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Antimicrobial potency of mistletoe (*Scurrula ferruginea* (Roxb. Ex Jack) Danser) extracts from orange plant and its antioxidant activities

Wahyu Dwisa Putra, Nurmiati nurmiati *, Periadnadi periadnadi and Sofia Nur Yenni Safitri Tanjung

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis, Padang 25163, Indonesia.

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Abstract

Orange mistletoe (*Scurrula ferruginea* (Roxb. ex Jack) Danser) is a common mistletoe found in Indonesia and often used as a herbal drink. This mistletoe has active compound such as terpenoids, tannins, saponins and flavonoids acts as antimicrobial and antioxidant. This research was conducted at the Microbiology Research Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. The method used in this research was experiment method with CRD nested pattern with 3 repetitions. The aims of this study were to clarifying the antimicrobial potency of orange mistletoe extract, to determining the MIC and MLC of the orange mistletoe extract, and to determining the antioxidant activity of orange mistletoe extract. The results showed that the greatest antimicrobial potency was found in the fresh extract of orange mistletoe which significantly different against Staphylococcus aureus (14.05 mm), Escherichia coli (10.57 mm) and Candida albicans (7.00 mm). The MIC of fresh extract against S. aureus was 12.5%, E. coli was 25%, and no MIC values against C. albicans, then no MLC values were found. The highest antioxidant activity value of *Scurrula ferruginea* was found in the fresh extract (118.22 µg/mL).

Keywords: Antimicrobial; Antioxidant; MIC; MLC; Polyphenol; *Scurrula ferruginea*

1. Introduction

Indonesia is rich in biodiversity, which makes it possible to develop drugs or medicinal raw materials that function as antimicrobial and antioxidant substances with low cytotoxicity. One of them is a mistletoe plant, namely *Scurrula ferruginea* (Roxb. ex Jack) Danser. This mistletoe can be found on several host plants, one of which is Citrus sinensis. Orange (Citrus sinensis) is a medicinal plant that contains antimicrobial compounds include flavonoids, essential oils, saponins, and terpenoids (Selawati, 2019).

People usually consume mistletoe as a herbal drink to treat various diseases such as a cough medicine, cancer, diuretic, pain reliever and postnatal care (Fazriah et al., 2007).

Phytochemicals are chemical compounds that occur naturally in plants and are responsible for color and other organoleptic properties. There are as many as 4,000 different phytochemicals that have the potential to influence diseases such as cancer, stroke or metabolic syndrome. Some of these phytochemicals are saponins, tannins, alkaloids, steroids, glycosides, carbohy- drates, flavonoids, phlobatannins, and terpenoids (Bano, 2007).

Antimicrobials as chemical substance that have the property or ability to kill or inhibit the growth of germs, while the toxicity to humans is relatively small. Antimicrobial substances will interfere with the process of folic acid formation, resulting in non-functional folic acid and disruption of metabolism in microbial cells (Adrianto et al., 2014).

* Corresponding author: Nurmiati

In this research, 3 test bacteria were used, namely S. aureus, E. coli, and C. albicans. S. aureus is one of the most common gram-positive bacteria causing poisoning in food products. E. coli is a gram-negative bacteria that causes diarrhea. C albicans is a fungal pathogen that causes infections. People usually add lime juice when consuming tea. The addition of lime juice into tea at least 0.2% can increase the antioxidant activity of tea steeping (Sudjatini, 2016).

In this research, the lime used is Citrus aurantifolia. According to Raharjo (2004), citric acid acts as sequestrants/chelators, namely antioxidants that are able to bind metals that catalyze oxidation reactions. While ascorbic acid/vitamin C acts as an oxygen scavanger which functions to bind oxygen so that it does not support the oxidation reaction in the material. Enzymatic antioxidants are endogenous antioxidants, consisting of the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). While non-enzymatic antioxidants are found in vegetables and fruits, which include reduced glutathione (GSH), vitamin C, E, β-carotene, flavonoids, isoflavones, flavones, antosionin, catechins, and isocatechins, and lipoic acid. These phytochemical compounds protect cells from oxidative damage caused by free radicals (Zulaikhah, 2017).

2. Materials and Methods

2.1. Materials

The materials that used consist of fresh and dry leaves of *Scurrula ferruginea*, pure cultures of E. coli ATCC 25922, S. aureus ATCC 25923 and C. albicans, Nutrient Agar (NA), Potato Dextrose Agar (PDA), Mueller Hinton Agar (MHA), Sabouraud Dextrose Agar (SDA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Broth (SDB) medium, aquadest, alcohol, spirits, Phenol-ciocalteu reagen, DPPH solution, Natrium carbonate (Na_2CO_3), methanol, Vit C, Gallic acid, Chloramphenicol and Fluconazole.

2.2. Sample collection

Scurrula ferruginea sample was collected at Taeh Bukik Subdistrict, Lima Puluh Kota Regency. Sampling was done by picking fresh plant part then wash trougly, put in a plastic bag and bring the sample to the laboratory.

2.3. Extract treatment

Scurrula ferruginea sample was extracted in fresh, boiled and brewed treatment. For fresh extract treatment, fresh leaves was mashed in a mortar and filtered using sterile gauze, then centrifuged for 5 minutes at 10,000 rpm. For boiled treatment, fresh leaves was air-dried for 3 to 4 days, then dry sample was measured at 2 g then boiled with 100 ml of water until it reaches a volume of 50 ml and let cool. For brewed and brewed with lime treatment, dry sample was measured at 2 g then infused with 50 ml of boiled water and let cool (add 5 ml of Citrus aurantifolia for brewed with lime treatment).

2.4. Medium preparation

NA, PDA, MHA, SDA, MHB, AND SDA each medium was measured for 5 g, 10 g, 9.5 g, 16.5 g, 5.5 g and 7.5 g respectively, then put each medium into each Erlenmeyer and add distilled water until the volume reaches 250 ml, then heated on a hot plate until boiled and continue to sterilized using an autoclave at 121°C temperature and 1 atm pressure for 15 minutes.

2.5. Preparation of test microbial suspension

Each pathogenic microbe was taken one needle and inoculated in NaCl 0.9, then homogenized with vortex until the turbidity is equivalent to Mc Farland's 0.5 standard solution.

2.6. Antimicrobial activity test

2.6.1. Disc Diffusion

Based on Poelongan et al (2006), MHA and SDA medium was poured into each petri dish about 15 ml aseptically and allowed to solidify. Then the microbial suspension smeared on the surface of the media with a sterile cotton swab. After that, dip the disc paper aseptically into each sample, then place it on the surface of the media using sterile tweezers and incubate for 24 hours. Furthermore, observations and measurements of the diameter of the microbial-free area formed around the disc were done by using a caliper.

2.6.2. Dilution Method

Determination MIC and MLC was determined by the Dilution Method. Diluted mistletoe extract in SDB/MHB medium to create variation concentrations 50%, 25%, 12.5%, 6.25%, 3.125%, 1.6%, 0.8 %, 0.4%, 0.2% and 0.1%. Then added 1 ml of each test microbial suspension in test tubes and incubated at 37°C for 24 hours. Observe the turbidity of the solution, take 1 ml of the solution from a clear tube then pour plate with SDA/MHA medium and incubated for 24 hours. MLC was determined by the least concentration in the dilution results which did not show any growth of the test microbes after being cultured on SDA/MHA medium (Lennete et al., 1991, as cited in Fatisa, 2013).

2.6.3. Determination of antioxidant activity (IC50) using the DPPH method

The antioxidant activity test was done by using the DPPH free radical scavenging effect method (1.1-Diphenyl-2-Picryl-Hydrazine). The DPPH method refers to Molyneux (2004). S.ferruginea extract solution was reacted with DPPH solution. Antioxidant activity was analyzed using a spectrophotometer with a wavelength of 517 nm. The linear regression curve equation and IC50 value were calculated using the equation:

$$Y = ax+b.$$

2.6.4. Gallic Acid Standard Curve

The standard curve for gallic acid used refers to Singleton et al (1999), Standard solutions of gallic acid were prepared with various concentrations of 0, 50, 100, 150 and 200 ppm. 1 ml of standard gallic acid solution was reacted with 1 ml of Folin-Ciocalteu reagent and homogenized. After 5 minutes, 1 ml of sodium carbonate (Na_2CO_3) solution was, then add aquadest until the volume reach 10 ml, then homogenize and incubated for 90 minutes, the absorbance value was measured at wavelength 725 nm. The linear regression curve equation is calculated using equation: $Y = ax + b$

2.6.5. Determination of Polyphenols Levels

Determination of phenolic compounds in *Scurrula ferruginea* extract was carried out using the Folin-Ciocalteu Assay. 1 ml of *Scurrula ferruginea* sample was reacted with 1 ml of Folin- Ciocalteu, to make the pH of the solution 10, at 5 minutes intervals, 1 ml of 13% Natrium Carbonate was added to the mixture and made up with distilled water until the volume reached 10 ml. The reaction was stored in the dark for 90 minutes and the absorbance value was measured using a spectrophotometer at the wave of 765 nm.

2.7. Data analysis

The data obtained in the diffusion method was analyzed by nested pattern, if the result between each treatment significantly differences, then data analysis was continued by DMRT test at the 5% level. Meanwhile, the results of the dilution method, antioxidant activity, total phenolic content were analyzed descriptively.

3. Results and discussion

3.1. Antimicrobial Activity of *Scurrula ferruginea*

Based on the results of the antimicrobial activity test of *Scurrula ferruginea* extract in 143 diffusion method were incubated for 24 hours, the results were obtained as follows:

Table 1 The average diameter of Inhibition zone in each treatment

No	Sample extract	Inhibitory zone average diameter (mm)		
		S. *aureus*	**E. *coli***	**C. *albicans***
1	Fresh extract	14.05 [a]	10.57 [a]	7.00 [a]
2	Dried boiled	12.60 [b]	7.93 [b]	6.00 [b]
3	Dried brewed	10.67 [d]	7.00 [c]	6.00 [b]
4	Dried brewed with lime	11.37 [c]	7.20 [b]	6.00 [b]

Note: The numbers followed by lowercase letters that are not the same in the column are significantly different at the 5% level of Duncan's test

From Table 1 it can be seen that all treatments of orange mistletoe extract can inhibit the growth of tested microbes. The average diameter of the inhibition zone of the four orange mistletoe extract against S. aureus ranged from 10.67-14.05 mm, E. coli ranged from 7.00- 10.57 mm, and C. albicans ranged from 6.00-7.00 mm. The results of analysis of variance showed that each treatment have a significantly different effect on S. aureus. Then the treatment of dried boiled and dried brewed with lime did not have a significantly different effect on E. coli. However, it was significantly different from the fresh extract and dried brewed treatment. As for C. albicans, dried boiled, dried brewed, and dried brewed with lime treatment did not give a significantly different effect. The largest inhibition zones for S. aureus, E. coli, and C. albicans were found in the fresh extract treatment. The fresh extract produced a higher inhibition zone when compared to the boiled and brewed method. This is caused by the rupture of plant cell walls containing active compounds due to the grinding process and it is hoped that these substances can come out of the plant cell vacuole (Harbone, 1996). As reported by Diba et al., (2021), orange mistletoe have antibacterial content in the form of terpenoids, tannins, saponins and flavonoids. Drying and heating can also affect the antibacterial content of plants. According to Gunawan and Mulyani (2004), the drying method causes some of the active compounds in the leaves to evaporate so that when the activity test is carried out, the active substances in the leaves will decrease, causing the resulting inhibition zone to be small. Davidson et al., (1993) also stated that antibacterial compounds will evaporate and disappear when heated. The descrease of antimicrobial activity in boiled treatment can be influenced by the amount of antimicrobial content in the extract. This is supported by Yanti et al., (2016) the higher concentration of the extract, the content of active substances will be greater. Boiling treatment can reduce the antibacterial substances so that not able to inhibit microbes. Ismarani (2012), states that one of the chemical properties of tannins is soluble in water, so when they are dissolved in hot water, their solubility will increase. Total phenol in the brewed treatment was thought to be lower than the other extract. This is because the brewing process can cause damage to phenolic compounds resulting in a decrease in phenol levels. The temperature and time that used to this treatment is not too long, so that the solubility of active compound in extract will not maximal. This is in accordance with the opinion of Farida (2002) which states that phenol damage can be caused by environmental factors such as light, temperature, and oxygen. The size of the inhibition zone formed was influenced by the addition of 5% lime juice to the dried brewed of orange mistletoe. Lime has antibacterial properties that are able to interact with the antibacterial content of mistletoe, thus forming an inhibition zone. In the research of Razak et al., (2013) which stated that lime juice has the presence of chemical compounds in essential oils, including phenol. Phenol has the ability to act as an antibacterial by denaturing proteins and being able to damage the cytoplasm of cells.

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC)

Based on the research that has been done on the MIC and MLC of *Scurrula ferruginea* against the tested microbes, the results were obtained as follows:

Table 2 Value of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of orange mistletoe extract against S. aureus, E. coli, and C. albicans, - is for indicates no MIC/MLC value

Test Microbe	MIC Value (%)	MLC Value (%)
Staphylococcus aureus	12.5	-
Escherichia coli	25	-
Candida albicans	-	-

Based on Table 2. it can be seen that the fresh extract of orange mistletoe was able to inhibit S. aureus at a concentration of 12.5%, E. coli at a concentration of 25%, and no MIC value for C. albicans. Base on Jawetz et al., (1995), MIC is the lowest concentration of an antibiotic or antimicrobial which can inhibit the growth of certain microbes. No MIC value for C. albicans is thought to be due to the thick and complex cell wall of C. albicans. Accordance with Mutiawati (2016), who stated that the cell wall of Candida albicans is dynamic with a layered structure, consisting of several different types of carbohydrates (80- 90%), namely mannan (polymers of mannose), α-glucans, and chitin. The other basic elements are protein (6-25%) and fat (1-7%). The dilution method has no MLC value. This difference in growth occurs because there is a difference in concentration. According to Widyawati (2013) the way antimicrobial substances work in inhibiting bacteria is influenced by the concentration of these antimicrobial substances. This is supported by the opinion of Pelczar and Chan (1988), the higher the concentration of an antimicrobial substance, the greater the antimicrobial power. This means that many microbes will be killed more quickly when the concentration of the substance is higher.

3.3. Results of polyphenols and antioxidants activity

Based on research that has been carried out on polyphenol levels and antioxidant activity of orange mistletoe extract with the Damping Effect method on DPPH Free Radicals (1,1 – Diphenyl-2-PicrylHidrazil). The results are shown in Table 3 below:

Table 3 Levels of polyphenols and antioxidants in fresh extract, dried boiled, dried brewed,and dried brewed with lime of orange mistletoe

Sample Extract	Polyphenols (mg GAE/g)	Antioxidants activity (µg/ml)
Fresh extract	73.57	118.22
Dried boiled	71.23	118.86
Dried brewed	53.23	119.78
Dried brewed with lime	58.40	119.01

From Table 3, it can be seen that all orange mistletoe extracts have antioxidant activity. Fresh extract had the highest antioxidant activity (118.22 µg/ml) and polyphenol level (73.57 mg GAE/g). Antioxidant activity is influenced by the contribution of phenolic compounds. Phenolic compounds can act as antioxidants by breaking free radical chains directly. The smaller the IC50 value, the stronger the antioxidant activity (Khadijah et al., 2017). According to Larson (1988), phenolic components such as flavonoids which are known as primary antioxidants from plants are polar. Flavonoid levels are lower than phenolic levels because flavonoids are part of phenolics (Rambi et al., 2016). The antioxidant value of the fresh sample was higher than that of the dry sample. The antioxidant value obtained is in line with the polyphenol value. Based on Rivai et al., (2011) on the effect of drying method on the quality of meniran herbs, where dry samples of meniran plants contain less flavonoid compounds than fresh samples. The addition of 5% lime (Citrus aurantifolia) juice adds to the antioxidant content of the mistletoe extract. This is in accordance with Waisnawi et al., (2022) which states that in lime there are polyphenols and tannins which also function as antioxidants. This is because lime contains citric acid and ascorbic acid (vitamin C), each of which functions as an antioxidant (Rukmana, 2003).

4. Conclusion

Based on the research result, it can be concluded that: 1. The greatest antimicrobial potency was found in the fresh extract which significantly different against S. aureus (14.05 mm), E. coli (10.57 mm) and C. albicans (7.00 mm). 2. The MIC of fresh extract against S. aureus was 12.5%, E. coli was 25%, and no MIC values against C. albicans, then no MLC values were found. 3. The highest antioxidant activity value was found in the fresh extract (118.22 µg/mL) of orange mistletoe, followed by dried boiled (118.86 µg/mL), dried brewed with lime (119.01 µg/mL), and dried brewed (119.78 µg/mL).experiments and/or point out those that are underway.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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