Advances in Engineering Research, volume 209
International Joint Conference on Science and Engineering 2021 (IJCSE 2021)

Production and Quality Test Nganjuk Shallot Extract as a Basis of Aromatherapy Products

Mirwa A.Anggarani[1,*] Aris Rudi Purnomo[2] Lailatul Wakidah[3]

[1]*Department of Chemistry, Universitas Negeri Surabaya, Surabaya, Indonesia*
[2]*Department of Science, Universitas Negeri Surabaya, Surabaya, Indonesia*
[3]*Department of Chemistry, Universitas Negeri Surabaya, Surabaya, Indonesia*
[*]*Corresponding author. Email: mirwaanggarani@unesa.ac.id*

ABSTRACT
Shallot (Allium cepa L.) is a common root used as both a spices for cooking and a traditional ingredient. This plant is known to have medicinal properties, especially medications for breathing and inflammation. This research was conducted to know the quality of shallot extract is reviewed from specific and non-specific parameters as the basis of an aromatherapy product. It is known that shallot extract contains alkaloid, flavonoid, triterpenoid, steroid, tannin, saponin and phenolic. The aromatherapy product quality, from a nonspecific parameter, which covers a total amount of ashes of 10.2%, water content of 2%, microbial contamination of <104 CFU/gr, a residue of microbial heavy by <10 ppm, a deflation of Cd <0.30 ppm. Aromatherapy quality is based on shallot, which are within the specific parameters of shallot extract temperature <27.5 °C, whereas the shallot extract pH is 7.5.

Keywords: *Shallot, Aromatherapy, Specific and Non-specific parameters.*

1. INTRODUCTION

As an agricultural country, Indonesia has abundance of potential crops and plantations. Nganjuk Regency is known as one of the agricultural areas successfully planted plants. One of which is shallot (Allium cepa L.) that has medicinal properties for breathing and inflammation. It is rich in bioactive compounds of flavonoid, saponin, essential oil, allicin and quercetin functioning as bactericidal and fungicidal. The essential oil consists of cycloaliin, methylaliine, kaempferol, quercetin and floroglusi [1]. These compounds are able to damage cell walls and cytoplasmic membrane, degenerate cell proteins, and inhibit the work of enzymes in cells. It also has the potential for damaging the veil and blocking the virus from attaching to host cell receptors [2].

Flavonoid compounds have antibacterial activity by bonding nucleophilic amino acids in proteins and inactivation enzymes [3]. A flavonoid antibacterial would hinder bacteria growth by damaging cell walls and cytoplasm membranes. Antibacterial substances would hinder the formation or transport of individual components to the cell wall, resulting in weak structures coupled with the disappearance of cell walls and the release of contents that would eventually kill and inhibit the growth of those bacteria [4]. Shallot also contain vitamin c, potassium, fiber, folic acid, calcium and iron. Shallot also contain a natural growth additive that includes both auxin and gibberellin. Shallot based aliin has an antiseptic effect, so it is often used as a traditional medicine.

Aliin is converted by alliinase enzymes to go further into pyruvic acid, ammonia and allicin as bacteriologic antimicrobe [5]. The shallot antiviral and antibacterial activity is supported by the enrichment of quercetin and kaempferol, which are compounds of the flavonoid family [6]. As antioxidants, they neutralize free radicals and reactive oxygen species (ROS) produced by human cells. Quercetin has resistance to H1N1 and H3N2 viruses. The mechanism is through reduction of the m-RNA transcription process of the hemagglutinin protein in virus-infected cells. Hemagglutinin proteins play a key role in helping the virus enter the host cell [7]. Kaempferol also has a similar effect, one that can inhibit the lowering of the pathogenicity of influenza virus H9N2 [8].

Aromatherapy is a method of treatment through the medium of fragrances derived from certain plant ingredients. Initially, aromatherapy is formed from essential fluid. However, as it grows, there are various

forms of aromatherapy starting with essential oils, incense, wax, salt, massage oil, and soap [9]. According to their kinds, the aromatherapy can be used as room deodorizers, massage oils, body odour and room deodorizers [10]. Aromatherapy is a way of harnessing natural oils extracted from plants with a view to improving both physical and psychological health. The oil used is essential oil made of a variety of herbs, flowers, roots, fruit and trees growing [11]. Aromatherapy plays a role in relaxing the mind and alleviating stress. It certainly has to do with more orderly emotional states. The state of human emotion is governed by the brain in the limbic system. The limbic system is a combination of the limbic lobe and subcortical nuclei, the amygdala, the nuclear septales, the hypothalamus, the ephytalamus, the thalamus nucleus, and the ganglia basalis. In limbic systems not only manage one another [12].

In meeting general standards of herbal extract and of traditional medicinal quality requirements according to the food and drug control board, a standardized shallot extract is required. Standardization measures to obtain uniform raw materials that can eventually guarantee the plant's pharmacological effects. Some of the parameters studied in this study are non-specific and specific parameters. Non-specific parameters tested include total ash levels, water levels, microbial contamination, and heavy metals; whereas the specific parameters for which to be tested are the chemical properties of extract (phytochemical screening test) and organoleptic.

2. METHODS

2.1 Extraction Using Maceration

Extraction is done using the maceration method. The roots of fresh shallot are peeled and then dried until water level is reduced. Dried shallot extract is added to the macerator's tools while occasionally stirred. The maceration process takes nine hours. The macerate is evaporated with a vacuum or low-pressure evaporator until obtained a viscous extract. Rendemen is calculated between the weight of the dry simplicia and the weight of the extract gained through the weighing process.

$$\% \text{ rendemen} = \frac{b2}{b1} \times 100\% \quad \text{.....(1)}$$

Note:

b1 = weight of the extracted material, b2 = weight of the obtained material

2.2 Test of Specific Parameters and Non-Specific Parameters

Phytochemical screening test is used to illustrate the properties of chemical compounds in shallot extract. These included alkaloid, tannin, triterpenoid, saponin, steroid, flavonoid, quinone and essential oil. Testing the materials is done by seeing changes in colour after the mixing of retraction. The testing of non-specific parameters included physical and chemical properties. The physical properties are temperature and pH, whereas the chemical property is heavy metal content (As, Cd, Pb). Total bacteria is also measured to determine the biological aspect of the product.

3. RESULTS AND DISCUSSION

3.1 Extraction Using Maceration

In this research, the sample used was local shallot that came from Nganjuk, East Java. Extraction is necessary as a process of extracting chemical compounds in shallot using solvents and the correct method [13]. Shallot extract is produced by the maceration method. This method is chosen because it is simpler than any other extraction method. Repetition of the stirring or shuffle shuffle is intended to keep the concentration of the extracted material balanced. A comparison of simplisia with a high solvent can give a lot of extracts [14].

A dry simplicia powder is obtained from a sample of fresh sliced and washed shallot and then dried in the sun's hot sun to dry. Next, it is blended smooth and sifted to uniform size. Halting the sample until it was formed was aimed at increasing the surface area of the sample, increasing the extraction process and increasing the time of maceration. The basic principle of maceration in the research was by soaking the siliconic powder of shallot into the polar solvents of water. Water solvents are chosen as polar and so, later on, when the extract is consumed by society it tends to be safe. During maceration, the solvent diffuses into the sample and dissolves compounds that have the same polarity with the likes dissolve like in principle [15].

3.2 Test of Specific Parameters

The specific parameters test is phytochemical screening which is the first step to determine the active ingredients which are secondary metabolites in plants [16]. The aim of the phytochemical screening of this research is to test the quality of substances used in shallot such as alkaloid, flavonoid, tannin, phenolic, steroid, saponin and triterpenoid. From this test, it can be seen that the water solvent's ability to attract the compounds contained in the extract of shallot.

Table 1. Phytochemical test results of shallot extract

No.	Phytochemical Test	Result	Color
1.	Alkaloid		
	• Dragendorf	+	White precipitate
	• Mayer	+	Orange precipitate
	• Wagner	+	Brown precipitate
2.	Flavonoid	+	Red
3.	Triterpenoid	+	Brownish red color is formed between surfaces
4.	Steroid	+	Blackish purple
5.	Tannin	+	Greenish brown
6.	Saponin	+	Foam formed

Alkaloid tests showed positive results in three reagents. First, there is the formation of white-colored precipitate in which Dragendorf reduction is applied. In this reaction there is a ligand replacement in which nitrogen which has a lone pair of electrons on the alkaloid forms a coordinate covalent bond with the K^+ ion from potassium tetraiodobismutate to produce a potassium-alkaloid complex which precipitates [17]. Alleged reactions of alkaloid test with Dragendorff reagent shown in the following reaction [18].

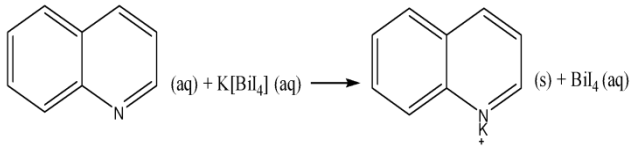


Figure 1 Alleged reactions of alkaloid test with dragendorff.

Next, orange precipitate is formed when reacted with Mayer reagent and brown precipitate for Wagner reagent. Alleged reactions of alkaloid tests with Mayer and Wagner reagents are shown in the following reaction [18].

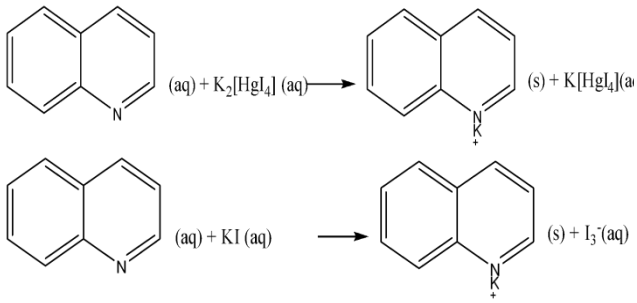


Figure 2 Alleged reactions of alkaloid tests with mayer and wagner reagents.

The next test is for flavonoid compounds. Most common in shallot is a flavonoid chemical compound [19]. If a sample extract has a flavonoid compound, after adding to the Mg metal and HCl has a gram of red or orange flavylium [20]. The addition of magnesium and hydrochloric acid in flavonoid tests would cause the fusion of existing flavonoid substances to create a red reaction typical of flavonoid [21]. The chemical reactions that occur are as follows:

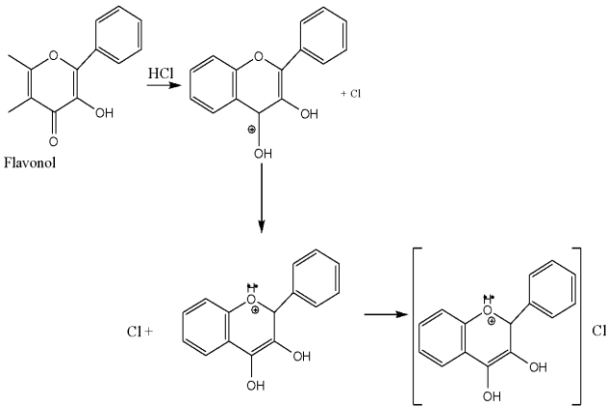


Figure 3 Red reaction typical of flavonoid.

Next is testing the triterpenoid compound. The triterpenoid is a compound whose carbon skeleton is derived from 6 units of isoprene and biosynthetically derived from acyclic C30 hydrocarbons, namely squalene. It is a cyclic structure mostly of alcohol, aldehyde, or carotene acid [22]. The result of this research is an extract of positive shallot containing a triterpenoid marked by a brownish-red ring between the surface. The reactions are as follows:

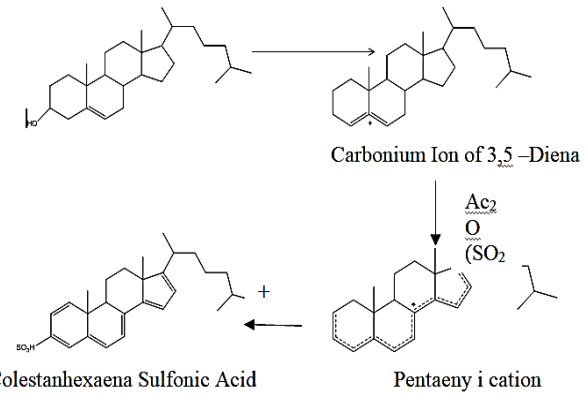


Figure 4 Triterpenoid Reaction.

Next test is a steroid compound. Steroids are a group of compounds having four integrated rings. These substances have certain physiological effects. The compound has several uses for plants as the regulators of growth (sescertenoid abisin and gibberellin), carotenoid for dyes and plays a role in helping out photosynthesis process [22]. In this research, the shallot extract indicates that positive contains compound steroid characterized by blackish purple colour.

The next test tannin compound. Tannin are very complex organic substance made up of phenolic compounds. Tannins are composed of a group of substances that are prevalent in the plant world, including the bark, stems and fruits [23]. Results from this research suggest that shallot are positive for tannin which is indicated by the formation of a greenish brown color. This color formation occurs due to the formation of complex compounds between Fe metal and tannins [22]. The reactions are as follows:

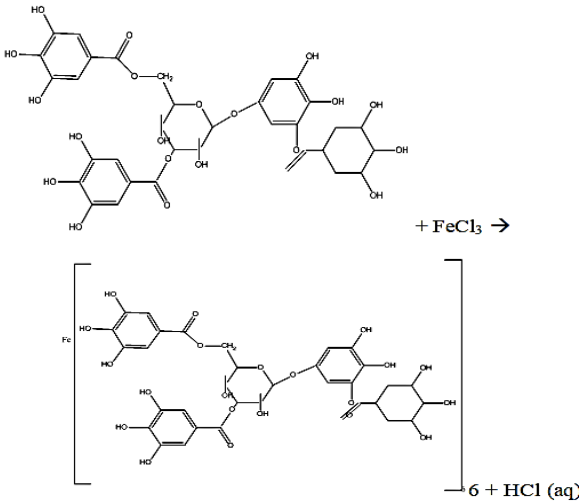


Figure 5 Tannin reaction.

The next test is saponin compound. Saponin is a compound that has hydrophilic and hydrophobic clusters. Saponin is whipped up a froth because of the hydrophilic clusters that are tied to the water and the hydrophobic binds the air. In misel structures, the polar cluster faces outward while the non-polar cluster faces inward. It's the foaming state [24]. In this research, shallot extract of positive contains saponin, indicated by the formation of a stable sponge. The foam that exists is hydrolytic glycoside to glucose and other compounds that form foam [24]. The reactions are as follows:

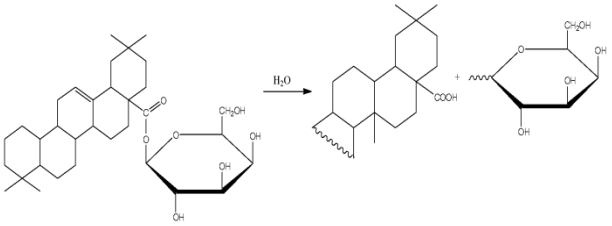
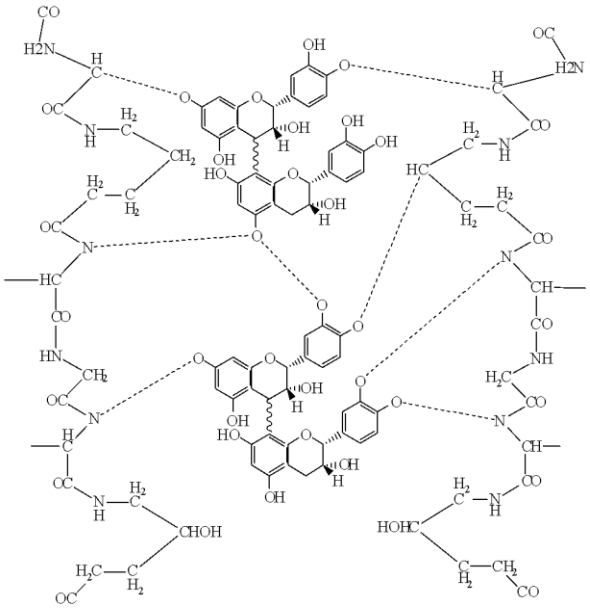


Figure 6 Saponin reaction.

The next test is phenolic compounds. Fenol compounds include compounds derived from plants, which have the same chemical characteristic of aromatic rings containing one or two hydroxyl fertilizers. Fenol compounds tend to be easy to dissolve in water because they can generally bond with sugar as glycoside, and they are usually present in vakuola cells [22]. In this research, shallot extract positive contains phenolic compounds. Phenolic react with $FeCl_3$ 1% to form deep red, purple, blue, or black because $FeCl_3$ reacts with aromatic –OH groups [17]. The resulting color is a complex of iron (III) hexafenolate. The Fe3+ ion experiences orbita hybridization d2sp3, so the Fe3+ (4s0d5) has 6 empty orbial cells filled with electron-pair donors, namely the oxygen atom in phenolic compounds that has lone electron pairs [17]. These reactions are as follows



3.3 Test of Non-Specific Parameters

Table 2. Physical and chemical properties of shallot extract.

No.	Test Description	Sample Result	Regulatory Limit	Unit	Method
	Physical Properties				
1.	Temperature	27.5	Air Temp. ± 3	°C	SNI 06-6989.23-2005
2.	pH	7.5	6.5 – 8.5	pH Unit	SNI 06-6989.11-2004

No.	Test Description	Sample Result	Regulatory Limit	Unit	Method
	Chemical Properties				
1.	Arsenic, As	0.0031	<0.0031	mg/L	APHA 3113B-2017
2.	Cadmium, Cd	<0.001	<0.001	mg/L	SNI 6989.16-2009
3.	Lead, Pb	<0.002	<0.002	mg/L	SNI 6989.8-2009
	Bacteria				
1.	Total Bacteria	0	0	CFU/100 mL	IKM-EI-SML-30 (*Membrane Filter*)

Non-specific standardization is a standardization to learn about the security and stability of shallot extract from the pharmacological side. With standardization it is expected that public trust levels rise in the knowledge of the medicinal benefits of natural materials [25]. To determine whether or not the quality extracted from shallot would be used in this research, the properties of physics and chemistry of shallot extract are tested.

3.4 Physical and Chemical Properties

Temperature testing according to the SNI 06-6989.23-2005 method is carried out by immersing a thermometer in the sample for 2-5 minutes until the value is stable. In testing the physical properties of the shallot extract, it shows that the temperature detected is at 27.5 °C. The temperature corresponds to room temperature.

In chemical properties, there are 2 types of tests, namely the pH test and the heavy metal contamination test. According to SNI 06-6989.11-2004 The pH measurement method is based on the measurement of the hydrogen ion activity simultaneously potentiometry or electrometry using a pH meter. Based on the results shown in (Table 2), the pH of shallot extract is 7.50. Where the results have already met a regulatory limit according to SNI 06-6989.11-2004, namely between 6.5 – 8.5.

Next, test metal contamination. An important metal contamination test was made because of the impurities of shallot extracts has a very bad impact on human health. The impact depends on where the metal is tied to the body. The danger of the heavy metal is that when it is absorbed by the body it is indestructible but settles down and can only escape through the excretion process. Heavy metal concentrations above lead, arsenic and cadmium can cause toxicity to health [26]. This research examined three kinds of heavy metals as Arsenic (As), Cadmium (Cd) and Lead (Pb).

The first test was arsenic (As) metal level. Arsenic is a carcinogen compound, which can trigger the onset of cancer [27]. Another effect of arsenic compounds is on skin tissues of keratosis as well as pigmentation disorders. According to APHA 3113B-2017, determination of Arsenic (As) concentration using standard American Public methods Health Association (APHA) with Atomic Absorption Spectroscopy (AAS). The method used for this metal is the Direct Air Acetylene Flame method. The results of the parametric test on (Table 2) show that the extract of shallot contains arsenic (As) metal contamination much as 0.0031 mg/L. Based on these results, shallot extract meets the regulatory limits according to APHA 3113B-2017, which is <0.0031 mg/L.

The second test was cadmium (Cd) metal level. Cadmium is considered a dangerous contamination of plants because they are absorbed through roots and disseminated onto plant tissue. Cadmium is a type of heavy metal that can accumulate long periods in the liver and kidneys. Metal ties with the active side of the enzyme in cadmium can induce a toxic property, causing enzyme activity to be compromised. Besides the effects on the organ of elimination, the heavy metal of cadmium also affects bone and reproductive system. The older half of cadmium causes the metals to accumulate and the toxicity in the organs [27]. According to SNI 6989.16-2009 The cadmium content in shallot extract was determined spectrophotometrically based on the absorption of radiation sources against cadmium atoms in the maximum wavelength of 228.8 nm. The results of the parametric test on (Table 2) show that the extract of shallot contains cadmium (Cd) metal contaminants of <0.001 mg/L. Based on these results, shallot extract meets the regulatory limits according to SNI 6989.16-2009, which is <0.001 mg/L.

The third test was lead (Pb) metal level. Lead is considered a dangerous contaminant for plants because it is easily absorbed through the roots and spread to plant tissues. Lead (Pb) contamination can lead to neurological and mental disorders, especially during childhood growth [27]. According to SNI 6989.8-2009, cadmium content in shallot extract was determined spectrophotometrically based on the absorption of radiation sources against cadmium atoms in the maximum wavelength. The cadmium content in shallot extract was determined spectrophotometrically based on the absorption of radiation sources against cadmium atoms in the maximum wavelength of 228.8 or 217.0 nm. The results of the parametric test on Table 2 show that the extract of shallot contains lead (Pb) as much as <0.002 mg / L. Based on these results, shallot extract meets the regulatory limits according to SNI 6989.8-2009, which was <0.002 mg / L. Overall, it can be said

that the extract of shallot on this research meets the requirements for the contamination of the heavy metals specified by BPOM RI.

3.5 Bacterial Contamination

The next test is bacterial contamination. For food to function properly, quality of food must be taken into consideration, including the availability of essential substances (nutrients) and prevention of contamination leading to health problems [28]. Unsafe food can cause a variety of illnesses such as diarrhoea, hepatitis, lead poisoning, cholera, amebiasis, typhus, trachoma and dysentery [29]. In qualifying for purity extract, there was a bacterial contamination test of shallot extract. This test involves determining the number of microorganisms allowed.

Shallot extract with a certain volume is filtered using a cellulose filter membrane with a diameter of 47 mm with a pore size of 0.45 micrometres which keeps the bacteria present in the sample. Bacterial colonies growing in the petri dishes were observed and counted. The results of the parametric test on (Table 2) show that shallot extract does not contain any contamination of bacteria. Based on these results, shallot extract meets the limit of the regulations set by (SK Dirjen Pom No: 03726/B/SK/VII/89), which was <106 colonies/g.

4. CONCLUSION

The aromatherapy product quality, from a nonspecific parameter, which covers a total amount of ashes of 10.2%, water content of 2%, microbial contamination of <104 CFU/gr, a residue of microbial heavy by <10 ppm, a deflation of Cd <0.30 ppm. Aromatherapy quality is based on shallot, which are within the specific parameters of shallot extract temperature <27.5 °C, whereas the shallot extract pH is 7.5. Based on the phytochemical test it is known that shallot extract contains alkaloid, flavonoid, triterpenoid, steroid, tannin, saponin and phenolic.

AUTHORS' CONTRIBUTIONS

Mirwa Adiprahara Anggarani: concieved and planned the experiment and wrote the manuscript. Aris Rudi: review and finishing the manuscript. Lailatul: data analysis and complete manuscript.

REFERENCES

[1] S. Wibowo, Cultivation of Onions, Shallots, Garlic, Onions, Penebar Swadaya, 2008.

[2] R. Harazem, S. A. El-Rahman, A. El-Kenawy, Evaluation of Antiviral Activity of Allium Cepa and Allium Sativum Extracts Against Newcastle Disease Virus, Alexandria Journal for Veterinary Sciences, 61(1) (2019) 108–18. DOI: http://dx.doi.org/10.5455/ajvs.29663

[3] S. Ahmadi, Z. Rajabi, M. V. Marandi, Evaluation of the Antiviral Effects of Aqueous Extracts of Red and Yellow Onions (Allium Cepa) against Avian Influenza Virus Subtype H9N2, Iranian Journal of Veterinary Science and Technology, 10(2) (2018) 23–27. DOI: http://dx.doi.org/10.22067/veterinary.v2i10.74060

[4] WHO, WHO Traditional Medicine Strategy, Geneva, 2013.

[5] R. Rukmana, Shallot, Penerbit Kanisius, 1994.

[6] D.N.A Tagoe, H.D. Nyarko and R. Akpaka, A Comparison of The Antifungal Properties of Onion (Allium Cepa), Ginger (Zingiber Officinale) and Garlic (Allium Sativum) Against Aspergillus Flavus, Aspergillus Niger and Cladosporium herbarum, Research Journal of Medicinal Plant, 5(3) (2011) 281–87. DOI: http://dx.doi.org/10.3923/rjmp.2011.281.287

[7] W. Wu, R. Li, X. Li, J. He, S. Jiang, S. Liu, and J. Yang, Quercetin As an Antiviral Agent Inhibits Influenza a Virus (IAV) Entry, Viruses, 8(1) (2016) 6. DOI: https://doi.org/10.3390/v8010006

[8] R. Zhang, X. Ai, Y. Duan, M. Xue, W. He, C. Wang, T. Xu, M. Xu, B. Liu, and C. Li, Kaempferol Ameliorates H9N2 Swine Influenza Virus-Induced Acute Lung Injury By Inactivation of TLR4/MyD88-Mediated NF-KB and MAPK Signaling Pathways, Biomedicine & Pharmacotherapy, 89 (2017) 660–72. DOI: http://dx.doi.org/10.1016/j.biopha.2017.02.081

[9] Rahmi, Aromatherapy, PT. Gramedia Pustaka Utama, 2012.

[10] H. Sunarjo, P. Soedomo, Cultivation of Shallots (Allium Ascalonicum L.), Sinar Baru, 1989.

[11] J. Buckle, Care of The Elderly (Chapter 15)—Essential Oils in Practice, Clinical Aromatherapy, 2nd Edition. Elsevier Ltd., 2003.

[12] G. Lee, H. Bae, Therapeutic Effects of Phytochemicals and Medicinal Herbs on Depression, BioMed Research International, (2017) 1–11. DOI: https://doi.org/10.1155/2017/6596241

[13] E. Rahayu, B.V.A Nur, Shallot, Penebar Swadaya, 1996.

[14] R. Voight, Book of Introduction to Pharmaceutical Technology, Gadjah Mada University Press, 1994.

[15] N. S. Ncube, A. J. Afolayan, A. I. Okoh. Assessment Techniques of Antimicrobial

Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends, African Journal of Biotechnology, 7(12) (2008) 1797–1806. DOI: http://dx.doi.org/10.4314/ajb.v7i12.58804

[16] S. Purwati, S. V. T. Lumowa, Samsurianto Samsurianto, Phytochemical Screening of Saliara Leaf (Lantana Camara L) as Pest Suppressant Vegetable Pesticides and Disease Incidence in Horticultural Plants in East Kalimantan, Proceedings of Seminar Kimia, 2017, 153–58.

[17] Haryati, N. Aini, C. Saleh, Toxicity and Antibacterial Activity Test of Red Leaf Extract (Syzygium Myrtifolium Walp) against Staphylococcus Aureus and Escherichia Coli Bacteria, Jurnal Kimia Mulawarman, 2016, pp. 35–39. URL: http://jurnal.kimia.fmipa.unmul.ac.id/index.php/JKM/article/view/43

[18] M. Nafisah, S. Tukiran, N. Hidayati. Phytochemical Screening Test on Hexane, Chloroform and Methanol Extracts from the Patikan Kebo Plant (Euphorbiae Hirtae), Proceedings of Seminar Nasional Kimia, 2014, pp. 281–82. URL: https://docplayer.info/33407200-Uji-skrining-fitokimia-pada-ekstrak-heksan-kloroform-dan-metanol-dari-tanaman-patikan-kebo-euphorbiae-hirtae.html#tab_1_1_2

[19] C. L. T. Chang, Y. Lin, A.P. Bartolome, Y. C. Chen, S. C. Chiu, and W. C. Yang, Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology, and Ppotential Application of Selected Plants and Compounds, Evidence-Based Complementary and Alternative Medicine, (2013) 1–33. DOI: https://doi.org/10.1155/2013/378657

[20] D. Septyaningsih, Isolation and Identification of Main Components of Red Fruit Seed Extract (Panduus Conoideus Lamk), Skripsi, 2010.

[21] T. Robinson, High Plant Organic Content, ITB, 1995.

[22] J. B. Harbone, Phytochemical Method: Guide to Modern Methods of Analyzing Plants, ITB, 1987.

[23] A. Fitriyani, L. Winartim, S. Muslichah, Nuri, Anti-Inflammatory Test of Red Betel Leaf Methanol Extract on White Rats, Traditional Medicine Magazine, 16 (2011) 34–42.

[24] S. Marliana, V. Suryanti, Suyono, Phytochemical Screening and Thin Layer Chromatography Analysis of Chemical Components of Chayote (Sechium Edule Jacq. Swartz) in Ethanol Extract, Biofarmasi, 3 (1) (2005) 26–31. DOI: http://dx.doi.org/10.13057/biofar/f030106

[25] A. Saifudin, V. Rahayu, Y. H. Teruna, Standardization of Natural Medicines, Graha Ilmu, 2011.

[26] Depkes RI, General Standard Parameters of Medicinal Plant Extracts, Ministry of Health of the Republic of Indonesia, 2000.

[27] H. A. Cahyanto, Heavy Metal Content in Raw Materials of Spice Products from Markets in Pontianak City, Majalah BIAM, 11(2) (2015) 57–62. URL: http://ejournal.kemenperin.go.id/bpbiam/article/download/2050/1618

[28] N. Amaliyah, Healthy Food and Beverage, Deepublish, 2017.

[29] T. Cahya, M. Amir, R. T. Manalu, Test of Ice Cube Microbial Contamination at Beverage Sellers in the Market Environment of Jagakarsa District, South Jakarta, Sainstech Farma, 12(2) (2019) 78–84.