FOOD
RESEARCH

Physicochemical, antioxidant, and enzyme inhibitory properties of Bignay (*Antidesma bunius* L. Spreng) and Duhat (*Syzygium cumini* L.) extracts microencapsulated with β-cyclodextrin

*Flores, F.P. and Caro, M.A.D.

Institute of Food Science and Technology, University of the Philippines Los Baños, College, Laguna, Philippines 4031

Abstract

Seasonal tropical fruits with health-promoting properties are commonly dehydrated to preserve their bioactive potential. Despite the commercial and industrial appeal of spray drying, the significant capital outlay may deter small-scale applications associated with small volumes of seasonal fruits. In this study, inclusion complexation using β-cyclodextrin (bCD) was applied to ethanolic extracts of two common seasonal fruits, duhat and bignay, and the resulting powders were characterized using spectrophotometric, electron microscopy, colourimetric, and gravimetric methods. Electron microscopy images of microcapsules and changes in FTIR spectra indicate successful encapsulation. Despite having different concentrations of monomeric anthocyanins, phenolic contents and radical scavenging activities, the inclusion complexes possess equal encapsulation efficiencies and retention of bioactive components with antioxidant and enzyme inhibitory activities. The results of this study can be used to design small-scale encapsulation strategies for indigenous, seasonal fruits.

1. Introduction

Syzygium cumini L. ("Duhat") and *Antidesma bunius* L. Spreng ("Bignay") are tropical, fruit-bearing trees endemic to South and Southeast Asia. Some varieties of these fruits are found in the wild and possess ethnobotanical importance; cultivated varieties find increasing use in the processing of jams, candies, and wine. Recently, many authors reported the health-promoting potential for these two fruits arising from anthocyanins and other phenolic compounds. For instance, Quiming *et al.* (2016) reported that *A. bunius* fruits have flavonoids and tannins that impart a-glucosidase, iron chelating and free radical scavenging activities. The *in vivo* bariatric potential of *S. cumini* and *A. bunius* seed extracts that contain saponins, tannins, polyphenols, phlobatannins, steroids and terpenoids were proven with reduced serum insulin and improve lipid profiles after ingestion (Sampath *et al.*, 2013; Grijaldo *et al.*, 2019).

The fruit extracts can also be encapsulated to extend the availability of the fruits and their bioactive compounds. Although spray drying is a commercially viable encapsulation technique, the costs of capital outlay may limit small-scale processors from using this technology. Thus, less energy-intensive encapsulation

techniques, such as inclusion complexes may be more suitable. Inclusion complexes form when hydrophobic molecules replace the water molecules present in the cyclodextrin cavity even in highly aqueous systems (Abarca *et al.*, 2016). However, previous studies suggest that the colourless hemiketal, rather than the reddish forms, are complexed with cyclodextrin and that complexation of cyanidin-3-*O*-glucoside with α-cyclodextrin is not favoured (Fernandes *et al.*, 2013). More recently, however, the capacity of β-cyclodextrin (bCD) to protect crude fruit extracts from gastrointestinal and thermal degradation was demonstrated (Fernandes *et al.*, 2018).

Inclusion complexes prepared with bCD have been reported to stabilize phenolic compounds and anthocyanins present in fruit extracts (Fernandes *et al.*, 2013; Shao *et al.*, 2014; Aguilera *et al.*, 2016) with low to intermediate encapsulation efficiencies (Barba *et al.*, 2015; Abarca *et al.*, 2016). However, data regarding the retention of antioxidant and enzyme inhibitory activities of the inclusion complexes are scarce. Thus, this present investigation aimed to characterize the microcapsules prepared with bCD and fruit extracts and quantify the efficiency of encapsulation in terms of anthocyanins, phenolics and antioxidant activity. The results of this

*Corresponding author.
Email: *fpflores@up.edu.ph*

study may impact current approaches to developing cost-effective food ingredients or health-promoting supplements.

2. Materials and methods

2.1 Materials

Duhat "Giant", Accession Number 6375, Pangasinan (1990) and bignay "Jumbo", Accession Number 6328, Batangas (1990) samples were collected from the National Plant Genetics Research Laboratory (NPGRL) of the Institute of Plant Breeding (IPB), University of the Philippines Los Baños (UPLB). Three batches of each fruit were obtained in a single fruiting season (May-July). Fresh fruits (at least 80% maturity index per bunch, i.e., >80% red mature and fully mature) were cleaned of stems and leaves and blanched for 5 mins by immersing in boiling water. Seeds were removed using a fabricated pulper/finisher.

Folin-Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, and sodium acetate were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol and ethanol were sourced from RCl Lab Scan Ltd. (Bangkok, Thailand). Hydrochloric acid, potassium chloride, and sodium carbonate were procured from Merck (Darmstadt, Germany) while bCD (1st grade, min. 98% purity) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Preparation of bCD-fruit extract inclusion complexes

Based on preliminary tests, 2 hrs of extraction with 80% (v/v) aqueous ethanol were found sufficient to recover anthocyanins and phenolic compounds. Subsequently, each fruit pulp was extracted using those conditions at a ratio of 1:10 (fruit solids: solvent) in a water bath maintained at 40°C. The extracts were decanted and filtered using a Whatman No. 1 under 33 kPa to 50 kPa vacuum pressure. The fruit extract (5 mL) and 5 g of bCD were placed in a Petri dish and kneaded continuously for 15 mins to make a paste, and subsequently dried at 60°C in a convection oven until constant weight. The dry powder was stored in glass containers and kept in a freezer at -20°C to minimize any physicochemical change until further analyses.

2.3 Water activity, moisture content and colour properties of the inclusion complex powders

Water activity was evaluated for each 1 g powder sample using Novasina LabShift (Lachen, Switzerland). Readings were obtained after the constant temperature was achieved. Moisture content was evaluated using oven drying at 105°C and gravimetric analysis. The colour values of the textured proteins were determined using a Konika Minolta BC-10 colourimeter. The instrument was calibrated with a white tile, and colour values were expressed in CIE-Lab parameters as $L*$ (0 = black and 100 = white), $a*$ (-a/+a denotes greenness or redness), and $b*$ (–b/+b denotes blueness or yellowness). Values were expressed as the mean of the measurements taken from three random surface locations. Chroma and hue angle were determined using equations (1) and (2):

$$Chroma = \sqrt{(a*)^2 + (b*)^2} \qquad (1)$$

$$Hue\ Angle = tan^{-1}\left(\frac{b*}{a*}\right) \qquad (2)$$

2.4 Fourier Transform Infrared (FTIR) analysis of the inclusion complex powders

The Fourier Transform Infrared (FTIR) spectra of bCD and bCD-fruit extract complexes were obtained using an Infrared Fourier Transform spectrometer (ABB model MB 3000, USA). The spectral range used was 485 – 8500 cm^{-1} with a resolution of 0.7 cm^{-1}. The samples were pelletized before measurement.

2.5 Scanning electron microscopy of the inclusion complex powders

Particle morphology was determined using FEI Teneo (FEI Co., Hillsboro, OR) field emission scanning electron microscopy with 2 kV acceleration voltage and Everhart-Thornley secondary electron detector.

2.6 Encapsulation efficiency and retention of total monomeric anthocyanins, phenolic content and radical scavenging activity of rehydrated powders relative to extracts

The ethanolic extracts were diluted appropriately and powder samples were rehydrated with distilled water at a ratio of 1:5 mass: volume, vortexed for 60 s and centrifuged at 1066×g for 15 mins. The supernatant fluid was used as a test sample. Equations (3) and (4) were used to calculate individual encapsulation efficiencies (where X = monomeric anthocyanins or phenolics content) and individual retention values (where X = monomeric anthocyanins, phenolics content or antioxidant activity), respectively.

$$Encapsulation\ efficiency\ (EE_X) = \frac{X_{total} - X_{surface}}{X_{total}} \times 100\% \qquad (3)$$

$$Retention = \frac{X_{rehydrated\ powder} \times amount\ BCD\ used\ in\ inclusion\ complexation}{X_{extract} \times volume\ used\ in\ inclusion\ complexation} \times 100\% \qquad (4)$$

Where X = total monomeric anthocyanins (ppm cyanidin-3-*O*-glucoside equivalents), total phenolic content (ppm gallic acid equivalent) or antioxidant activity (ppm ascorbic acid equivalent)

The total monomeric anthocyanin content of extracts and powder samples was measured using the pH differential method (Flores *et al.*, 2013). The

absorbances at 520 nm and 700 nm were measured using a UV-Vis spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). Values were reported as ppm cyanidin-3-*O*-glucoside equivalent (C3G).

A modified Folin-Ciocalteu method (Flores *et al.*, 2013) was used to measure total phenolics content with modifications. Briefly, 0.5 mL of the test sample was mixed with 0.5 mL of 2 N Folin-Ciocalteu's phenol reagent along with 1 mL aqueous sodium carbonate solution (20%). After 5 mins, 8 mL of distilled water were added. The final mixture was vortexed for 1 min and stored for 60 mins to allow sufficient colour development. The absorbance was read at $\lambda = 765$ nm. The TPC was calculated from a standard curve of gallic acid (0-100 ppm). Values were reported as ppm gallic acid equivalent (GAE).

The DPPH radical scavenging activity of the extracts was determined using an adapted method (Dela Cruz and Flores, 2020). Approximately 2 mL of 0.150 mM DPPH methanolic solution were mixed with 2 mL of the extract, ascorbic acid (100 μg/mL) or methanol (blank). The mixture was then vortexed for 15 s and stood for 30 mins at room temperature in the dark before its absorbance was measured at $\lambda = 517$ nm. For powders, 50 mg of each sample were mixed with 5 mL of 50 % (w/v) methanol. The solution was vortexed for 10 mins and centrifuged at $1066 \times g$ for 15 mins. One millilitre of the supernatant fluid was diluted 5-fold using distilled water and mixed with 1 mL of freshly prepared 0.150 mM DPPH methanolic solution. The resulting solution was stood for 30 mins at room temperature in the dark before its absorbance was measured at $\lambda = 517$ nm. A calibration curve was generated with ascorbic acid. The DPPH antioxidant activity was calculated as the percentage rate relative to the blank and reported as ppm ascorbic acid equivalent (AAE).

2.7 Alpha-glucosidase inhibitory assay of rehydrated powders relative to extracts

A modified colourimetric method was also used (Flores *et al.*, 2013). Crude extracts were diluted 2-fold, while rehydrated powders were diluted 5-fold. Type I α-glucosidase from *Saccharomyces cerevisiae* (100 μL of 0.35 U/mL in 100 mM phosphate-buffered saline or PBS, pH 6.9) were added to 50 μL of the diluted extracts and incubated at 37°C for 10 mins in a Taitec Personal-10, Tokyo, Japan water bath. Next, 100 μL of 1.5 mM p-nitrophenyl-α-*D*-glucopyranoside (dissolved in 100 mM PBS, pH 6.9) were added and incubated for 20 mins. The reaction was terminated by the addition of 1 mL of 1 M sodium carbonate. Absorbance was read at $\lambda = 400$ nm. Results are presented as per cent inhibition of reactants with test sample relative to without.

2.8 Statistical analysis

Extractions and preparation of inclusion complexes were conducted twice and the resulting samples were pooled. Means of triplicate measurements were analyzed using PROC TTEST of SAS University Edition (Cary, NC) at $\alpha = 0.05$.

3. Results and discussion

Bignay extract contained less monomeric anthocyanin equivalents than Duhat (366 vs. 879 mcg C3G equivalents/g fruit), but the resulting bCD complex was significantly darker with a bluer component than bCD-Duhat (Table 1). These results are consistent with the redder duhat extract and bluish black bignay extract, suggesting different anthocyanin profiles in the fruits.

Table 1. Moisture indexes and color properties of duhat and bignay extracts microencapsulated with bCD

	Duhat	Bignay
Moisture content (% wb)	10.02±1.5	10.07±0.93
Water activity	0.59±0.01	0.56±0.01
Color Parameters		
L	92.6±0.2*	89.9±0.1
a	1.7±0.1	3.0±0.1*
b	0.57±0.11*	-0.13±0.06
Chroma	1.8±0.1	3.0±0.1*
Hue Angle	0.32±0.06*	-0.05±0.02

Values within the rows with asterisks (*) are significantly different at p<0.05

Morphological examination of bCD showed irregularly shaped particles and sheets of various sizes (Figure 1). In contrast, the inclusion complex particles appear as dented spheres with maximum dimensions of 10 mm. The particles appear different from spray-dried mixtures of bCD and saffron anthocyanin extracts, which morphologically resemble the host material (Ahmad *et al.*, 2018). Simulated molecular interactions between anthocyanins and bCD usually result in flat to globular shapes (Fernandes *et al.*, 2014). Changes in the drying rate and final moisture content between convection and spray drying may modulate the hydrophobic and hydrophilic interactions between the guest and host molecules. The FTIR spectrum of the host material (Figure 1) shows six characteristic peaks associated with bCD (Xie *et al.*, 2019): O-H stretching (3294 cm^{-1}), C-H stretching (2916 cm^{-1}), H-O-H bending (1636 cm^{-1}), C-C stretching (1149 cm^{-1}), antisymmetric C-O-C stretching (1018 cm^{-1}) and symmetric C-O-C stretching (941 cm^{-1}) that was also associated with skeletal vibration of the α-1,4 glycosidic bond (Gomes *et al.*, 2014). Changes in intensity and shifts in wavenumbers are generally regarded as evidence of inclusion complexation. For instance, the intensity of the peaks observed at 3294, 2916 and 1018 cm^{-1} was attenuated for both bCD-fruit
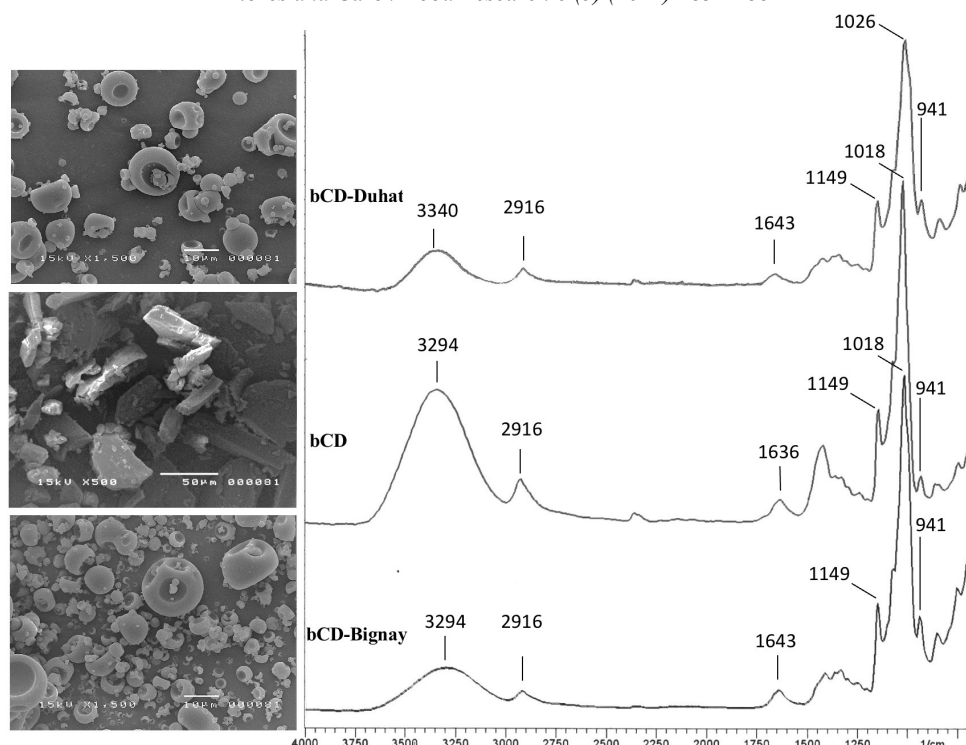
Figure 1. Scanning electron microscopy images and FTIR spectra of microcapsules prepared with bCD and fruit extracts of duhat and bignay

mixtures. The signal at 1643 cm⁻¹ was less intense with bCD-Duhat. Broad and less intense signals were similarly observed in mixtures of cyclodextrin and anthocyanins (Ahmad *et al.*, 2018; Xie *et al.*, 2019). In contrast, the relative intensity of the peak observed at 941 cm⁻¹ for the mixtures was significantly greater than the native bCD, indicative of changes in the toroid structure of bCD due to the presence of the phenolic compounds. Shifts in wavenumbers were observed for 3294 (bCD-Duhat), 1636 (bCD-Duhat and bCD-bignay), and 1018 cm⁻¹ (bCD-Duhat). Thus, mixtures of bCD and fruit extracts can form inclusion complexes.

Besides different monomeric anthocyanin equivalents, the ethanolic duhat extract contains less phenolic content (1213 vs. 1370 mcg GAE/g fruit) and greater antioxidant activity (265 vs 254 ppm AAE). Duhat extract exerted a slightly higher (74%) but not statistically different α-glucosidase inhibitory activity (Figure 2) than that of bignay (68%). Both extracts exerted low inhibitory activity (<15%) against α-amylase (data not shown). Following inclusion complexation, the extents of retention of monomeric anthocyanins (~45%), phenolic content (75%) and antioxidant activity (96%) were statistically equivalent ($p>0.05$). Similarly, the encapsulation efficiencies based on monomeric anthocyanins (~95%) and phenolic content (82%) were also statistically equivalent ($p>0.05$). There was no loss of activity against α-glucosidase for the microcapsules.

The C3G values were comparable to 547 mcg/g bignay and ~200 mcg/g duhat reported in the literature (Jorjong *et al.*, 2015; Branco *et al.*, 2016). However, the phenolic content of bignay and duhat extracts were lower than reported in similar studies (Banerjee *et al.*, 2005; Lim, 2012). This is more likely attributable to the slightly nonpolar extraction solvent and the cultivar used in this study. Ethanol was chosen because it can extract higher concentrations of phenolic compounds that are sparingly soluble in water. Regardless, the radical scavenging activities of the extracts were within the range of 80-100% (Banerjee *et al.*, 2005; Quiming *et al.*, 2016) and the extracts even had greater α-glucosidase
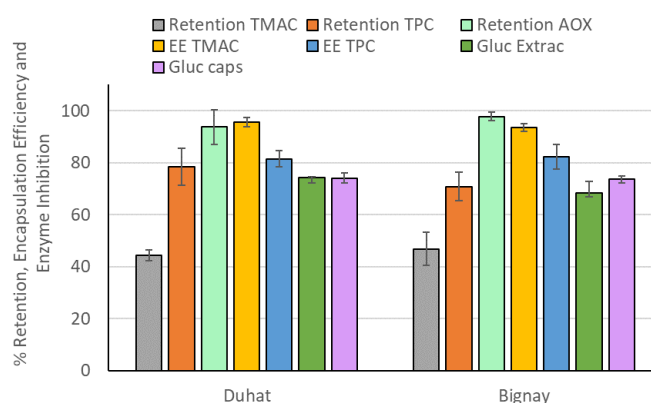

Figure 2. Retention, encapsulation efficiencies and glucosidase inhibitory activities of extracts and microcapsules prepared with bCD and fruit extracts of duhat and bignay. For each fruit sample, the bars refer to (from left to right): retention of total monomeric anthocyanins (Retention TMAC), retention of total phenolic content (Retention TPC), retention of DPPH radical scavenging activity (Retention AOX), encapsulation efficiency of total monomeric anthocyanins (EE TMAC), encapsulation efficiency of total phenolic content (EE TPC), α-glucosidase inhibitory activity of extracts (Gluc Extrac) and α-glucosidase inhibitory activity of the microcapsules (Gluc caps).

inhibitory activity than similar samples (20-40%) used in the same study (Quiming *et al.*, 2016). Fruit extracts can thus be incorporated into inclusion complexes without significant loss in enzyme inhibitory activity. This can impact current efforts in controlling postprandial glucose concentrations.

4. Conclusion

Inclusion complexation was successfully applied to duhat and bignay extracts with high retention of bioactive properties, especially in terms of enzyme inhibition. Results of this study can impact current approaches in microencapsulation that may be commercially feasible but require significant capital outlay. Future studies can be performed to determine the *in vitro* release profiles of the bioactive host molecules and evaluate the impact of different extraction methods. These may lead to more food and health-promoting applications of anthocyanin-containing inclusion complexes.

Conflict of interest

The authors declare no conflict of interest.

References

Abarca, R.L., Rodríguez, F.J., Guarda, A., Galotto, M.J. and Bruna, J.E. (2016). Characterization of betα-cyclodextrin inclusion complexes containing an essential oil component. *Food Chemistry,* 196, 968-975. https://doi.org/10.1016/j.foodchem.2015.10.023

Aguilera, Y., Mojica, L., Rebollo-Hernanz, M., Berhow, M., de Mejía, E.G. and Martín-Cabrejas, M.A. (2016). Black bean coats: New source of anthocyanins stabilized by β-cyclodextrin copigmentation in a sport beverage. *Food Chemistry,* 212, 561-570. https://doi.org/10.1016/j.foodchem.2016.06.022

Ahmad, M., Ashraf, B., Gani, A. and Gani, A. (2018). Microencapsulation of saffron anthocyanins using beta-glucan and betα-cyclodextrin: Microcapsule characterization, release behaviour and antioxidant potential during in-vitro digestion. *International Journal of Biological Macromolecules,* 109(1), 435-442. https://doi.org/10.1016/j.ijbiomac.2017.11.122

Banerjee, A., Dasgupta, N. and De, B. (2005). In vitro study of antioxidant activity of *Syzygium cumini* fruit. *Food Chemistry,* 90(4), 727-733. https://doi.org/10.1016/j.foodchem.2004.04.033

Barba, C., Eguinoa, A. and Maté, J.I. (2015). Preparation and characterization of β-cyclodextrin inclusion complexes as a tool of a controlled antimicrobial release in whey protein edible films. *LWT - Food Science and Technology,* 64(2), 1362-1369. https://doi.org/10.1016/j.lwt.2015.07.060

Branco, I.G., Moraes, I.C.F., Argandoña, E.J.S., Madrona, G.S., dos Santos, C., Ruiz, A.L.T.G., de Carvalho, J.E. and Haminiuk, C.W.I. (2016). Influence of pasteurization on antioxidant and *in vitro* anti-proliferative effects of jambolan (*Syzygium cumini* (L.) Skeels) fruit pulp. *Industrial Crops and Products,* 89(1), 225-230. https://doi.org/10.1016/j.indcrop.2016.04.055

Dela Cruz, J.D. and Flores, F.P. (2020). Evaluation of physicochemical characteristics, in vitro release and antioxidant properties of β-carotene and β-cyclodextrin inclusion complexes with rice as food matrix. *The Philippine Agricultural Scientist,* 103(4), 337-348.

Fernandes, A., Ivanova, G., Bras, N.F., Mateus, N., Ramos, M.J., Rangel, M. and de Freitas, V. (2014). Structural characterization of inclusion complexes between cyanidin-3-*O*-glucoside and β-cyclodextrin. *Carbohydrate Polymers,* 102(1), 269-277. https://doi.org/10.1016/j.carbpol.2013.11.037

Fernandes, A., Rocha, M.A.A., Santos, L., Bras, J., Oliveira, J., Mateus, N. and de Freitas, V. (2018). Blackberry anthocyanins: β-cyclodextrin fortification for thermal and gastrointestinal stabilization. *Food Chemistry,* 245(1), 426-431. https://doi.org/10.1016/j.foodchem.2017.10.109

Fernandes, A., Sousa, A., Azevedo, J., Mateus, N. and de Freitas, V. (2013). Effect of cyclodextrins on the thermodynamic and kinetic properties of cyanidin-3-*O*-glucoside. *Food Research International,* 51(2), 748-755. https://doi.org/10.1016/j.foodres.2013.01.037

Flores, F.P., Singh, R.K., Kerr, W.L., Pegg, R.B. and Kong, F. (2013). Antioxidant and enzyme inhibitory activities of blueberry anthocyanins prepared using different solvents. *Journal of Agricultural and Food Chemistry,* 61(18), 4441-4447. https://doi.org/10.1021/jf400429f

Gomes, L.M., Petito, N., Costa, V.G., Falcao, D.Q. and de Lima Araujo, K.G. (2014). Inclusion complexes of red bell pepper pigments with β-cyclodextrin: Preparation, characterisation and application as natural colorant in yogurt. *Food Chemistry,* 148(1), 428-436. https://doi.org/10.1016/j.foodchem.2012.09.065

Grijaldo, S.J.B., Quiming, N.S., Nicolas, M.G. and Alvarez, M.R.S. (2019). *In vivo* hypoglycemic activities of male and female *Antidesma bunius* (L.) Spreng. in alloxan-induced diabetic mice. *Oriental Journal Of Chemistry,* 35(4), 1398-1406. https://doi.org/10.13005/ojc/350421

Jorjong, S., Butkhup, L. and Samappito, S. (2015). Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from northeastern Thailand. *Food Chemistry,* 181(1), 248-255. https://doi.org/10.1016/j.foodchem.2015.02.093

Lim, T.K. (Ed.) (2012). *Antidesma bunius*. In Edible Medicinal and Non-Medicinal Plants. Vol. 4. Fruits, p. 220-224. Germany: Springer Science+Business Media B.V. https://doi.org/10.1007/978-94-007-4053-2_27

Quiming, N., Asis, J., Nicolas, M., Versoza, D. and Alvarez, M. (2016). *In vitro* and α-glucosidase inhibition and antioxidant activities of partially purified *Antidesma bunius* fruit and *Gynura nepalensis* leaf extracts. *Journal of Applied Pharmaceutical Science,* 6(5), 97-101. https://doi.org/10.7324/JAPS.2016.60515

Sampath, S., Narasimhan, A., Chinta, R., Nair, K.R., Khurana, A., Nayak, D., Kumar, A. and Karundevi, B. (2013). Effect of homeopathic preparations of *Syzygium jambolanum* and *Cephalandra indica* on gastrocnemius muscle of high fat and high fructose-induced type-2 diabetic rats. *Homeopathy,* 102(3), 160-171. https://doi.org/10.1016/j.homp.2013.05.002

Shao, P., Zhang, J., Fang, Z. and Sun, P. (2014). Complexing of chlorogenic acid with β-cyclodextrins: Inclusion effects, antioxidative properties and potential application in grape juice. *Food Hydrocolloids,* 41(1), 132-139. https://doi.org/10.1016/j.foodhyd.2014.04.003

Xie, J., Xu, Y., Shishir, M.R., Zheng, X. and Chen, W. (2019). Green extraction of mulberry anthocyanin with improved stability using β-cyclodextrin. *Journal of the Science of Food and Agriculture,* 99 (5), 2494-2503. https://doi.org/10.1002/jsfa.9459