

RESEARCH ARTICLE

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DOI: 10.21705/mcbs.v1i1.3 **α -/ β -Glucosidase and α -Amylase Inhibitory Activities of Roselle (*Hibiscus sabdariffa* L.) Ethanol Extract**Marisca Evalina Gondokesumo¹, Hanna Sari W. Kusuma², Wahyu Widowati³¹Faculty of Pharmacy, Surabaya University, Surabaya, Indonesia²Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia³Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

Background: Diabetes mellitus is a metabolic disease, characterized by hyperglycemia due to disturbance in both insulin secretion and function. One of the therapeutic approaches is to reduce blood glucose levels by inhibiting α -/ β -glucosidase and α -amylase involved in carbohydrate digestion. Thus, inhibition of these enzymes play important role in the treatment of diabetes mellitus. Roselle (*Hibiscus sabdariffa* L.) has been known to have several medicinal properties and potency as an antidiabetic agents. This research aimed to observe antidiabetic properties of roselle ethanol extract (REE) towards α -glucosidase, β -glucosidase and α -amylase.

Materials and Methods: REE was done with maceration technique using diluent of 70% ethanol. Antidiabetic properties were measured by inhibitory activity of α -amylase, α -glucosidase and β -glucosidase.

Results: REE was able to inhibit α -/ β -glucosidase and α -amylase in the highest concentration with inhibition percentage of 72.68, 47.34 and 73.08% respectively, and were comparable with Acarbose of 81.49, 50.97, 73.08%. The median inhibitory concentration (IC₅₀) of α -/ β -glucosidase and α -amylase of REE were 15.81, 41.77, 18.09 μ g/mL respectively, and Acarbose were 9.45, 22.57, 3.64 μ g/mL respectively.

Conclusions: REE inhibits α -/ β -glucosidase and α -amylase.

Keywords: Roselle, Acarbose, α -glucosidase, β -glucosidase, α -amylase, antidiabetic

Introduction

Diabetes mellitus (DM) is a metabolic disease, characterized by hyperglycemia due to disturbance in both insulin secretion and function.¹ Type 1 DM is a type with defective insulin secretion by pancreatic β -cells, and type 2 DM is a type with insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β -cell dysfunction.² DM has postprandial phase indicated by increased blood glucose levels, which is called postprandial hyperglycemia.³

Antihyperglycemia is commonly used to reduce hyperglycemia prostaglandins via inhibition of responsible enzymes in glucose absorption that hydrolyze carbohydrates, such as α -/ β -glucosidase and α -amylase.⁴ Glucosidase is an enzyme involved in complex carbohydrate dissociation in the small intestine that play its role to cleave glycosidic bonds, resulting in glucose release from the non-reducing end of an oligo- or poly-saccharide chain involved in glycoprotein biosynthesis.⁴

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Common drugs are expensive or generate adverse effects. Common adverse effects of enzyme-inhibitory drugs, like Acarbose, are excessive inhibition of pancreatic α -amylase, which can result in abdominal distention, flatulence and diarrhea.⁵ Thus, natural medicine is proposed to be an alternative. Traditional herbal medicine is believed to possess hypoglycemic activity. There are more than 800 species of plants reported to contain hypoglycemic properties.⁶

Roselle (*Hibiscus sabdariffa* L) has been used as a medicinal beverage in many countries such as Australia, India, Myanmar, Thailand, Senegal, France, Gambia, Nigeria, Greece, Saudi Arabia, Sudan, Latin America, Panama, Indonesia, Malaysia and China.⁷ Bioactive compounds is documented in roselle such as alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols and tannins.⁸ Roselle extract demonstrate anti-insulin resistance properties due to its activity in reducing hyperglycemia and hyperinsulinemia, as well as decrease in Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL).⁹ Roselle extract also play role in lowering blood glucose levels in streptozotocin-induced diabetic mice, it is comparable with glibenclamide.^{6,10,11} Therefore our current study was carried out to evaluate possible effectivity of Roselle extract to inhibit α/β -glucosidases and α -amylase.

Materials and methods

Preparation roselle extract

Extraction was performed based on maceration method.^{12,13} Roselle calyx were collected from Cimenyan, West Java, Indonesia. The plant was identified by herbarium staff of Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. Dried and milled roselle calyx were soaked in 70% distilled ethanol and filtered every 24 hours. The process was performed until colorless filtrate was gained, then filtrate was evaporated to obtain ethanol extract. Roselle ethanol extract (REE) was stored at -20°C.

α -glucosidase inhibitory activity assay

The α -glucosidase inhibitor activity was tested with the modified method.^{1,14,15} Mixture reaction consists of 4-Nitrophenyl α -D-glucopyranoside (N1377, Sigma-Aldrich, St.Louis, USA), phosphate buffer (pH 7.0) and sample solution. After mixed well, α -glucosidase was added,

incubated at 37°C for 30 minutes, then Na₂CO₃ was added. The 4-Nitrophenyl α -D-glucopyranoside was measured at 400 nm wavelength with microplate reader. The inhibition percentage of α -glucosidase was calculated according to equation 1:

$$\% \text{ inhibition} = \frac{(C-S)}{C} \times 100$$

C : absorbance of control

S : absorbance of sample

β -glucosidase inhibitory activity assay

The β -glucosidase inhibitory activity assay was conducted using modified method.¹ Mixture solution contained 4-Nitrophenyl β -D-glucopyranoside (N7006, Sigma-Aldrich), phosphate buffer (pH 7.0) and sample solution. Mixture reaction was incubated at 37°C for 5 minutes, added with enzyme mixture and incubated for 15 minutes. Reaction was stopped by addition of Na₂CO₃. Absorbance of 4-Nitrophenyl β -D-glucopyranoside was measured at 400 nm wavelength. The inhibition percentage of β -glucosidase was calculated according to equation 1.

α -amylase inhibitory activity assay

The α -amylase inhibitory activity assay was performed using a modified method.¹⁶ Sample was introduced into sample well, dimethyl sulfoxide (DMSO) was used as a blank. Furthermore, α -amylase enzyme (A7595, Sigma-Aldrich) was added into each well, except for blank well. Then the mixture was incubated at 37°C for 10 minutes, and added with starch solution in each well, while control well was added with phosphate buffer. Another incubation at 37°C for 15 minutes was conducted. Enzymatic reaction was stopped by adding acidic iodine solution in each well. The absorbance was measured at 565 nm wavelength. The inhibition percentage of α -amylase was calculated according to equation 1.

Statistical analysis

Data was presented as mean \pm standard deviation. To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ were considered as statistically significant, along with Duncan Post-Hoc Test significant and 95% confidence interval. The median inhibitory concentration (IC₅₀) was measured to determine the inhibitory activities of α/β -glucosidase, α -amylase, according to linear regression. SPSS version 20.0 program was used for statistical analysis.

Results

α -glucosidase inhibitory activity

The α -glucosidase inhibitory of REE and Acarbose can be seen in Table 1 and Figure 1, and while the value of IC_{50} can be seen in Table 2.

α -glucosidase activity of REE and Acarbose occurred in concentration-dependent manner in which higher α -glucosidase inhibition present in higher concentration of sample. Figure 1 shows that at the highest concentration (37.5 μ g/mL), Acarbose generated higher inhibitory activity, compared with REE. The IC_{50} value of Acarbose 14.65 μ M or 9.45 μ g/mL was more active than REE 15.81 μ g/mL (Table 2). Inhibition of α -glucosidase is expected to normalize blood glucose level.¹⁷ Based on statistical analysis, both REE and Acarbose had significant α -glucosidase inhibition activity differences at each concentration, higher concentration increased the activity ($p<0.05$).

β -glucosidase inhibitory activity

The β -glucosidase is a carbohydrate hydrolizing enzyme associated with metabolic disorder such as DM. Inhibition of carbohydrate hydrolizing enzymes is used as therapeutic approach to decrease hyperglucose level and improve hyperglycemic.^{1,18} The β -glucosidase inhibitory of REE and Acarbose can be seen in Table 3 and Figure 2, the IC_{50} value can be seen in Table 4. The result of β -glucosidase activity in REE and Acarbose showed that inhibitory activity was associated with concentrations in which higher β -glucosidase inhibition present in higher concentration of

Table 1. Effect concentrations toward α -glucosidase inhibitory activity (%) of REE and Acarbose

Concentrations (μ g/mL or μ M)	Inhibitory Activity (%)	
	REE	Acarbose
37.5	72.68 \pm 1.56 ^e	81.49 \pm 1.19 ^e
12.5	46.38 \pm 2.72 ^d	44.94 \pm 0.54 ^d
6.25	41.63 \pm 0.74 ^c	38.21 \pm 0.32 ^c
3.125	38.79 \pm 0.26 ^b	34.86 \pm 0.43 ^a
1.563	33.16 \pm 1.00 ^a	32.82 \pm 0.83 ^a
0.781	32.48 \pm 1.05 ^a	32.44 \pm 2.82 ^a

The data are presented as mean \pm standard deviation. Different superscript small letters (a,b,c,d,e) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at $p<0.05$. Each treatment of samples was performed in triplicate.

sample. Inhibition percentage of Acarbose with percentage 50.97 \pm 3.78 was comparable with REE 47.34 \pm 2.69 (Table 3). As shown in Table 4, Acarbose was more active in inhibiting β -glucosidase, the IC_{50} value 34.96 μ M or 22.57 μ g/mL was lower compared to REE 41.77 μ g/mL. Statistical analysis showed REE and Acarbose had significant differences in β -glucosidases inhibition activity at each concentration, higher concentration increased β -glucosidase inhibition ($p<0.05$).

α -amylase inhibitory activity

Pancreatic α -amylase is an enzyme in the digestive system and catalyses the initial step in starch hydrolysis.¹⁹ Inhibition of intestinal α -amylase delays the starch and oligosaccharides degradation to monosaccharides prior to absorption, which later decreases the glucose absorption, as well as postprandial blood glucose level.^{20,21} The α -amylase activity showed inhibitory activity in concentration-dependent manner in which increasing concentrations generated higher inhibitory activity. Inhibitory activity was lower in REE compared to Acarbose (Table 5 & 6, Figure 3) with inhibition percentage 73.08 \pm 3.85 of REE while Acarbose 77.08 \pm 4.77. Acarbose was more active in inhibiting α -amylase, the IC_{50} value 5.64 μ M or 3.64 μ g/mL was more active compared to REE 18.09 μ g/mL. Based on statistical analysis, both REE and Acarbose had significant difference in α -amylases inhibition activity at each concentration, higher concentration increased α -amylase inhibition ($p<0.05$).

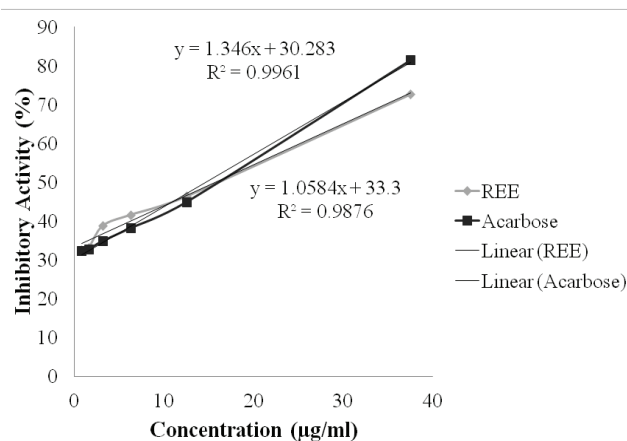


Figure 1. The α -glucosidase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 μ g/mL.

Table 2. The median inhibitory activity (IC₅₀) of α -glucosidase of REE and Acarbose

Sample	Equation	R ²	IC ₅₀ (μ g/mL/ μ M)	Average IC ₅₀ (μ g/mL/ μ M)
REE Test 1	Y = 1.0166x + 33.096	0.9866	16.63 μ g/mL	
REE Test 2	Y = 1.0296x + 33.828	0.9845	15.71 μ g/mL	
REE Test 3	Y = 1.1288x + 32.976	0.9802	15.08 μ g/mL	
Average	y = 1.0584x + 33.3	0.9876	15.77 μ g/mL	15.81 \pm 0.78 μ g/mL
Acarbose Test 1	Y = 1.3975x + 29.574	0.9977	14.62 μ M	
			(9.43 μ g/mL)	
Acarbose Test 2	Y = 1.3267x + 31.097	0.9870	14.25 μ M	
			(9.43 μ g/mL)	
Acarbose Test 3	Y = 1.3139x + 30.179	0.9955	15.09 μ M	
			(9.74 μ g/mL)	
Average	y = 1.346X + 30.283	0.9961	14.64 μ M	14.65 \pm 0.42 μ M
			(9.45 μ g/mL)	

The IC₅₀ value was presented as mean \pm standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

Table 3. Effect concentrations toward β -glucosidase inhibitory activity (%) of REE and Acarbose

Concentrations (μ g/mL or μ M)	Inhibitory Activity	
	REE	Acarbose
37.5	47.34 \pm 2.69 ^d	50.97 \pm 3.78 ^c
12.5	23.47 \pm 1.49 ^c	32.54 \pm 3.17 ^d
6.25	21.66 \pm 0.62 ^{bc}	23.81 \pm 0.23 ^c
3.125	19.56 \pm 0.99 ^{ab}	22.35 \pm 1.06 ^c
1.563	16.92 \pm 3.32 ^a	15.60 \pm 1.00 ^b
0.781	16.39 \pm 0.85 ^a	10.60 \pm 1.93 ^a

The data are presented as mean \pm standard deviation. Different superscript small letters (^{a,ab,b,bc,c,d,e}) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at $p < 0.05$. Each treatment of samples was performed in triplicate.

Discussion

The result of present study showed that REE possessed antidiabetic activity, based on inhibitory activity of α -/ β -glucosidase and α -amilase. These results indicate the antidiabetic properties of REE was lower than Acarbose. The α -/ β -glucosidase are carbohydrate hydrolyzing enzymes that take place in metabolic disorder such as DM.

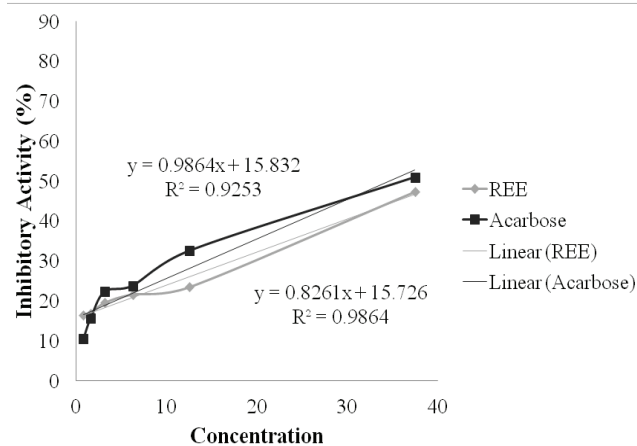


Figure 2. The β -glucosidase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 μ g/mL.

Glucosidase cleaves the glycosidic bond that release glucose from the non-reducing end of an oligo- or poly-saccharide chain involved in glycoprotein biosynthesis.²² Inhibition of carbohydrate hydrolyzing enzymes is therefore used as therapeutic approach to decrease hyperglycemia.²³ This data is in accordance with previous study that the aqueous extract of roselle has the inhibitory activity of α -glucosidase as well as lowering blood glucose levels in DM animal models.²⁴

Table 4. The median inhibitory activity (IC₅₀) of β -glucosidase of REE and Acarbose

Sample	Equation	R ²	IC ₅₀ (μ g/mL/ μ M)	Average IC ₅₀ (μ g/mL/ μ M)
REE Test 1	Y = 0.9265x + 14.298	0.9722	38.53 μ g/mL	
REE Test 2	Y = 0.8285x + 15.843	0.9652	41.23 μ g/mL	
REE Test 3	Y = 0.7235x + 17.036	0.9944	45.56 μ g/mL	
Average	y = 0.8264x + 15.726	0.9864	41.48 μ g/mL	41.77 \pm 3.55 μ g/mL
Acarbose Test 1	Y = 1.0059x + 16.285	0.9240	33.52 μ M (21.64 μ g/mL)	
Acarbose Test 2	Y = 1.0905x + 15.473	0.9075	31.66 μ M (20.43 μ g/mL)	
Acarbose Test 3	Y = 0.8627x + 15.738	0.9381	39.71 μ M (25.63 μ g/mL)	
Average	y = 0.9864x + 15.832	0.9253	34.63 μ M (22.35 μ g/mL)	34.96 \pm 4.22 μ M (22.57 μ g/mL)

The IC₅₀ value was presented as mean \pm standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

Table 5. Effect concentrations toward α -amylase inhibitory activity (%) of REE and Acarbose

Concentrations (μ g/mL or μ M)	Inhibitory Activity	
	REE	Acarbose
34.29	73.08 \pm 3.85 ^c	77.08 \pm 4.77 ^d
11.43	41.88 \pm 6.45 ^b	58.82 \pm 5.88 ^c
5.71	38.00 \pm 4.00 ^b	55.83 \pm 3.82 ^c
2.86	25.49 \pm 3.92 ^a	52.67 \pm 4.16 ^c
1.43	22.42 \pm 2.78 ^a	43.86 \pm 1.75 ^b
0.71	20.24 \pm 2.73 ^a	35.45 \pm 0.92 ^a

The data are presented as mean \pm standard deviation. Different superscript small letters (^{a,b,c,d}) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at $p < 0.05$. Each treatment of samples was performed in triplicate.

Roselle ethanol extract reduced the blood glucose levels on diabetic mice was comparable with n-hexane and ethyl acetate extract of roselle.⁶ The ability might be due to presence of dietary phytochemicals in roselle such as anthocyanins, flavonoids and phenolic acids.²⁵ Roselle also has variety content bioflavonoid such as prunin, myricitrin, 6-hydroxyl-flavonoids, 6-hydroxyluteolin, apigenin, and luteolin, that can lower blood glucose, and have inhibitory

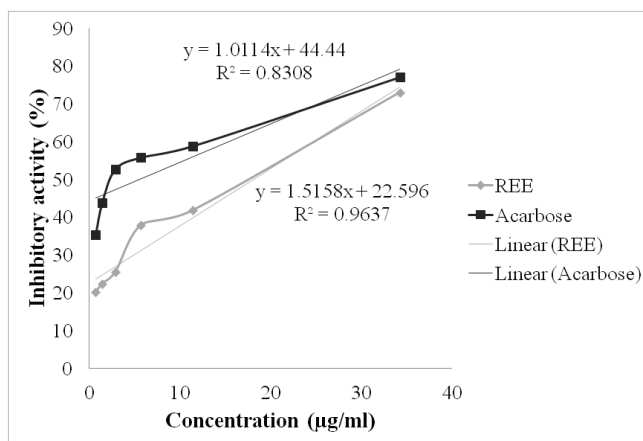


Figure 3. The α -amylase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 μ g/mL.

activity of α -glucosidase.²⁴ Flavonoid contents of REE are flavonols (gossypetin) and the anthocyanins.²⁵ Flavonoid and isoflavonoid glycosides are found in human small intestine, in which α - and β -glucosidase inhibitory present to inhibit carbohydrate catalyses²⁶ that suggests contribution of flavonoid content as antidiabetic effects.

In this study, REE has α -amylase inhibitory activity. Inhibition of α -amylase will promote retardation of starch hydrolysis, that reduces digestion and absorption rate

Table 6. The median inhibitory activity (IC₅₀) of α -amylase of REE and Acarbose

Sample	Equation	R ²	IC ₅₀ (μ g/mL/ μ M)	Average IC ₅₀ (μ g/mL/ μ M)
REE Test 1	Y = 1.5748x + 22.019	0.9858	17.77 μ g/mL	
REE Test 2	Y = 1.4414x + 23.607	0.8631	18.31 μ g/mL	
REE Test 3	Y = 1.5512x + 22.161	0.9454	18.18 μ g/mL	
Average	Y = 1.5158x + 22.596	0.9637	18.07 μ g/mL	18.09 \pm 0.28 μ g/mL
Acarbose Test 1	Y = 0.8853x + 42.773	0.8291	8.16 μ M (5.26 μ g/mL)	
Acarbose Test 2	Y = 1.0349x + 46.243	0.7196	3.63 μ M 2.10 μ g/mL	
Acarbose Test 3	Y = 1.1141x + 44.305	0.8909	5.11 μ M (3.29 μ g/mL)	
Average	Y = 1.0114x + 44.44	0.8308	5.49 μ M (3.54 μ g/mL)	5.64 \pm 2.31 μ M (3.64 μ g/mL)

The IC₅₀ value was presented as mean \pm standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

of carbohydrates, resulting in decreased post-prandial hyperglycemia.¹⁵ Flavanoids content in roselle play key role in α -amylase inhibitory activity.²⁷ Inhibition of α -amylase preserve β -cell integrity and function by removing free radicals could enhance protection against the progression of insulin resistance in type 2 DM.²⁸ Previous studies also showed that roselle extract has potency to inhibit α -amylase activity, sugars and starch absorption, which may assist in weight loss.²⁹

Flavonoids present in roselle, are well known to contain medicinal properties such as antiinflammatory, antioxidant and antidiabetic.^{30,31} Phenolic and flavonoids content in roselle are suggested to play role in inhibitory activity of both α -amylase and α -/ β -glucosidase.^{32,33} Interactions between chemical compound of REE and enzymes causes alteration in the enzyme's molecular configuration, as well as hydrophilic and hydrophobic properties, that will decrease enzyme activities.¹⁰ Active components of REE compete with the substrate in binding to active site of enzyme prevent the oligosaccharides breakdown to disaccharides.³⁴

Conclusion

REE is a promising antidiabetic agent to inhibit α -/ β -glucosidase and α -amylase. Further researches regarding

identification and development of bioactive compounds contained in roselle, are encouraged.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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References

1. Widowati W, Maesaroh M, Fauziah N, Erawijantari PP, Sandra F. Free Radical Scavenging and Alpha/Beta-Glucosidase Inhibitory Activities of Rambutan (*Nephelium lappaceum* L.) Peel Extract. *Indones Biomed J.* 2015; 7(3): 157-62.
2. Cheplick S, Kwon YI, Bhowmik P, Shetty K. Phenolic Linked Variation in Strawberry Cultivars for Potential Dietary Management of Hyperglycemia and Related Complications of Hypertension. *Bioresour Technol.* 2010; 101(1): 404-13.
3. Manaharan T, Palanisamy UD, Ming CH. Tropical Plant Extracts as

- Potential Antihyperglycemic Agents. *Molecules*. 2012; 17(5): 5915-23.
4. Al-Zuhair S, Dowaidar A, Kamal H. Inhibitory Effect of Dates-Extract on α -Amylase and α -Glucosidase. *J Biochem Tech*. 2010; 2(2).
 5. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, *et al*. Natural Compounds for Cancer Treatment and Prevention. *Pharmacol Res*. 2009; 59(6): 365-78.
 6. Rosemary R, Rosidah R, Haro G. Antidiabetic Effect of Roselle Calyces Extract (*Hibiscus sabdariffa* L.) in Streptozotocin Induced Mice. *Int J PharmTech Research*. 2014; 6(5): 1703-11.
 7. Tzu L, Hui H, Chang C, Ming C, Ming C, Chau J. *Hibiscus sabdariffa* L. Extract Reduces Serum Cholesterol in Men and Women. *Nutr Res*. 2007; 27: 140-5.
 8. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou TH. Phytochemical Analysis, Purification and Identification of *Hibiscus* Anthocyanins. *J Pharm Chem Biol Sci*. 2015; 3(2): 156-68.
 9. Peng CH, Chyau CC, Chan KC, Chan TH, Wang CJ, Huang CN. *Hibiscus sabdariffa* Polyphenolic Extract Inhibits Hyperglycemia, Hyperlipidemia, and Glycation-Oxidative Stress while Improving Insulin Resistance. *J Agric Food Chem*. 2011; 59(18): 9901-9.
 10. Adisakwattana S, Ruengsamran T, Kampa P, Sompong W. In Vitro Inhibitory Effects of Plant-Based Foods and Their Combinations on Intestinal α -Glucosidase and Pancreatic α -Amylase. *BMC Complement Altern Med*. 2012; 12: 1-8.
 11. Widowati W, Widyanto RM, Laksmiawati DR, Erawijantari PP, Wijaya L, Sandra F. Phytochemical, Free Radical Scavenging and Cytotoxic Assay of *Cucumis Melo* L. Extract and β -Carotene. *J Advanced Agricultur Technol*. 2015; 2(2).
 12. Widowati W, Mofez T, Risdian C, Ratnawati H, Tjahyani S, Sandra F. Apoptosis and Antioxidant Activities of *Catharanthus roseus* [L] G. Don Extract on Breast Cancer Cell Line. *Indones J Cancer Chemoprevent*. 2010; 1(2): 99-107.
 13. Widowati W, Wijaya L, Wargasetia T, Yellianty Y, Laksmiawati D. Antioxidant, Anticancer and Apoptosis-Inducing Effects of Piper Extracts in HeLa Cells. *J. Exp Integr Med*. 2013; 3(3): 225-30.
 14. Widowati W, Ratnawati H, Retnaningsih C, Lindayani L, Rusdi D, Winarno W. Free Radical Scavenging and α -Glucosidase Inhibitor Activity of *Mucuna pruriens* L. *JFI*. 2011; 5(3): 117-24.
 15. Soeng S, Evacuasiy E, Widowati W, Fauziah N. Antioxidant and Hypoglycemic Activities of Extract and Fractions of Rambutan Seeds (*Nephelium lappaceum* L.). *Biomed Eng*. 2015; 1(1): 13-8.
 16. Bhutkar MA, Bhise SB. In Vitro Assay of Alpha Amylase Inhibitory Activity of Some Indigenous Plants. *Int J Chem Sci*. 2012; 10(1): 457-62.
 17. Adnyana K, Abuzaid AS, Iskandar EY, Kurniati NF. Pancreatic Lipase and α -Amylase Inhibitory Potential of Mangosteen (*Garcinia mangostana* Linn) Pericarp Extract. *Int J Med Res Health Sci*. 2015; 4(4): 796-8.
 18. Elya B, Basah K, Mun'im A, Yuliasuti W, Bangun A, Septiana EK. Screening of α -Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae and Rubiaceae. *J Biomed Biotechnol*. 2012; 2012: 1-6.
 19. Wu C, Shen J, He P, Chen Y, Li L, Zhang L, *et al*. The α -Glucosidase Inhibiting Isoflavones Isolated from *Belamcanda chinensis* Leaf Extract. *Rec Nat Prod*. 2012; 6(2): 110-20.
 20. Sudha P, Smita S, Shobha Y, Ameeta R. Potent Alpha-Amylase Inhibitory Activity of Indian Ayurvedic Medicinal. *BMC Complement Altern Med*. 2011; 11(5): 1-10.
 21. Karthic K, Kirthiram K, Sadasivam S, Thayumanavan B. Identification of α -Amylase Inhibitors from *Syzygium cumini* Linn Seeds. *Indian J Experiment Biolog*. 2008; 46(9): 677-80.
 22. Sangeetha R, Vedesree N. In Vitro α -Amylase Inhibitory Activity of the Leaves of *Thespesia populnea*. *ISRN Pharmacol*. 2012; 2012: 1-4.
 23. de Melo EB, Gomes AdS, Carvalho I. α - and β -Glucosidase Inhibitors: Chemical Structure. *Tetrahedron*. 2006; 62(44): 10277-302.
 24. Palanisamy U, Manaharan T, Teng L, Radhakrishnan A, Subramainiam T, Masilamani T. Rambutan Rind in the Management of Hyperglycemia. *Food Res Inter*. 2011; 44(7): 2278-82.
 25. Wang SC, Lee SF, Wang CJ, Lee CH, Lee WC, Lee HJ. Aqueous Extract from *Hibiscus sabdariffa* Linnaeus Ameliorate Diabetic Nephropathy via Regulating Oxidative Status and Akt/Bad/14-3-3y in an Experimental Animal Model. *Evid Based Complement Alternat Med*. 2011; 2011: 1-9.
 26. Formagio A, Ramos D, Vieira M, Ramalho S, Silva M, Zarate N, *et al*. Phenolic Compounds of *Hibiscus sabdariffa* and Influence of Organic Residues on Its Antioxidant and Antitumoral Properties. *Braz J Biol*. 2015; 75(1): 69-76.
 27. Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJC, Morgan MRA, *et al*. Deglycosylation of Flavonoid and Isoflavonoid Glycosides by Human Small Intestine and Liver β -Glucosidase Activity. *FEBS J*. 1998; 436(1): 71-5.
 28. Kwon YI, Apostolidis E, Shetty K. Evaluation of Pepper (*Capsicum annum*) for Management of Diabetes and Hypertension. *J Food Biochem*. 2007; 31(3): 370-85.
 29. Sama K, Murugesan K, Sivaraj R. In Vitro Alpha Amylase and Alpha Glucosidase Inhibition Activity of Crude Ethanol Extract of *Cissus arnottiana*. *Asia J Plant Sci Res*. 2012; 2(4): 550-3.
 30. Apostolidis E, Kwon YI, Shetty K. Inhibitory Potential of Herb, Fruit, and Fungal-Enriched Cheese Against Key Enzymes Linked to Type 2 Diabetes and Hypertension. *Innov Food Sci Emerg Technol*. 2007; 8(1): 46-54.
 31. Humadi SS, Istudor V. Quantitative Analysis of Bio-Active Compound in *Hibiscus sabdariffa* L. Extracts. *Note I Quantitative Analysis of Flavonoids*. *Farmacia*. 2008; 56(6): 699-707.
 32. Mungole A, Chaturvedi A. *Hibiscus sabdariffa* L. a Rich Source of Secondary Metabolites. *Int J Pharm Sci Rev Res*. 2011; 6(1): 83-7.
 33. Mai T, Thu N, Tien P, Van Chuyen N. Alpha-Glucosidase Inhibitory and Antioxidant Activities of Vietnamese Edible Plants and Their Relationships with Polyphenol Contents. *J Nutr Sci Vitaminol*. 2007; 53(3): 267-76.
 34. Ramkumar K, Thayumanavan B, Palvannan T, Rajaguru P. Inhibitory Effect of *Gymnema montanum* Leaves on α -Glucosidase Activity and α -Amylase Activity and Their Relationship with Polyphenolic Content. *Med Chem Res*. 2010; 19(8): 948-61.
 35. Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, *et al*. Yeast Alpha Glucosidase Inhibitory and Antioxidant Activities of Six Medicinal Plants Collected in Phalaborwa, South Africa. *S Afr J Bot*. 2010; 76(3): 465-70.