadilah, S., Mu'nisa, A., & Rachmawaty. (2018). Analisis Fitokimia Air Rebusan Daun Kayu Jawa (*Lannea coromandelica*). *Bionature*, *19*(1), 73–77.

Ferdinan, A., Izzamaulana, Y., & Justicia, A. K. (2023). Uji Aktivitas Mukolitik Ekstrak Etanol Akar Bajakah (*Spatholobus littoralis* Hassk.) Secara in Vitro In Vitro Test of The Mucolytic Activity of The Ethanol Extract of Bajakah (*Spatholobus littoralis* Hassk .) Root. *Journal Pharmasci*, *8*(2), 173–178.

Fitriyanti, R., Emmawati, E., & Yuliantini, A. (2022). Analisis Antosianin dari Buah dengan Berbagai Macam Pelarut Menggunakan Metode Spektrofotometeri UV-VIS. *Jurnal Health Sains*, *3*(7), 812–818.

Fretes, F. de, Messakh, S. T., & Saogo, I. D. M. (2020). Manajemen Keluarga Terhadap Penanganan ISPA Berulang Pada Balita di Puskesmas Mangunsari Salatiga. *Jurnal Sains Dan Kesehatan*, *2*(4), 275–281. https://doi.org/10.25026/jsk.v2i4.144

Hariana, H. A. (2013). *262 Tumbuhan Obat dan Khasiatnya*. Cibubur: Penebar Swadya.

Kurniawati, D., Fitrianingsih, S. P., & Suwendar. (2020). Studi Literatur Aktivitas Mukolitik Beberapa Tanaman Genus Hibiscus dan Genus Piper terhadap Mukus Usus Sapi. *Prosiding Farmasi*, *6*(2), 97–100.

Ladeska, V., Dwitiyanti, & Dilar, E. A. (2020). Aktivitas Mukolitik Fraksi Etanol Herba Meniran (*Phyllanthus niruri* L) Pada Mukosa Usus Sapi Secara In Vitro. *Uhamka Repository* .

Liu, H., Wang, W., & Gao, X. (2020). Comparison of The Efficacy of Ambroxol Hydrochloride and N-acetylcysteine in The Treatment of Children with Cronchopneumonia and Their Influence on Prognosis. *Experimental and Therapeutic Medicine*, *20*(6), 1–1. https://doi.org/10.3892/etm.2020.9260

Mahmoudi, G. A., Astaraki, P., Mohtashami, A. Z., & Ahadi, M. (2015). N-acetylcysteine Overdose After Acetaminophen poisoning. *International Medical Case Reports Journal*, *8*, 65–69. https://doi.org/10.2147/IMCRJ.S74563

Martin, A., Swarbrick, J., & Cammarata, A. (2016). *Farmasi Fisik: Dasar-Dasar Kimia Fisik Dalam Ilmu Farmasetik Edisi Ketiga*. UI-Press.

Mulyono. (2009). *Membuat Reagen Kimia di Laboratorium*. Jakarta: Bumi Kasara.

Nerdy, N., & Manurung, K. (2018). Mucolytic Activity of Rosella (*Hibiscus sabdariffa* L.) Calyces Edxtract on Cow Intestinal Mucus. *Jurnal Kedokteran Hewan - Indonesian Journal of Veterinary Sciences*, *12*(2), 43–46. https://doi.org/10.21157/j.ked.hewan.v12i2.9621

Nofriyaldi, A., Adlina, S., & Rusmayanti, W. W. (2022). Formula dan Uji Aktivitas Antibakteri Sediaan Masker Gel Peel-Off Ekstrak Buah Kapulaga (*Amomum compactum*) terhadap Bakteri Propinium acnes. *Seminar Nasional Penelirian Dan Pengabdian 2022*, 39–50. https://ejurnal2.poltekkestasikmalaya.ac.id/index.php/prosidingpenelitian/article/view/96

Nofriyaldi, A., Nur Endah, S. R., & Mutiara, A. P. (2023). Efektivitas Daya Antibakteri Ekstrak Etanol Buah Kapulaga (*Amomum compactum* Sol. Ex Maton) Terhadap Pertumbuhan Propionibacterium acnes. *Media Informasi*, *19*(1), 67–74. https://doi.org/10.37160/bmi.v19i1.123

Pakadang, S. R., Sinala, S., Salasa, A. M., Ratnah, S., Rosmala Dewi, S. T., & Hilaria, M. (2020). Potential of Miana Leaf Extract as Expectorant (Profile Place of Growing, Antioxidant, Sputum Contaminants, Antibacterial, MIC, MKC Expectorant). *Majalah Obat Tradisional*, *25*(2), 94. https://doi.org/10.22146/mot.52500

Raaman, N. (2006). *Phytochemical Techniques*. India: New India Publishing Agency.

Raghu, G., Berk, M., Campochiaro, P. A., Jaeschke, H., Marenzi, G., Richeldi, L., Wen, F.-Q., Nicoletti, F., & Calverley, P. M. A. (2020). The Multifaceted Therapeutic Role of N-Acetylcysteine (NAC) in Disorders Characterized by Oxidative Stress. *Current Neuropharmacology*, *19*(8), 1202–1224. https://doi.org/10.2174/1570159x19666201230144109

Rambe, R., Rani, Z., & Thomas, N. A. (2021). Uji Efektivitas Mukolitik Ekstrak Umbi Bawang Dayak (*Eleutherine bulbosa* (Mill) Urb). *Journal Syifa Sciences and Clinical Research*, *3*(2), 71–77. https://doi.org/10.37311/jsscr.v3i2.7041

Riskesdas. (2018). *Laporan Riskesdas 2018 Nasional*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Rowe, R. C., Sheskey, P. J., & Quinn, M. E. (2009). *Handbook of Pharmaceutical Excipient*. UK: Pharmaceutical Press.

Rumagit, H. M., Runtuwene, M. R., & Sudewi, S. (2015). Uji Fitokimia dan Uji Aktivitas Antioksidan dari Ekstrak Etanol Spons Lamellodysidea herbacea. *Pharmacon*, *4*(3), 2302–2493.

Saminan. (2016). Efek Obstruksi Pada Saluran Pernapasan Terhadap Daya Kembang Paru. *Jurnal Kedokteran Syiah Kuala*, *16*(1), 36–39. http://jurnal.unsyiah.ac.id/JKS/article/download/5010/4441

Sari, K., Sutoyo, S., & Hidajati, N. (2019). Uji Aktivitas Mukolitik Ekstrak Metanol Tumbuhan Paku Perak (*Pityrogramma calomelanos*). *Prosiding Seminar Nasional Kimia*, 172–178.

Sulistanti, E., Pratama, I. S., Hidayati, A. R., & Wirasisya, D. G. (2022). Uji Aktivitas Mukolotik Rebusan Herba Putri Malu (*Mimosa pudica* L.) secara In Vitro. *Jurnal Medika Udayana*, *11*(7), 56–60. https://doi.org/eum

Susiami, S., & Mubin, M. F. (2022). Peningkatan Bersihan Jalan Nafas Pada Anak Balita Penderita ISPA Dengan Terapi Uap Air Dan Minyak Kayu Putih Di Poliklinik AKPOL Semarang. *Ners Muda*, *3*(1). https://doi.org/10.26714/nm.v3i1.7089

Sutoyo, S., Tukiran, & Sari, N. I. K. (2020). In Vitro Mucolytic Activity Assay of The Acetone Extract of The Silver Fern (*Pityrogramma calomelanos*). *Atlantis Press*, *390*, 137–140. https://doi.org/10.2991/icracos-19.2020.29

Syaputra, R. A., Aini, S. R., & Juliantoni, Y. (2021). Aktivitas Mukolitik Sirup Ekstrak Etanolik Biji Kapulaga (*Amomum compactum* Sol. Ex Maton) pada Mukus Usus Sapi Secara In Vitro. *Unram Medical Journal*, *10*(1), 384–390. https://doi.org/10.29303/jku.v10i1.504

Tenório, M. C. D. S., Graciliano, N. G., Moura, F. A., de Oliveira, A. C. M., & Goulart, M. O. F. (2021). N-acetylcysteine (NAC): Impacts on Human Health. *Antioxidants*, *10*(6). https://doi.org/10.3390/antiox10060967

Tiwow, V. A. (2015). Analisis Aliran Fluida Netonian Pada Pipa Tidak Horizontal. *JSPF Jilid*, *11*(1), 104–108.

Wahyuni, S., Vifta, R. L., & Erwiyani, A. R. (2018). Kajian Aktivitas Antibakteri Ekstrak Etanol Daun Jati Belanda (*Guazuma ulmifolia* Lamk) Terhadap Pertumbuhan Streptococcus mutans. *Jurnal Inovasi Teknik Kimia*, *3*(1), 25–30. https://doi.org/10.31942/inteka.v3i1.2122

Wahyuningtyas, A., Suyatno, & Hidajati, N. (2016). Uji Aktivitas Mukolitik Senyawa Flavonoid Hasil Isolasi dari Ekstrak Diklorometana Batang Tumbuhan Paku *Chingia sakayensis*. *Prosiding Seminar Nasional Kimia Dan Pembelajarannya*, 13–20.

Wati, L. K. (2017). Formulasi Sirup Ekstrak Daun Sendok (*Plantago mayor* L.) Sebagai Ekspektoran dengan Parameter Uji Mukolitik. *Jurnal Ilmiah As-Syifaa*, *9*(1), 43–50. https://doi.org/10.33096/jifa.v9i1.240

WHO. (2020). *Severe Acute Respiratory Infections Treatment Centre*. Word Health Organization.

Windriyati, Y. N., Murrukmihadi, M., & Junita, N. R. (2019). Aktivitas Mukolitik In Vitro Ekstrak Etabolik Herba Meniran (*Phyllanthus niruri* L) Terhadap Mukosa Usus Sapi. *Jurnal Ilmu Farmasi & Farmasi Klinik*, *3*, 94–100.

THIS PAGE INTENTIONALLY LEFT BLANK