Antioxidant, Antibacterial, and Antidiabetic Activities of Roselle (*Hibiscus sabdariffa*) Extracts

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Abstract

Hibiscus sabdariffa L., also referred to as roselle, is commonly utilized in the pharmaceutical and food industries. Roselle contains bioactive compounds such as phenolics, alkaloids, tannins, flavonoids, saponins, and organic acids, which have pharmacological properties, such as antioxidant, antibacterial, immune booster, antidiabetic, anti-inflammatory, and anti-hypertensive properties. There are many studies regarding the pharmacological activities of roselle extract and its applications. However, there has been no research to study the effectiveness of the solvent in testing roselle petal extracts against antibacterial, antioxidant, and antidiabetic activities, simultaneously. This research used two kinds of polar solvents, dH_2O and ethanol, with various concentrations for antibacterial activity test by five pathogenic bacteria, for antioxidant test by the DPPH method, and for antidiabetic test by the alpha-glucosidase inhibition method. The result showed that the ethanol extract of roselle had higher antibacterial activity compare to the roselle water extract. Antioxidant activity of roselle ethanol extract at 20% concentration had the highest activity 69.75 ± 0.002%; while, the 100% concentration of roselle water extract had the highest antioxidant activity 138.73 ± 0.013%. antidiabetic activity of roselle ethanol and water extract at 100% concentration had the highest activity 1,195.44 ± 0.007% and 1,552.49 ± 0.069%, respectively.

Keywords: antibacterial activity, antioxidant activity, antidiabetic activity, roselle, pharmacological bioprospection

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Introduction

Hibiscus sabdariffa L (roselle) is commonly utilized in the pharmaceutical and food industries. Roselle seeds are often used as an oil producer for cosmetics, powdered seeds and leaves are often used as animal feed, and roselle flower petals are used as herbal beverage products (Da-Costa-Rocha *et al.*, 2014). Roselle petal contains phytochemical compounds, such as phenolics, alkaloids, tannins, flavonoids, saponins, and organic acids that have pharmacological activities including antioxidant, antibacterial, immune booster, antidiabetic, anti-inflammatory, and antihypertensive (Alaa, 2012; Brown *et al.*, 2019; Herdiani & Wikurendra, 2020; Wang *et al.*, 2011). Many studies regarding the pharmacological activity of roselle extract and its applications have been conducted (Izquierdo-Vega *et al.*, 2020; Shruthi *et al.*, 2016; Qi *et al.*, 2005). However, there has been no research on the effectiveness of the solvent extract of roselle for antimicrobial, antioxidant, and antidiabetic activities, simultaneously.

Márquez-Rodríguez *et al.* (2020) reported that the roselle ethanol extract inhibited the expansion of food spoilage pathogenic bacteria, including *Escherichia coli, Salmonella*

typhimurium, Staphylococcus aureus, Listeria monocytogenes, and *Bacillus cereus*. Additionally, Abou-Arab *et al*. (2011) stated that the ethanol extract of roselle with the addition of 1% citric acid showed higher antioxidant activity than using other solvents. The high levels of polyphenolic compounds in the roselle petal extract are known to be effective in reducing blood glucose levels in diabetes mellitus rats (Rosemary & Haro, 2014; Herdiani & Wikurendra, 2020). The aim of this study was to analyze the pharmacological profile of roselle extract on an antibacterial, an antioxidant, and an antidiabetic test simultaneously by extracting roselle petals using various concentrations of polar solvents (water and ethanol). The results of this study are expected to be as scientific information regarding the pharmacological bioprospection of roselle petal extract as a source of antibacterials, antioxidants, and antidiabetics.

Materials and Methods

Extraction of Roselle Petals. The 100 g of roselle flower petals were collected and then extracted by the maceration method using distilled water or 96% ethanol as solvent with a concentration of 100% (100 g roselle petals: 100 mL solvent). The 100% extracts were poured into dilution bottles of 200 µL, 400 µL, 600 µL, and 800 µL, respectively, and subsequently added with sterile distilled water until each solution amounted to 1 mL. So, the extracts obtained had respective concentrations of 20%, 40%, 60%, and 80%. The extracts obtained were stored at room temperature.

Antibacterial Activity. The antibacterial activity test was carried out using the disk diffusion method (Manguntungi *et al*., 2020). Roselle petal extracts in water or ethanol are used in concentrations of 20%, 40%, 60%, 80%, and 100%. Five pathogenic bacteria used in this test were *E. coli* ATCC 25922, PIDT *Proteus* obtained from IPB University, *Salmonella tyhposa* obtained from Research Center for Chemistry, Indonesian Institute of Sciences, *S. aureus* ATCC 25923, and *Listeria monocytogenes* obtained from Universitas Gadjah Mada. The area of the inhibition zone was then measured in mm to determine the antibacterial activity of the roselle petal extracts.

Antioxidant Activity. The antioxidant test against DPPH free radicals was used with a slight modification (Zahratunnisa *et al*., 2017). Roselle petal extracts in water or ethanol were used in concentrations of 20%, 40%, 60%, 80%, and 100%. Vitamin C with a concentration of 50 ppm was used as a positive control. All samples that had been incubated for 30 minutes at 27^{o}C were measured for absorbance values using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Antidiabetic Activity. An antidiabetic test using the alpha-glucosidase inhibition method was conducted (Yuniarto & Selifiana, 2018). Roselle petal extracts in water or ethanol were used in concentrations of 20%, 40%, 60%, 80%, and 100%. Acarbose with a concentration of 100 ppm was used as a positive control. The extracts were measured with ELISA Reader at a wavelength of 200 nm.

Analysis Data. At the initial stage, the data are analyzed for normality. If the results are normal and homogeneous, then a comparative analysis can be carried out between groups using a one-way ANOVA test with a 5% confidence interval.

Results

Pharmacological Activity of the Roselle Petals Extract using Ethanol Solvent.

In this study, Gram-positive (*S. thyposa, S. aureus,* and *L. monocytogenes*) and Gram-negative bacteria (*E. coli* and PIDT *proteus*) were used as test bacteria for the antibacterial activity of the roselle flower petals' ethanol extracts. The various concentrations of the roselle petal ethanol extracts against the five pathogenic bacteria showed different zones of inhibition. The results of the antibacterialactivity test of the ethanolic extracts of roselle petals are presented in Table 1. As a positive control, the antibiotic ampicillin with a concentration 0.5 µg/µL was used. As a negative control, 96% ethanol was used.

Based on the results of the data analysis in Table 1, compared to a positive control, the roselle ethanol extract at 80% concentration had the highest antibacterialactivity against *S. aureus* and *L. monocytogenes* with inhibition zones of 12.67±1.15 mm and 23.00±0.00 mm,

respectively. The 100% concentration of roselle petal ethanol extracts were able to inhibit *E. coli* and PIDT *Proteus* with inhibition zones of 17.00±0.00 mm and 14.00±0.00 mm, respectively. While, the highest value of antibacterialactivity against *S. tyhposa* was found in the control (+) with an area of inhibition zone of 21.00±0.00 mm.

Table 1. The results of the antibacterial test of roselle petal extracts with ethanol as a solvent

Concentration of roselle flower petal extracts	Antibacterial activity of roselle petal extracts with ethanol as a solvent (mm)				
	E. coli	PIDT *Proteus*	*S. tyhposa*	*S. aureus*	*L. monocytogenes*
20%	7.00 ± 1.00^{b}	7.00 ± 0.00^{c}	3.33 ± 1.15^{b}	3.33 ± 1.15^{bc}	7.67 ± 0.00^{b}
40%	12.67 ± 2.88^{c}	4.67 ± 1.15^{b}	7.00 ± 0.00^{c}	6.33 ± 0.57^{d}	9.00 ± 0.00^{b}
60%	12.00 ± 1.73^{c}	8.33 ± 0.57^{d}	12.00 ± 0.00^{d}	12.00 ± 1.73^{e}	12.00 ± 0.00^{c}
80%	16.00 ± 0.00^{d}	11.00 ± 0.00^{e}	12.67 ± 1.15^{d}	**12.67 ± 1.15^{e}**	**23.00 ± 0.00f**
100%	**17.00 ± 0.00^{d}**	**14.00 ± 0.00^{f}**	18.00 ± 0.00^{e}	2.00 ± 0.00^{b}	21.00 ± 0.00^{e}
C (+) Ampicillin	14.67 ± 1.15^{cd}	9.00 ± 0.00^{d}	**21.00 ± 0.00^{f}**	4.00 ± 0.00^{c}	15.00 ± 0.00^{d}
C (−) ethanol 96%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

An antioxidant test of roselle petal ethanol extracts as a solvent is shown in Table 2. The antioxidant activity of roselle petal extracts using ethanol as a solvent with varying concentrations in inhibiting DPPH free radicals showed significantly different results among experimental groups.

Based on the results of the data analysis in Table 2, compared to vitamin C with a concentration of 50 ppm as a positive control, all the extracts have stronger antioxidant activities than vitamin C and ethanol as a negative control. The 20% concentration of roselle ethanol extract has the highest antioxidant activity 69.75±0.002% no significant difference with the 40% concentration compared to concentrations of 60%, 80%, 100%, and a positive control (vitamin C).

Table 2. Antioxidant test results of roselle petals extracts with ethanol as a solvent

Concentration of roselle flower petal extract	Antioxidant activity of roselle petal extract with ethanol as a solvent (%)
20%	**69.75 ± 0.002^{f}**
40%	68.66 ± 0.001^{f}
60%	66.67 ± 0.000^{e}
80%	59.10 ± 0.001^{d}
100%	54.13 ± 0.001^{c}
C (+) Vitamin C	49.00 ± 0.001^{b}
C (−) Ethanol 96%	0.00 ± 0.00^{a}

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

Roselle petal extracts using ethanol as a solvent showed antidiabetic activity by inhibiting alpha-glucosidase in 30 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside. The results of the antidiabetic test of the ethanol extracts of roselle petal are shown in Table 3.

Based on the result of the data analysis in Table 3, it is shown that, compared to acarbose with a 100 ppm concentration as a positive control except for a 20% concentration of roselle petal ethanol extracts, all treatments showed strong antidiabetic activities . The 100% concentration of roselle petal ethanol extracts had the highest antidiabetic activity, 1,195.44±0.007% compared to other treatment groups. All treatment groups showed significant differences.

Table 3. antidiabetic test results of roselle petal extracts with ethanol as a solvent

Concentration of roselle flower petal extract	Antidiabetic activity of roselle petal extracts with ethanol as a solvent (%)
20%	251.56 ± 0.005[b]
40%	526.14 ± 0.020[d]
60%	798.08 ± 0.005[e]
80%	1,019.42 ± 0.013[f]
100%	**1,195.44 ± 0.007[g]**
C (+) Acarbose	490.59 ± 0.074[c]
C (−) Ethanol	0.00 ± 0.00[a]

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

Pharmacological Activity of Roselle Extract using Water Solvent.

Roselle petals extracted using water were tested on *E. coli*, PITD *proteus*, *S. tyhposa*, *S. aureus*, and *L. monocytogenes* showed an antibacterial activity (Table 4). As a positive control and negative control, the antibiotic ampicillin with a concentration of 0.5 µg/µL and water were used, respectively.

Based on the results of the data analysis in Table 4, the roselle petal water extract at 80% and 100% concentrations were able to inhibit *E. coli* with the highest inhibition zone area of 7.00±0.00 mm. While, the roselle petal water extracts at 40% and 60% concentrations had the highest antibacterial activity against PIDT *Proteus* with an inhibition zone 5.33±0.57 mm. The highest value of microbial activity against *S. tyhposa* and *S. aureus* was found in the 100% treatment, 6.00±1.73 mm and 9.00±0.00 mm, respectively. The positive control treatment had the highest antibacterial activity with an inhibitory zone of 6.00±0.00 mm against *L. monocytogenes*.

Table 4. The results of the antibacterial test of roselle petal extracts with water as a solvent

Concentration of roselle flower petal extract	Antibacterial activity of roselle petal extracts with water as a solvent (mm)				
	E. coli	**PIDT *Proteus***	***S. tyhposa***	***S. aureus***	***L. monocytogenes***
20%	5.67 ± 1.15[bc]	3.33 ± 1.15[b]	3.33 ± 0.57[b]	4.00 ± 0.00[b]	2.00 ± 0.00[b]
40%	6.00 ± 0.00[bc]	**5.33 ± 0.57[d]**	3.67 ± 1.15[b]	5.67 ± 0.57[cd]	2.33 ± 0.57[b]
60%	5.33 ± 2.88[bc]	**5.33 ± 0.57[d]**	4.33 ± 0.57[bc]	6.33 ± 0.57[d]	3.67 ± 0.57[c]
80%	**7.00 ± 0.00[c]**	4.00 ± 0.00[b]	4.67 ± 0.57[bc]	7.67 ± 1.15[e]	4.33 ± 0.57[c]
100%	**7.00 ± 0.00[c]**	4.33 ± 0.57[bcd]	**6.00 ± 1.73[c]**	**9.00 ± 0.00[f]**	5.33 ± 0.57[d]
C (+) Ampicillin	4.00 ± 0.00[b]	5.00 ± 0.00[bc]	5.00 ± 0.00[bc]	5.00 ± 0.00[bc]	**6.00 ± 0.00[d]**
C (−) water	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

Table 5. The results of the antioxidant test of roselle petal extracts with water as a solvent

Concentration of roselle flower petal extract	Antioxidant activity of roselle petal extract with water as a solvent (%)
20%	39.77 ± 0.128[c]
40%	12.88 ± 0.041[b]
60%	12.05 ± 0.006[b]
80%	52.02 ± 0.006[e]
100%	**138.73 ± 0.013[f]**
C (+) Vitamin C	49.00 ± 0.001[d]
C (−) Water	0.00 ± 0.00[a]

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

The antioxidant activity of roselle extract using water as solvent with various concentrations in inhibiting DPPH free radicals showed significantly different results between experimental groups (Table 5).

Based on the results of the data analysis in Table 5, the 100% concentration of roselle water extract had the highest antioxidant activity, 138.73 ± 0.013% compared to concentrations of 20%, 40%, 60%, 80%, positive (vitamin C), and negative control (water).

Roselle petals extracted using water showed antidiabetic activity by inhibiting alpha-glucosidase in 30 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside. The results of the antidiabetic test of roselle petal water extracts is shown in Table 6.

Based on the results of data analysis in Table 6, the 100% concentration of roselle petals water extract had the highest antidiabetic activity, 1,552.49±0.069% compared to other treatment groups. All treatment groups showed significant differences.

Table 6. Antidiabetic test results of roselle petal extracts with water as a solvent

Concentration of roselle flower petal extract	Antidiabetic activity of roselle petal extracts with water as a solvent (%)
20%	317.91 ± 0.005[b]
40%	657.46 ± 0.015[b]
60%	969.65 ± 0.008[e]
80%	1,243.03 ± 0.023[f]
100%	**1,552.49 ± 0.069[g]**
C (+) Acarbose	437.06 ± 0.176[c]
C (−) Water	0.00 ± 0.00[a]

Note: Numbers followed by the same letters in the same column show no significant difference in the one-way ANOVA test.

Discussion

Polar substances or compounds will dissolve in polar solvents, so in this study two types of polar solvents (distilled water and ethanol) with different concentrations showed a different effect on each test performed.

The antibacterial activity of the ethanol extracts and the distilled water extracts of roselle petals against each tested bacterial pathogen has different effectiveness (Tables 1 and 4). This difference in effectiveness is due to differences in the optimization of the types of bioactive compounds that can be extracted from roselle petals. Haeriah *et al.* (2018) reported the different levels of each bioactive compound contained in roselle petals was depend on variations of concentration, time of extraction, and the type and age of the plant, so that it will have a different effect on antibacterial activity. According to Márquez-Rodríguez *et al.* (2020) the high content of phenolic acids in the phase 1 fraction of roselle extract showed the most effective antibacterial activity in inhibiting food spoilage bacteria such as *E. coli*, *S. tyhposa*, *S. aureus*, *L. monocytogenes*, and *B. cereus*.

The roselle petal ethanol extracts had higher antibacterial activity in comparison to the roselle petal water extracts. Similarly, Alaa (2012) discovered that the antibacterial activity of ethanol extracts was greater than that of roselle water extracts against *Streptococcu*s mutant and *E. coli*, indicating that alcohol solvent was the most effective solvent in extracting roselle phenolic compounds that act as antibacterial. Bioactive compounds release antibacterial activity through various mechanisms, such as oxidative stress on cell membranes, inhibiting cellular division, and bacterial cell metabolic enzymes.

The antioxidant test of the ethanol extract of roselle petals showed that the 20% concentration of DPPH free radicals indicated a higher value than other concentrations; while, the 100% concentration of roselle petal water extracts had higher antioxidant activity than other concentrations. In addition, the antioxidant activity of the roselle petals water extracts were higher than the roselle ethanol extracts (Tables 2 and 5). This is indicated that water extracts is more effective as an antioxidant. Yang *et al.* (2012) reported the result of roselle petals water

extract have higher antioxidanrt activity compared to 30% and 60% roselle ethanol extract. Alaa (2012) stated that roselle petals contain phenolic compounds related to their ability as antioxidants. In addition, roselle extract also contains steroid compounds, alkaloids, tannins, terpenoids, flavonoids, and saponins (Aryati *et al*., 2020). Phenol and flavonoid compounds contained in roselle water extract have the most effective ability to chelate Fe^{2+} metal, which acts as a highly reactive free radical (Alaa, 2012).

Ethanol and water of roselle petals extract at 100% concentration (Tables 3 and 6) showed better antidiabetic activity than acarbose. Acarbose is used as a synthetic drug in diabetics by inhibiting the alpha-glucosidase enzyme, which plays a role in the absorption and digestion of glucose (Yuniarto & Selifiana, 2018). The content of bioactive compounds in roselle extract acts as an antioxidant to ward off free radicals, which are also associated with the cause of diabetes mellitus and diabetes complications. The content of polyphenolic compounds in roselle water extract can reduce the effect of insulin resistance and hyperglycemia (Ajiboye *et al*., 2015). Furthermore, Wang *et al*. (2011) discovered that roselle extracts had a potential effect in improving diabetic nephropathy by increasing antioxidants and regulating the Akt/bad/14-3-3γ signal associated with cell apoptosis caused by hyperglycemia.

Based on the results of the study, it concluded that the ethanol and water extracts with varying concentrations inhibited the pathogenic bacteria. Each treatment concentration of ethanol and water extracts of roselle petals had a different antibacterial activity on each bacterium. The roselle petal ethanol extracts showed higher antibacterial activity than the roselle petals water extracts.

The antioxidant test on water extract or roselle petals against DPPH free radicals was stronger than the ethanol extract of roselle petals; while, the antidiabetic test of water extracts was more effective than the ethanol extracts of roselle petals. The higher the concentration of roselle petals water extract, the higher the antioxidant and an antidiabetic activity.

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References

Abou-Arab, A. A., Abu-Salem, F. M., & Abou-Arab, E. A. (2011). Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). *Journal of American Science, 7*(7), 445-456.

Alaa, G. A. H. (2012). Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts. African Journal of Food Science, 6(21), 506-511. https://doi.org/10.5897/AJFS12.099.

Aryati, D. L., Rohadi, & Pratiwi, E. (2020). Aktivitas Antioksidan Ekstrak Kelopak Bunga Rosela (*H. sabdariffa* L.) Merah Pada Berbagai Suhu Pemanasan. *Jurnal Teknologi Pangan Dan Hasil Pertanian*, *15*(1), 1-9. https://doi.org/10.26623/jtphp.v13i1.1845.kodeartikel

Brown, F., González, J., & Monan, M. (2019). Pharmacognostic, Physicochemical and Phytochemical Analysis of Two Cultivars from *Hibiscus sabdariffa* L. in Cuba. *Open Access Library Journal, 6*(12), 1-8. https://doi.org/10.4236/oalib.1105894.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus sabdariffa* L.–A phytochemical and pharmacological review. *Food chemistry, 165*, 424-443. Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2014.05.002

Haeriah, H., Djide, N., Alam, G., & Sartini, S. (2018). Sinergitas Aktivitas Antibakteri dari Kelopak Bunga Rosella dan Kitosan Terhadap Staphylococcus aureus. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)(e-Journal), 4*(2), 93-97. https://doi.org/10.22487/j24428744.10590.

Herdiani, N., & Wikurendra, E. A. (2020). Efek Antioksidan Ekstrak Kelopak Rosella Terhadap Glukosa Darah Tikus Diabetes Mellitus Tipe 2. *An-Nadaa: Jurnal Kesehatan Masyarakat (e-Journal), 7*(2), 89-93. https://ojs.uniska-bjm.ac.id/index.php/ANN/article/view/3321.

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., Vargas-Mendoza, N., Gómez-Aldapa, C. A., ... & Madrigal-Santillán, E. (2020). Organic acids from Roselle (*Hibiscus sabdariffa* L.)—A brief review of its pharmacological effects. *Biomedicines, 8*(5), 100.

Manguntungi, B., Saputri, D. S., Mustopa, A. Z., Ekawati, N., Nurfatwa, M., Prastyowati, A., ... & Fidien, K. A. (2020). Antidiabetic, Antioxidants and Antibacterial Activities of Lactic Acid Bacteria (LAB) from Masin (Fermented Sauce from Sumbawa, West Nusa Tenggara, Indonesia). *Annales Bogorienses*, *24*(1), 27-34.

Márquez-Rodríguez, A. S., Nevárez-Baca, S., Lerma-Hernández, J. C., Hernández-Ochoa, L. R., Nevárez-Moorillon, G. V., Gutiérrez-Méndez, N., ... & Salas, E. (2020). In vitro antibacterial activity of *Hibiscus sabdariffa* L. phenolic extract and its in situ application on shelf-life of beef meat. *Foods, 9*(8), 1080.

Qi, Y., Chin, K. L., Malekian, F., Berhane, M., & Gager, J. (2005). Biological characteristics, nutritional and medicinal value of roselle, Hibiscus sabdariffa. *Circular-urban Forestry Natural Resources and Environment, 604*, 1-2.

Ramadhanti, E., Rustini, & Marlina. (2020). A Review: Antibacterial Activity of Roselle (*Hibiscus sabdariffa*) Calyx Extract against Pathogenic Bacteria. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 2319-7676. https://doi.org/10.9790/3008-1505075560.

Rosemary, R., & Haro, G. (2014). Antidiabetic effect of roselle calyces extract (*Hibiscus sabdariffa* L.) in streptozotocin induced mice. *International Journal of PharmTech Research, 6*(5), 1703-1711.

Shruthi, V. H., Ramachandra, C. T., Nidoni, U., Hiregoudar, S., Naik, N., & Kurubar, A. R. (2016). Roselle (Hibiscus sabdariffa L.) as a source of natural colour: a review. *Plant Archives, 16*(2), 515-522.

Wang, S. C., Lee, H. J., Lee, S. F., Wang, C. J., Lee, C. H., & Lee, W. C. (2011). Aqueous extract from hibiscus sabdariffa linnaeus ameliorate diabetic nephropathy via regulating oxidative status and Akt/Bad/14-3-3γ in an experimental animal model. *Evidence-Based Complementary and Alternative Medicine*, *2011*. https://doi.org/10.1093/ecam/nep181.

Yang, L., Gou, Y., Zhao, T., Zhao, J., Li, F., Zhang, B., & Wu, X. (2012). Antioxidant capacity of extracts from calyx fruits of roselle (*Hibiscus sabdariffa* L.). *African Journal of Biotechnology*, *11*(17), 4063-4068.

Yuniarto, A., & Selifiana, N. (2018). Aktivitas Inhibisi Enzim Alfa-glukosidase dari Ekstrak Rimpang Bangle (Zingiber cassumunar Roxb.) secara In vitro. *MPI (Media Pharmaceutica Indonesiana)*, *2*(1), 22–25. https://doi.org/10.24123/mpi.v2i1.1299.

Zahratunnisa, N., Elya, B., & Noviani, A. (2017). Inhibition of Alpha-glucosidase and antioxidant test of stem bark extracts of Garcinia fruticosa Lauterb. *Pharmacognosy Journal*, 9(2).