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Formulation of Polyherbal Carbonated Beverage based on *Halalan Thoyyiban* Principle

Puteri Shazleen Izreena Mohd Shahrin[1], Norhayati Muhammad[1,2]* and Nur Fazira Abdul Rahim[1]

[1]Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, UTHM- Campus (Pagoh Branch), Pagoh Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia

[2]Institut Ahli Sunnah Wal Jamaah, Universiti Tun Hussein Onn Malaysia, 86400, Parit Raja, Batu Pahat, Johor, Malaysia

ABSTRACT

Herbal products usually have less palatability on their taste even though they are healthy and nutritious. Thus, this study aims to formulate polyherbal carbonated beverages, followed by the evaluation of sensory, physicochemical properties and their compliances with the *halalan thoyyiban* principle. Five formulations were prepared by varying the amount of water and polyherbal extracts that were designated using Design Expert 6.0.4 software. The most accepted formulation was formulation 1 (F1), containing 12.50% polyherbal extract and 87.50% water. F1 was characterised for its physicochemical properties, including carbon dioxide volume, pH, and total acidity, with 3.0 g/L, 4.5, and 1.7%, respectively, and these findings have complied with the *halalan thoyyiban* principle. The antioxidant properties were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant potential (FRAP), as well as total phenolic content (TPC), and it exhibited 1.25 mg GAE/mL,1.08 mM Fe (II) and 0.39 mg GAE/mL respectively. Eight Halal Control Point (HCP) were identified along with the production. Moreover, the beverage was found to have antioxidant properties and nutritional content (carbohydrate (0.3%) and energy content (2.0 kcal/100g)), which can give health benefits to the consumer and proven safe for consumption (LC_{50} =1066.60 μg/mL). To conclude, the formulated polyherbal carbonated beverage is accepted by the consumer and complies with the *halalan thoyyiban* principle.

Keywords: Antioxidant, carbonated beverage, *halalan thoyyiban*, polyherbal

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E-mail addresses:
aw170060@siswa.uthm.edu.my (Puteri Shazleen Izreena Mohd Shahrin)
norhayatim@uthm.edu.my (Norhayati Muhammad)
gw180043@siswa.uthm.edu.my (Nur Fazira Abdul Rahim)
* Corresponding author

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INTRODUCTION

Malaysia has a wide range of plant species that pose medicinal properties. The herbal plants can be combined as polyherbal to give a more significant effect. This combination can give a more desirable therapeutic effect because the individual plant phytochemical components are insufficient to achieve the beneficial effects (Pandey et al., 2013). *Orthosiphon stamineus* (misai kucing), *Phyllanthus niruri* (dukung anak) and *Strobilanthes crispus* (pecah beling) are herbal plants in a polyherbal combination that exhibited the synergistic effect on 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. It was proven in a previous study that a combination of these three herbs has similar antioxidant properties and showed a synergistic interaction effect (Rahim et al., 2018). According to Capecka et al. (2005), these herbs are an excellent source of phenolic compounds, which can help prevent various degenerative diseases and exhibit antioxidant effects, making them suitable to be combined as a polyherbal. However, nowadays, people usually refuse to consume herbal plants because they have less palatability even though they are healthy and nutritious. It is due to the presence of antioxidants phytochemicals in the herbal plants or vegetables, which give a noticeable bitter taste (Drewnowski & Gomez-Carneros, 2000).

A formulation is needed to develop a new herbal-based product with high palatability. In Malaysia, there has been a growth in interest in natural herbs for medicinal purposes that indirectly led to rapid growth in the herbal product industry. Herbal products have grown in popularity over the last decade and are now utilised by about 20% of the population, contributing to people's health care (Ya'akob, 2018). In addition, according to Singh (2018), herbal products can meet the tastes and preferences of consumers, which causes many products to have been developed nowadays, such as food supplements (Ekor, 2014), teas (Alexieva et al., 2019) and snacks (Wangcharoen et al., 2006). In this study, the polyherbal carbonated beverage has been developed by formulating the amount of polyherbal extract and water as well as the ingredients. In addition, carbon dioxide gas was added to the beverage by the carbonation process to enhance its taste. In Malaysia, carbonated drinks have become popular because they have a sharp, refreshing taste and quench thirst (Kendra, 2018). The herbal-based carbonated beverage is a novel concept that has the potential to add a unique sparkle effect to the herbal beverage when combined. This concept can increase the consumer preference toward the herbal product. In this study, the best formulation of the product was chosen based on the overall acceptability of the sensory evaluation. The sensory characteristics of food can influence consumer preference of food substances (Kostyra et al., 2016).

In addition, it is important to meet the *halalan thoyyiban* principle's requirements in food production. From an Islamic perspective, *halal* means permissible or allowable and *thoyyiban* is defined as pure, safe, wholesome, and healthy (Yahya et al., 2016). It means that the products identified as *halalan thoyyiban* should not be identified as delicious and pure only. They should also be beneficial to the body and do not cause any harm.

Unfortunately, the food industry has neglected *halalan toyyiban* compliance, which causes many concerns about the questionable status of *halal* food (Salahudin et al., 2015). Thus, the polyherbal carbonated beverage was formulated based on consumer preferences by conducting a sensory evaluation test and developed according to the *halalan thoyyiban* principle, which is safe, healthy and gives health benefits for human consumption, providing antioxidant properties.

MATERIALS AND METHODS

Materials

In this study, *O. stamineus*, *P. niruri* and *S. crispus* leaves were used in the form of dried powder. These herbs were purchased from Ethno Resources Sdn Bhd, Sungai Buloh, Selangor. Sucralose, citric acid and sodium benzoate were purchased from Thong Sheng Food Technology Sdn Bhd, Pulau Pinang. DPPH, gallic acid, methanol, Folin-Ciocalteu's phenol reagent have been purchased from (Sigma Aldrich, USA). Sodium carbonate has been purchased from Samchun Chemical Corporation (Pyeongtaek, Korea). Sodium acetate buffer, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate and iron (III) chloride have been purchased from Sigma Aldrich (St. Louis, MO, USA). All the reagents are food-grade procured from local sources.

Preparation of Plant Materials

O. stamineus, *P. niruri* and *S. crispus* leave powders were used to develop the polyherbal formulations. The herbal extract preparation was done according to Chanthasri et al. (2018). with slight modification. First, an amount of 100 g of each powder (130–150 mesh) was heated slowly by boiling with one litre of distilled water until the volume of the mixture reduced to about a third of the original volume. Then, each type of powder was immersed in hot water at a range of 80°C to 90°C for 20 minutes, as Wijaya (2019) suggested. As a result, it was proven that the optimum condition for extraction herbal for functional drink production was 90°C and 21 minutes, which is expected to give higher antioxidant activity. Then, the extracts were filtered separately using a muslin cloth and sterile grade no. 1 filter paper (Whatman, 1004125-DS, Mexico). Finally, to develop the polyherbal mixture, each extract was mixed in a proportion by referring to the optimised formulation based on antioxidant activity conducted from the previous study (Rahim et al., 2018), as shown in Table 1.

Formulation of Polyherbal Carbonated Beverage

The components used were the filtered water and the polyherbal extract, and the mixture proportion was designated using Simplex Lattice mixture design of Design Expert 6.0.4 and as shown in Table 2. The low and high values used for filtered water in the formulation

Table 1
The optimized formulation of polyherbal combination based on antioxidant activity

Plant samples			Antioxidant activities		
O.stamineus	*P. niruri*	*S. crispus*	DPPH	TPC	FRAP
75.42%	0.62%	23.96%	91.23%	141.851 µg GAE/g	148.516 µg/mL

Table 2
Design layout and experimental results

Formulation	Volume of filtered water (%)	Volume of polyherbal extract (%)
F1	87.5	12.5
F2	83.75	16.25
F3	80 .00	20.00
F4	95.00	5.00
F5	91.25	8.75

Notes: (**F1**) formulation 1, (**F2**) formulation 2, (**F3**) formulation 3, (**F4**) formulation 4, (**F5**) formulation 5

were 80% and 95%, respectively. Meanwhile, the low and high values used were 5% and 20% for the polyherbal extract, respectively. The mixture was standardised at 100% of the formulation.

Carbonation Process

The formulated polyherbal beverages were carbonated using a carbonator machine (Soda Xpress, Malaysia). Therefore, the beverage was needed to be pre-chilled first to ensure the temperature was at the range of 2 to 4°C. This condition helps ease the absorption of carbon dioxide (CO_2) into the beverage (Abdulkareem et al., 2011).

Sensory Analysis

Sensory attributes including colour, aroma, taste, sweetness, sourness, sparkling sensation and overall acceptability were determined by hedonic test (untrained panellists). The hedonic test was used to evaluate sensory characteristics and determine the consumer's acceptability for polyherbal carbonated beverages. The samples of five formulations with the control (pure polyherbal extract) were presented in sensory cups coded of F1, F2, F3, F4, F5 and control.

Physicochemical Analyses

The beverage was analysed for the physicochemical characteristics, including the colour, pH, total acidity, and carbon dioxide volume. A colorimeter (MiniScan EZ, USA) was used to measure the beverage's colour. The pH was estimated using a digital pH meter (Eutech pH 700, India), calibrated according to the method AOAC (2005). The total titratable acidity

of the sample was analysed by a titration method according to AOAC (2000) with slight modification, and the acidity was expressed as citric acid (%). Total acidity was calculated by using Equation 1. A thermometer (SK Sato, Japan) and pressure gauge (Ashcroft, USA) were used to determine the volume of carbon dioxide in the sample. The pressure reading at the constant temperature was converted to the volume of carbon dioxide using a carbonation chart (Thongrote et al., 2016).

$$Total\ acidity\ (\%) = \frac{volume\ of\ 0.1\ M\ NaOH\ (ml) x\ 0.064\ x100}{volume\ of\ sample\ (ml)} \quad [1]$$

Evaluation of Polyherbal Carbonated Beverage with the *Halalan Toyyiban* Principle Compliance

In order to evaluate the *halalan thoyyiban* compliance, the *halal* status of ingredients used in the beverage development was identified. In addition, the beverage was evaluated to have antioxidant and nutritional content as health benefits. Also, the toxicity test was conducted to ensure the beverage was safe to be consumed.

Antioxidant Properties. The antioxidant properties of the formulated polyherbal carbonated beverage were determined by using DPPH, FRAP and TPC assays. In addition, the antioxidant activity of the beverage was compared with the control (polyherbal extract).

DPPH Assay. The DPPH assay was carried out according to BehnamNik & Vazifedoost (2020) with minor modifications. The DPPH solution 0.004% was prepared by adding 4 mg DPPH in 100 mL methanol. Then, 0.5 mL of the drink sample was mixed with 3.5 mL of 0.004% prepared DPPH solution. After that, the mixed sample was placed in the dark at room temperature (27°C) for 40 minutes, and then, the sample light absorbance was read at 517 nm. The absorbance values of the samples were compared to the standard curve of the gallic acid. The gallic acid standard curve was drawn in the concentration range of 0.02 and 0.1 mg/mL. The experiment was done in triplicate.

FRAP Assay. The FRAP assay was used with slight modifications (Benzie & Strain, 1996). Briefly, the FRAP reagent was prepared by mixing 25 mL of 300 mMol/L acetate buffer (pH 3.6), 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L hydrochloric acid and 20 mmol/L Ferric chloride hexahydrate and then incubated in a water bath at 37°C for five minutes (Memmert/WNB14, Germany). An amount of 300 μL of FRAP reagent was mixed with 10 μL of sample and 30 μL of distilled water. Then, the absorbance of the mixture was measured at 593 nm. The FRAP values of samples were determined using the iron (II) sulphate standard curve (0.2 to 1.0 mM). The antioxidant activity of the sample was expressed as mM iron (II) equivalent.

TPC Assay. The content of total phenolic compounds in the carbonated polyherbal beverage was determined by Folin-Ciocalteu colourimetric method. This total phenolic content was measured following BehnamNik and Vazifedoost (2020) with slight modifications. First, an amount of 0.5 mL of drink samples and 2.5 mL of Folin-Ciocalteu reagent 1 N were mixed in the test tube. After that, 2 mL of 7.5% sodium carbonate solution was added to the mixture after ten minutes and then incubated in the dark at room temperature for one hour. The absorbance was measured at 760 nm. For TPC determination, the absorbance values of the samples were compared to the standard curve of the gallic acid. The gallic acid standard curve was drawn in the concentration range of 0.04 and 0.4 mg/mL. The experiment was done in triplicate.

Nutritional Content Determination. The polyherbal carbonated beverage was analysed for the presence of the total carbohydrates (AOAC, 2005), protein, energy (Nielsen, 2010) and fat content (Bench et al., 2010).

Toxicity Assay. The beverage was tested on its toxicity by using brine shrimp lethality assay with slight modification (Asaduzzaman et al., 2015). Firstly, the brine shrimp eggs, *Artemia salina,* were hatched in artificial seawater prepared by dissolving 38 grams of sea salt in one litre of distilled water in a container with a light source for 24 hours. After 24 hours, the light source was turned off to separate the empty egg float from the brine shrimp. The beverage was prepared at 1600μg/mL concentration as a stock solution by diluting 32 mg of the beverage in 200 μL distilled water and was made up the volume to 20 mL with seawater. By using serial dilution, the concentration of 10 μg/mL, 100 μg/mL, 250 μg/mL, 500 μg/mL, and 1000 μg/mL were made up with the seawater. Ten nauplii were introduced into five mL of the sample in a separate test tube for each concentration. After 24 hours, the test tube was inspected, and the number of deaths of nauplii and the percentage of death in each tube was counted using Equation 2. The concentration killing 50% of the nauplii (LC_{50}) was determined using probit analysis and the linear regression method from the plotted graph.

$$\%\, Death = \frac{Number\ of\ dead\ nauplii}{Total\ number\ of\ nauplii\ placed\ in\ a\ test\ tube} \times 100 \qquad [2]$$

Statistical Analysis

The results were expressed as means±standard deviation to show variations in the various experimental. Statistical analyses for the formulation were conducted using Design Expert 6.0.4 (State-Ease Inc., MN, USA). The sensory analysis and antioxidant assay results were analysed by one-way analysis of variance (ANOVA) using commercial statistical

software IBM SPSS Statistics 26 of Tukey-LSD. Differences are considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Sensory Evaluation

F1 showed the significantly ($p < 0.05$) highest mean value for overall acceptance with an 8.2±1.17 mean value compared to other samples. F2 had a total of 7.9±1.36 mean score as the second-highest, followed by F3 with a 6.84±1.49 mean score. However, samples coded F4 and F5 were less favourable by panellists with a mean score of 3.8±2.25 and 4.3±1.89, respectively, as shown in Table 3. The overall acceptability of this beverage is assumed to be affected by the colour and taste attributes of the product. Polyherbal carbonated beverage with a lighter colour than the control with the sweetness and sourness, neither too high nor too low was preferred. According to Saint-Eve et al. (2009), the use of carbon dioxide in the beverage can enhance the flavour. Thus, the sourness in the sample was described due to carbon dioxide content, since F1 accounted for 12.5% of the polyherbal extract, which this amount is neither too high nor too low, resulting in stable sweetness and sourness of the sample. Statistical analysis of data obtained from questionnaires completed by the panellists indicated that the samples had significant differences ($p < 0.05$) among the others for all attributes. F1 was selected to be evaluated for physicochemical properties and its compliance with the *halalan thoyyiban* principle since this formulation obtained the highest overall acceptance among formulations.

Physicochemical Properties

The results for the physicochemical properties of polyherbal carbonated beverages are shown in Table 4. The pH of carbonated polyherbal beverage was 4.5±0.01. The lower pH of the beverage could be attributed to the addition of CO_2 in the sample, which affects the

Table 3
Mean scores for sensory evaluation

Attributes	F1	F2	F3	F4	F5	Control
Colour	6.3±2.12^{b}	6.1±2.29^{b}	4.8±1.92^{c}	7.5 ±1.22^{a}	6.6±1.50^{ab}	1.8±1.59^{d}
Aroma	7.0 ±1.46^{ab}	7.1±1.66^{a}	7.9±1.01^{a}	5.9±1.75^{c}	6.1±1.40^{bc}	2.64±2.66^{d}
Taste	8.0±1.34^{a}	6.8±1.55^{bc}	7.2±2.16^{ab}	4.3±2.16^{d}	6.1±1.47c	2.08±1.52^{e}
Sweetness	7.4±1.23^{ab}	7.1±1.47^{bc}	6.4±1.47^{c}	8.0±0.92^{a}	7.8±1.68^{ab}	5.0±0.00^{d}
Sourness	8.0±0.96^{a}	7.1±1.52^{b}	5.8±2.01^{c}	6.4±1.6b^{c}	5.9±1.67^{c}	5.0±0.00^{d}
Sparkling sensation	7.8±1.45^{a}	7.3±1.47^{ab}	6.7±1.70^{b}	8.0±1.17^{a}	7.8±1.17^{a}	5.0±0.00^{c}
Overall acceptance	8.2±1.17^{a}	7.9±1.36^{a}	6.8±1.49^{b}	3.8±2.25^{c}	4.34±1.89^{c}	1.7±1.10^{d}

Notes: a-d Means with different lowercase superscript letters are significantly different ($p < 0.05$)

Table 4
Physicochemical properties of polyherbal carbonated beverage

Parameters	Experimental value
pH	4.5±0.01
Total acidity	1.7±0.12 %
Carbon dioxide volume	3.0±0.0 g/L
Colour	L* = 33.00±0.49, a*= 4.10±0.13 and b* = 24.30± 0.71

concentration of the carbonic acid present in the drink (Aslam et al., 2016). Meanwhile, citric acid, an acidulant, gives sourness and increases thirst-quenching effects in the beverage (Steen, 2005). Moreover, the amount of 1.7±0.12 % for total acidity resulted from the polyherbal carbonated beverage. Similar reason with the pH, the addition of citric acid resulted in a high total of acidity could be noticed. The increasing pH value of carbonated beverages is linked to their total acidity (Abdulkareem et al., 2011). Furthermore, the CO_2 volume of the beverage contained 3.0± 0.0 g/L of each 330 mL of bottle drink. The volume for a typical carbonated soft drink ranged between 1 to 5 volumes (2-10 g/L) CO_2 (Abu-Reidah, 2020). Thus, the result for the CO_2 volume of polyherbal carbonated beverages was within the range of the standard carbonated drink. The results for the colour measurement are L* = 33.00±0.49, a*= 4.10±0.13 and b* = 24.30± 0.71. The polyherbal carbonated beverage can be described as brighter, less red and more yellow than control (L* = 9.71±0.14, a* = 1.73±0.16 and b* = 8.09±0.51). It might be because of the addition of water to the polyherbal extract resulting in a lighter colour.

Compliance of *Halalan Thoyyiban* Principle

Identification of Halal Control Point (HCP). Based on Figure 1, eight HCP were identified in developing polyherbal carbonated beverages. HCP 1 begins with the supply of raw materials and ingredients. All the raw materials and ingredients used were purchased from the company that obtained the Halal certificates for their product. Halal certification is an important component in the food and beverages industry in Malaysia as it indicates that the food product is assured in terms of religious compliance, safety, and hygiene. HCP 2, HCP 4, and HCP 6 were involved in the process of mixing the ingredients. During the mixing of ingredients, it was necessary to make sure all ingredients were not contaminated with the *haram* materials to ensure the product was processed in a *halal* manner from raw materials until consumed by the consumers.

On the other hand, HCP 3, HCP 5 and HCP 8 were involved in the equipment used during the process that should be cleaned and not contaminated with any *haram* materials. In addition. the place to store the product was separated from the non-*halal* sources. According to Islamic Law, *halal* foods should be free from contamination and not contain *haram* ingredients during preparation, processed, transported, or stored using any appliance

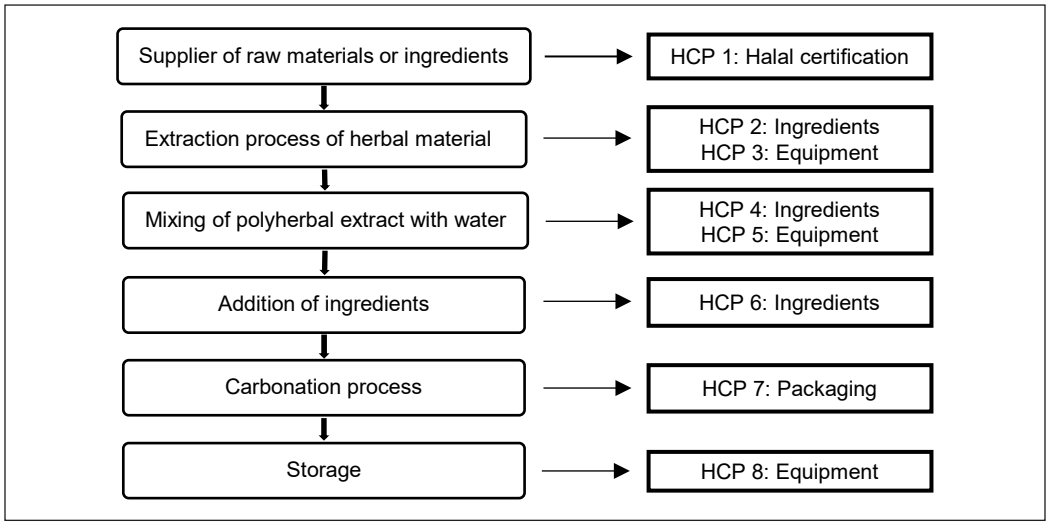

Figure 1. Halal Control Point (HCP) of the process

or facility (Hassan & Awang, 2009). Other than that, the food must also have no direct contact with any non-*halal* materials. Therefore, with the presence of unlawful materials, the polyherbal carbonated beverage can be considered *haram*.

HCP 7 was identified in the carbonation process. During this process, the beverage mixture was transferred into the bottle to allow the carbonation process in the capped bottle. Therefore, the polyherbal carbonated beverage packaging bottle came from *halal* materials and was not contaminated with non-*halal* sources. On the other hand, carbon dioxide addition was not considered as HCP because CO_2 is one of *halal* additive with E-Numbers "E290,' which this additive has been verified its status as *halal* (JAKIM, 2006). So, to simplify, all HCP were successfully identified in the development of polyherbal beverages. Therefore, this product can be considered *halal* as it was not involved with non-*halal* sources along the process.

Antioxidant Properties. The results for the antioxidant properties of the beverage are shown in Table 5.

DPPH Radical Scavenging Activity. The antioxidant activity referred to as DPPH radical scavenging activity of polyherbal carbonated beverage had obtained a lower value (1.25±0.18 mg GAE/mL) than the control (13.41±0.59 mg GAE/mL). As shown in Table 5, the scavenging activity of polyherbal carbonated beverages and control was significantly different as the *p*-value was less than 0.05. It might be due to the addition of water in polyherbal carbonated beverages, causing the concentration of the sample to become lower than the control. It was noticed that, with an increase in the amount of polyherbal extract,

Table 5
Antioxidant properties for polyherbal carbonated beverage and control

Sample	DPPH (mg GAE/mL)	FRAP (mM Fe (II))	TPC (mg GAE/mL)
Control Polyherbal	13.41±0.59[a]	1.42 ± 0.03[a]	3.12 ± 0.18[a]
Carbonated beverage	1.25±0.18[b]	1.08 ± 0.04[b]	0.39 ± 0.22[b]

Notes: [a,b] Different letters at each column indicates significant different ($p < 0.05$)

the antioxidant level was shown to rise. It might be because the increases in the amount of polyherbal extract in the sample caused the concentration of the sample to increase, which led to the stronger action of the scavenging activity (Rusmana et al., 2017). Thus, the higher the amount of polyherbal extract in the formulation, the higher the antioxidant activity of the sample.

Ferric Reducing Antioxidant Power (FRAP). The polyherbal carbonated beverage was found to have a significantly lower ($p < 0.05$) FRAP value, which is 1.08 mM Fe (II) equivalent compared to the control 1.42 mM Fe (II) equivalent as presented in Table 5. This result revealed that the control had stronger electron donors and was able to minimise the oxidised intermediates of the lipid peroxidation process. Thus, polyherbal carbonated beverages can be considered to have lower antioxidant activity than the control. The control is 100% pure polyherbal extract, which has more bioactive components, such as phenolic content that correlated with the higher antioxidant properties.

Total Phenolic Content (TPC). Based on Table 5, the control had significantly higher ($p < 0.05$) phenolic content of 3.12 mg GAE/mL than the polyherbal carbonated beverage (0.39 mg GAE/mL). Thus, the control can be considered to have higher antioxidant properties than the beverage. It might be because the polyherbal extract has a higher total phenolic content than the polyherbal carbonated beverage. Both edible and non-edible plants have phenolic compounds, which give the biological effects of antioxidant activity (Kahkonen et al., 1999). A study showed that *O. stamineus* aqueous extract has higher antioxidant properties than other herbal plants, such as *P. niruri*, *S. crispus,* and *S. rebudiana,* which might be because the total phenolic content plant is in the appropriate range (Rahim et al., 2018). According to Hasmida et al. (2015), a higher concentration of the phenolic compound can benefit from preventing the oxidative activities of plant extract. Therefore, it showed that the antioxidant activities for the plant extracts were affected by their phenolic content.

Nutritional Contents. Food products that comply with the *halalan thoyyiban* principle must be healthy and nutritious. Besides providing antioxidant properties, the amount of sugar or carbohydrate content needs to be considered; hence all consumers can drink this beverage, including diabetic patients, when it is proven with low sugar or carbohydrate

content. Therefore, the nutritional contents of the beverage were determined to comply with the principle, as shown in Table 6. Based on the result, the beverage contains 0.3% of carbohydrates. According to Kregiel (2015), sucrose, glucose or fructose is used as natural carbohydrate sweeteners in soft drink. Due to the sugar content in the regular soft drink, the total carbohydrate can be determined as one of the nutritional compositions of the beverage. However, in this study, sugar or sucrose was not included in the formulation of polyherbal carbonated beverages and replaced by sucralose. Therefore, it resulted in a low total carbohydrate in the beverage with only 0.3%. Typically, carbonated beverages have about 10% to 12% sugar content (Abu-Reidah, 2020). Thus, the polyherbal carbonated beverage can be considered lower nutritious than regular carbonated beverages. It indicates that the developed polyherbal carbonated beverage is healthier than regular soft drink as its formulation has no sugar content, which compliance with the *halalan thoyyiban* concept. It is agreed with Hassan (2011) that stated the combination of *halal* and *tayyib* must ensure that the food is *halal*, safe and healthy. Furthermore, the result revealed that the beverage has no protein or fat content. It is because the formulation of the sample does not involve protein and fat sources. Moreover, the beverage contains 2.0 kcal/100 g of energy content. The energy content of soft drinks per 100 mL ranged from 0 kcal to 55 kcal (Iglesias et al., 2016). Thus, the energy content for polyherbal carbonated beverages lies within the range.

Table 6
Nutritional content of polyherbal carbonated beverage

Nutritional content	Amount
Carbohydrate	0.3%
Protein	0.0%
Fat	0.0%
Energy	2.0 kcal/100g

Toxicity. Food products that comply with the *halalan thoyyiban* principle must be safe to consume and not harmful to our bodies. A toxicity test was performed to determine the LC_{50} of the polyherbal carbonated beverage to determine its safety. There was a positive linear relationship between mortalities of brine shrimps and concentration of polyherbal carbonated beverage where, as increasing the concentration, the rates of mortalities also increased. The percentages of mortality of brine shrimp nauplii for concentration 10 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL of polyherbal carbonated beverage were 7%, 23%, 33%, 43% and 47% respectively. In this study, LC_{50} of polyherbal carbonated beverage was found at 1066.60 µg/mL concentration. Based on Clarkson's toxicity criterion, a sample with LC_{50} more than 1000 µg/mL is non-toxic (Clarkson et al., 2004). Thus, the polyherbal carbonated beverage used in this study was found to have non-toxic properties as deduced from their LC_{50} values (Dosumu et al., 2010). Therefore, the polyherbal carbonated beverage was found to be safe regarding brine shrimp toxicity assay and since polyherbal carbonated beverage was produced at relatively low concentration due to the combination of polyherbal extract and water.

CONCLUSION

The addition of carbon dioxide by the carbonation process can enhance the taste of the polyherbal beverage. The most accepted formulation was F1 (12.50% polyherbal extract and 87.50% water). Its physicochemical properties were followed the standard values. The polyherbal carbonated beverage exhibited antioxidant properties and is also nutritious, fulfiling the *halalan thoyyiban* principle as it provides health benefits. Moreover, LC_{50} of polyherbal carbonated beverage was found at 1066.60 µg/mL, which can be declared non-toxic and safe for consumption. Thus, the beverage can be considered to comply with the *halalan thoyyiban* principle. Therefore, it can be concluded that the formulated polyherbal beverage is not only accepted by the consumer but was also complied with the *halalan thoyyiban* principle.

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