Meilinda Mustika *et al*, International Journal of Pharmaceutical Sciences and Medicine (IJPSM), Vol.9 Issue. 2, February- 2024, pg. 105-111

ISSN: 2519-9889
Impact Factor: 5.9

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY FROM STEEPING HERBAL TEA COMBINATION FIG LEAF (Ficus *carica* L.) AND SUNGKAI LEAF (*Paronema canesescens* Jack)

Meilinda Mustika*; Nurmalia; Henni Rosiani

School of Pharmceutical Science Padang, West Sumatera, Indonesia
Email: meipiai46@stifarm-padang.ac.id

DOI: 10.47760/ijpsm.2024.v09i02.010

Abstract:

Herbal tea is made from a mixture or single form of leaf, seeds, or roots of various types of plants that have properties in help treatment. The combination of Ficus carica (fig) leaf and Peronema canescens Jack (sungkai) leaf is one of the innovative ingredients that can be used in herbal teas. This study aims to determine the antioxidant and hedonic activity of herbal tea steeping a combination of fig leaf, sungkai leaf, and their combinations with various weight variations, namely (70:30), (50:50), (30:70). Antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, then analyzed using a UV-Visible Spectrophotometer. To find out the level of consumer preference for herbal tea brewing, a hedonic test was carried out. From the antioxidant activity test, the IC_{50} values of fig leaf, sungkai leaf, and the combination were 25.4885, 30.7039, 18.0717, 21.0907, and 25.0547 µg/mL respectively. Hedonic tests were performed on panelists and analyzed using the Friedman test results obtained p-value>0.05. It can be concluded that all formulas have very strong antioxidant activity, and there is no significant effect between the 5 formulas on the color, smell, and taste of the brew

Keywords: antioxidants, fig leaf, sungkai leaf, hedonic, herbal tea

1. Introduction

In everyday life we cannot be free from exposure to free radicals. Sources of free radicals can come from cigarette smoke, fried or burned foods, excessive sun exposure, vehicle fumes and air pollution. Free radical is an atom or group of atoms that has one or more unpaired electrons in the outermost orbital, so it has a reactive nature and the atom will always look for electrolyte pairs that can bind in order to stabilize itself by continuously attacking cells in the body (Alfian *et al.*, 2018). So, compounds that are antioxidants are needed to ward off these free radicals in the body.

Antioxidants are compounds that can inhibit, prevent, or slow down the occurrence of lipid oxidation processes caused by exposure to the impact of free radicals, before the destruction of vital molecules in the body. The mechanism of action of antioxidants is to neutralize free radicals by donating their electrons without disturbing their function at all because free radicals can act as electron acceptors. Compounds that have antioxidant activity have a variety of sources, where the mechanism of action is also very varied. The combination of two or more herbs produces a synergistic antioxidant effect, thereby increasing the sensitivity of antioxidant activity (Bag &; Chattopadhyay, 2015). In the study of Mustika *et al*., (2023) the combination of fig leaf and roselle flowers (2: 1) in tea bags has the highest antioxidant activity compared to the single-form

Fig plants with the name *Ficus carica* L. is one of the plants of the Moraceae Family which is widely distributed in tropical and subtropical regions. However, in Indonesia, the cultivation of this plant is still small due to the lack of public knowledge about the efficacy of this fig plant for health. In folk medicine, it is reported that fig

ISSN: 2519-9889
Impact Factor: 5.9

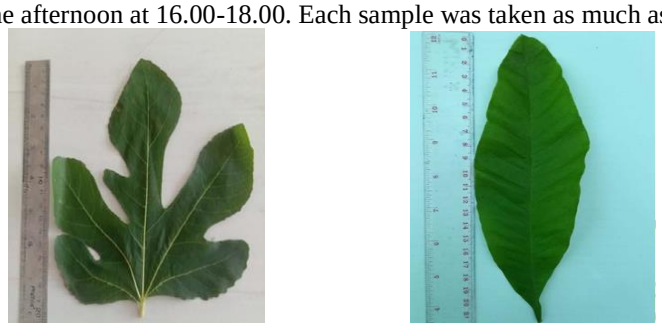
leaf (*Ficus carica* L.) is used to treat disorders of the respiratory tract, and gastrointestinal tract and to treat pain and inflammation (Mawa *et al.*, 2013). This is supported by previous research obtained fig leaf water extract (*Ficus carica* L .) has antioxidant activity with an IC_{50} value of 3.6976 µg/mL (Agustina, 2017)

The sungkai plant that has the name *Peronema canescens* Jack is one of the plants of the Lamiaceae Family. Sungkai plants are widespread in Indonesia, and the decoction of sungkai leaf has been widely used by people as a treatment. Empirically, sungkai leaves have been used as malaria drugs, immunomodulators, and headache drugs (Yani, 2013). In previous studies, it was reported that the n-butanol fraction of sungkai leaf has antioxidant activity with an IC_{50} value of 42,219±19,440 µg/mL (Fadlilaturrahmah, 2021).

Herbal tea is a preparation in the form of a decoction of flowers, leaf, seeds, roots, or dried fruits of a plant used for health and medicinal purposes. Herbal tea is used therapeutically in various forms of traditional medicine and is a popular global beverage (Poswal *et al.*, 2019). Based on the description, researchers are interested in formulating a combination herbal tea of fig leaf (*Ficus carica* L.) and sungkai leaf (*Peronema canescens* Jack), as well as conducting antioxidant activity tests and hedonic tests on these formulations.

2. Material and Methods

The samples used were fig leaf taken from local farmers in Tilatang Kamang District, Agam Regency, West Sumatra Province, and sungkai leaf taken in Nanggalo District, Padang City, West Sumatra Province (**Figure.1**). Sample collection in the afternoon at 16.00-18.00. Each sample was taken as much as 500 g.



(a) (b)

Figure. 1: (a) Tin leaf, (b) sungkai leaf used in this study

Plant identification at the Herbarium of Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Andalas University, West Sumatra. Fig leaf and sungkai leaf are washed thoroughly with running water, then withered at room temperature for 18 hours with occasional turning, then chopped and dried using an oven with a temperature of 50° C for 2 hours, grind the dried leaves using a blender, powder size sieving is carried out with a 60 mesh sieve.

Ethanol 96% p.a, Iron (III) Chloride, Magnesium Powder, Potassium Iodide, Methanol p.a, Hydrochloric Acid, and Gallic Acid was obtained from Merck, *2,2-diphenyl-1-picrylhydrazyl (*DPPH) from sigma-Aldrich. Activity of antioxidant using DPPH method. The absorbance of the sample was measured with a UV-Vis spectrophotometer at the maximum absorption wavelength DPPH.

2.1 Phytochemical Screening

Phytochemical screening aims to identification of secondary metabolite in .tin leaf and sungkai leaf

Alkaloid

50 g of each simplicia, was added 1 mL HCl 2 N and 9 mL aquadest, then heated and filtered. Inserted into 3 test tubes, each added Mayer, Wagner, and Dragendroff reagents.

Flavonoid

50 mg of each simplicia, plus 5 mL of aquadest is heated for 5 minutes and filtered. Filtrate plus a small amount of Mg powder, 1 mL concentrated HCl. If a red, yellow, or orange color is formed, it indicates the presence of flavonoid compounds.

ISSN: 2519-9889
Impact Factor: 5.9

Phenol

50 mg of each simplicia, plus 5 mL of aquadest is heated 5 minutes and filtered. The filtrate is added with 3-4 drops of 5% FeCl3, if a green to black-blue color is formed, indicating the presence of phenol compounds.

Tannins

50 mg of each simplicia, plus 5 mL of aquadest is heated for 5 minutes and filtered. Filtrate plus 10% gelatin solution, if a precipitate of white color is formed, it indicates the presence of tannin compounds.

Saponins

50 mg of each simplisia, plus 10 mL of warm aquadest, shaken vigorously, if foam forms when added 1 drop of HCl 2 N foam is still present then the powder contains saponins.

Steroids and Terpenoids

50 mg of each simplisia, macerated with 10 mL of n-hexane for 1 hour then filtered. The filtrate is evaporated, and the rest is added 10 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid. If the formation of blue indicates the presence of steroid compounds, while the formation of red indicates the presence of terpenoid compounds.

2.2 Formulation of herbal tea in tea bag

Table 1: The formulation of herbal tea from tin leaf, sungkai leaf, and its combination

Formula	Fig leaf (g)	Sungkai leaf (g)	Weight comparison (%)
I	2,8	0	100:0
II	0	2,8	0:100
III	1,96	0,84	70:30
IV	1,4	1,4	50:50
V	0,84	1,96	30:70

Figure. 2: Fig leaf dan sungkai leaf in tea bag

Each formula is put into a tea bag (**Figure. 2.**). Next brew with boiling water as much as 140 mL, cover and leave for 6 minutes (**Figure. 3.**) (SNI, 2013).

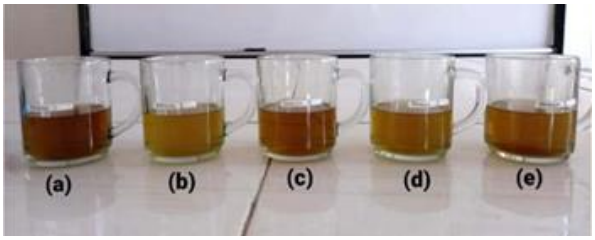


Figure. 3: Steeping of herbal tea (a) F I, (b) F II, (c) F III, (d) F IV, (e) F V

ISSN: 2519-9889
Impact Factor: 5.9

2.3 Determination of antioxidant activity of steeping

Activity of antioxidant using DPPH method. The absorbance of the sample was measured with a UV-Vis spectrophotometer at the maximum absorption wavelength DPPH

Preparation of DPPH Solution 30 µg/mL

10 mg DPPH, then dissolved with methanol p.a up to 50 mL in volumetric flask, then shaken until homogeneous so that a DPPH solution with a concentration of 200 µg/mL is obtained. The mother solution was pipetted as much as 7.5 mL and then diluted with methanol p.a to 50 mL, then shaken homogeneously so that a DPPH solution with a concentration of 30 µg/mL was obtained.

Blanks solution generation and DPPH wavelength optimization

3.8 mL DPPH solution (30 µg/ml.) is pipetted into a vial coated with aluminum foil. Then add methanol p.a as 0.2 mL and homogenize. Then incubated in a dark room for 30 minutes. Determine the absorption spectrum of the maximum wavelength of DPPH solution using a UV-Vis spectrophotometer at a wavelength of 400-800 nm.

Preparation of gallic acid comparison solution

10 mg gallic acid dissolve with methanol p.a up to 100 mL in volumetric flask, so that a concentration of 100 µg / mL is obtained. Then made a concentration series of 1, 2, 3, 4, and 5 µg/mL. To determine the activity of antioxidants, each concentration is pipetted as much as 0.2 mL of sample solution and inserted into the vial then add 3.8 mL of DPPH solution 30 µg/mL. The mixture is homogenized and then measure the absorption with a UV-Vis spectrophotometer at the maximum wavelength DPPH.

Antioxidant activity of steeping tin leaf herbal tea, breech leaf, and their combinations

100 mg of herbal tea stepping, then dissolved with methanol p.a up to 100 mL in volumetric flask, so that a concentration of 1000 µg / mL is obtained. Then dilution of the tea solution to a concentration of 100 µg / mL is carried out. After that, a series solution of concentrations of 5, 10, 15, 20, 25 µg/mL in 10 mL volumetric flask.

2.4 Hedonic test

Hedonic tests were conducted on 6 panelists. There were 5 samples tested, with test parameters, namely color, smell, and taste. Testing was conducted in the time range of 14.00-16.00.

To determine the level of consumer preference for the combination of fig leaf and sungkai leaf, a hedonic test was carried out. The parameters tested are color, aroma, and taste. The hedonic test uses a measurement scale that is a reference for assessing favorability, whereas the hedonic scale consists of 4 types of scales including the 3, 5, 7, and 9 scales. In this study, a scale of 5 was used. The 5-scale hedonic test is the most ideal measurement method for herbal tea products because it is relatively simple but has a fairly good sensitivity in analyzing the scores given by panelists (Triandini &; Wangiyani, 2022). The questionnaire data from the panelists' assessment was then analyzed statistically with the Friedman test.

2.5 Data Analysis

The calculation of antioxidant activity is included in the equation of the line y = a + bx with concentration (µg/mL) as abscissa (x-axis) and % value of antioxidant activity as ordinate (y-axis). The IC50 value obtained from the line equation. Meanwhile, hedonic testing is analyzed using SPSS 20.

3. Result and Discussion

Plant identification was carried out at the Herbarium (ANDA) of Andalas University, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University (UNAND) Padang, West Sumatra. The purpose of sample identification is to ensure the correctness of the identity of the sample used in the study so as to avoid errors in sampling. The test results proved that the samples used were fig plants (*Ficus carica* L.) and sungkai plants (*Peronema canescens* Jack).

ISSN: 2519-9889
Impact Factor: 5.9

Fig leaf and fresh sungkai leaf are made into simplicia powder with several stages including washing, withering, knitting, drying, grinding, sieving so that simplisia powder with a fine degree of fineness is obtained. From 500 g each fig leaf and fresh sungkai leaf, 172 g of tin simplisia powder and 276 g of sungkai leaf simplisia powder were obtained. There are differences in the weight of simplisia powder obtained due to differences in the morphology of fig leaf and sungkai leaf. Fig leaf have a bright green color, wavy and 3-5 fingers, thick, coarse-hairy on the upper surface and soft hairy on the lower surface (Joseph & Raj, 2011). The leaf of sungkai are lanceolate shape with a length of 8-12 cm, width 2-3.5 cm and thinner (Ningsih &; Ibrahim, 2013).

Identification of secondary metabolite in fig leaf and sungkai leaf simplicia carried out phytochemical tests (**Table 1**.). Alkaloid testing showed positive results on fig leaf and sungkai leaf simplisia. This is shown by the occurrence of a chemical reaction when simplisia is added to mayer reagents with the formation of white deposits which are predicted to occur because nitrogen atoms in alkaloids react with potassium ions in mayer reagents (Yanti *et al.*, 2021). In tests with the Wagner reagent also showed positive results, the occurrence of a chemical reaction between nitrogen atoms in alkaloids reacted with potassium ions in the brown Mayer reagent. However, with dragendroff reagents both samples did not show positive results. In flavonoid testing, it showed positive results in the formation of red to mauve red waarna with the addition of concentrated magnesium and HCl powder. This reaction causes the reduction of flavonoid compounds, causing a red color. The positive results of saponins are marked by the formation of foam, this is due to the presence of hydrophilic and hydrophobic groups where the formation of foam is due to hydrophilic groups binding to water while hydrophobic binds to air (Sangi &; Kumaunang, 2013). A positive result in a phenol test tested with the addition of $FeCl_3$ causes a blackish or dense green color, because phenol reacts with Fe^{3+} ions to form complex compounds. Furthermore, tannin testing was carried out by adding gelatin solution, a positive result of tannins produced a white precipitate. Because tannins are phenol compounds that can form effective strong complexes with proteins and some macromolecules. Positive results of steroids and terpenoids are characterized by the presence of red, blue or purple color. Positive steroids are characterized by the formation of red color, while positive terpenoids are characterized by the formation of blue. If a purple color is formed, it is stated that the sample is positive for steroids and terpenoids. Color change is formed due to oxidation of steroid compounds or terpenoids through the formation of conjugated double bonds (Hanani, 2015).

Table 1. Phytochemical screening test results of fig leaf and sungkai leaf

seconds metabolite	simplicia	
	Fig leaf	Sungkai leaf
Alkaloid		
Mayer	(+)	(+)
Wagner	(+)	(+)
Dragendorff	(+)	(+)
Flavonoid	(+)	(+)
Phenol	(+)	(+)
Saponins	(+)	(+)
Tannins	(+)	(+)
Steroids	(+)	(-)
Terpenoid	(+)	(-)

Note: (+) there is a content of a sequel metabolite
(-) there is no secondary metabolite content

Testing the antioxidant activity of steeping tin leaf herbal tea, sungkai leaf, and their combinations using the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) method analyzed using a double-beam UV-Vis spectrophotometer. Testing with the DPPH method is based on reduced purple color due to reduced DPPH by antioxidant compounds in the sample. The antioxidant activity of the sample is shown by the absorption inhibition of DPPH at wavelengths of 515-517 nm. This is because DPPH has a strong absorbance at these wavelengths with a dark

ISSN: 2519-9889
Impact Factor: 5.9

violet color. In this study, DPPH solution with a concentration of 30 µg/mL produced a maximum absorption of 0.631 at a maximum wavelength of 515.5 nm.

In determining antioxidant activity, gallic acid is used as a comparison because it functions as a secondary antioxidant, namely by capturing free radicals and preventing chain reactions. Gallic acid was chosen as a comparison because in previous studies gallic acid proved to be more efficient than other comparisons, namely ascorbic acid and quercetin (Maesaroh &; Al-Anshori, 2018). In testing the antioxidant activity of gallic acid, absorption results were obtained 0.592; 0,545; 0,498; 0,450; and 0.399. Based on the absorbance results obtained, it can be known that the greater the concentration, the smaller the concentration. The IC value of $_{50}$ or free radical fighting activity of 50% was obtained at a concentration of 6.6380 µg/mL. The results obtained showed antioxidant activity with very strong intensity due to IC values of < 50 µg/mL.

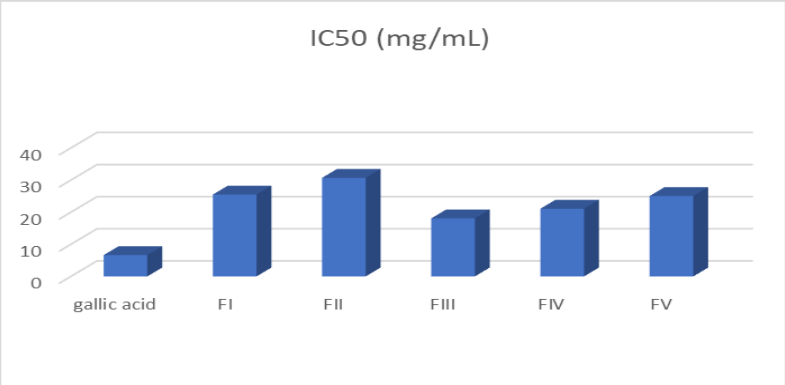


Figure 4. IC_{50} value of steeping fig leaf, sungkai leaf and combination

The results of testing the antioxidant activity of steeping herbal tea obtained IC_{50} formulas I, II, III, IV and V respectively amounted to 25.4885 µg / mL, 30.7039 µg / mL, 18.0717 µg / mL, 21.0907 µg / mL, 25.0547 µg / mL respectively (**Figure 4**.). Based on the results obtained showed that steeping 5 herbal tea formulas of tin leaf, sungkai leaf and their combinations have a very strong intensity of antioxidant activity. Of the five formulas, formula III was obtained with a ratio of fig leaf and sungkai leaf (70:30) the most powerful. The smaller the IC50 value, the stronger the antioxidant activity (Molyneux, 1999). Some flavonoid group compounds contained in fig leaf and sungkai leaf are rutin, quercetin and luteolin. These compounds are flavonoid compounds that play a role in antioxidant activity. Supported by previous research Vaya & Mahmood (2006) and Ulfa research (2022), it was found that the total flavonoid content in fig leaf ethanol extract was 681 mg/kg extract, while the total flavonoid content in sungkai leaf ethanol extract was 49,341 mg QE/g extract.

To determine the level of consumer preference for the combination of fig leaf and breech leaf, a hedonic test was carried out. The parameters tested are color, aroma and taste. The hedonic test uses a measurement scale that is a reference for assessing favorability, where the hedonic scale consists of 4 types of scales including the 3, 5, 7, 9 scales. In this study, a scale of 5 was used. The hedonic test scale 5 is the most ideal measurement method for herbal tea products, because it is relatively simple but has a fairly good sensitivity in analyzing the scores given by the panelists. Hedonic tests were conducted on 6 panelists, then the questionnaire data from the panelists' assessments were then analyzed statistically with the Friedman test. The results of the Friedman test obtained *a p-value of* >0.05 which means that H 0 $_{is}$ accepted, so it can be concluded that there is no significant difference between the 5 formulas on color, odor, and steeping taste (Triandini &; Wangiyani, 2022).

ISSN: 2519-9889
Impact Factor: 5.9

4. Conclusion

Based on the study, it can be concluded that steeping herbal tea tin leaf, sungkai leaf, and their combinations have antioxidant activity with a very strong category. Of the five formulas, the combination of fig leaf and sungkai leaf (70:30) is the highest, antioxidant activity. In the hedonic test, there was no significant effect between the five formulas on the color, smell, and taste of steeping.

References

1. Agustina, E., 2017. Test the activity of antioxidant compounds from Tiin leaf extract (ficus carica linn) with water solvent, methanol and methanol-water mixture. *Chlorophyll: Journal of Biological and Applied Sciences*, 1*(1)*, pp.38-47
2. Alfian, M.A.J., Sunarno, S., Zulfikar, M.F. and Rifai, A., 2018. The content of antioxidants and cholesterol in broiler meat (galus gallus domestica) as a result of supplementation in feed from gotu kola leaf meal and red spinach. *Bulletin of Anatomy and Physiology*, *3*(1), pp.126-132.
3. Standardization National Indonesia. 2013. *Packaged Dry Tea.* SNI 3836:2013
4. Bag, A. and Chattopadhyay, R.R., 2015. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PloS one*, *10*(7): 1-17.
5. Ministry of Health of the Republic of Indonesia. 2000. *General Standard Parameters of Medicinal Plant Extracts.* Jakarta, Indonesia
6. Fadlilaturrahmah, F., Khairunnisa, A., Putra, A.M. and Sinta, I., 2021. Test of sunscreen and antioxidant activity of sungkai leaf ethanol extract (Perenema canescens Jack). *Scientific Journal of Ibn Sina*, *6*(2): 322-330.
7. Hanani, E. 2015. *Phytochemical analysis.* Jakarta : EGC Medical Book Publisher.
8. Joseph, B. and Raj, S.J., 2011. Pharmacognostic and phytochemical properties of Ficus carica Linn–An overview. *International journal of pharmtech research*, *3*(1): 8-12
9. Maesaroh, K., Kurnia, D. and Al Anshori, J., 2018. Comparison of DPPH, FRAP and FIC antioxidant activity test methods against ascorbic acid, gallic acid and quercetin. *Chimica et natura acta*, *6*(2): 93-100.
10. Mawa, S., Husain, K. and Jantan, I., 2013. Ficus carica L.(Moraceae): phytochemistry, traditional uses and biological activities. *Evidence-Based Complementary and Alternative Medicine*, *20(13): 1-8.*
11. Mustika, M., Nurjana, F.N. and Sarina, G., 2023. Steeping the Combination of Fig Leaf (Ficus carica L.) and Roselle Flowers (Hibiscus sabdariffa L.) As a Potential Natural Antioxidant. *Higean Journal of Pharmacy*, 15*(2)*: 134-145.
12. Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, *26*(2): 211-219.
13. Ningsih, A. and Ibrahim, A., 2013. Antimicrobial activity of n-hexan extract of sungkai leaf (peronema canescens. Jack) against some bacteria by klt-bioautography method. *Journal of Tropical Pharmacy and Chemistry*, *2*(2): 76-82.
14. Poswal, F.S., Russell, G., Mackonochie, M., MacLennan, E., Adukwu, E.C. and Rolfe, V., 2019. Herbal teas and their health benefits: a scoping review. *Plant Foods for Human Nutrition*, *74(3)*: 266-276.
15. Sangi, M.S., Momuat, L.I. and Kumaunang, M., 2012. Toxicity test and phytochemical screening of palm frond grain flour (Arenga pinnata). *Scientific Journal of Science*, 127-134.
16. Triandini, I.G.A.A.H. and Wangiyana, I.G.A.S., 2022. Mini-review uji hedonik pada produk teh herbal hutan. *Jurnal Silva Samalas*, 5(1): 12-19.
17. Ulfa, S.F. 2022. Determination of Total Phenolic and Total Flavonoid Content of Sungkai Leaf Ethanol Extract (*Peronema canescens* Jack) and its Chemometric Grouping Based on FTIR Spectrum, Thesis, Andalas University. Padang, Indonesia
18. Utami, Y.P., Umar, A.H., Syahruni, R. and Kadullah, I., 2017. Standardization of simplisia and ethanol extract of leilem leaf (Clerodendrum minahassae, Teisjm. & Binn.). *Journal of Pharmaceutical and medicinal sciences*, *2*(1): 32-39.
19. Yani, A.P., 2013. Local wisdom of the use of medicinal plants by the Lembak Eight Tribe in Central Bengkulu Regency, Bengkulu. *Proceedings of SEMIRATA 2013*, *1*(1).
20. Yanti, S.N., RS, A. and Chandra, V.E., 2021. Study of Secondary Metabolites in Sambal Orange Juice (Citrus microcarpa Bunge) Originating from Kalimas Village, West Kalimantan. *Journal of Pharmaceutical And Sciences*, *4*(2): 105-110.

