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

Sesilia Rante Pakadang¹, Santi Sinala*, Alfrida Monica Salasa¹, St Ratnah¹, Sisilia Teresia Rosmala Dewi¹, Maria Hilaria²
¹Poltekkes Kemenkes Makassar, Indonesia
²Poltekkes 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 be 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 pneumonia, Klebsiella pneumonia, 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 (Ibrahim, 2012). Research by Altiner et al (2009) of bacteria found in acute cough phlegm namely Streptococcus pneumonia, Haemophilus influenza, Haemophilus parainfluenza 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 influenza, 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, Seratia marcescens, Hafnia alvei, Staphylococcus aureus, Proteus, Enterobacter aerogenes, Acinetobacter baumannii, Seratia marcescens, Hafnia alvei, Staphylococcus aureus, Proteus.

*Corresponding author: Santi Sinala
Email: santisinala@poltekkes-mks.ac.id
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.) 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 pneumonia, Klebsiella pneumonia (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 Ibn Sina Hospital Makassar in June - July 2017). Sputum samples were obtained from patients diagnosed as acute non-tuberculosis airway infections at the Ibn 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%; 0.125%; 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 Freezeer 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
Potential of Miana Leaf Extract as Expectorant (Profile Place of Growing Traditional Medicine Journal, 2020)

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

| Test Material | Source of Test Material | IC50 | 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

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

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

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

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

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

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

Information: *Streptococcus pneumoniae; Klebsiella pneumonia; 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%
Traditional Medicine Journal, 25(2), 2020

**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\(^{+}\) | 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\(^{abcd}\) | 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\(^{gh}\)  | 16.00   | 19.00   |
| 9  | EDM 1.5%      | 3  | 17.0000 | .00000         | 17.0000\(^{gh}\)  | 17.00   | 17.00   |
| 10 | EDM 1.75%     | 3  | 15.0000 | .00000         | 15.0000\(^{i}\)   | 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    | 3  | 20.0000 | .00000         | 20.0000\(^{abcd}\) | 20.00   | 20.00   |
| 15 | 0.004%        | 3  | 19.0000 | .00000         | 19.0000\(^{degh}\) | 19.00   | 19.00   |
| 16 | buyromheksin  | 3  | 17.0000 | .00000         | 17.0000\(^{ghi}\)  | 17.00   | 17.00   |
| 17 | 0.01%         | 3  | 25.0000 | .00000         | 25.0000\(^{gh}\)  | 25.00   | 25.00   |
| 18 | 0.05%         | 3  | 25.0000 | .00000         | 25.0000\(^{gh}\)  | 25.00   | 25.00   |

Information: \(^{abdefghi}\) 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, Altimer et al (2009) found bacteria in acute cough phlegm namely Streptococcus pneumonia, Haemophilus influenza, Haemophilus parainfluenza, and Moraxella catarrhalis. Parhuis (2004) in BP4 Medan found the bacteria that cause lower respiratory tract infections are Streptococcus viridans and Staphylococcus aureus. Research by Ziyade and Yagi (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 baumanii, 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 turned out to have a mold content. These conclusions are 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).
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 multinfected, the dose given is MKC (Bannister, B., Gillespie, S., Jones, J., 2006). Therefore the recommended dosage of miana leaf extract as an anti-i-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 (Franson, 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 glycosylcic 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 (Karлина 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 teicic acid. This teikat acid functions as a way to exit and enter ions from and into bacterial cells (Scott JR and Barnett TC, 2006). Lipoteikolat acid which is a kind of teicic 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 on the fungal 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 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 gives 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 pneumonia, Klebsiella pneumonia, 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.

REFERENCES
Abdollahzadeh SH, Masouf RY, Mortazavi H, Moghaddam MH, Roozbahani N, Vahedi M. Antibacterial and Antifungal Activities of Punica Granatum Peel Extracts Against Oral Pathogens. Tehran University of Med Sci J Dentistry. 2011;8(1):1-6.
Altiner A, Wilm S, Daubener W, Bormann C, Pentzek M, Abholz H, et al. Sputum colour for diagnosis of a bacterial infection in patient with acute cough. Scandinavian journal of primary health care 2009:1-4
Alshawsh, M., Abdulla, M., Ismail, S., Amin, Z. (2012) Free Radical Scavenging, Antimicrobial and Immunomodulatory Activities of Orthosiphon stamineus. Journal of Molecules ISSN 1420-3049, 5385-5395.
Bannister, B., Gillespie, S., Jones, J. (2006) Infection Microbiology and Management. Third edition, Published by Blackwell Publishing Asia Pty Ltd, Victoria Australia.
Baratawidjaja, K.G., Rengganis, I. (2010) Imunologi Dasar. edisi ke-10. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia, hal 21,32-43,62-63,74,19,401-402,518-519,524,546-547,651,649,681.
Cushnie TPT, Lamb AJ. Antimicrobial Activity of Flavonoids. Int J Antimicrob Agents. 2005; 26: 343-356
Danusantoso, H., 2000, Anamnesis Penyakit Paru, In: Rachmah L, editor, Buku saku Ilmu Penyakit Paru, Hipokrates, Jakarta, p.7-12
Farming.id, 2017, Green Indonesian Project, https://farming.id/faktor-faktor-yang-mempengaruhi-pertumbuhan-dan-perkembangan-tanaman/
Hanani, E, Mun’im A, Sekarini, R, dan Wiryowidagdo, S. (2006) Uji aktivitas antioksidan beberapa sponse laut dari kepulauan Seribu, Jurnal Bahan Alam Indonesia, vol 5, no.1 Jan 2006 (in Press).
Hanani,E., 2016, “Analisis Fitokimia” Penerbit : Buku Kedokteran EGC; Jakarta.
Ibrahim M. Sputum culture [homepage on the Internet]. 2012 [updated 2012 Aug 13].
Available from: http://emedicine.medscape.com/article/2 119232-overview.

Islam B, Khan AN, Khan AU. Dental Caries: From Infection to Prevention. Med Sci Monit. 2007; 13 (11):196-203.

Karлина CY, Ibrahim M, Trimulyono G. Aktivitas Antibakteri Ekstrak Herba Krokot (Portulaca oleracea L.) terhadap Staphylococcus aureus dan Escherichia coli. Lentera Bio. 2013; 2 (1):87-93.

Kemenkes RI (2013) Pedoman Teknologi Formulasi Sediaan Berbasis Ekstrak vol 2, Badan Pengawasan Obat dan Makanan, Jakarta. Hal 5-12.

Mutatiukum, D., Alegantina, S. dan Astuti, Y. (2012) Standardisasi Simpilis dari Buah Miana (Plectranthus Scutellaroides (L) R.Bth ) yang Berasal dari 3 Tempat Tumbuh Menado, Kupang dan Papua, Buletin Penelitian dan Pengembangan Kesehatan. Vol 38. No.1 hal 1-16

Pakadang, SR., 2015, Potensi Ekstrak Daun Miana (Coleus scutellaroides (L) Benth) sebagai Imunomodulator terhadap Tikus Model yang Terinfeksi Mycobacterium tuberculosis, Disertasi, Universitas Airlangga, Surabaya.

Pakadang, SR., 2014, Sensitivitas M. Tb terhadap Ekstrak Etanol Daun Miana (C. Scutellaroides (L.) Benth), Jurnal Farmasi dan Bahan Alam ISSN 2338-0616 vol 2, no. 2, Sept 2014

Pakadang, SR, Dewi, STR, Lopak, Y., 2015 Ethnofarmakologi Tumbuhan Obat untuk Tuberkulosis Pada Suku Toraja di Sulawesi Selatan, oral presentasi pada simposium nasional kesehatan masyarakat ke-1, FKM, Universitas Airlangga, Surabaya.

Pakadang, SR. dan Karim, D., 2016, Inventorization and ethnopharmacology plant for treatment of infectious diseases in Gowa and Maros district of South Sulawesi Province, International Conference and Workshop on Pharmacy and Statistics; Current Development of Medicinal Plants and Biostatistics by Tadulako University, Palu, Indonesia

Panggalo, JT., Porotuo, J, Buntuian, V., 2013, Identifikasi Bakteri Aerob pada Penderita Batuk Berdahak di Poliklinik Interna BLU RSUP Prof. dr. RD Kandou, Manado, Journal e-Biomedik (eBM), Volume 1, Nomor 1, Maret 2013, hlm. 408-413

Ringel, E., 2009, Pendekatan terhadap pasien dengan penyakit paru. In: Officer DK, editor, Buku Saku Hitam Kedokteran Paru, alih bahasa, Melfia Wati, PT Indeks, Jakarta, p.10

Richardson MD., Warnock, DW., 2003, Fungal Infection Diagnosis and Management, third edition, Blackwell Publishing Massachusetts, USA. p 19, 29-30

Ryan, KJ., Ray, CG., 2004, Sherris Medical Microbiology. An introduction to infectious disease, fourth edition, M. Crawn Hill, Medical Publishing Division, New York, USA. p.661

Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of Medicinal Plants. J Pharmacog Phytochem. 2013; 1 (6): 168-182.

Scott JR, Barnett TC. Surface Proteins of GramPositive Bacteria and How They Get There. Annu Rev Microbiol. 2006; 60: 397-423.

Tan, H.T., Rahardja, K. (2010). Obat-Obat Penting: Khasiat, Penggunaan dan Efek-Efek Sampingnya, Edisi Kelima, PT. Elex Media Komputindo, Jakarta. Hal 5.

Vasconcelos LCS, Sampaio FC, Sampaio MCC, Pereira MSV, Higino JS, Peixoto MHP. Minimum Inhibitory Concentration of Adherence of Punica granatum Linn (Pomegranate) Gel Against S. mutans, S. mitis, and C albicans. Braz Dent J. 2006;17(13):223-227.

Venkatesha, S., Rajaiah, R., & Berman, B. (2011) Immunomodulation of Autoimmune Arthritis by Herbal C AM. Evidence Based Complementary and Alternative Medicine, 1-13.

Ziyade N, Yagci A. Improving sputum culture result for diagnosis of lower respiratory tract by saline washing. Marmara medical journal. 2010;23:30-6.