INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease caused by an increase in blood glucose levels due to insufficient insulin production in the pancreas or because the body cells experience insulin resistance. The risk of death of individuals with T2DM is twice that of individuals who do not suffer from T2DM [1]. In 2014, there were 422 million people worldwide experiencing T2DM, and this amount is expected to increase to 2045 by 629 million people [2]. To date, the treatment of patients with T2DM has been very challenging. The administration of insulin and drugs such as sulfonylurea, metformin, and meglitinide apparently still has side effects such as hypoglycemia, fever, nausea, vomiting, diarrhea, and decreased appetite [3].

Treatments with medicines sourced from plant ingredients, often called herbal medicines, has become an alternative in reducing the side effects of using conventional medicines. Herbal medicines are proven to have antidiabetic activity and therapeutic properties in traditional treatment systems. In addition, herbs are easy to find, easy to process, and have no side effects [4]. Coccinia grandis (L.) Voigt is a plant that has long been used as an antidiabetic. The flavonoids and phenolics contained in the Coccinia grandis (L.) Voigt plant show the ability to inhibit the activity of α-amylase and α-glucosidase enzymes, regenerate β cells in the pancreas, and have been shown to reduce blood glucose levels in diabetic rats [5-7]. Recently, there have been many studies on combinations of plants that have the same properties to be used as healthy beverages because they are more practical and more economical, for example, a combination of Limonium algarvense and Camellia sinensis L. [8] and a combination of C. sinensis black tea and Averrhoa bilimbi L. [9]. These combinations were proven to increase the activity of individual plants.

Averrhoa bilimbi L. is a plant that has traditionally been used to treat several diseases such as sore throat, eye pain, toothache, scabies, fever, hyperdipsia, and diabetes mellitus [10]. The phytochemical content in Averrhoa bilimbi L. includes flavonoids, steroids, triterpenoids, glycosides, proteins, fats, and vitamins A, B, and C [9]. In streptozotocin-induced rats, Averrhoa bilimbi L. extract showed significant results in reducing blood glucose levels [11].

Based on the literature search, to date, the combination of Coccinia grandis (L.) Voigt leaves and Averrhoa bilimbi L. fruits as an antidiabetic health beverage has never been reported. This research was conducted to make water extracts from the combination of Coccinia grandis (L.) Voigt leaves and Averrhoa bilimbi L. fruits at various mass ratios and various sample masses. The resulted extracts were then tested for antidiabetic activities in vitro.

MATERIALS AND METHODS

Materials

Coccinia grandis (L.) Voigt leaves and Averrhoa bilimbi L. fruits were harvested from North Kuta, Bali, Indonesia, in February 2019. The leaves and fruits were identified in Pharmacognosy Laboratory, Pharmaceutical Biology Department, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. ethanol (96%), methanol, aluminum chloride, potassium acetate, and phosphate buffer pH 7.4 were purchased from Merck. Quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-amylase, potassium iodate, starch, gallic acid, Folin-Ciocalteu reagent, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Sigma Aldrich. Acarbose (Glucobay) was purchased from PT. Bayer Indonesia.

Preparation of combined healthy beverages

Coccinia grandis (L.) Voigt leaves and Averrhoa bilimbi L. fruits were washed and dried for 24 h at room temperature. The leaves and fruits were cut into small pieces and then dried using an oven at 50°C for
Table 1: Total flavonoid content of healthy beverages at various mass ratios and amount of powdered plant samples

| Samples     | Ratio | Total flavonoid content (mg QE/g d.m.) |
|-------------|-------|----------------------------------------|
|             |       | 1 g                                   | 2 g                                   | 3 g                                   | 4 g                                   | 5 g                                   |
| GG          | 1:0   | 134.91±2.63<sup>a</sup>                | 115.56±2.60<sup>a</sup>               | 117.33±2.23<sup>a</sup>               | 108.40±7.22<sup>ab</sup>              | 91.66±3.22<sup>ab</sup>               |
| GG+AB       | 3:1   | 93.28±3.05<sup>b</sup>                 | 98.46±1.44<sup>b</sup>                | 93.56±4.73<sup>ab</sup>               | 87.45±1.64<sup>ab</sup>               | 88.04±0.70<sup>ab</sup>               |
| GG+AB       | 1:1   | 98.00±9.16<sup>f</sup>                 | 74.09±2.74<sup>bc</sup>               | 61.47±5.21<sup>bc</sup>               | 99.46±3.90<sup>f</sup>                | 82.68±2.05<sup>bc</sup>               |
| GG+AB       | 1:3   | 135.14±0.18<sup>b</sup>                | 75.18±2.32<sup>bc</sup>               | 90.64±0.38<sup>bc</sup>               | 57.11±0.60<sup>b</sup>                | 50.50±0.48<sup>b</sup>                |
| AB          | 0:1   | 12.34±5.13<sup>b</sup>                 | 67.21±2.13<sup>bc</sup>               | 79.04±2.90<sup>bc</sup>               | 103.32±2.27<sup>ab</sup>              | 86.19±0.128<sup>ab</sup>              |

Total flavonoid content values were represented in the mean±standard error of the mean (n=3). Different letters indicate significant difference based on Tukey test (p<0.05). CG: Coccinia grandis L. Voigt; AB: Avicennia alba L. QE: Quercetin equivalent, d.m.: Dry matter.

The determination of α-amylase inhibitory activity

The determination of total flavonoid content (TFC)

The determination of total phenolic content (TPC)

The determination of DPPH antioxidant activity

Antioxidant activity test was performed based on Attanayake et al. [12] using DPPH solution. The sample (20 µl) was diluted with 480 µl of distilled water and added with 200 µl of DPPH solution in 0.004% ethanol. The mixture was shaken strongly, then allowed to stand at room temperature for 30 min. Ethanol was used as a blank sample. The absorbance of the sample was measured at a wavelength of 765 nm. The resulting mixture was incubated at 27°C for 30 min. The absorbance was measured using a UV-visible spectrometer (Thermo Scientific Genesys 10 UV-Vis) at 415 nm. The flavonoid content was calculated using standard calibration of quercetin solution in the range of 0–25 µg (y=0.0092x+0.003, R²=0.9979). The results are expressed as quercetin equivalent micrograms (mg QE)/g dry matter.

The determination of α-amylase inhibitory activity

The determination of total flavonoid content

A total of 30 µl of the sample was mixed with 50 µl (400 µl/ml) of starch in phosphate buffer (0.25 M; pH 7.0). After 30 min being incubated at 37°C, 10 µl α-amylase (50 µg/ml in phosphate buffer) was added to the mixture, then followed by adding 10 µl of 0.25 M phosphate buffer. Afterward, 50 µl of 0.1 N iodine solution and 1300 µl of distilled water were added to the mixture. Absorbance measurement using a UV-visible spectrometer was performed at a wavelength of 660 nm. All tests were carried out in triplicate. Similar to the DPPH antioxidant activity, the α-amylase inhibitory activity was also expressed as the mean±SEM of the inhibition concentration (IC<sub>50</sub>).

Data analysis

All research data are expressed as the mean±SEM, and all experiments were carried out in triplicate. Significant differences were assessed by the normality test, followed by the analysis of variance (ANOVA) test. If there were significant differences when the value of p<0.05, the test was followed by the Tukey test. The statistical analysis was performed using SPSS v.24 statistical analysis (IBM).

RESULTS AND DISCUSSION

TFC

Flavonoids are secondary metabolites of plants that function as antioxidants to ward off free radicals [15]. The addition of AlCl<sub>3</sub> solution in the experiment was intended to form a complex compound that causes a shift in the wavelength toward the visible. It was marked by a change in the color of the sample from light yellow to a brighter yellow [16]. The intensity of the color formed was then measured using a UV-visible spectrometer at a wavelength of 415 nm.

Table 1 shows the results of TFC of water extracts at various combination ratios and total sample masses. Among all combinations, the sample that had the highest total flavonoid was CG+AB with a ratio of 1:3 (135.14±0.18 mg QE/g d.m.). In general, the addition of AB in the combination samples could increase the TFC. Flavonoid is thermally stable with time and temperature so that during high temperature in the decocation process, it did not cause unfavorable degradation and contributed well to CG [17]. The results of the analysis using ANOVA (p<0.05) showed significantly different values for each sample. The results of further tests using the Tukey test showed that each sample had a significant difference in total flavonoids.

The TFC values in this study varied. The higher the total mass of powdered samples, the lower the total flavonoid they had. When extracting 1 g of sample powder using 100 ml of water, it was found that the flavonoid compound which escaped from the sample was completely absorbed by water. This happens because of the balance between the substances that come out and the amount of water added. When the mass of the powdered sample increased and the water used to extract it remained, reabsorption of flavonoid substances occurred. The pharmacokinetic activity of flavonoids is closely related to its functional groups so that it arises from other chemical components present in the extract, resulting in total flavonoid instability at each concentration [16]. The choice of solvent greatly influenced the flavonoid content in the samples. Solvents such as methanol and ethanol have been more widely used for the extraction of phenolics and flavonoids from plants compared to...
Table 2: Total phenolic content of healthy beverages at various mass ratios and amount of powdered plant samples

| Samples  | Ratio | Total phenolic content (mg GAE/g d.m.) |
|----------|-------|---------------------------------------|
|          |       | 1 g                                   |
| CG       | 1:0   | 41.52±3.68                            |
|          |       | 2 g                                   |
|          | 3 g   | 17.36±1.40                            |
|          | 4 g   | 13.45±0.33                            |
|          | 5 g   | 13.13±1.14                            |
| CG+AB    | 3:1   | 24.89±1.70                            |
|          |       | 15.78±0.49                            |
|          | 16.84±0.23                            |
|          |       | 14.29±0.93                            |
|          | 15.66±1.45                            |
| CG+AB    | 1:1   | 32.82±0.20                            |
|          |       | 21.37±2.35                            |
|          | 24.68±0.46                            |
|          |       | 22.04±1.06                            |
|          | 20.42±0.89                            |
| CG+AB    | 1:3   | 48.48±3.09                            |
|          |       | 27.08±1.14                            |
|          | 17.60±0.23                            |
|          |       | 15.11±0.04                            |
|          | 15.08±0.03                            |
| AB       | 0:1   | 24.75±1.86                            |
|          |       | 27.08±1.14                            |
|          | 17.74±0.88                            |
|          |       | 23.19±0.25                            |
|          | 20.42±0.89                            |

Total phenolic content values were represented in the mean±standard error of the mean (n=3). Different letters indicate a significant difference based on Tukey test (p<0.05). CG: Coccinia grandis; Voigt; AB: Averrhoa bilimbi; L.; GAE: Gallic acid equivalent; d.m.: Dry matter.

Table 3: Antioxidant and inhibition of α-amylase activity values of healthy beverages at various mass ratios

| Samples  | Ratio | Antioxidant activity, IC_{50} (mg/ml) | Inhibition of α-amylase, IC_{50} (mg/ml) |
|----------|-------|-------------------------------------|----------------------------------------|
| CG       | 1:0   | 8.04±0.03                           |
|          |       | 0.40±0.03                           |
| CG+AB    | 3:1   | 4.45±0.45                           |
|          |       | 2.73±0.30                           |
| CG+AB    | 1:1   | 6.18±0.66                           |
|          |       | 1.09±0.26                           |
| CG+AB    | 1:3   | 7.60±0.79                           |
|          |       | 1.50±0.37                           |
| AB       | 0:1   | 4.00±0.08                           |
|          |       | 0.52±0.25                           |
| Acarbose  | -     | -                                   |
|          |       | 3.86±0.07                           |

IC_{50} values were represented in the mean±standard error of the mean (n=3). Different letters indicate a significant difference based on Tukey test (p<0.05). CG: Coccinia grandis; Voigt; AB: Averrhoa bilimbi; L.; C_{50}: Inhibitory concentration.

TPC Phenolic is a group of bioactive compounds that are found in plants and have free radical scavenging activity [21]. The determination of TPC in the sample was done using a Folin-Ciocalteu solution and added with sodium carbonate so that it changes color from yellow to blue. The blue discoloration is caused by the formation of phenolic ions in the sample solution. Phenolate ions are only present in base solutions, whereas Folin-Ciocalteu reagents and products produced are unstable under alkaline conditions [22]. The blue color formed is more concentrated, which is equivalent to the concentration of phenolic ions formed. This means that the greater the concentration of phenolic compounds, the more phenolic ions that reduce heteropoly acid so that the resulting blue color becomes more concentrated [23].

The results of the TPC test are shown in Table 2. The TPC of CG+AB (1 g) with a ratio of 1:3 was higher than that of individual extracts (CG and AB). The increase was caused by an increase in AB mass, which contributed to the addition of phenolic amounts in CG+AB. In addition, the stability of phenolic compounds in CG+AB (1:3) also caused an increase in total phenolic levels. Phenolic compounds are less degraded at high temperatures or long extraction times [17]. Increased extraction temperatures can increase the solubility of phenolic compounds and increase the rate of mass transfer [18]. Maizura et al. reported that the combination of Polygonum minus and Zingiber officinali had a TPC of 132.0 mg GAE/g extract, which shows the potential as significant free radical scavengers [21]. Similar to total flavonoids, total phenolic levels in this study also declined with increasing mass of plant powder samples. The mechanism of phenolic readsoption back to the plant matrix is one thing responsible for this phenomenon. Statistical analysis using ANOVA (p<0.05) showed significantly different values for each sample. The results of further tests using the Tukey test showed that each sample had a total phenolic difference.

DPPH antioxidant activity The antioxidant can delay or inhibit cell damage from free radicals that exist in biological systems or that are produced in various metabolic processes. In addition, antioxidants play a central role in health care to prevent various chronic diseases as well as premature aging [24]. The results of the antioxidant activity assay showed that all extracts have the ability to inhibit DPPH free radicals (Table 3). The ability of the extracts to scavenge DPPH free radicals was characterized by fading the purple-colored solution [25]. This color fading decreased the absorbance value of visible light on UV-visible spectrometers. The lower the absorbance value means, the higher the antioxidant activity possessed.

Table 3 shows that the CG+AB with a ratio of 3:1, 1:1, and 1:3 had antioxidant activity (IC_{50}) of 4.45±0.45, 6.18±0.66, and 7.60±.07 mg/ml, respectively. These IC_{50} values are smaller (stronger antioxidant activity) compared to individual CG samples, which are 8.04±0.03 mg/ml. This indicates that the addition of AB to CG can increase antioxidant activity, with the best combination being at a ratio of 3:1. Statistical analysis using ANOVA (p<0.05) showed significantly different values for each sample. The results of further tests using the Tukey test showed that some samples had differences in antioxidant activity.

The antioxidant potential of plant extracts is influenced by phenolic and flavonoid compounds. Indeed, phenolic can directly reduce oxidative stress [26]. It has been reported that phenolic and flavonoid compounds have a high scavenging ability associated with the hydroxyl groups present in these compounds [27]. Antioxidant activity is used to prevent chronic diseases by protecting organisms from the production of reactive oxygen species. Thus, it is important to replace synthetic antioxidants with natural antioxidants because of its lower toxicity and side effects [28]. Nedamani et al. showed that the combination of green tea (C. sinensis), rosemary (Rosmarinus officinalis), and oak fruit (Quercus brant) extracts had better activity than butylated hydroxytoluene. It was also reported that there was a direct relationship between total phenolic and antioxidant activity of combined extracts [29].

α-Amylase inhibitory activity Inhibiting the activity of enzymes that hydrolyze carbohydrates such as α-amylase is an efficient way to reduce postprandial blood glucose levels [30]. Inhibition of these enzymes is very important in overcoming hyperglycemia associated with T2DM [31]. Inhibition of α-amylase contributes to improving the symptoms of T2DM and delays glucose absorption due to the slow digestion of starch [32]. Drugs such as acarbose, miglitol, and voglibose have the ability to inhibit the activity of the α-amylase enzyme. However, the side effects produced by these synthetic drugs are very disturbing [33].

The results of this study indicate that all extracts have the ability to inhibit the performance of the α-amylase enzyme, as shown in Table 3. The inhibition of α-amylase activity of CG extract...
was greater [IC_{50}=0.40±0.03 mg/ml] than that of AB extract [IC_{50}=0.52±0.253 mg/ml]. Meanwhile, GG+AB extracts with ratios of 3:1, 1:1, and 1:3 had IC_{50} values of 2.73±0.30, 1.09±0.26, and 1.50±0.37 mg/ml, respectively. Among all these combinations, the sample that had the highest α-amylase enzyme inhibitory activity was GG+AB (1:1) with an IC_{50} value of 1.09±0.26 mg/ml. The flavonoid and phenolic contents found in GG and AB have the potential to inhibit the activity of the α-amylase enzyme in diabetic rats [34,35]. Flavonoid and phenolic compounds present in the GG and AB also act as antioxidants in protecting pancreatic cells from oxidation damage and are able to regenerate damaged beta cells of the pancreas so that they can function properly [5,11]. In addition to flavonoids, these compounds play a role in improving glucose tolerance, stimulating glucose uptake in peripheral tissues, and regulating the activity of enzymes involved in carbohydrate metabolism [36]. The results of the analysis using ANOVA (p<0.05) showed significantly different values for each sample, which then were confirmed by the Tukey test.

This study used acarbose as a positive control with an IC_{50} value of 3.86±0.07 mg/ml. This shows that the combination samples had better α-amylase inhibitory activity compared to positive control and could be used as innovative healthy beverages for the treatment of T2DM. Contrary to research by Rodrigues et al., health drinks combined with sealavender and green tea did not show an α-amylase inhibitory activity but had a better α-glucosidase enzyme inhibiting activity compared to acarbose [8].

CONCLUSION

In this study, the combination of Coccinia grandid (L.) Voigt leaves and Averrhoa bilimbi L. fruit showed good properties as healthy beverages. The combination samples showed higher total flavonoid and total phenolic values compared to individual samples. Antioxidant activity and α-amylase inhibition tests showed that the combination samples have excellent potential as anti diabetic healthy beverages. This research can be used as a basis for further research in the framework of the exploration of plants that have efficacy as anti diabetic. Further testing on animals is highly recommended to understand the mechanisms of the action of these water extracts.

ACKNOWLEDGMENT

The authors would like to thank the Science and Health Laboratory, University of Dhyana Pura, for providing the facilities to carry out the research.

AUTHORS’ CONTRIBUTIONS

I Made Wisnu Adhi Putra conceived the research, provided the methods, and authored the manuscript. Olyn Tien Ate conducted experiment in the laboratory and analyzed the obtained data. I Gusti Ayu Wita Kusumawati and Ni Wayan Nursini analyzed the obtained data and authored the manuscript.

CONFLICTS OF INTEREST

All authors have none to declare.

FUNDING

This research received financial assistance from The Research and Community Service Institution, University of Dhyana Pum, through the Hibah Unggulan Perguruan Tinggi (Higher Education Excellent Grants) 2019 with contract number: 036/UNDHIRA-LP2M/PN/2019.

REFERENCES

1. Upadhyay J, Polyzos SA, Perakakis N, Thakkar B, Paschou SA, Katsiki N, et al. Pharmacotherapy of Type 2 diabetes: An update. Metabolism 2018;78:13-42.
2. Assaad Khalil SI, Megallas MH, Rohoma KH, Ismail H, AbouSeif M, Kharbouh I, et al. Prevalence of Type 2 diabetes mellitus in a sample of the adult population of Alexandria, Egypt. Diabetes Res Clin Pract 2018;144:63-73.
3. Gómez-Huelgas R, Gómez Peralta F, Rodriguez Mañas L, Formiga F, Pujol Domingo M, Mediavilla Bas M. Treatment of Type 2 diabetes mellitus in elderly patients. Rev Clin Esp 2018;218:74-88.
4. Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants used for diabetes mellitus. J Acute Dis 2013;2:196-200.
5. Attanayake AP, Jayatilaka KA, Pathirana C, Mudduwa LK. Antihyperglycemic activity of Coccinia grandid (L.) Voigt in streptozotocin induced diabetic rats. Indian J Tradit Knowl 2015;14:376-81.
6. Waisundara VY, Watawana MI, Jayawardena N. Costus speciosus and Coccinia grandid: Traditional medicinal remedies for diabetes. J Sri L J Biol 2015;9:1-5.
7. Mohammed SI, Chopda MZ, Patil RH, Vishwakarma K, Maheshwari VL. In vivo anti diabetic and antioxidant activities of Coccinia grandid leaf extract against streptozotocin induced diabetes in experimental rats. Asian Pac J Trop Dis 2016;6:298-304.
8. Rodrigues MJ, Oliveira M, Neves V, Ovelheiro A, Pereira CA, Neng NR, et al. Coupling sea lavender (Limonium algarveense Erben) and green tea (Camellia sinensis (L.) Kuntze) to produce an innovative herbal beverage with enhanced enzymatic inhibitory properties. S Afr J Sci 2019;120:87-94.
9. Angrrai T, Febrianti F, Aisman, Ismanto SD. Black tea with Averrhoa bilimbi L extract: A healthy beverage. Agric Agric Sci Proc 2016;9:241-52.
10. Khanam Z, Sam KH, Zakaria NH, Ching CH, Bhat IU. Determination of polyphenolic content, HPLC analyses and DNA cleavage activity of Malaysian Averrhoa carambola L. fruit extracts. J King Saud Univ Sci 2015;27:331-7.
11. Kurup SB, Mini S. Averrhoa bilimbi fruits attenuate hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. J Food Drug Analy 2017;25:360-8.
12. Attanayake AP, Jayatilaka KA, Mudduwa LK, Pathirana C. In vivo antihyperlipidemic, antioxidative effects of Coccinia grandid (L.) Voigt (Cucurbitaceae) leaf extract: An approach to scrutinize the therapeutic benefits of traditional Sri Lankan medicines against diabetic complications. Int J Pharm Sci Res 2016;7:3949-58.
13. Bi W, Shen J, Gao Y, He C, Peng Y, Xiao P. Ku-jin tea (Acer tataricum subsp. gymnola or A. tataricum subsp. theifenum), an underestimated functional beverage rich in antioxidant phenolics. J Funct Foods 2016;24:75-84.
14. Neugr & Radu GD, Albu C, Paun G. Antioxidant activity, acetylcyanidinesterase and tyrosinase inhibitory potential of Palmoniana officinalis and Centarium umbellatum extracts. S Afr J Sci 2018;114:578-85.
15. Aryan S, Banerji MK, Danekul K, Kunwar P, Guirung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel) 2019;8:1-12.}

27
25. Loganathan K, Bai VN. High frequency in vitro plantlet regeneration and antioxidant activity of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal: An important medicinal plant. Asian Pac J Reprod 2014;3:241-4.

26. Jain DP, Pancholi SS, Patel R. Synergistic antioxidant activity of green tea with some herbs. J Adv Pharm Technol Res 2011;2:177-83.

27. Rahman MM, Habib MR, Hasan MA, Al Amin M, Saha A, Mannan A. Comparative assessment on in vitro antioxidant activities of ethanol extracts of *Averrhoa bilimbi*, *Gymnema sylvestre* and *Capsicum frutescens*. Pharmacogn Res 2014;6:36-41.

28. Rodrigues MJ, Soszynski A, Martins A, Rauter AP, Neng NR, Nogueira JM, *et al*. Unravelling the antioxidant potential and the phenolic composition of different anatomical organs of the marine halophyte *Limonium algarvense*. Ind Crops Prod 2015;77:315-22.

29. Ranjbar Nedamani E, Sadeghi Mahoonak A, Gorbani M, Kashaninejad M. Evaluation of antioxidant interactions in combined extracts of green tea (*Camellia sinensis*), rosemary (*Rosmarinus officinalis*) and oak fruit (*Quercus brantii*). J Food Sci Technol 2015;52:4565-71.

30. Kazeem MI, Adamson JO, Oggunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. Biomed Res Int 2013;2013:527570.

31. Baessa M, Rodrigues MJ, Pereira C, Santos T, da Rosa Neng N, Nogueira JM, *et al*. A comparative study of the in vitro enzyme inhibitory and antioxidant activities of *Butea monosperma* (Lam.) Taub. and *Sesbania grandiflora* (L.) Poiret from Pakistan: New sources of natural products for public health problems. S Afr J Bot 2019;120:146-56.

32. Jemaa HB, Jemia AB, Khlifi S, Ahmed HB, Slama FB, Benzarti A, *et al*. Antioxidant activity and α-amylase inhibitory potential of *Rosa canina* L. Afr J Tradit Complement Altern Med 2017;14:1-8.

33. Panagai K, Josephine GI. Alpha-amylase and alpha-glucosidase inhibitory effects of *Calliandra haematocephala* and its potential role in diabetes mellitus. Asian J Pharm Clin Res 2018;11:429-32.

34. Chowdhury SS, Uddin GM, Muntahana N, Hossain M, Hasan SM. In-vitro antioxidant and cytotoxic of hydromethanolic extract of *Averrhoa bilimbi* L. Fruits. Int J Pharm Sci Res 2012;3:2263-8.

35. Meenatchi P, Purushothaman A, Maneemegalai S. Antioxidant, antiglycation and insulinotropic properties of *Coccinia grandis* in vitro: Possible role in prevention of diabetic complications. J Tradit Complement Med 2017;7:54-64.

36. Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM. Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. Pharmacogn Res 2010;2:215-20.