Anti-Inflammatory and Mucolytic Activity Test of Ethanol Extract Fennel Leaf (*Foeniculum vulgare* Mill.)

Syifatul Lutviani, Ita Nur Anisa*, Andreanus A. Soemardji

Faculty of Pharmacy, Jenderal Achmad Yani University, West Java, Indonesia
**Corresponding author. Email: ita.nuranisa@gmail.com*

ABSTRACT
Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disease characterized by chronic bronchitis, airway thickening, and emphysema. There are several main mechanisms of COPD, namely chronic inflammatory processes in the airways, oxidative stress, and disturbances in the balance between proteolytic and antiproteolytic. Fennel is one of the plants that has been widely used in traditional medicine including as an anti-inflammatory, antioxidant, and cough reduction. This study was conducted to determine the potential of fennel leaf ethanol extract to be used in COPD therapy. The ethanol extract of fennel leaf was obtained by maceration which was carried out for 3×24 hours. The method used to determine anti-inflammatory activity in vitro is membrane stabilization by induction of a hypotonic solution. The mucolytic testing was carried out in vitro using cow intestinal mucus. The ethanol extract of fennel leaf in various concentrations (25,75 µg/mL; 51,50 µg/mL; 103 µg/mL; 206 µg/mL; 412 µg/mL) showed membrane stabilization and mucolytic activity. The ethanol extract of fennel leaf at a concentration of 412 µg/mL gave 53,5357% inhibition and could reduce the viscosity most. The results of the statistical test showed the ethanolic extracts of leaf with a concentration of 25,75 µg/mL; 51,5 µg/mL; 103 µg/mL; 206 µg/mL; 412 µg/mL have % inhibition and decrease in viscosity significantly different ($p < 0,05$) compared to the control group. Based on the results, the ethanol extract of fennel leaf had hemolysis inhibition and mucolytic activity, but it is not equivalent (not effective) to the comparison group.

Keywords: *COPD, Inflammation, Mucolytic, Foeniculum vulgarae, Fennel.*

1. INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disease characterized by chronic bronchitis, airway thickening, and emphysema. According to World Health Organization, in 2020 COPD be the third leading cause of death in the world. There are several main mechanisms of COPD, namely chronic inflammatory processes in the airways, oxidative stress, and disturbances in the balance between proteolytic and autoproteolytic. Chronic inflammation of the airways is caused by inflammatory cells entering the lungs in response to cigarette smoke. Oxidative stress can lead to impaired cell function until proteolytic and anti-proteolytic disorders occur. Inflammation that occurs in COPD is caused by the entry of foreign particles or heavy metals from cigarettes [1]. Inflammation that occurs can be in the form of pulmonary and systemic inflammation which can be characterized by increased markers of inflammation in the blood circulation [2]. The markers or cells that have been known to play a role in the inflammatory process in COPD are macrophages, neutrophils, and CD8+ T cells [3].

Oxidative stress is a major mechanism in the pathogenesis of COPD. Increased oxidative stress in the body can worsen comorbidities, cause bone damage, and muscle weakness, accelerate lung aging and cause DNA damage [4]. Coughing up phlegm is also a symptom of a respiratory tract infection, characterized by symptoms of phlegm in large quantities, thick sputum, usually slightly yellow or green in color. Mucus secretion is stimulated by the presence of diseases of the respiratory tract such

J. Sukweenadhi and F. Setiawan (eds.), *Proceedings of the Conference on Natural Resources And Life Sciences 2022 (NRLS-BIO 2022)*, Advances in Biological Sciences Research 38,
https://doi.org/10.2991/978-94-6463-322-1_18

as bronchitis, chronic obstructive pulmonary disease, and asthma as a form of inflammatory response. If the mucus is released too much, then the function of the respiratory tract will be disrupted. Treatment of cough with phlegm can be done by giving synthetic mucolytic drugs such as ambroxol, acetylcysteine, etc. However, these synthetic drugs have side effects such as gastrointestinal disturbances, feelings of dizziness, and sweating [5].

Fennel is one of the plants that has been widely used in traditional medicine including as an anti-inflammatory, antioxidant, and cough reduction. The fennels is contains secondary metabolites of phenols, flavonoids, saponins, and tannins. Phenol and flavonoid as antiinflammation [6] and saponin tannin as mucolytic [7].

2. MATERIAL AND METHOD

2.1. Material

Simplicia of fennel leaf, cow intestinal mucus, human red blood cell, buffer phosphate pH 7,4 and 7,4, acethylcystein, methylprednisolone, NaCl 0,9%, hypotonic solution (NaCl 0,25%) and ethanol 96%.

2.2. Method

2.2.1. Making Simplicia of fennel leaf

The leaf of the fennel plant used were young leaf that were two months old and obtained from the Manoko Lembang medicinal plant garden, West Bandung. Fennel leaf were washed and sorted wet then dried at 50°C in the oven, and then sorted dry and mashed using a blender.

2.2.2. Characterization of fennel leaf Simplicia

Characterization of fennel leaf Simplicia, namely macroscopic, phytochemical screening, water content, drying shrinkage, ash content, acid insoluble ash content, water-soluble ash content, water, and ethanol soluble content.

2.2.3. Making Ethanol Extract of Fennel Leaf

The leaf powders were extracted using 96% ethanol by maceration at room temperature for 3x24 hours. The obtained macerate is then filtered and evaporated using a rotary evaporator then evaporated again in a water bath at 50° C until a thick consistency is obtained.

2.2.4. Phytochemical Screening of Ethanol Extract Fennel Leaf

Phytochemical screening consists of checking out the material of alkaloids, polyphenols, flavonoids, saponins, tannins, quinones, coumarin, steroids-triterpenoids, and mono-sesquiterpenes.

2.2.5. Anti-Inflammatory Test

HRBC method was used for the estimation of anti-inflammatory activity in vitro. The blood used the author's blood who has not taken any NSAIDs or steroids for two weeks before the experiment [8]. This blood solution was centrifuged at 3000 rpm for 10 minutes and the packed cells were separated. The packed cells were washed with isosaline solution. To make a 10% v/v erythrocyte suspension, 1 mL of RBC (Red Blood Cells) was taken and isosaline was added until 10 mL. This HRBC suspension was used for the estimation of the anti-inflammatory property. Different concentrations of extract, reference sample, comparison (methylprednisolone 5 ppm), and control were separately mixed with 1 mL of phosphate buffer pH 7,4, 2 mL of hyposaline, and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%. The absorbance obtained is then calculated as %inhibition of hemolysis.

$$\%\ Inhibition = \left(\frac{Abs\ Control - Abs\ Sample}{Abs\ \ Control}\right) x\ 100\%$$

2.2.6. Mucolytic Test

Cow intestinal mucus solution in buffer phosphate pH 7 was incubated at a temperature of 37°C and measured for initial viscosity. Consisted of 10 mL of ethanol extract fennel leaf from various concentration, aquades, and acethylcysteine (0,1%) were separately mixed with 90 mL of 20% cow intestinal mucus solution in buffer phosphate pH 7 who has been incubated. The test sample is re-incubated at a temperature of 37°C and measured for final viscosity.

3. RESULT AND DISCUSSION

3.1 Characterization of fennel leaf Simplicia

Macroscopic examination of fresh leaf and fennel leaf Simplicia including shape, color, smell, and taste. The result was tabulated in table 1. Characterization of fennel leaf Simplicia, namely macroscopic, phytochemical screening, water content, drying shrinkage, ash content, acid insoluble ash content, water-soluble ash content, water, and ethanol soluble content. The result was tabulated in table 2.

Table 1. Results of macroscopic examination of fresh fennel leaf and simplicia powder

Characterization	Result	
	Fresh leaf	Powder
Shape	Linear or awl	Powder
Colour	Light green	Green
Smell	Spesific	Spesific
Taste	Spicy	Spicy

Table 2. Result of characterization examination of Fennel leaf Simplicia

Characterization	Content ± SD	Indonesian Herbal Pharmacopoeia
Water content	8,67% v/w	<10,00% v/w
Ash content	16,16% w/w	≤13,10% w/w
Water-soluble ash content	8,19% w/w	-
Acid insoluble ash content	14,68% w/w	≤2,70% w/w
Water soluble content	33,72% w/w	≥12,30% w/w
Ethanol soluble content	8,11% w/w	≥5,40% w/w
Drying shrinkage	5,78% w/w	<10,00% w/w

n= 3

3.2 Phytochemical Screening of Simplicia and Ethanol Extract of Fennel Leaf

Phytochemical screening was carried out using the color reagent method. The result of the above test showed that Simplicia and ethanol extract of fennel leaf were positive for polyphenols, flavonoids, saponins, tannins, quinones, coumarin, steroids-triterpenoids, and mono-sesquiterpenes.

3.3 Anti-Inflammatory Activity

The % inhibition data obtained were then tested for normality and homogeneity. Based on the results of normality and homogeneity tests, p-value were obtained at 0,082 and 0,063 respectively. by looking at the p-value that is more than 0,05, the data is declared to be normally distributed and homogeneous. Data analysis continued with ANOVA and LSD tests in post-Hoc analysis to determine differences between treatment groups. The results showed that all groups of fennel leaf ethanol extract were significantly different from the control group, which means that all fennel leaf ethanol extracts had anti-inflammatory activity. When compared with the comparison group, all concentrations of fennel leaf ethanol extract had significant differences, meaning that all fennel leaf ethanol extract concentrations were not equal (not as effective) as the comparison. The higher the concentration of the extract, the greater the percentage of inhibition. This is supported by the value of r = 0.935, which means that the concentration of the extract and % inhibition has a strong and directly proportional correlation. Extract ethanol from fennel leaf at different concentrations showed stabilization towards HRBC membrane. The percentage inhibition of extract ethanol fennel leaf at concentration 412 ppm was higher than that of concentrations. The result was tabulated in table 4. Factors that can influence hemolysis inhibitory activity in ethanolic extract of fennel leaf are secondary metabolites, while secondary metabolites suspected of having membrane stabilizing activity are tannins, steroids, saponins [6] and flavonoids [9].

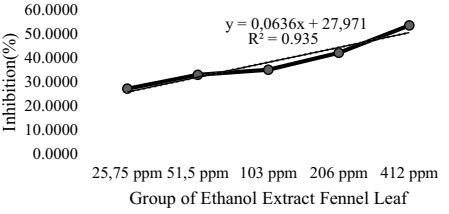

Figure 1. Graph of Relationship between Extract Concentration and %Inhibition

3.4 Mucolytic Activity

The viscosity data obtained were then tested for normality and homogeneity. based on the results of normality and homogeneity tests, p values were obtained at 0,149 and 0,932 respectively. by looking at the p-value that is more than 0,05, the data is declared to be normally distributed and homogeneous. Data analysis continued with ANOVA and LSD tests in post-Hoc analysis to determine differences between treatment groups. The results showed that all groups of fennel leaf ethanol extract were significantly different from the control group, which means that all fennel leaf ethanol extracts had mucolytic activity. When compared with the comparison group, all concentrations of fennel leaf ethanol extract had significant differences, meaning that all fennel leaf ethanol extract concentrations were not equal (not as effective) as the comparison. The higher the concentration of the extract, the greater the percentage of inhibition. This is supported by the value of r = -0.8362, which means that the concentration of the extract and viscosity has a strong and inverse correlation. The ethanol extract of fennel leaf at different concentrations showed a decrease in the viscosity of cow intestinal mucus and the greatest decrease in viscosity was found in the ethanol extract of fennel leaf with a concentration of 412 ppm. The result was tabulated in table 5. Factors that can influence mucolytic activity in ethanolic extract of fennel leaf are secondary metabolites, while secondary metabolites suspected of having membrane stabilizing activity are tannins, flavonoids, saponins [7].

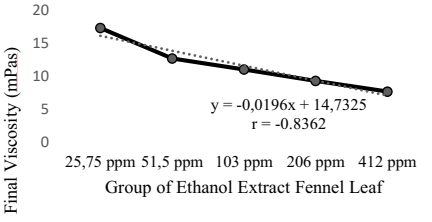

Figure 2. Graph of Relationship between Ethanol Extract Fennel Leaf and Cow Intestinal Mucus Viscosity

4. CONCLUSION

The extract ethanol of fennel leaf has anti-inflammatory and mucolytic activity, but is not effective if compared with methylprednisolone and acethylcycstein as comparison. The extract Ethanol of Fennel Leaf has the potential to be used as a therapy in COPD but further research is needed by in vivo method to determine the effectiveness or potential side effect of fennel leaf ethanol extract as a therapy in COPD.

AUTHORS' CONTRIBUTIONS

INA conceived the original screening and research plans. AAS supervised the experiments. SL and INA performed most of the experiments. INA and AAS critically interpreted the data. SL wrote the manuscript.

ACKNOWLEDGMENTS

This research was supported and facilitated by Jenderal Achmad Yani University, Cimahi, West Java, Indonesia.

REFERENCES

[1] Virlando Suryadinata, R. (2018). *Pengaruh Radikal Bebas Terhadap Proses Inflamasi pada Penyakit Paru Obstruktif Kronis (PPOK) Effect of Free Radicals on Inflammatory Process in Chronic Obstructive Pulmonary Disease (COPD)*. 1–12. https://doi.org/10.2473/amnt.v2i4.2018.317-324

[2] Nanda A., Oea K., Irvan M., Yeesi S. S. (2016). Degrees Relations & Event Exacerbations COPD Patients with components of Metabolic Sydrome. Jurnal Respirasi Indonesia, 36(1), 33-40.

[3] Yudhawati, R., & Prasetiyo, Y. D. (2018a). *Imunopatogenesis Penyakit Paru Obstruktif Kronik* (Vol. 4, Issue 1).

[4] Barnes, P. J. (2020). Oxidative stress-based therapeutics in COPD. In *Redox Biology* (Vol. 33). Elsevier B.V. https://doi.org/10.1016/j.redox.2020.101544

[5] Leboe, D. W., Ningsi, S., & Annur, M. (N.D.). *Uji Aktivitas Mukolitik Ekstrak Etanol Daun Tembelekan (Lantana Camara Linn.) Secara In Vitro*.

[6] Dewi, B. A., Setianto, R., & Rosita, F. (2020). Uji Aktivitas Tanaman Pangotan (Microsorium beurgerianum (Miq.) Ching) Sebagai Antiinflamasi Secara Invitro dengan Metode HRBC (Human Red Blood Cell) Activity Test of Pangotan (Microsorium beurgerianum (Miq.) Ching) as an Invitro Anti-inflammatory with the HRBC (Human Red Blood Cell) Method. In *Jurnal Ilmiah Kesehatan* (Vol. 1, Issue 2).

[7] Fisheri Kurniati, N., Winda Suwandi, D., & Yuniati, S. (2018). Aktivitas Mukolitik Kombinasi Ekstrak Etanol Daun Kemangi dan Ekstrak Etanol Daun Sirih Merah (Mukolitic Activity of Combination of Ethanol Extract of Basil Leaves and Ethanol Extract of Red Betel Leaves). *Pharmaceutical Sciences and Research*, *5*(1), 7–13.

[8] Leelaprakash, G., & Dass, S. M. (n.d.). Invitro Anti-Inflammatory Activity Of Methanol Extract Of Enicostemma Axillare. In *Int. J. Drug Dev. & Res* (Vol. 3, Issue 3). http://www.ijddr.in

[9] Ihsan, H., Pratama, I. S., & Hanifa, N. I. (2021). Uji Aktivitas Antiinflamasi Infusa Bunga Pukul Empat Secara In Vitro. *Acta Pharmaciae Indonesia : Acta Pharm Indo*, *9*(1), 21. https://doi.org/10.20884/1.api.2021.9.1.3683