

## Potential sources of anti-obesity compound – hydroxycitric acid among some of the underutilized fruits in the Philippines

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### Abstract

Hydroxycitric acid (HCA), a derivative of citric acid, is one of the promising food components with reported anti-obesity properties. HCA is currently extracted only from its principal source, *Garcinia cambogia*, which is not readily available in the country. Herewith, the study aims to identify potential sources of HCA among local underutilized fruits to increase their current commercial use. A total of twenty underutilized fruits generally rich in citric acid were screened via histochemical staining to detect the presence of HCA. Those samples that produced the characteristic reddish orange colour complexes indicative of HCA have undergone further experiments including extraction (via water extraction method), quantification (via spectrophotometry), and identity validation (via thin layer chromatography) to determine the actual HCA sources. Out of the 20 local fruits tested, HCA was found only from *Tamarindus indica* L. (tamarind) flesh at 3.731±0.046 g HCA/100 g fresh weight and from *Garcinia binucao* (batuan) flesh and seeds at 3.447±0.059 g HCA/100 g fresh weight and 1.241±0.009 g HCA/100 g fresh weight, respectively. Meanwhile, most of the other fruits tested were found to contain merely citric acid. This study could bring new ways in utilizing batuan and tamarind fruits as functional ingredients in nutraceuticals and value-added products, thereby increasing its current health and economic significance.

## 1. Introduction

Obesity is one of the most serious and the fastest-growing public health problems throughout the industrialized world (Kim, Lee, Kim *et al.*, 2008). Obesity develops when energy intake exceeds energy expenditure, thus the result is the storage of excess energy in adipose tissue (Kim and Park, 2011). According to WHO (2020), about 39% of the world's adult population were overweight and 13% were obese in 2016. Consequences include obese people incur at least 25% higher health care expenditures than a healthy person (Withrow and Alter, 2011). Moreover, excess weight gain reduces insulin sensitivity which in turn is a huge risk factor to metabolic syndrome (Weiss and Gepstein, 2019).

In the Philippines, there is also an alarming increase in the prevalence of overweight and obesity in adults from 16.6% in 1993 to 31.1% in 2015 (FNRI, 2016). Unfortunately, this health problem is not only confined to adults as data from the NHANES show that childhood

obesity in the United States has tripled since 1980 (Kim and Park, 2011) and was nearly doubled since 2003 among below 19 years old Filipino children (FNRI, 2016). This population of overweight children will most likely remain obese in their adulthood (Kim and Park, 2011), making the problem persists, if not worsen, over time.

At present, the global strategy may involve a combination of therapies including reducing energy intake, increasing energy expenditure, behavioural modification, pharmacotherapy and even surgery to counteract obesity development (Celleno *et al.*, 2007). The use of medicinal plant extracts for weight loss is a rapidly growing therapeutic area, which has been embraced by the public since nutraceuticals were found to help deteriorate the progression of oxidative stress and inflammation, hence restrict the inception of obesity (Slavin, 2005). Moreover, there is also increasing evidence that isolated functional compounds from plants contain promising therapeutic effects including appetite control and body fat reduction in animal studies (Astell

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et al., 2013). The current proposed nutraceuticals are particularly those with claimed effects on dietary fat bioavailability, antioxidative and anti-inflammatory effects, modification of lipogenesis and pre-adipocyte maturation, increased lipid metabolism, and satiety (Cope, 2019).

One of the dietary components with a promising weight loss effect is hydroxycitric acid (HCA). HCA is a derivative of citric acid (a citric acid with a hydroxyl group at the second carbon) that is found in a variety of tropical plants including *Garcinia cambogia* (Yamada et al., 2007). HCA is a compound that was first extracted from sugar beet by Lipmann in 1883 followed by Lewis and Neelakantan (1965) from peels of the *Garcinia cambogia*. In 1969, Watson and Lowenstein reported that HCA can inhibit the action of ATP citrate lyase, a key enzyme in fatty acid synthesis, thus, preventing fat accumulation in the body. This compound is commonly extracted from *Garcinia cambogia* having 16–18% of the acid per fresh pulp weight (Jayaprakasha and Sakariah, 1998) and 48-49% of the acid per 100g dried pulp (Edirisinghe et al., 2015).

There were already several published studies on the effects of HCA on the prevention and treatment of obesity (Talpur et al., 2003; Roy et al., 2004; Asghar et al., 2007; Kim, Lee, Kim et al., 2008; Kim, Kim, Kim et al., 2008). However, all these studies used only HCA extracted from its principal source, *Garcinia cambogia*. Having promising results in counteracting obesity, it is therefore of interest to identify potential local fruits sources of this compound, especially that *Garcinia cambogia* is not readily available in the country. The nutritional, economic, and sociocultural potential of neglected and underutilized fruits have yet to be fully exploited and are suffering from a lack of research interest (Beccaro et al., 2015). Therefore, studies on these plants may be of interest in the search for novel sources of functional dietary components for nutraceuticals (Donno et al., 2015), including HCA. Furthermore, the utilization of local underutilized fruits represents an opportunity for local growers since their crops will also be more of interest to investors yielding additional income (Coelho et al., 2018).

The objective of the study is to identify potential sources of HCA, particularly among the underutilized fruits in the Philippines identified by Coronel (2011). Specifically, this study aims to isolate, quantify, and validate the HCA to be obtained from these fruits. Being a derivative of citric acid and sourced mainly from *Garcinia cambogia*, it was speculated that HCA can also be found in fruits with a significant amount of citric acid and those under the genus *Garcinia*, with seasonality

being taken into consideration. The results could increase the health and economic utilization of the underutilized fruits found to be natural sources of HCA, which could spark the idea of developing new and improved product lines.

## 2. Materials and methods

### 2.1 Selection and preparation of materials

The fruits included in the study together with some of its maturity indices were presented in Table 1. Batuan and katmon were obtained from a backyard garden in Los Baños, Laguna while the rest of the fruits were purchased from a local market in Biñan, Laguna and RC Fruit Conservation Farm in Mabacan, Calauan Laguna. Most of the fruits used in the study were at their respective ripened stage and were free of any extensive defects or damages.

All experiments were done in triplicates and were conducted in the BioAssay Laboratory of the Institute of Human Nutrition and Food – College of Human Ecology, UPLB. All chemicals and reagents used were of analytical grade and were purchased from Sigma-Aldrich (Singapore).

### 2.2 Screening via histochemical staining

The histochemical staining technique depends on the specific reaction between the sample and/or food component of interest (Rao et al., 2014). In this screening test, NaVO<sub>3</sub> was used to detect the presence of HCA since it specifically produces a reddish orange colour complex once it reacted with HCA in the sample (Antony et al., 1999). The protocol was modified from Val et al. (2008).

Three to six pieces of each fruit were used for this screening process, using one to two pieces per replicate. The fruits were washed initially with tap water to remove adhering contaminants such as soil and leaves and to minimize the microbial load. It was then disinfected by soaking in 10 ppm hypochlorous acid (HOCl) solution for 20 s to further minimize the microbial load. Further washing in running potable water was done to remove excess chlorine on the fruits. Afterwards, the fruits were cut cross-sectionally. Each half was then applied with 5% sodium metavanadate (NaVO<sub>3</sub>) (Sigma-Aldrich, Singapore), enough to cover the exposed flesh, peels, and seeds. Fruit(s) that may be a potential source of HCA should form a distinct reddish orange colour on its surface. This colour complex is unique and specific for HCA since the yellow colour will be formed when citric acid was taken as a sample (Antony et al., 1999). Moreover, this colour complex is directly proportional to

Table 1. Fruits included in the study and some of its maturity indices

Scientific Name	Common Name	Skin Color	Size per piece	Weight per piece
1. <i>Malpighia glabra</i> L.	Barbados cherry (Acerola)	Red	2.2-2.4cm dia	2.6-3.8 g
2. <i>Garcinia binucao</i>	Batuan (Binukaw)	Green	4.3-4.5cm dia	30.0-35.0 g
3. <i>Rheedia edulis</i>	Berba (Mameyito)	Green	2.2-2.5cm dia	8.2-9.5 g
4. <i>Averrhoa bilimbi</i> L.	Bilimbi (Kamias)	Yellow green	4.5-5.5cm length	18.0-20.0 g
5. <i>Citrofortunella microcarpa</i>	Calamondin (Calamansi)	Green	2.2-2.5cm dia	9.5-10.2 g
6. <i>Eugenia uniflora</i> L.	Cayenne Cherry (Pitanga)	Dark red	2.1-2.4cm dia	3.8-4.2 g
7. <i>Garcinia xanthochymus</i>	Gamboge Tree (Yellow Mangosteen)	Yellow	5.8-6.4cm dia	110.0-125.0 g
8. <i>Vitis Vinifera</i>	Grapes (Ubas)	Greenish yellow	2.3-2.5cm length	4.2-5.5 g
9. <i>Psidium guajava</i>	Guava (Bayabas)	Yellow green	4.5-5.0cm dia	60.0-75.0 g
10. <i>Cucumis melo</i> L.	Honey Dew	Greenish yellow	15.0-16.5cm dia	1.2-1.5 kg
11. <i>Mangifera indica</i> L.	Indian mango	Green	7.5-8.5cm length	120.0-140.0 g
12. <i>Dillenia philippinensis</i>	Katmon	Light green	6.0-6.5cm dia	70.0-85.0 g
13. <i>Citrus limon</i> L.	Lemon	Yellow	6.0-6.5cm length	120.0-130.0 g
14. <i>Citrus aurantifolia</i>	Lime (Dayap)	Green	4.5-5.0 diameter	65.0-75 g
15. <i>Triphasia trifolia</i>	Limeberry (Limonsito)	Dark red	1.0-1.2cm length	0.8-1.2 g
16. <i>Cynometra cauliflora</i> L.	Namnam	Yellowish brown	6.6-7.5cm length	38.0-45.0 g
17. <i>Psidium guajava</i>	Native guava (Bayabas)	Yellow green	4.5-5.0cm dia	60.0-75.0 g
18. <i>Mangifera altissima</i> Blanco	Paho	Green	3.6-4.2cm length	15.0-20.0 g
19. <i>Tamarindus indica</i> L.	Tamarind (Sampalok)	Brown	9.0-11.0cm length	30.0-45.0 g
20. <i>Syzygium samarangense</i>	Wax Apple (Makopa)	Light red	6.0-7.0cm length	50.0-65.0 g

the quantity of HCA (i.e., darker colour means more HCA). The fruits (flesh, seed, or peel) that produced colour complex indicative of the presence of HCA were investigated further (extraction, quantification, and validation of HCA).

### 2.3 Isolation of HCA via water extraction method

Methods developed for the isolation of HCA from fruits were based on the fact that HCA is known to be soluble in water and alcohol (Lewis and Neelakantan, 1965) since HCA is relatively polar because of two hydroxyl and three carboxyl groups present in its structure (Hida *et al.*, 2005). In this study, the water extraction method was used for its practicality and efficiency as recommended by a similar study conducted in batuan fruits (Bainto *et al.*, 2018).

The following procedure was based on the published protocol of Krishnamurthy *et al.* (1982). A fresh set of fruits that produced colour complex indicative of HCA were obtained. These fruits were initially cleaned as described earlier. The parts of interest (flesh, seed, or peel) were obtained, cut into smaller pieces, and then stored in clean containers.

To isolate the HCA from the samples using this method, 200 g of the flesh/seed/peel from the fruit was obtained and 600 mL of distilled water was added. This was allowed to boil in an autoclave at 115°C for 15

mins. The extract was then allowed to cool then decanted through several folds of cheesecloth and filtered on a funnel with filter paper. The residue was washed with distilled water and then the filtrate was collected. The combined filtrate (~600 mL) was concentrated to about 100 mL on an electric stove and then treated with 200 mL of ethanol while stirring. The resulting precipitate (pectinous material) was then removed by filtration.

The acidic filtrate obtained was then neutralized by the cautious addition of 40% KOH, with careful stirring. The heavy oily liquid formed was allowed to settle for a few mins and the supernatant was discarded. Using 60% ethanol (two portions of 100 mL), the oily liquid was washed. Then, another washing with absolute alcohol (one portion of 100 mL) was done, letting the suspension stand for 60 mins. Another portion of 100 mL of absolute ethanol was added and this was allowed to stand overnight. Afterwards, the ethanol was decanted, and the hygroscopic semisolid residue (HCA salt) was obtained. This was dried in a drying oven (Memmert GmbH + Co. KG, Germany) at 80°C to remove traces of ethanol. To recover the semisolid HCA sample, a minimum amount of water was added to dissolve it. The concentrated liquid was then transferred to a clean container and stored in a freezer.

#### 2.4 Quantification of HCA via spectrophotometry

For HCA quantification, the following procedure was based on the protocol of Antony *et al.* (1999). Exactly 0.2 g of the isolated extract (pulp/seed/peel) from each fruit was obtained. This was dissolved in 5 mL of 1 N H<sub>2</sub>SO<sub>4</sub> and diluted to 25 mL with distilled water. The solution was filtered into a 50 mL volumetric flask and diluted to volume. For the HCA standard, food grade *Garcinia cambogia* extract (80% HCA) was used (Naturewise, United States). Exactly 0.4 g of the standard was dissolved in 10 mL 1 N H<sub>2</sub>SO<sub>4</sub> followed by the addition of 50 ml distilled water. The solution was transferred into a 100 mL volumetric flask and then diluted to volume. This served as the stock solution for the standard.

In the analysis using spectrophotometry, 5% NaVO<sub>3</sub> was used. From the standard stock solution, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL and 0.7 mL were dispensed individually in 50 mL volumetric flasks and then diluted to volume. Exactly 0.9 mL of 5% NaVO<sub>3</sub> was added to each standard solution to allow colour development. The colour of the solutions turned yellow at first, then only some of the samples change their colour to reddish orange after 20 mins of incubation. The assays were read at 467 nm (Antony, 2003) using a spectrophotometer (Labtronics, India). A calibration graph was plotted against the concentration of HCA and absorbance. For the sample solutions, the same procedures as that of the standard solution were applied. Blank solutions were also prepared. The concentration of HCA in the samples was then computed using the formula:

$$\text{HCA content } \left( \frac{\text{g}}{100 \text{ g}} \right) = \frac{\text{HCA in solution} \times \text{weight of collected extract} \times \text{dilution factor}}{\text{weight of sample}} \times 100$$

#### 2.5 Identity validation of HCA via thin-layer chromatography

The following procedure was based on a related study conducted by Bainto *et al.* (2018) and was done in triplicates using different TLC plate per run. Standard solutions of pure citric acid (Sigma Aldrich, Singapore) and HCA were prepared to serve as the basis for analysis. For citric acid standard, 300 mg of chemically pure citric acid was dissolved in 10 mL 0.1 N HCl. While for HCA standard, 380 mg of food-grade *Garcinia cambogia* extract was dissolved in the same solvent. The solutions were then filtered to remove the suspended solids. For the sample extracts, 500 mg of crude extract from each of the sample fruit pulp/seed/peel was dissolved in 10 mL 0.1 N HCl. It was then filtered to remove residues. The filtrate was directly used in the thin layer chromatography analysis.

Precoated TLC plates prewashed with methanol were used as the stationary phase while methanol-water

solution (6:2 v/v) was used as the mobile phase. The standards (citric and HCA) and samples were then spotted onto the TLC plates. After spotting, the spots were allowed to dry. The prepared plates were then allowed to develop inside the saturated chamber. The solvent was allowed to run up to 7.5 cm from the point of sample injection. The running time was approximately 30 mins.

After the development, the plates were heated using a hot air blower to dry the samples and the mobile phase. To visualize the spots, 5% NaVO<sub>3</sub> was sprayed in the developed chromatogram. The chromatogram was then heated again using a hot hair blower until dried. The colour of the spots was noted, and the retention fraction (R<sub>f</sub>) values of the standards and samples were computed for comparison. The R<sub>f</sub> value was computed using the formula:

$$\text{Retention Factor} = \frac{\text{distance travelled by the sample (cm)}}{\text{distance travelled by the solvent (cm)}}$$

#### 2.6 Statistical analysis

Results were expressed as mean±standard error of the mean (SEM). The data were analysed using one-way analysis of variance (ANOVA) for differences between samples and *post-hoc* analysis Tukey test at p<0.05 using Statistical Package for the Social Sciences (SPSS) version 20.

### 3. Results

#### 3.1 Screening via histochemical staining

Results showed out of all the fruit samples, only batuan flesh and seeds and tamarind flesh produced the characteristic reddish orange colour complex indicative of HCA (Figure 1). Other samples showed no change in colour, which include Barbados cherry, yellow mangosteen, grapes, honeydew, lime berry, namnam, and wax apple, indicating trace to no HCA and/or citric acid was present. On the other hand, the development of a yellow colour complex was observed for some fruits, namely bilimbi, calamondin, Indian mango flesh, lemon, lime, and paho flesh, indicative of significant amounts of citric acid. Interestingly, blue-black colour complexes were observed from berba, cayenne cherry, guava, Indian mango seeds and peels, katmon, native guava, and paho seeds, which might suggest the presence of other compounds reactive to NaVO<sub>3</sub> or potentially concentrated amounts of HCA or citric acid.

#### 3.2 Extraction and spectrophotometric quantification of HCA

Given the results of histochemical staining, those that produced the characteristic reddish orange colour complex (batuan flesh and seeds and tamarind flesh)

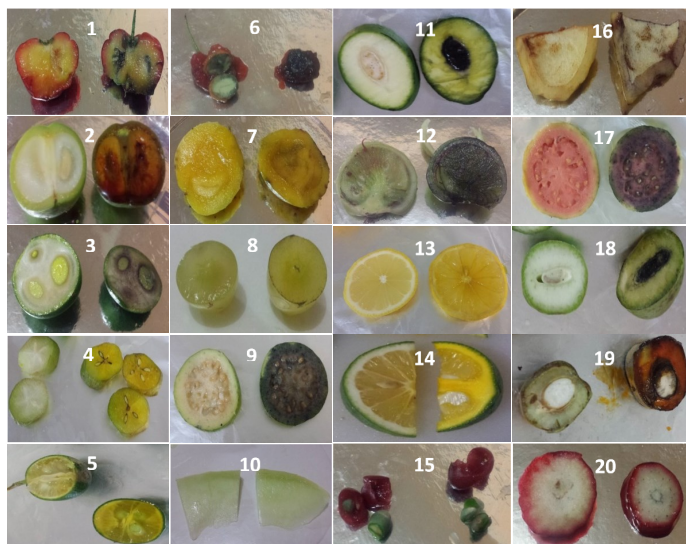


Figure 1. Colour complex formed from the fruits before (left) and after (right) histochemical staining. 1 – Barbados cherry, 2 – Batuan, 3 – Berba, 4 – Bilimbi, 5 – Calamondin, 6 – Cayenne cherry, 7 – Yellow mangosteen, 8 – Grapes, 9 – Guava, 10 – Honeydew, 11 – Indian mango, 12 – katmon, 13 – Lemon, 14 – Lime, 15 – Limeberry, 16 – Namnam, 17 – Native guava, 18 – Paho, 19 – Tamarind, 20 – Wax apple

were included in the extraction and quantification of HCA. The study also included the seeds and peels of Indian mango and flesh of native guava fruit in the succeeding experiments to confirm the identities of the compound present that reacted to  $\text{NaVO}_3$  producing the blue-black colour complexes. These additional fruits to be tested were chosen based on practicality and availability of fruits at the time of the experiment.

In this experiment, a reddish orange colour complex was formed when HCA reacted with  $\text{NaVO}_3$  and the intensity of this colour complex was directly proportional to the quantity of HCA present in the sample (Antony *et al.*, 1999). Figure 2 shows the assays of the samples tested.



Figure 2. Solutions containing different sample extracts before (top) and after (below) addition of 5%  $\text{NaVO}_3$ . 1 – Blank solution, 2 – Indian mango peel, 3 – Indian mango seeds, 4 – Native guava flesh, 5 – Batuan seeds, 6 – Batuan flesh, 7 – Tamarind flesh

The concentrations of HCA from the different samples were determined using the standard curve. The established standard curve has a  $R^2$  value equal to 0.999 indicating that the obtained set of data has a good linear relationship. Figure 3 shows the HCA amounts from each sample tested. Tamarind flesh had significantly higher HCA content at  $3.731 \pm 0.046$  g/100 g fresh weight as compared with batuan flesh at  $3.447 \pm 0.059$  g/100 g fresh weight and batuan seeds at  $1.241 \pm 0.009$  g/100 g fresh weight. Conversely, the extracts from Indian mango peel and seeds, and native guava flesh have significantly lower values as compared with those of batuan and tamarind extracts, ranging only from 0.033 g/100 g to 0.083 g/100 g fresh weight. This is suggestive of trace to no HCA content among these samples, especially that it was observed that these three samples produced yellow-coloured solutions upon addition of 5%  $\text{NaVO}_3$  prior to quantification (Figure 2) instead of the characteristic reddish orange colour specific to HCA.

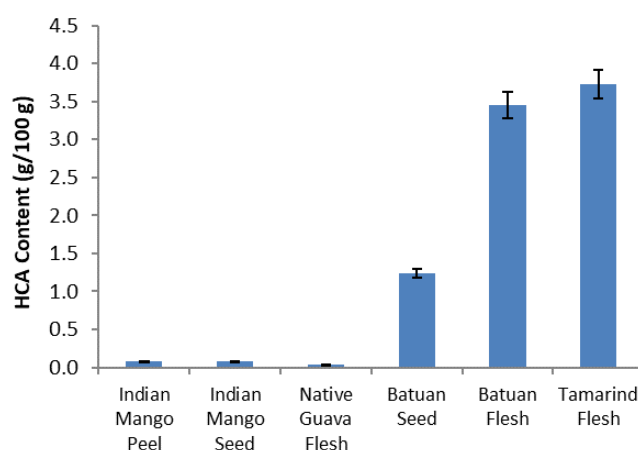


Figure 3. HCA content (g/100 g fresh sample) of the sample extracts

### 3.3 Validation of the identity and purity of HCA via thin-layer chromatography

Thin-layer chromatography was employed to confirm the identity and purity of the isolates from the different samples tested. Food grade *Garcinia cambogia* and chemically pure citric acid were used as standards. The spots were visualized by spraying with 5%  $\text{NaVO}_3$  on the developed chromatogram (Figure 4). Two coloured spots were observed from the chromatogram, reddish orange- and yellow-coloured spots. According to Antony *et al.* (1999), HCA produces red orange spots while citric acid produces yellow spots upon reaction with  $\text{NaVO}_3$ . Sample extracts obtained from batuan seeds and flesh, and tamarind flesh produced red orange colour spots while the rest of the samples (Indian mango peel and seeds, and native guava pulp) produced yellow-coloured spots which support the earlier observations that these samples might be concentrated sources of citric acid and not HCA. Furthermore, the retention

factor ( $R_f$ ) of the spots were also computed relative to the solvent front of the chromatogram. The results for this part of the study were summarized in Table 2.

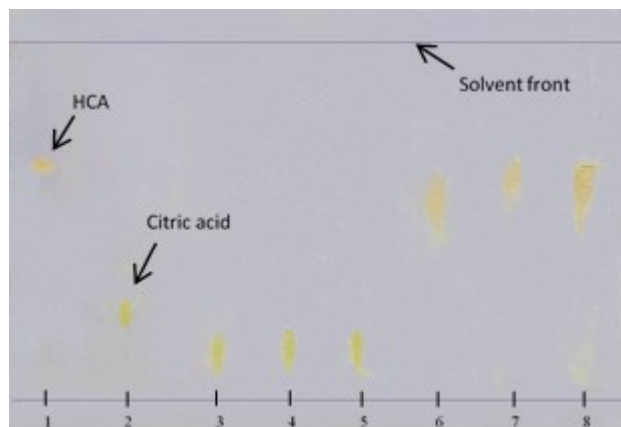


Figure 4. Sample developed chromatogram. 1 - HCA standard, 2 - Citric acid standard, 3 - Indian mango peel, 4 - Indian mango seed, 5 - Native guava flesh, 6 - Batuan seed, 7 - Batuan flesh, 8 - Tamarind flesh

Table 2. Summary of the results of thin layer chromatography

Standard/Sample	$R_f$ Values	Colour of the	Identity
(1) HCA standard	$0.67 \pm 0.03^a$	Red orange	HCA
(2) Citric acid standard	$0.26 \pm 0.03^b$	Yellow	Citric Acid
(3) Indian mango peel	$0.19 \pm 0.05^b$	Yellow	Citric Acid
(4) Indian mango seed	$0.20 \pm 0.03^b$	Yellow	Citric Acid
(5) Native guava flesh	$0.20 \pm 0.04^b$	Yellow	Citric Acid
(6) Batuan seeds	$0.63 \pm 0.06^a$	Red orange	HCA
(7) Batuan flesh	$0.67 \pm 0.05^a$	Red orange	HCA
(8) Tamarind flesh	$0.65 \pm 0.06^a$	Red orange	HCA

\*Means followed by the same superscript are not significantly different at  $p < 0.05$ .

There was a significant difference between the  $R_f$  values of HCA and citric acid standards, thus these values can be used as a basis to distinguish the identity of the sample extracts being studied. From the samples, it was observed that the yellow-coloured spots and  $R_f$  values of the extracts from Indian mango peel, Indian mango seed, and native guava flesh were matching with the characteristics of the citric acid standard. On the other hand, reddish orange spots and  $R_f$  values comparable to that of the HCA standard were obtained from the batuan seeds, batuan flesh, and tamarind flesh extracts. However, given the standard error of the mean being relatively high, particularly for the CA standard and samples, spot colour difference could still be considered a reliable parameter in identifying the presence and purity of hydroxycitric acid in the chromatogram (Bainto *et al.*, 2018). These data validated that the extracts from the batuan seeds, batuan flesh, and tamarind flesh were certainly HCA.

Thin-layer chromatography strengthens the soundness of the HCA quantification procedure via spectrophotometry. In terms of purity, all of the samples produced certain streaks and smears on the developed chromatogram which could be caused by some contaminants and/or too much injected sample (over spotting).

#### 4. Discussion

The availability of HCA is limited by the restricted habitat of plants as well as the difficulty of stereoselective organic synthesis. Only certain species of plants are known to produce HCA, and their growth is limited to tropical and semitropical areas. These facts suggest that the production of natural HCA is restricted by plant distribution area and climate (Hida *et al.*, 2005). With this, Philippines, being a tropical country, is a great area for potential fruit sources of HCA as it can support the growth and proliferation of these fruits.

The present study showed that some of the local underutilized fruits in the country can be good sources of this anti-obesity compound. The results validated the reports of Bainto *et al.* (2018) and Tantengco *et al.* (2018) wherein one of the organic acids that can be isolated from *Garcinia binucao* (batuan) is HCA. The computed values of HCA from batuan fruit were also comparable with what has been observed by Bainto *et al.* (2018). On the other hand, HCA yield and methods on HCA extraction/quantification from tamarind are not yet well documented in the present literature. The results of this study provided information on this knowledge gap by showing that *Tamarindus indica* (tamarind) flesh contains HCA at  $3.731 \pm 0.046$  g/100 g fresh weight, at par with the HCA content of batuan, a close relative of *Garcinia cambogia*. This finding is in line with the report of Nair and Khidse (2016) wherein tamarind has been known to suppress the appetite by increasing the serotonin neurotransmitter in animals, which is one of the mechanisms of action of HCA in weight control.

Other fruits tested in this study showed little to no signs of HCA content as it does not produce the characteristic colour complex specific to HCA-vanadate reaction during histochemical staining. Those fruits that produced blue-black colour complex, namely Indian mango peel and seed and native guava flesh, were confirmed to be citric acid, possibly in high and concentrated amounts since mangoes, especially unripe ones, were found to have major organic acids in the form of citric and malic acids (Medlicott and Thompson, 1985). On the other hand, organic acids in guava are predominantly citric acid, with significant amounts of malic acid and lactic acid (Chan *et al.*, 1971). The more recent study also showed that the major organic acids in

guava flesh have been found to be citric, together with malic, glycolic, acetic and lactic acids (Kocher, 2011). The obtained data and values from the present experiment (yellow spots, negligible HCA content, and  $R_f$  values close to citric acid standards) agree with the stated acid components of mango and guava fruits.

#### 4. Conclusion

Out of the 20 locally available fruits tested, only *Garcinia binucao* (batuan) seeds and flesh and *Tamarindus indica* (tamarind) flesh were the potential sources of HCA. Tamarind flesh contains the highest HCA content among the three, followed by batuan flesh, then by batuan seeds. This study could bring new ways in utilizing batuan and tamarind fruits as functional ingredients in nutraceuticals and value-added products, thereby increasing its current health and economic significance.

#### Conflict of interest

The authors declare no conflict of interest.

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