Usadha: Journal of Pharmacy. Vol. 3, No. 2, (2024). ISSN 2827-9905
Available online at: https://jsr.lib.ums.ac.id/index.php/ujp

UJI AKTIVITAS ATIOKSIDAN DAN ANTI-AGING EKSTRAK ETANOL DAUN KESUM (*Polygonum minus* HUDS.) SECARA *IN VITRO*

IN VITRO ANTIOXIDANT AND ANTI-AGING ACTIVITY OF KESUM LEAVES (*Polygonum minus* HUDS.) ETHANOL EXTRACT

Aji Winanta[1]*, Salwa Dwi Agustin S[1], Dhiva Andarika Fitri[1], Tresna Sriwahyuni Gumilang[1]
[1]Department of Pharmaceutical Biology, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Jalan Brawijaya, 55183, Yogyakarta, Indonesia
*E-mail correspondence : ajiwinanta@umy.ac.id

Dikirim : 30 April 2024, Disetujui : 28 Mei 2024, Diterbitkan : 31 Mei 2024

Abstrak

Proses penuaan atau yang disebut dengan *aging* adalah suatu proses biologis yang dapat terjadi secara alami pada manusia. Proses penuaan pada kulit ditandai dengan kulit menjadi kasar atau bersisik, kering, timbul noda hitam, kusam, bahkan muncul keriput pada kulit. Penelitian dilakukan untuk mengetahui aktivitas biologis daun kesum (*Polygonum minus* Huds.) sebagai bahan aktif anti-aging secara *in vitro*. Proses pembuatan ekstrak menggunakan metode maserasi menggunakan pelarut etanol 70%. Selain itu, dilakukan identifikasi metabolit sekunder menggunakan uji skrining fitokimia. Aktivitas antioksidan diukur menggunakan pengujian peredaman radikal bebas *2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)* (ABTS). Efek penghambatan enzim pendegradasi kulit (anti-aging) dilakukan dengan mengukur persentase penghambatan enzim kolagenase. Berdasarkan skrining fitokimia, ekstrak etanol daun kesum memiliki kandungan senyawa flavonoid, saponin, dan tanin. Hasil aktivitas antioksidan daun kesum memiliki nilai IC_{50} sebesar 41,46 µg/mL dan merupakan kategori antioksidan sangat kuat. Hasil penghambatan enzim kolagenase memberikan hasil bahwa pada konsentrasi 50, 100, dan 200 µg/mL dapat menghambat kerja kolagenase sebesar 83%, 82%, dan 82%. Berdasarkan hasil penelitian yang telah dilakukan, didapatkan bahwa ekstrak etanol daun kesum yang diuji memiliki aktivitas antioksidan dan berpotensi sebagai anti-aging.

Kata Kunci: *Polygonum minus* Huds., daun kesum, antioksidan, metode ABTS, anti penuaan, enzim kolagenase

Abstract

Skin aging is a biological process in humans characterized by the skin becoming rough or scaly, dry, black spots appearing, dull, and even wrinkles. The study was conducted to determine the biological activity of kesum leaves (Polygonum minus Huds.) as an anti-aging active ingredient in vitro. The process of making the extract using the maceration method. Secondary metabolites were identified using a phytochemical screening test. Antioxidant activity was measured using the free radical scavenging test of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The inhibitory effect of skin-degrading enzymes was measured by the percentage of inhibition of the collagenase enzymes. Based on phytochemical screening, the ethanolic extract of kesum leaves contains flavonoids, saponins, and tannins. The results of the antioxidant activity of kesum leaves have an IC_{50} value of 41.46 g/mL and are included in the category of strong antioxidants. The results of collagenase enzyme inhibition showed that at concentrations of 50, 100, and 200 g/mL, collagenase activity could inhibit collagenase activity by 83%, 82%, and 82%. Based on the

results of the research that has been carried out, it was found that the ethanol extract of the tested kesum leaves has antioxidant activity and has the potential to anti-aging.

Keywords: *anti-aging, antioxidant, Polygonum minus* Huds*., collagenase enzyme*

INTRODUCTION

The skin organ will experience the aging process as we age. Aging is a biological process and naturally occurs in humans. The skin's aging process is characterized by the skin becoming rough or scaly, dry, with black spots, dullness, and even wrinkles appearing on the skin (Swastika et al., 2013). Research on anti-aging is currently an exciting topic for humankind. In a survey conducted by JakPat and ERHA Age Corrector in April 2021, 76% of Indonesian women see premature aging as a severe problem, and about 60% of female respondents feel less confident because of the symptoms of premature aging they experience.

Anti-aging products in this modern era have become one of the most widely used cosmetic products and one of the most sought-after cosmetics by the public, especially women. In general, cosmetics are synthetic and herbal, each with advantages and disadvantages. However, synthetic cosmetics have drawbacks that can harm individual health and the environment (Chen, 2009). Therefore, researchers are attracted to research using natural ingredients to find their anti-aging potential.

One of the plants suspected of having anti-aging activity is kesum leaves (*Polygonum minus* Huds.). The compounds thought to be contained in kesum leaves are flavonoids, phenolics, tannins, steroids, saponins, alkaloids, and beta-carotene. Results of phytochemical screening of the methanol fraction of kesum leaves were positive for containing high alkaloids, flavonoids, phenols and terpenoids so that their antioxidant activity was high (Wibowo et al., 2009). Previous studies regarding the antioxidant activity of kesum leaves extract found that the methanol extract of kesum leaves had antioxidant activity that approaches the antioxidant activity of vitamin C. Kesum leaves methanol extract has a very strong antioxidant because it has an IC_{50} <50 ppm which is 20.632 ppm (Purwaningsih et al., 2018).

Antioxidant compounds are useful as anti-aging because they can protect the body from free radicals by donating hydrogen from its hydroxyl group to free radicals (Arifin and Ibrahim, 2018). Antioxidants have various benefits for skin health, such as antiaging, UV protection, and protection from ROS due to oxidative stress (Haerani et al., 2018). UV light can trigger the formation of Reactive Oxygen Species (ROS) by expressing genes and proteins that can trigger skin damage and skin cancer (Hart and Norval, 2018). Dermal enzymes like collagenase and elastase, which can break down and degrading collagen and elastin can indirectly be activated after the skin is exposed to photoaging stressor (Chatatikun and Chiabchalard, 2017; Popoola et al., 2015). Collagenase is a protease enzyme which can be responsible for collagen degradation. Collagenase inhibiting compounds benefits the skin to prevent loss of elasticity, so using compound that inhibit collagenase is potent in preventing aging (Thring et al., 2009).

Therefore, research on anti-aging activity should be developed using kesum leaf extract (*Polygonum minus* Huds.) to obtain empirical evidence on the benefits of kesum leaves as anti-aging. This study is expected to provide more information about the benefits of kesum leaves to the community.

RESEARCH METHODOLOGY

Materials

Kesum leaves (*Polygonum minus* Huds.) collected from Sambas, West Kalimantan, Indonesia, were determined in Biological Laboratory, UAD with No. 292/Lab.bio/B/VII/2022; ethanol 70% (JT-Baker, USA), ethanol p.a, ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma), $K_2S_2O_8$, quercetin, Collagenase Activity Assay Kit (Sigma-Aldrich).

Extraction

Kesum leaves ethanol extract was prepared by maceration method using 70% ethanol with a ratio of 1:10. Maceration was carried out for 5 x 24 hours, while occasionally stirring, then filtered using filter paper. The liquid extract from the maceration was then concentrated using a water bath at 50ºC to obtain a thick extract (Geraldine and Hastuti, 2018).

Phytochemical screening

Saponin test. The saponin test was carried out by observing froth formation in the water after the reagent was added. Two mL of sample was added with 2 mL of distilled water, then shaken vigorously and one drop of 2N HCl was added (Harborne, 1996).

Alkaloid test. Mix 50 mg of extract with a few mL of HCl and filter. Then the solution is tested by adding one or two drops of different reagents in different tubes. The reagents used are Mayer, Wagner and Dragendorff (Raaman, 2006).

Tannin test. Two mL of the extract was added with 2 mL of 1% $FeCl_3$, then shaken (Harborne, 1996).

Steroid test. Two mL of the extract was added to 2 mL of n-hexane, then shaken. The n-hexane layer was added with Liebermann-Burchard reagent (Harborne, 1996).

Flavonoid test. Kesum leaves ethanol extract sample was added with a few drops of 10% NaOH reagent (Harborne, 1996).

Antioxidant activity test with ABTS method

Amount 25 mg of kesum leaf extract and 25 mg of quercetin, each dissolved in a volumetric flask using pro-analytical ethanol to 25 mL and homogenized to obtain a stock solution of 1000 ppm. ABTS stock solution was prepared by (A) weighing 7.1015 mg ABTS and (B) weighing 3.5 mg $K_2S_2O_8$, then (A) and (B) each were dissolved in 5 mL of distilled water. Solutions (A) and (B) were mixed in the dark and incubated for 16 hours. Then, the volume was made up to 25 mL using pro-analytical ethanol. Two mL of ABTS solution was pipetted. The volume was adjusted to 10 mL with pro-analytical ethanol and measured with a UV-Vis spectrophotometer with a 400-800 nm wavelength. A 1000 ppm kesum leaves extract stock solution was pipetted in 200 µL, 400 µL, 600 µL, and 800 µL, and the volume was made up to 10 mL with pro-analytical ethanol to obtain concentrations of 20 ppm, 40 ppm, 60 ppm, and 80 ppm. Pipetted 1000 ppm quercetin stock solution, each 40 µL, 60 µL, 80 µL, and 100 µL and made up the volume to 10 mL with pro-analytical ethanol to obtain concentrations of 4 ppm, 6 ppm, 8 ppm, and 10 ppm (Jatmiko and Mursiti, 2021).

The antioxidant capacity and the percentage of inhibition to inhibit free radicals' activity are determined by the equation:

$$\% \text{ Inhibition} = \frac{control\ enzyme\ slope - sample\ slope}{control\ enzyme\ slope}\ x\ 100\% \quad \text{.....(1)}$$

The percentage of free radical scavengers is made of a curve between percent free radical scavengers and solution concentration to get a linear regression equation. From this linear regression equation, the IC_{50} value will be determined. The calculation results are entered into a linear equation: (Agustina, 2017).

$$y = ax + b \quad \text{.....(2)}$$

Description:
a = gradient
b = constant
x = concentration (µg/mL)
y = inhibition percentage (%)

Collagenase enzyme inhibition test

Collagenase inhibition testing was carried out by following the manual procedure of the Sigma-Aldrich Collagenase Activity Assay Kit. The collagenase inhibition test was carried out by making a stock solution of 100.000 ppm by weighing a sample of 10 mg of kesum leaf extract and dissolving it in 100 µL of PBS. Then, dilute the stock solution to 1000 ppm by taking 10 µL of solution and make up to 1000 µL of PBS. The sample solution was made in three concentration series, which were 50 ppm, 100 ppm, and 200 ppm. Ten µL of each concentration series solution was taken, 2 µL Inhibitor (1 M, 1, 10-Phenanthroline), and 88 µL of Collagenase Assay Buffer was added. Enzyme control was made by taking 10 µL of Collagenase Kit and adding 90 µL of Collagenase Assay Buffer. Inhibitor control was made by taking 10 µL Collagenase Kit, 2 µL Inhibitor (1 M, 1, 10-Phenanthroline), and 88 µL Collagenase Assay Buffer. Then, add 100 µL of the reaction mixture containing 40 µL of Collagenase Substrate and 60 µL of Collagenase Assay Buffer to each well. Incubate for 5-15 minutes at 37°C and measure the absorbance using an ELISA reader at 345 nm. The data were means from three replicates (Utami et al., 2018).

The analysis of collagenase enzyme inhibition are determined by the equation:

$$\% \text{ Inhibition} = \frac{1 - control\ absorbance}{sample\ absorbance}\ x\ 100\% \quad \text{.....(3)}$$

RESULT AND DISCUSSION

Extraction

The preparation of 70% ethanol extract of kesum leaves was carried out using the maceration method. The purpose of using 70% ethanol in the maceration process is to remove all chemical components in the sample. This is because ethanol is a universal solvent that can attract compounds that dissolve in non-polar solvents to become polar solvents (Snyder et al., 1997). The percentage (%) of the yield obtained from the kesum leaves (*Polygonum minus* Huds.) 70% ethanol extract was 9.28%.

Phytochemicals screening

The results of the phytochemical screening that had been carried out stated that the 70% ethanol extract of kesum leaves contained saponins, tannins, and flavonoids. Flavonoid

compounds have antioxidants either directly by donating hydrogen ions to neutralize toxic effects caused by free radicals or indirectly by increasing the expression of endogenous antioxidant genes (Kusuma, 2015). Saponins also have benefits as antioxidants by increasing the formation of SOD and catalase (Aripasha et al., 2015). Likewise, tannins, which belong to the polyphenol group, also can be an antioxidant, whereas the –OH group contained in tannins can reduce free radicals (Wrasiati et al., 2011).

Antioxidant activity test with ABTS method

The antioxidant activity of kesum leaves (*Polygonum minus* Huds.) was carried out using the ABTS method as measured by a UV-Vis spectrophotometer. The ABTS method reacts faster between radical ABTS and antioxidant compounds. ABTS can also provide a more specific absorbance value at visible wavelengths and ABTS can be dissolved in water or organic solvents so that it can detect compounds that have hydrophilic and lipophilic properties (Jatmiko and Mursiti, 2021).

The positive control used in this antioxidant research was quercetin. Quercetin was chosen as a comparison because quercetin is a flavonoid compound commonly found in plants; besides that, quercetin has very strong antioxidant activity (Widyasari et al., 2019). The results of the study proved that the higher the sample concentration, the higher the inhibition percentage. This is because the flavonoid content in the sample is higher, so it can donate hydrogen atoms to free radicals (Indranila and Ulfah, 2015)

ABTS method antioxidant activity test was carried out based on the ability of antioxidant compounds to donate proton radicals to stabilize free radical compounds. The linear regression equation obtained from the comparison of quercetin is y = 5.15x + 9.2 (Figure 1) and the linear regression equation of the kesum leaves ethanol extract is y = 0.41x + 33 (Figure 2).

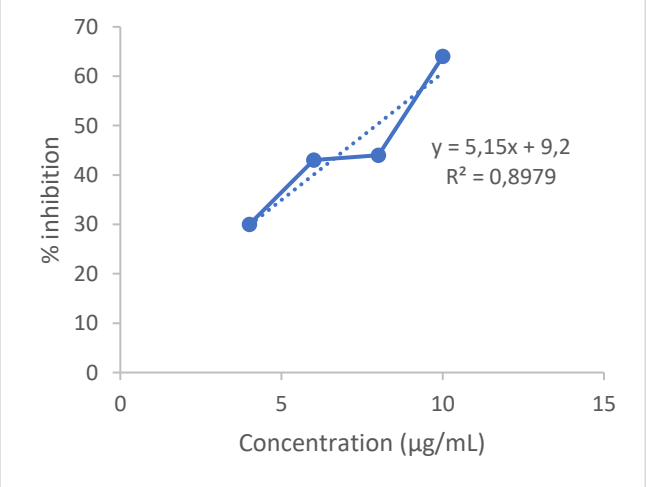

Figure 1. The relationship between inhibition percentage and quercetin concentration

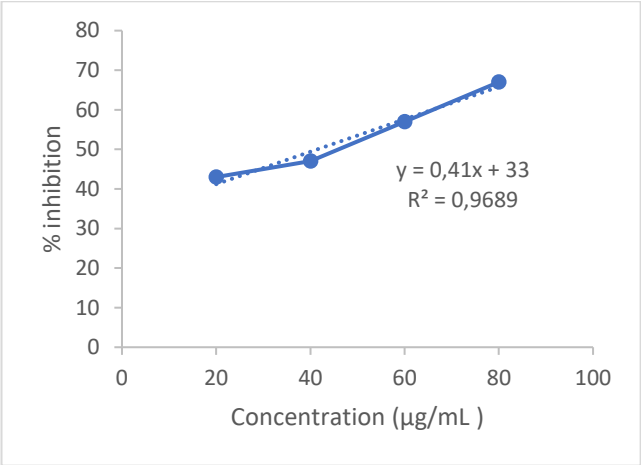

Figure 2. The relationship between inhibition percentage and kesum leaves concentration

The IC_{50} value indicates the strength of an antioxidant activity. The IC_{50} value indicates the concentration value of a sample solution required to reduce 50% of ABTS free radical activity (Imrawati et al., 2017). The sample of kesum leaf ethanol extract has an IC_{50} value of 41.46 µg/mL, which is included in the category of strong antioxidant because it is in the range <50 (Agustina, 2017). This strong antioxidant activity results from the content of compounds such as flavonoids, which have hydroxyl groups that can donate hydrogen atoms to Reactive Oxygen Species (ROS) and have hydroxyl ketone groups that can act as metal chelators that act as catalysts in lipids (Rezaeizadeh et al., 2011).

Collagenase enzyme inhibition test

Factors that can cause aging are factors such as exposure to free radicals. During the aging process, there is an imbalance between collagen production and collagen degradation. The imbalance that occurs is a decrease in collagen production while the collagenase enzyme will increase. Free radicals can cause an increase in the collagenase enzyme, resulting in collagen damage (Hooda, 2015). However, unwanted aging can be prevented by inhibiting free radicals with antioxidants and inhibiting collagenase activity.

Collagenase inhibition was tested by following the manual procedure of the Sigma-Aldrich Collagenase Activity Assay Kit. The test was carried out using 3 series of concentrations of kesum leaves ethanol extract, namely 50, 100, 200 µg/mL. The inhibition of the collagenase enzyme was observed by measuring the absorption at a wavelength of 345 nm.

Absorbance values obtained from readings using an ELISA reader obtained the percentage inhibition of collagenase enzyme activity from the ethanol extract of kesum leaves 50 ppm, 100 ppm, and 200 ppm by 83%, 82%, and 82% (Figure 3). These results indicate no effect of concentration on the percentage of enzyme inhibition. Regarding collagenase inhibitory activity, kesum leaves had a higher inhibitory activity than the inhibitor (1, 10-Phenanthroline, 1 M). This is because the secondary metabolites in kesum leaves, such as flavonoids, have the potential as endogenous antioxidants containing phenolic groups and are proven useful in preventing cell damage due to oxidative stress (Sumardika and Jawi, 2012). Free radicals cause collagen degradation through a chain molecular reaction that increases the formation of AP-1, which stimulates the transcription process of the matrix

metalloproteinase (MMP) enzyme, which plays a role in collagen degradation. The degradation process can be inhibited with the chemical compounds containing tannins, saponins, and flavonoids in kesum leaves. This can happen because tannins, saponins, and flavonoids have antioxidant properties. Antioxidant compounds can inhibit free radicals (Haerani et al., 2018). High antioxidant compounds can be free radical scavengers by consuming free radicals directly or oxidizing them with less effective and more stable radicals so that they can protect amino acids or collagen proteins. This causes an increase in the production of skin collagen, which can maintain its smoothness, elasticity and flexibility (Aizah, 2016), so that kesum leaves can have the potential as anti-aging for the skin.

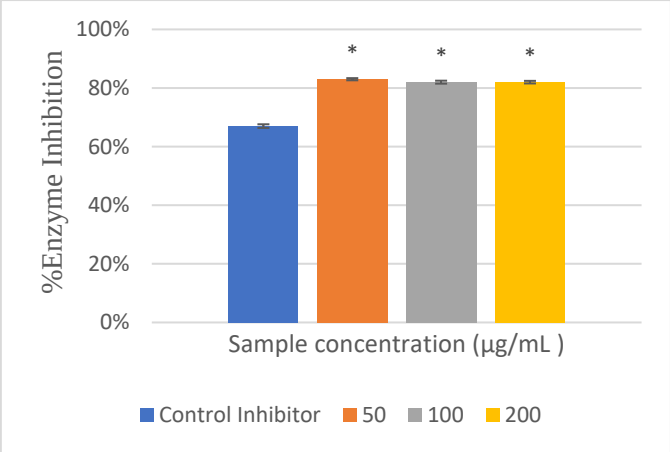

Figure 3. Enzyme inhibition percentage graph
*shows significant difference compared with control (P<0.05)

CONCLUSIONS

Based on the study result, kesum leaves provide very strong antioxidant activity in the ABTS test based on the IC_{50} value of 41.46 µg/mL. In addition, the results of the collagenase inhibition test on the ethanol extract of kesum leaves could inhibit the collagenase enzyme by 81%, 82%, and 83%. Based on these data, the ethanol extract of kesum leaves has the potential to be an activate ingredient in anti-aging cosmetics.

ACKNOWLEDGMENTS

Acknowledgments to the Cell Culture Laboratory and Pharmacy Study Program, Muhammadiyah University of Yogyakarta, for providing facilities for research.

REFERENCES

Agustina, E. (2017). Uji Aktivitas Senyawa Antioksidan dari Ekstrak Daun Tiin (*Ficus carica* Linn) Dengan Pelarut Air, Metanol dan Campuran Metanol-Air. *Klorofil*. 1(1), 38–47.

Aizah, S. (2016). Antioksidan Memperlambat Penuaan Dini Sel Manusia. *In: Prosiding Seminar Hasil Hayati IV Unversitas Nusantara PGRI Kediri*. 182–185.

Arifin, B. and Ibrahim, S. (2018). Struktur, Bioaktivitas dan Antioksidan Flavonoid. *Jurnal Zarah*, 6 (1), 21-29.

Aripasha, A., Andriana, D., and Purnomo, Y. (2015). Efek Dekok Daun Pulutan (*Urena lobata*) terhadap Kadar SOD (Superoxyde Dismutase) dan MDA (Malondialdehyde) Serum Tikus Model Diabetes Mellitus Tipe II. *Jurnal Kedokteran Komunitas*, 3(1), 304-311.

Kusuma, A.S.W. (2015). The Effect of Ethanol Extract of Soursop Leaves (*Annona muricata* L.) to Decreased Levels of Malondialdehyde. *Jurnal Majority,* 4(3), 14-18.

Chatatikun, M. and Chiabchalard, A. (2017). Thai Plants with High Antioxidant Levels, Free Radical Scavenging Activity, Anti-tyrosinase and Anti-collagenase Activity. *BMC Complementary and Alternative Medicine*, 17(487), 1-9.

Chen, Q. (2009). Evaluate the Effectiveness of the Natural Cosmetic Product Compared to Chemical-Based Products. *International Journal of Chemistry*, 1(2), 57-59.

Geraldine, E.T. and Hastuti, E.D. (2018). Formulation of Sunscreen Cream of Parijoto Fruit Extract (*Medinilla speciosa* Blume) and *In Vitro* SPF Value Test. *Jurnal Farmasi Sains dan Komunitas*, 15(2), 92-98.

Haerani, A., Chaerunisa, A.Y., and Subarnas, A. (2018). Artikel Tinjauan: Antioksidan untuk Kulit. *Farmaka*, 16(2), 135-151.

Harborne, J. B. (1996). *Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan*. Penerbit ITB.

Hart, P.H. and Norval, M. (2018). Ultraviolet Radiation-Induced Immunosuppression and its Relevance for Skin Carcinogenesis. *Photochemical & Photobiological Sciences,* 17(12), 1872-1884.

Hooda, R. (2015). Antiwrinkle Herbal Drugs-An Update. *Journal of Pharmacognosy and Phytochemistry*, 4(4), 277-281.

Imrawati, Mus, S., Gani, S.A., and Bubua, K.I. (2017). Antioxidant Activity of Ethyl Acetate Fraction of *Muntingia calabura* L. Leaves. *Journal of Pharmaceutical and Medicinal Sciences*, 2(2), 59–62.

Indranila, I. and Ulfah, M. (2015). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Karika (*Carica pubescens*) dengan Metode DPPH Beserta Identifikasi Senyawa Alkaloid, Fenol, dan Flavonoid. In: *Prosiding Seminar Nasional Peluang Herbal Sebagai Alternatif Medicine, Fakultas Farmasi Universitas Wahid Hasyim*, 105-111.

Jatmiko, M.P. and Mursiti, S. (2021). Isolation, Identification, and Activity Test of Flavonoid Compounds in Jamblang Leaves (*Syzygium cumini* L.) Skeel. as Antioxidants. *Indonesian Journal of Chemical Science*, 10(2), 129-138.

Popoola, A.R., Ganiyu, S.A., Enikuomehin, O.A., Bodunde, J.G., Adedibu, O.B., Durosomo, H.A. and Karunwi, O.A. (2015). Isolation and Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Nigeria. *Nigerian Journal of Biotechnology*, 29(2015), 1-10.

Purwaningsih, I., Sapriani, R., and Indrawati, R. (2018). Aktivitas Antioksidan Ekstrak Metanol Daun Kesum (*Polygonum minus* Huds.) Metode DPPH. *Jurnal Laboratorium Khatulistiwa*, 2(2), 161-165.

Raaman, N. (2006). *Phytochemical Techniques*. New India Publishing Agency.

Rezaeizadeh, A., Zuki, A.B.Z, Abdollahi, M., Goh, Y.M., Noordin, M.M., Hamid, M., and Azmi, T.I. (2011). Determination of Antioxidant Activity in Methanolic and Chloroformic Extracts of *Momordica charantia. African Journal of Biotechnology*, 10(24), 4932-4940.

Snyder, L.R., Kirkland, J.J., and Glajch, J.L. (1997). *Practical HPLC Method Development*. John Wiley & Sons.

Sumardika, I.W. and Jawi, I.M. (2012). Ekstrak Air Daun Ubi Jalar Ungu Memperbaiki Profil Lipid dan Meningkatkan Kadar SOD Darah Tikus yang Diberi Makanan Tinggi Kolesterol. *Jurnal Ilmiah Kedokteran Medicina*, 43(2), 67–70.

Swastika NSP, A., Mufrod, and Purwanto (2013). Antioxidant Activity of Cream Dosage Form of Tomato Extract (*Solanum lycopersicum* L.). *Traditional Medicine Journal*, 18(3), 132-140.

Thring, T.S.A., Hili, P., and Naughton, D.P. (2009). Anti-collagenase, Anti-elastase and Anti-oxidant Activities of Extracts from 21 Plants. BMC Complementary and Alternative Medicine, 9(27), 1-11.

Utami, S., Sachrowardi, Q.R., Damayanti, N.A, Wardhana, A., Syarif, I., Nafik, S., Arrahmani, B.C., Kusuma, H.S.W., and Wahyu, W. (2018). Antioxidants, Anticollagenase and Antielastase Potentials of Ethanolic Extract of Ripe Sesoot (*Garcinia picrorrhiza* Miq.) Fruit as Antiaging. *Journal of HerbMed Pharmacology*, 7(2), 88–93.

Wibowo, M.A., Anwari, S., Aulanni'am, and Rahman, F. (2009). Skrining Fitokimia Fraksi Metanol, Dietil Eter dan N-heksana Ekstrak Daun Kesum (*Polygonum minus* H.), *Jurnal Penelitian Universitas Tanjungpura*,16, 54-60.

Widyasari, E.M., Sriyani, M.E., Daruwati, I., Halimah, I., and Nuraeni, W. (2019). Karakteristik Fisikokimia Senyawa Bertanda 99mTc-Kuersetin. *Jurnal Sains dan Teknologi Nuklir Indonesia,* 20(1), 9-18.

Wrasiati, L.P., Hartati, A., and Yuarini, D.A.A. (2011). Kandungan Senyawa Bioaktif dan Karakteristik Sensoris Ekstrak Simplisia Bunga Kamboja (*Plumeria sp.*). *Jurnal Biologi*, 15(2), 39–43.