

**HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
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Judul Karya Ilmiah (Artikel)	: Potential of Miana Leaf Extract as Expectorant (Profile Place of Growing, Antioxidant, Sputum Contaminants, Antibacterial, MIC, MKC Expectorant)	
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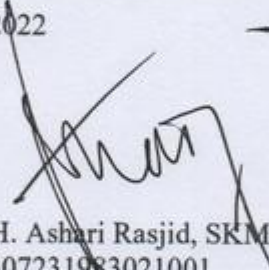
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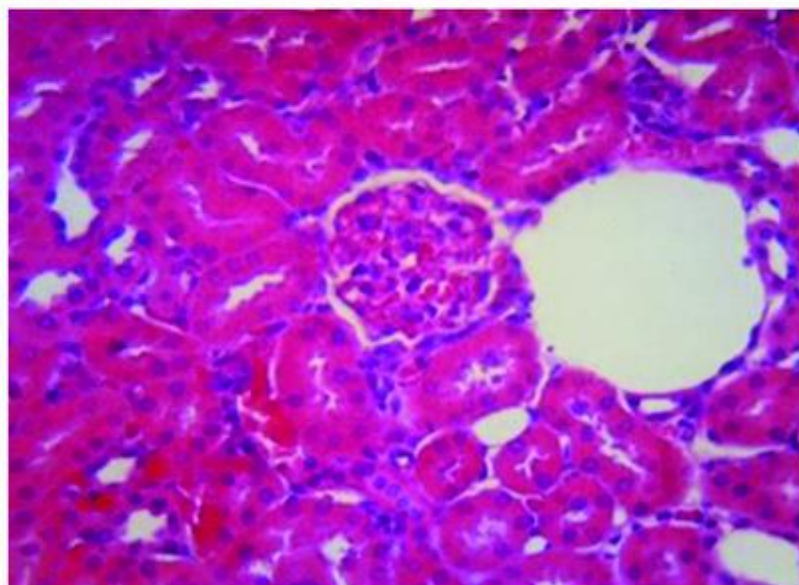
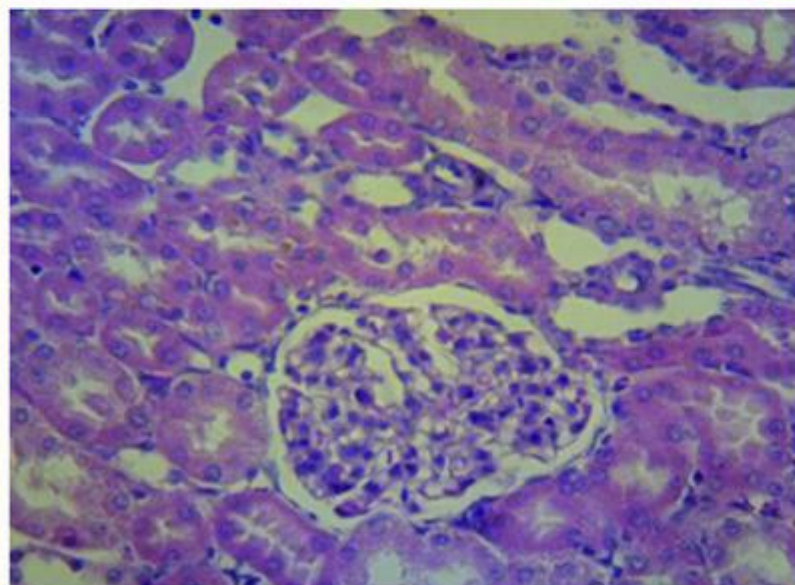
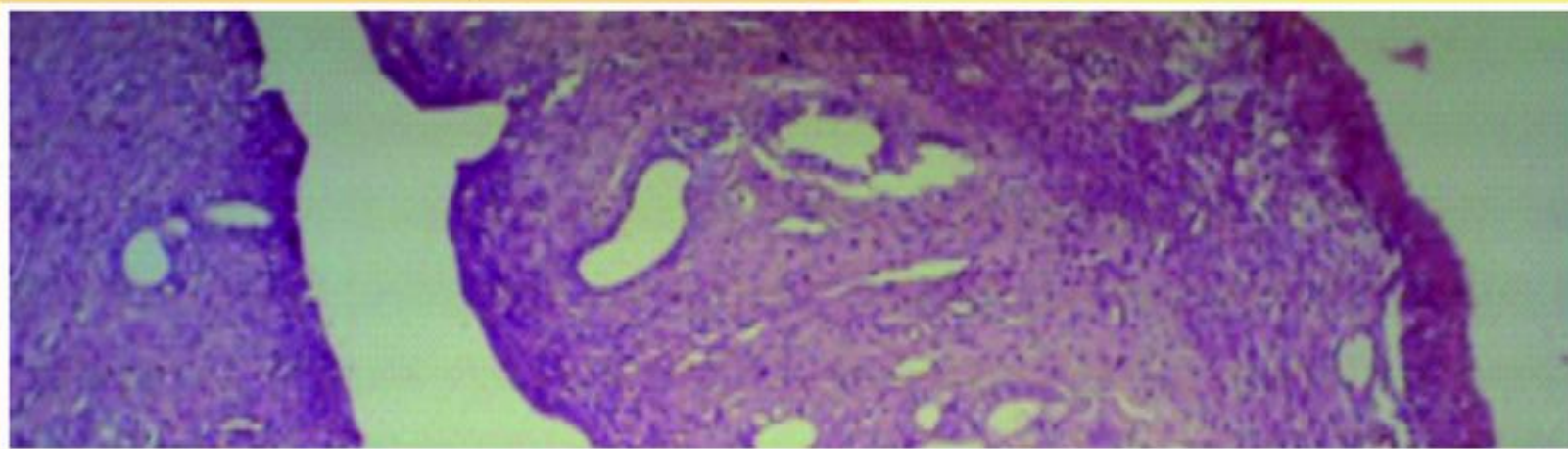


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Potential of Miana Leaf Extract as Expectorant (Profile Place of Growing, Antioxidant, Sputum Contaminants, Antibacterial, MIC, MKC Expectorant)

Sesilia Rante Pakadang¹, Santi Sinala^{1*}, Alfrida Monica Salasa¹, St Ratnah¹, Sisilia Teresia Rosmala Dewi¹, Maria Hilaria²

¹ Poltekkes Kemenkes Makassar, Indonesia

² Poltekkes Kemenkes Kupang, Indonesia

ABSTRACT

Research has been conducted on the treatment of phlegm cough with miana leaf extract in vitro (effective dose of miana leaf extract as an expectorant and antibacterial agent causing cough with phlegm). The study aims to compare the antioxidant activity of miana leaves from 3 locations where it grows, determine the types of contaminant bacteria in the sputum of cough sufferers, determine the minimum value of inhibitor concentration (MIC) and MKC of miana leaves against the test bacteria causing cough with phlegm, determine the effective dose of miana leaves that can used as a reference for coughing up phlegm and proving the potential of miana leaves as a sputum thinner. Miana leaf extraction is done by the juicer method. Antioxidant activity testing uses the DPPH method. Determination of test bacteria is done by isolating and identifying contaminant bacteria in the sputum sample of cough with phlegm. Testing the effectiveness of miana leaves against test bacteria is determined by the liquid dilution method. Expectorant activity testing was determined by measuring the viscosity of mucus viscosity of cow intestine treated with miana leaf extract. The results found that antioxidant activity was influenced by the location where miana leaves grew with an antioxidant potential of IC72 0.072 mg/ml - 0.76 mg/ml. Contaminant bacteria from sputum samples of cough patients are *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterobacter agglomerans*. MIC values for contaminant bacteria are 0.1% - 0.75% and MKC values are found between 0.25% - 1.75%. miana leaf extract has the potential as a sputum thinner at a concentration of 0.01% - 0.1%. The recommended dose of miana leaf extract as a cough with phlegm is 1.75% w/v.

Keywords: miana leaves; sputum contaminant bacteria; antioxidants; expectorants; MIC; MKC

INTRODUCTION

Coughing is a major complaint found in respiratory diseases. Coughing is one of the body's main physical defense mechanisms to clean the throat and respiratory tract from harmful antigenic material (Baratawijaya, 2010). Coughing with frequent and prolonged intensity can indicate a disorder or disease. Phlegm in cough indicates infection and inflammation of the respiratory tract (Ringel, 2009). Diseases that have symptoms in the form of cough with phlegm such as pneumonia, chronic obstructive pulmonary disease (COPD) acute exacerbation, acute bronchitis, bronchial asthma, and bronchiectasis (Danusantoso, 2000). Based on WHO reports that every year 4 of 13 million deaths in developing countries are caused by respiratory infections.

The most common pathogens detected by sputum culture are bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella species*

*Corresponding author : Santi Sinala
Email : santisinala@poltekkes-mks.ac.id

(Ibrahim, 2012). Research by Altiner *et al* (2009) of bacteria found in acute cough phlegm namely *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae* and *Moraxella catarrhalis*. Research by Parhusip (2004) in BP4 Medan, the most bacteria that cause lower respiratory tract infections are *Streptococcus viridians* and *Staphylococcus aureus*. Research by Ziyade and Yagci (2010) the most common bacteria in the culture of lower airway sputum are *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Based on the research of Panggalo *et al* (2013) bacteria found in sputum culture from patients with cough with phlegm in Manado General Hospital are *Streptococcus*, *Staphylococcus aureus*, *Proteus*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, *Serratia marcescens*, *Hafnia alvei*, *Staphylococcus aureus*, *Proteus*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, *Serratia marcescens*, *Hafnia alvei*, *Staphylococcus aureus*, *Proteus*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, *Serratia marcescens*, *Hafnia alvei*, *Staphylococcus aureus*, *Proteus*.

Treatment of phlegm cough can be done with sputum (expectorant) and antibacterial drugs from chemicals as conventional treatments and alternative treatments using herbal ingredients that are antibacterial, expectorant, and immunostimulant. The use of plants as an alternative treatment is caused by the ability of herbs to increase their response and immunity to disease. Alternative medicine can influence the formation of antibodies and immune complexes, change the inflammatory and anti-inflammatory balance, and regulate the pathogen response (Venkatesha *et al.*, 2011). There is currently an increasing interest in the investigation of medicinal plants for new antimicrobial discoveries, antioxidants, and other diseases (Alshawsh *et al.*, 2012).

Miana (*Coleus scutellarioides* (L.) Benth.) Is a plant that has been used empirically for the prevention and treatment of disease. Pakadang, SR. (2015) stated that the ethanol extract of miana leaves (*Coleus scutellarioides* (L.) Benth.), It is an immunomodulatory material against healthy model mice and infected with tuberculosis with mechanisms for increasing T lymphocyte proliferation, increasing T cells, increasing IFN- γ and TNF - α , and reduce the number of *Mycobacterium tuberculosis* colonies for mice infected with tuberculosis. In vitro test showed that *Mycobacterium tuberculosis* was sensitive to miana leaf extract in vitro on Lowenstein Jensen's medium obtained MIC values at 200 ppm (Pakadang, SR., 2014). A study conducted by traditional healers in Tana Toraja District shows that the Toraja people have used miana leaves to treat all types of coughs. In this case, asthma cough is characterized by a state of shortness of breath, cough with phlegm, dry cough (without phlegm) to cough accompanied by blood (Pakadang, SR., 2015). The activity of miana leaves as an antibacterial has been proven in vitro test against some cough-causing bacteria such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae* (Pakadang, SR., 2016). Research on ethnopharmacology by Pakadang, SR. and Karim, D. (2016) showed that the Bugis and Makassar tribes in South Sulawesi also used miana leaves as a medicine for phlegm and bleeding cough.

The main problem in the use of herbal medicine for miana leaves is the absence of effective doses to be used as a reference for use in the community. The purpose of this study was to determine the antioxidant activity of miana leaf extract from 3 different planting locations; determine the bacteria that cause cough with phlegm that is commonly found in clinical

laboratories; determine the value of minimum inhibitor concentration (MIC) and minimal killing concentration (MKC) of miana leaf extract against test bacteria that cause cough with phlegm; Proving the potential of miana leaf extract as a sputum thinner in vitro based on viscosity. Determine the effective dose of miana leaf extract that can be used as a reference for coughing up phlegm.

METHODOLOGY

Material

The material in this research is water extract of Miana leaf and the test sample was the sputum culture results of cough sufferers. Preparation of Test Bacteria from Phlegm of Cough Patients (test bacteria were isolated and identified from sputum samples of cough sufferers. Sputum samples were obtained from cough mucous patients at Ibnu Sina Hospital Makassar in June-July 2017). Sputum samples were obtained from patients diagnosed as acute non-tuberculosis airway infections at the Ibnu Sina Hospital in Makassar City in the period June - July 2017

Methods

Miana leaves are cleaned then the juice is taken using a juicer. Sari is made into a dry extract using freeze-drying. Furthermore, dry extracts are prepared into test materials with a dilution of 0.01% - 2.5%.

Antioxidant testing was carried out using the DPPH method (1,1-diphenyl-2-picrylhydrazyl).

Testing MIC and MKC values based on the liquid dilution method. (Miana leaf extract test material was made in series with 0.01%; 0.05%; 0.1%; 0.25%; 0.5%; 0.75%; 1%; 1.25%; 1.5%; 1.75%; 2%; 2.25%; 2.5%, negative control and positive control.

In vitro Mucus Viscosity Test (test material mixed with mucus from cow's intestine. The mixture was incubated under pH = 7 at 37°C for 1 hour. Test for viscosity of mucous intestinal cows after incubation was carried out using a Stormer viscometer. Viscosity of mucus was measured after extract treatment was administered at a temperature of 37°C for 1 hour. Miana leaves, the thickness of the test material was determined based on the thickness of the cow's intestinal mucus given the test material, positive control, and aquades (as negative control).

Determination of the effectiveness of Miana Leaf Extract Against Bacteria / mold test samples (effectiveness is determined based on the MIC value parameter. MKC and viscosity).

RESULTS AND DISCUSSION

The results can be seen in the attachment table below.

Based on statistical data processing results obtained by giving EDM 0.01% not significantly different from giving EDM 0.05%; 0.1%; 0.25%; 0.5% and bromhexine 0.004%. The results of giving 2.25% concentration showed that the thickness of mucus was not significantly different from the concentration of 2.5% (minimum viscosity obtained) but significantly different (lower viscosity) by administering negative control and positive control (bromhexine). This research uses miana leaf extract test material obtained from the results of the miana leaf juicer, in the form of miana leaf juice extract. Fresh *simplicia* squeeze is one of the extraction methods by crushing the *simplicia* and then squeezing (if necessary adding water) then filtered to obtain *simplicia* extract (Kemenkes RI, 2013). The juice extract is then made into a dry extract using a freeze dryer. The working principle of Freezer drying includes freezing the solution, granulating the frozen solution under ultra-high vacuum conditions with moderate heating so that the water in the frozen material will freeze and will produce an extract (Hanani, E., 2016).

Sources of *simplicia* miana leaves were obtained from 3 test material collection locations namely; Makassar City, Rappocini Subdistrict in Buakana Village (maintained in the home yard) as location 1 and Buakana Village (maintained outside the fence/roadside) as location 2. While location 3 was obtained from Kupang City. Selection of 3 locations for *simplicia* taking to determine the best quality of *simplicia* for use in testing. The quality expected in this case is the compound content and antioxidant activity of each test material.

Lately, the use of antioxidant compounds is growing rapidly both for food and treatment. Use as a drug is growing along with the increasing knowledge about the activity of free radicals against several degenerative diseases such as heart disease and cancer (Boer, 2000). Antioxidants are known to inhibit the work of free radicals. The results of the antioxidant activity test carried out by the DPPH method (1,1-diphenyl-2-picrylhydrazil) according to the data obtained the value of IC50 (inhibitory concentration 50%) for EDM is 0.022 mg/ml - 0.765 mg/ml. The next test is the effectiveness of EDM testing on test bacteria isolated from the sputum of cough sufferers and viscosity testing for testing expectorant activity.

The results of testing the antioxidant activity found that the location of the place of growth is very influential on the number and types

of active substance components contained in these plants. Antioxidant activity is strongly influenced by the active ingredient contained in the extract used as test material. Active substances that can provide antioxidant activity are the result of secondary metabolites from a plant extract. Comparison of natural conditions in Tana Toraja Regency (comparative EDM) is more conducive to plant growth compared to Makassar City so that the active substances contained in miana plants originating from Tana Toraja provide more efficient antioxidant activity, namely smaller IC50. According to the Farming id Green Indonesia Project, 2017 plant growth factors are determined by internal factors such as genes and plant hormones. While external factors that also influence are nutrition, sunlight, water and humidity, temperature, and soil. In this research, the place to grow DM1 and DM2 samples are Makassar City which is known that sunlight and temperature are quite high. And vice versa water and air humidity are quite low, accompanied by high pollution conditions as well as other Metropolitan Cities. So that the condition of the soil where it grows is not enough nutrients and nutrients for low plants. The opposite happened for DM3 (a sample from Kupang City) that was intentionally planted in a polybag so that plant nutrients were maintained. Maintenance was chosen as a cool location in Kupang City, Baumata Village, where natural conditions are still abundant in natural forests. This condition was chosen to approach the natural condition of the origin of the miana leaf, namely Tana Toraja Regency. High humidity conditions, allowing the presence of water absorbed by plants reduces evaporation thereby maintaining cell stability and cell elongation. Low air temperature affects the growth and development of plant cells because it is related to water absorption, photosynthesis, evaporation, and respiration in plants. High rainfall certainly affects the air temperature, humidity, and availability of water in the soil. The condition of Tana Toraja Regency which is dominated by mountainous areas and highlands makes the exposure to sunlight not optimal / less so that it supports high humidity and low temperatures. Sunlight is needed by plants for photosynthesis, but excessive amounts of sunlight will inhibit plant growth because light can damage the hormone auxin found at the end of the stem. Tana Toraja's natural conditions make conditions that allow the state of the soil to have better mineral, water, acidity and nutrient content, so that the soil becomes fertile. This soil fertility will support optimal plant development including the development of active substances in plants. So that

Table I. Antioxidant activity (IC50) of Miana leaf extracts from several growing locations

Test Material	Source of Test Material	(IC ₅₀)	Information
BU 1	Makassar City 1	0,517mg/ml	Planted on the roadside
BU 2	Makassar City 2	0,765mg/ml	Planted in the yard 1
BU 3	Makassar City 3	0,337mg/ml	Planted in the yard 2
BU 4	Kupang City NTT	0.072mg/ml	Planted in cool air areas
BU 5	Tana Toraja District	0,022mg/ml	Planted in a cool area in cultivation

Information: As a comparison, vitamin C was used with antioxidant activity (IC 50) of 0.015 mg/ ml

Table II. Pathogenic microorganisms (bacteria / mold / yeast) the results of isolation from the sputum of cough sufferers

Sample	Bacterial contaminants	Information
S 1	<i>Streptococcus pneumonia</i>	Recap the type of bacteria that contaminates the mucus of cough sufferers from Ibnu Sina Hospital Makassar
S 2	<i>Streptococcus pneumonia</i> <i>Klebsiella pneumoniae</i>	
S 3	<i>Streptococcus pneumonia</i> <i>Staphylococcus epidermidis</i>	
S 4	<i>Staphylococcus aureus</i> <i>Streptococcus pneumonia</i>	<i>Streptococcus pneumonia</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus epidermidis</i>
S 5	<i>Enterobacter agglomerans</i>	<i>Staphylococcus aureus</i>
S 6	<i>Staphylococcus epidermidis</i> <i>Streptococcus pneumonia</i>	<i>Enterobacter agglomerans</i> <i>Candida albicans</i>
S 7	<i>Staphylococcus epidermidis</i> <i>Streptococcus pneumonia</i>	
S 8	<i>Streptococcus pneumonia</i>	
S 9	<i>Candida albicans</i>	
S 10	<i>Staphylococcus aureus</i>	

Information: S1 - S10 is a sputum sample of a cough sufferer; Patogen yang ditemukan terdiri dari 5 jenis bakteri dan 1 jenis khamir

Table III. Effective Concentration of Miana (*Coleus scutellarioides* (L) Benth) Leaf Extract against Test Microorganisms

No.	Types of test bacteria	MIC	MKC
1	<i>Streptococcus pneumoniae</i>	0,1%	0,25%
2	<i>Klebsiella pneumonia</i>	0,25%	1,25%
3	<i>Staphylococcus aureus</i>	0,25%	0,5%
4	<i>Staphylococcus epidermidis</i>	0,25%	1,25%
5	<i>Enterobacter agglomerans</i>	0,75%	1,75%
6	<i>Candida albicans</i>	0,25%	0,75%

the development of active substances which are the result of metabolites is optimal. In addition to soil fertility, anthocyanins and total phenols in plants are also influenced by planting time and cooler temperatures. According to Reyes *et al* (2004) a longer number of planting days and cooler temperatures in Colorado produce tubers

with anthocyanin content and total phenol of 2.5- and 1.4-times higher than those grown in Texas.

This study took phlegm samples from cough sufferers at Ibnu Sina Hospital Makassar. Furthermore, sputum is isolated and identified in the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University to determine the

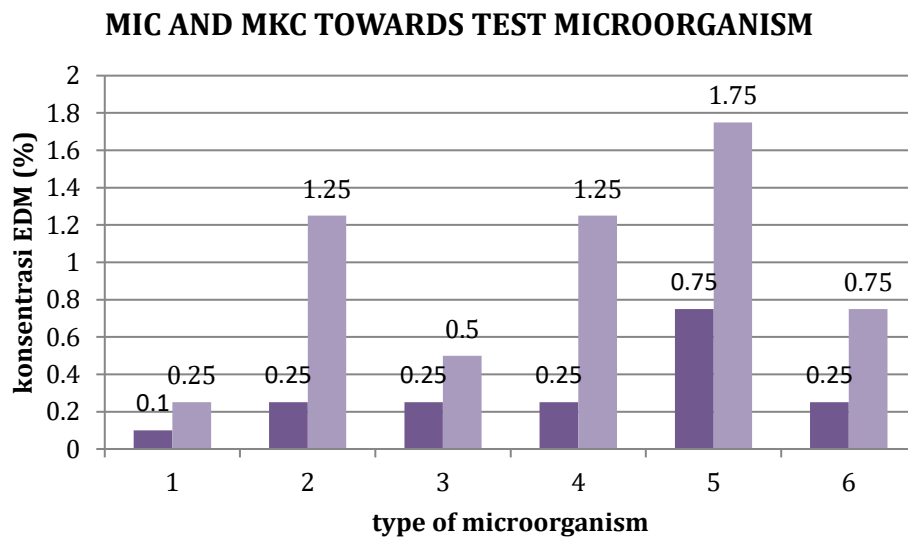


Figure 1. Histogram of MIC and MKC values of Miana Leaf Extract (*Coleus scutellarioides* (L) Benth) against Test Microorganisms

Information: *Streptococcus pneumoniae*; *Klebsiella pneumoniae*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Enterobacter agglomerans*; *Candida albicans*

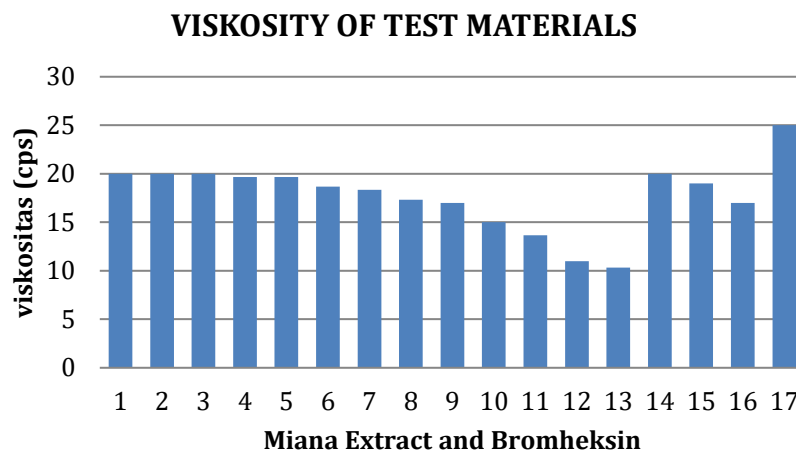


Figure 2. Average viscosity of the Test Material after treatment

Information: Test material 1: Miana leaf extract with concentration 0.01%; Test material 2: Miana leaf extract with concentration 0.05%; Test material 3: Miana leaf extract with concentration 0.1%; Test material 4: Miana leaf extract with concentration 0.25%; Test material 5: Miana leaf extract with concentration 0.5%; Test material 6: Miana leaf extract with concentration 0.75%; Test material 7: Miana leaf extract with concentration 1.00%; Test material 8: Miana leaf extract with concentration 1.25%; Test material 9: Miana leaf extract with concentration 1.5%; Test material 10: Miana leaf extract with concentration 1.75%; Test material 11: Miana leaf extract with concentration 2.0%; Test material 12: Miana leaf extract with concentration 2.25%; Test material 13: Miana leaf extract with concentration 2.5%; Test material 14: bromheksin with concentration 0.001%; Test material 15: bromheksin with concentration 0.01%; Test material 16: bromheksin with concentration 0.05%; Test material 17: mucus with concentration 20%

Table IV. Difference Test Results Between Test Materials Treatment Based on the Mann-Whitney Test on Viscosity

No	Test material	N	Mean	Std. Deviation	Median	Minimum	Maximum
1	EDM 0.01%	3	20.0000	.00000	20.0000 ^a	20.00	20.00
2	EDM 0.05%	3	20.0000	.00000	20.0000 ^{ab}	20.00	20.00
3	EDM 0.1%	3	20.0000	.00000	20.0000 ^{abc}	20.00	20.00
4	EDM 0.25%	3	19.6667	.57735	20.0000 ^{abcd}	19.00	20.00
5	EDM 0.5%	3	19.3333	.57735	19.0000 ^{abcde}	19.00	20.00
6	EDM 0.75%	3	18.6667	.57735	19.0000 ^{def}	18.00	19.00
7	EDM 1.00%	3	18.3333	1.15470	19.0000 ^{defg}	17.00	19.00
8	EDM 1.25%	3	17.3333	1.52753	17.0000 ^{defgh}	16.00	19.00
9	EDM 1.5%	3	17.0000	.00000	17.0000 ^{ghi}	17.00	17.00
10	EDM 1.75%	3	15.0000	.00000	15.0000 ^j	15.00	15.00
11	EDM 2.00%	3	13.6667	2.30940	15.0000 ^{jk}	11.00	15.00
12	EDM 2.25%	3	11.0000	.00000	11.0000^{kl}	11.00	11.00
13	EDM 2.5%	3	10.3333	.57735	10.0000 ^{kl}	10.00	11.00
14	bromheksin 0.004%	3	20.0000	.00000	20.0000 ^{abcde}	20.00	20.00
15	bromheksin 0.01%	3	19.0000	.00000	19.0000 ^{defgh}	19.00	19.00
16	bromheksin 0.05%	3	17.0000	.00000	17.0000 ^{ghi}	17.00	17.00
17	Cow intestinal mucus	3	25.0000	.00000	25.0000	25.00	25.00
	Total	51	17.7255	3.58931	19.0000	10.00	25.00

Information: ^{abcdeghi} the same superscript shows no difference between the test material (based on the Mann-Whitney test)) at $\alpha = 0.05$

type of pathogenic microorganisms that are the source of infection for cough sufferers. The test results (Table II) show that 10 sputum samples tested found 5 types of contaminant bacteria, namely: Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter agglomerans, and 1 type of yeast/fungus, namely Candida albicans. All types of bacteria found in this study turned out to often contaminate phlegm from cough sufferers that have been studied by many researchers before. Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and Klebsiella species (Ibrahim, 2012). Furthermore, Altiner *et al* (2009) found bacteria in acute cough phlegm namely Streptococcus pneumonia, Haemophilus influenza, Haemophilus parainfluenza, and Moraxella catarrhalis. Parhusip (2004) in BP4 Medan found the bacteria that cause lower respiratory tract infections are Streptococcus viridians and Staphylococcus aureus. Research by Ziyade and Yagci (2010) found that bacteria in the lower airway sputum are Haemophilus influenza, Pseudomonas aeruginosa and Streptococcus pneumoniae. Panggalo *et al* (2013) found bacteria in sputum culture in Manado General Hospital are

Streptococcus, Staphylococcus aureus, Proteus, Enterobacter aerogenes, Acinetobacter baumannii, Seratia marcescens, Hafnia alvei, Citrobacter diversus, E coli and Klebsiella ozaenae. Cough is a physiological mechanism that is useful for removing and cleaning the respiratory tract from sputum, foreign stimulants, and elements of infection. Thus coughing is a protection mechanism. Coughing is mainly caused by viral infections, such as common cold viruses, influenza, chickenpox, and also by inflammation of the branches and upper throat (bronchitis, pharyngitis). These viruses can damage the respiratory mucosa, thus creating an entry point for bacterial and viral infections, for example, Pneumococci and Haemophilus (Tan and Rahardja, 2010). This study also found mold, *Candida albicans*. Sputum taken as a sample in this study is ideal for fungal testing because it is taken from sputum in the morning and is processed immediately in the laboratory (Richardson and Warnock, 2003). *Candida albicans* is a common member of the oropharyngeal, gastrointestinal, and female genital flora (Ryan and Ray, 2004).

The next test is the effectiveness of miana leaf extract as an antibacterial against test bacteria found from the sputum samples tested. Tests are

carried out by the liquid dilution method to determine MIC (minimal inhibitory concentration) and MKC (minimal killing concentration). The test results show variations in MIC values for the 6 types of test microorganisms are 0.1% - 0.75%. The variation of MKC values for the 6 types of test microorganisms is 0.25% - 1.75%. So that in this study the MIC value was set at 0.75% and the MKC value was 1.75%. Based on the equality in the study, the MIC 0.75% is equivalent to 3.2 sheets or 1,056 grams of fresh miana leaves. MKC 1.75% is equivalent to 7.46 sheets or 2,463 grams of fresh miana leaves. MIC and MKC testing is a method of determining the minimum concentration to inhibit and kill (99.9%) a type of microorganism, so that it can be expressed as a minimum dose of miana leaf extract that provides antibacterial effectiveness against bacteria that cause coughing up phlegm. In chemotherapy MIC is the smallest dose of antimicrobial that can be given to patients who are supported by adequate phagocytic immune mechanisms. However, if the sufferer is found to be multiinfected, the dose given is MKC (Bannister, B., Gillespie, S., Jones, J., 2006). Therefore the recommended dosage of miana leaf extract as an anti-infective is 0.75% - 1.75% which is equivalent to 1.056 - 7.46 sheets of fresh miana leaves.

Test the viscosity of mucus using the viscosity parameters of the cow's intestinal mucus after being given test material treatment. Observation showed a decrease in viscosity compared to control after mixing the test material. This test refers to the use of toy leaves as a cough medicine in the community. Cough that is treated is cough with phlegm until the cough bleeds. Expectorant function of miana leaves is proven by sputum dilution after mixing with the test material. This is in line with modern treatment methods, namely to alleviate and reduce the frequency of coughs given symptomatic therapy with cough-relieving drugs. One of them is mucolytic which can help reduce the thickness of phlegm so that it is easily removed. Mucus is produced by the respiratory tract which is a complex liquid in the form of mucoprotein and mucopolysaccharide gel membranes. The composition of mucus is 95% water and 5% glycoprotein. The composition of mammalian intestinal mucus is 97.5% water, 0.8% protein, 0.73% other organic substances, and 0.88% organic salt (Frandsen, 1986).

The results of SPSS analysis for testing the viscosity data showed that the administration of EDM 0.01% was significantly different from negative control (mucus without EDM test material). However EDM 0.01% was not significantly different from EDM 0.05%; 0.1%; 0.25%; 0.5% and bromhexin 0.004%. This shows

that the administration of EDM 0.01% has a sputum-thinning effect or has function as an expectorant, because bromhexin is a pharmacologically proven expectorant drug. The administration of EDM 1% in this study has provided optimal expectorant effectiveness because it is not significantly different from 0.04% bromhexin administration. The mechanism of expectorantia that occurs in this case is the occurrence of decomposition of the mucus, increase hydrolysis of lysosomes and stimulate the mucus gland. Furthermore, it stimulates surfactant production, decreases the surface tension of the mucus so that the mucus adhesion properties in the bronchus and mucus dilution (Tan and Raharja, 2012). According to Mutiatikum (2010) active substances contained in EDM include flavonoids, tannins, saponins, terpenoids and kinon derivatives. Saponin is a compound that can function as a phlegm thinner because it is a surfactant that can reduce the surface tension of mucus.

EDM in this study is proven to function as an antioxidant, antibacterial and expectorant. The main active substances contained in EDM are flavonoids, saponins, tannins, terpenoids, essential oils are active ingredients that provide a mechanism of pharmacological activity. Flavonoids are the most dominant phenol compounds in plants as are miana. Flavonoids can function as antibacterial, anti-fungal, and anti-virus (Cushnie TPT and Lamb AJ, 2005). There are three kinds of antibacterial mechanism of flavonoids, namely inhibiting the synthesis of nucleic acids and inhibiting the function of the cytoplasmic membrane by damaging membrane fluidity in the hydrophilic and hydrophobic regions so that the fluidity of the outer layers and inner layers of the membrane will decrease. The third way is to inhibit energy metabolism. Besides flavonoids have the ability as an anti-glucosyltransferase (Vasconcelos, 2006). Saponins which are natural molecular weight products with high molecular weight (Johnson AM, 2013), are divided into 3 main groups namely triterpenoids, alkaloid steroids and glycosylic steroids (Saxena M, 2013). Saponins can form a stable foam in aqueous solutions such as soap. The mechanism of saponin as an antibacterial agent is by interacting with cholesterol in the cell membrane and causing the cell membrane to undergo lipid modification which will interfere with the ability of bacteria to interact with the membrane that has undergone such modification. The disruption of the interaction between bacteria by mutilation will cause the ability of bacteria to damage or interact with the host will be disrupted. When the cell membrane is disturbed, antibacterial

substances will be able to easily enter the cell and will interfere with metabolism until finally there is bacterial death (Karlina CY, 2013). The ability of tannin as an antibacterial can be seen from its action on the membrane. According to Vasconcelos *et al.*, Tannins can pass through cell membranes because tannins can precipitate on proteins (Abdollahzadeh SH, 2011). Tannins can also suppress the amount of some enzymes such as glucosyltransferase. In addition to antibacterial agents, the structure and composition of bacterial cells also have an important role in the antibacterial mechanism. The walls of Gram-positive bacteria have the teichoic acid found in peptidoglycan while the Gram-negative bacteria do not have the teichoic acid. This teichoic acid functions as a way to exit and enter ions from and into bacterial cells (Scott JR and Barnett TC, 2006). Lipoteichoic acid which is a kind of teichoic acid found in peptidoglycan which can bind to tannins, so that bacterial growth will be more easily inhibited by antibacterial components (Islam, B, 2007). *Candida albicans* are one of the yeasts that can be treated with several classes of compounds that are antifungal such as polyenes, the azoles, the allylamines, and the echinocandins (four major families) and miscellaneous groups such as flucytosine and griseofulvin. One mechanism that occurs in killing fungi is drug bound to sterols in the fungi cell membrane which causes the membrane defense function to disappear and cell contents will come out and metabolic disruption and cell death occur. When cell permeability is reduced, drugs can cause oxidation reactions and cell damage (Richardson and Warnock, 2003). Some chemical components in EDM such as flavonoids, saponins, and tannins can also cause lysis of microorganism cells including fungal cells. These properties allow EDM to damage the fungal cell membrane and cause fungal death including *Candida albicans*.

CONCLUSION

The antioxidant activity of miana leaf extract is influenced by the location of plant growth. Miana leaves from Makassar City provide antioxidant activity with IC₅₀ = 0.337 mg / ml; from the city of Kupang gave IC₅₀ values = 0.0712 mg / ml; from Tana Toraja Regency gave IC₅₀ values = 0.022 mg / ml. The bacteria that cause cough with phlegm are found are *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterobacter agglomerans* and the fungus *Candida albicans*. The minimum inhibitory concentration (MIC) value of miana leaf extract against test bacteria causing cough with phlegm is 0.1% -

0.75% w/v; and the minimum killing concentration (MKC) is 0.25% - 1.75% w/v. Miana leaf extract has the potential as a phlegm thinner based on a decrease in mucous viscosity with a concentration of 0.01% - 0.1% w/v. An effective dose of miana leaf extract that can be used as a reference for curing phlegm 1.75% w / v which is equivalent to 7 pieces of fresh miana leaves or 0.9 ml of juice extract.

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