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Physicochemical Characteristics of Butterfly Pea Flower Petals Steep Obtained at Different Steeping Temperature and Time

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Abstract

Flower is an important part of the plant containing phytochemical compounds, especially the phenolic acids, flavonoids, and anthocyanin that can be used as natural antioxidants. Phytochemical compounds are usually drawn by steeping the flower petals in hot water. Indeed, different temperatures and steeping times would affect the yield of phytochemicals obtained. The butterfly pea flower is one of edible flowers that brings antioxidant activity due to the presence of phytochemical compounds, namely the flavonoids and phenolic acids. Nevertheless, there have been limited studies dedicated to the search of steeping condition of butterfly pea flower petals in water. Thus, this study aimed to determine the time and temperature of steeping to produce the butterfly pea steep with preferred physicochemical characteristics. The result indicates that butterfly pea flower petals which undergo steeping at 60°C for 45 minutes produce antioxidant activity with an IC50 value of 251.89 ± 32.02 ppm. Furthermore, the butterfly pea flower steep produced had blue to purple colour with °Hue of 277.06 ±2.55, 1.23 ± 0.07 mg/L of total monomeric anthocyanin content, 6.83 ± 0.02 of pH and 138.42 ± 15.88 mg GAE / g sample of total phenolic content.

Keywords: antioxidant; butterfly pea flower; steep; temperature; time

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INTRODUCTION

Phytochemical compounds plants metabolites that are mainly found in leaves, roots, and flower with many functional properties. Polyphenols compounds are an example of phytochemical compounds characterized by at least one phenol unit in their molecular structure. Polyphenols divide into two main groups: flavonoids and phenolic acids. *Anthocyanins* are flavonoid compound compounds associated with the pigments found in plant tissue, including in edible flowers that possesses antioxidant activity (Kuspradini *et al*., 2016 and Gan *et al*., 2019).

Consumer awareness on food safety has promoted the use of natural colorings derived from edible flowers in food applications. Marigold (*Tagetes erecta*), hibiscus, roselle, and rose are some good examples of edible flowers. Tea drink of marigold flowers brewed at 100°C for 3 minutes contained the highest level of total phenolic content, 35.446 (mg GAE/g DW). In addition, marigold tea drinks brewed at 95°C for 5 minutes exhibited the highest total reducing capacity (Ferric Reducing Antioxidant Power, FRAP assay; 36.143 µmol FeSO4 /g DW) (Ngoitaku *et al*., 2016).

Another edible flower widely used nowadays is the butterfly pea flower (*Clitoria ternatea*), commonly known as *Bunga telang* in Indonesia. This flowering plant belongs to the *Fabaceae* family. The butterfly pea flower is a tropical flower which can be found in the garden or wildly grow as it is easy to grow and maintain. The leaves and roots of butterfly pea flowers have been used to treat conditions such as urinogenital disorders, animal stings, and anthelmintics. Besides leaves and roots, the flower has functional properties such as anxiolytic, anti-inflammatory, analgesic, anti-microbial, and antioxidant activity (Chusak et al., 2019, Saptarini et al., 2015; Ravindran, 2017; Jami et al., 2018). Butterfly pea flower has antioxidant activity due to its bioactive compounds such as anthocyanins, alkaloids, steroids, tannin, reducing sugars and flavonoids (Chusak et al., 2019, Saptarini et al., 2015; Ravindran, 2017; Jami et al., 2018). The deep blue colour of butterfly pea flower is also identified as rich in phytochemical compounds, mainly the anthocyanins. The aqueous extract, the butterfly pea flower exhibits good antioxidant activity. The percentage scavenging activity of butterfly pea flowers' aqueous extract reached 390.67 2.309% at only 25 g/mL compared to methanol extraction, which only exhibits 32.67±1.16% at the same concentration. Moreover, the total phenolic content of butterfly pea flower is 61.7±0.2 mg gallic acid equivalent/ g dry sample and IC50 of 32.67% scavenging activity per sample extract concentration (ug/ml) in 25µg/ml (Rabeta and Nabil, 2013 and Ramli and Rabeta, 2018). Under highly acidic conditions (pH 1-2), the colour of the pigment is red. The colour is purple to blue under weakly acidic to neutral conditions, but it is yellow-green under basic conditions (pH 8-14). Recently, artificial colouring was mostly replaced with the butterfly pea flower for its phenolic natural pigment with various colours (Chusak *et al*., 2019).

The phytochemicals compound can be extracted by steeping the flower in hot water as the standard method to prepare edible flower-based products (Hajiaghaalipour *et al*., 2016). The difference in steeping conditions would result in different yield and physicochemical characteristics phytochemicals obtained. There have been limited studies on the steeping condition of butterfly pea flower as it quickly produces blue colour when directly steeped in water. Therefore, this study intends to determine the time and temperature of steeping to produce the butterfly pea steep with preferred physicochemical characteristics.

MATERIALS AND METHOD

Materials and Equipment

Materials used in this research were butterfly pea flower (*Clitoria ternatea*) collected from Pluit, North Jakarta, water (Amidis), buffer solution pH 4 and pH 7 (Merck), NaOH (Merck), gallic acid (Merck), Folin-Ciocalteu reagent (Merck), Na_2CO_3 (Merck), DPPH (1,1-diphenyl-2-picrylhydrazyl), sodium acetate (Merck), HCl 37% (Smart Lab), ethanol (Smart Lab), potassium chloride (Merck). While the equipment used were glassware (Iwaki Pyrex), thermometer (Alla France), heater (Cimarec), table balance (O'Haus), analytical balance (Sartorius), spatula, pH meter (Metro Ohm), CR-400 chromameter (*Konica Minolta*), U-1800 UV–Vis spectrophotometer (Hitachi), quartz cuvette, bulp pump, filter cloth, sieve, aluminum foil, dark bottle, refrigerator (Sharp).

Butterfly Pea Flower Petals Steeping Process

The steeping process of butterfly pea flower petals was performed following the method conducted by Saptarini *et al.* (2015) with modifications. Firstly, the fresh butterfly pea flower was cut to form 5 mm strips. Then, 2.5 grams of the flower strips were introduced into 100 mL water for the steeping process. The steeping conditions (temperature and time) studied were 25°C for 12 hours, 24 hours, and 36 hours, 45°C for 1 hour, 2 hours, and 3 hours, and 60°C for 15 minutes, 30 minutes, and 45 minutes. The steeping process of 25°C was done inside the cupboard at room temperature and closed with aluminum foil. At the same time, water was heated to reach the steeping temperature of 45°C and 60°C. A Thermometer was used to check the water's temperature before adding the flower strips. The temperature is periodically checked and maintained every 5 minutes. After each treatment, flower strips were removed through filtration using a filter cloth and sieve. The steep was stored in the dark bottles, let to cool to room temperature and kept in the refrigerator for further analysis, namely pH, colour, antioxidant characteristics, total phenolic content, antioxidant activity, and monomeric anthocyanin content.

Determination of pH

The pH value of butterfly pea flower steep was measured using a pH meter based on AOAC method number 960.19. The analysis was done with three replications. Before measuring the sample's pH value, the pH meter was calibrated using buffer reference solution pH four and seven standards. Prior to pH measurement, the steep sample was equilibrated to room temperature. The electrode was submerged into the sample for pH measurement. Accordingly, it must be rinsed with distilled water and wiped before and after measurement (AOAC, 2005 and Amaolo, 2017).

Determination of Colour

Chromameter CR-400 "Konica Minolta" was used to evaluate the butterfly pea flower's steep colour. A CIELAB system with L**a***b** as parameters used for assessment of colour. The analysis was done to each sample in triplicates. The chromameter was calibrated prior to colour analysis by testing it first on the standard white colour. The sample was put in the designated container or using a Petri dish, subjected to chromameter, and measured three times. The value measured was L*, *a**, and *b**, then °Hue will be

calculated using equation 1 (Nielsen, 2010). Then the °hue value was converted into colour using the Munsell colour system, as shown in Table 1.

$$\text{Hue } (H^*) = \tan^{-1}(\frac{b^*}{a^*}) \quad (1)$$

Table 1. Munsell color system (Kuehni, 2010)

H^*	Color
18° - 54°	Red
54° - 90°	Yellow-red
90° - 126°	Yellow
126° - 162°	Green – yellow
162° - 198°	Green
198° - 234°	Blue – green
234° - 270°	Blue
270° - 306°	Blue – purple
306° - 342°	Purple
342° - 18°	Red - purple

Determination of Antioxidant Activity (IC_{50})

Because DPPH act as free radicals, thus, DPPH is selected as a suitable representative method for evaluating radical scavengers due to its rapidity and sensitivity. The butterfly pea flower steeps were analyzed for their antioxidant activity using DPPH method following Hajiaghaalipour *et al*. (2016) and Safdar et al. (2016) modification. The analysis was done with three replications.

Prior to analysis, 0.1mM DPPH solution was prepared by dissolving 10 mg of DPPH in 25.4 mL ethanol and followed by diluting the solution to 0.02mM. The resulting solution was stored in the dark room for 30 minutes before analysis. A carefully measured 2 mL of 0.1mM DPPH solution was added to 1 mL of butterfly pea flower steep as the sample in a test tube. The samples were prepared with different concentrations by diluting them with ethanol. A control sample was made using 1 mL of ethanol and mixed with 2 mL of 0.02mM DPPH solution in a test tube. In addition, a blank solution was made with the same treatment, substituting the DPPH with ethanol. Then each test tube was covered with aluminum foil and incubated 30 minutes prior to reading with spectrophotometer testing at 516 nm. The absorbance obtained was then introduced into Equation 2 to calculate the radical scavenging activity (RCA) of DPPH (Hajiaghaalipour *et al*., 2016 and Safdar *et al*., 2016):

$$RCA\ (\%): \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100\% \quad (2)$$

where:
$Abs_{control}$ = the absorbance of control
Abs_{sample} = the absorbance of extract/sample

The value of IC_{50} was determined by plotting the scavenging activity of DPPH against concentration of sample (i.e., linear regression: y = ax + b), where Y-axis was %inhibition and X-axis was extract concentration. The IC_{50} value was defined as the effective concentration that could scavenge 50% of the initial DPPH (i.e., 50% inhibition).

Determination of Total Phenolic Content (TPC)

Total phenolic compound was determined using Folin-Ciocalteu method adapted from Ngoitaku *et al*., (2016); meanwhile, the calculation was adapted from Kupina *et al*, (2018). The analysis was done with three replications. First, a standard curve should be prepared. Prior to TPC measurement, a standard curve was prepared by plotting the absorbance versus several concentrations of gallic acid solution containing Folin-Ciocalteu reagent. An exactly weighed 10 mg gallic acid was dissolved in 10 ml water, and the parent solution was diluted into several concentrations of 100 ppm, 80 ppm, 60 ppm, 40 ppm, and 20 ppm. Then 0.1 mL of gallic acid was taken and added with 0.2 mL of 10% Folin-Ciocalteu reagent, mixed well-using vortex. The mixture was added with 0.8 mL of 700 mM sodium carbonate solution and incubated for 2 hours at room temperature. After 2 hours, the absorbance of the mixture was measured at 765 nm wavelength using a spectrophotometer. For the blank solution, the 0.1 mL of standard gallic acid was replaced with 0.1 mL ethanol and added with 0.2 mL 10% Folin-Ciocalteu reagent, then added 0.8 mL of 700 mM sodium carbonate solution.

The phenolic content of the sample was determined by preparing a 0.1 mL sample and addition of 0.2 mL of 10% Folin-Ciocalteu reagent. The mixture was completely mixed using a vortex before further added with 0.8 mL of 700 mM sodium carbonate solution. The mixture's absorbance was determined at 765 nm wavelength after incubation at room temperature for 2 hours. The blank preparation followed the same procedure of blank making in the standard curve determination. The absorbance obtained used in the calculation the TPC (mg GAE/g sample) using Equation 3 as follows:

$$TPC = \frac{A-b}{m} \times \frac{V \times df}{W \times 1000} \times 100 \quad (3)$$

where:
A = absorbance
b = the y-intercept of the calibration curve
m = the slope of the calibration curve
V = the volume of the sample test solution (100 mL)
df = dilution factors
W = weight of tested sample (g)
1000 = conversion factors from mL to L

Determination of Total Monomeric Anthocyanin Content (TMAC)

The total monomeric anthocyanin content of the butterfly pea flowers steeps were quantified based on the pH-differential method adapted from Sutharut and Sudarat (2012). The analysis was done with three replications. The pH differential method was based on the reaction of anthocyanin that show oxonium colour

at pH 1.0 and colourless hemiketal at pH 4.5, which allows the accurate and rapid measurement of the total anthocyanins, even when there are interfering compounds such as a polymer of degraded pigment. As pH changes, anthocyanin pigment's structure undergoes reversible transformations, reflecting in contrast in different absorbance spectrums.

A carefully measured 2 mL sample was taken and transferred into a 10 mL volumetric flask. The volume was adjusted with potassium chloride buffer (pH1.0) and sodium acetate buffer (pH 4.5), resulting in 2 dilution samples. After being equilibrated for 15 min, 520 and 700 nm wavelength was used to measure the diluted samples' absorbance. Distilled water was used and measured with the same wavelength for the blank solution. Equation 4 calculates the diluted sample's absorbance (A). Subsequently, equation 5 was used to calculate the monomeric anthocyanin content (g/L) in the sample.

$$A = (A_{700-}A_{520})_{pH\ 1.0} - (A_{520-}A_{700})_{pH\ 4.5} \quad (4)$$

$$TMAC = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (5)$$

where:

MW = molecular weight (cyanidin-3-glucoside is 449.2 g/L)

DF = dilution factor

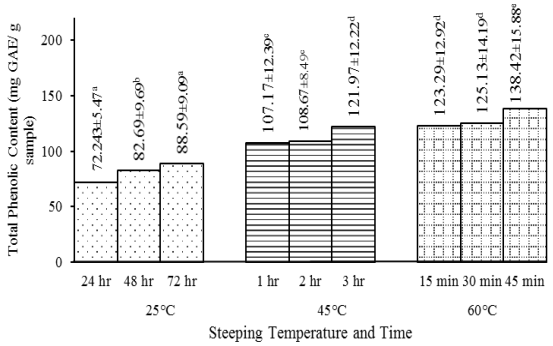
ε = molar absorptivity (cyanidin-3-glucoside is 26,900 L mol^{-1} cm^{-1})

RESULTS AND DISCUSSION

Total Phenolic Content of Butterfly Pea Flower Steep on The Different Steeping Temperature and Time

The phenolic compound was characterized based on the presence of an aromatic ring in its structure with at least one hydroxyl group (Nollet and Uribe, 2018). The measurement of total phenolic content was based on the reaction of phenols compound present in the sample with Folin-Ciocalteu reagent in the presence of sodium carbonate solution, forming a blue colour complex. Statistically, there was a significant effect of steeping temperature and time on the steep total phenolic content of butterfly pea flower, as demonstrated in Figure 1.

The total phenolic content of butterfly pea flower steep obtained from steeping at 60°C for 45 minutes was significantly higher than those obtained at other steeping conditions. Exactly, a higher temperature and a prolonged steeping time would result in a higher phenolic content (Nollet and Uribe, 2018). However, when the steeping temperature was too high and/or the steeping time was too long, lower recovery yields were attained, which was most probably due to thermal degradation of some phenolic compounds.



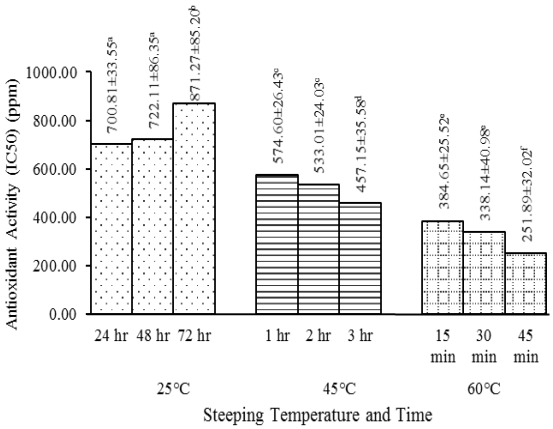
Note: Different superscript indicates significant differences ($p<0.05$)

Figure 1. Total phenolic content of butterfly pea flower steep on the different steeping temperature and time

Based on Rabeta and Nabil (2013), the steeping condition of butterfly pea flower at 25°C for 1 hour has total phenolic content of 18.5 to 20.7 mg GAE per gram sample. The result was comparable to butterfly pea flower steep, which has higher total phenolic content when applied for more a prolonged time. According to Spigno *et al.* (2007), the temperature of 45°C is preferable to 60°C when a long time of extraction occurs. However, it is advised not to steep butterfly flower petals for longer than 8 hours as the phenol compounds will degrade. In addition, it could interfere with the results because of thermal degradation or polymerization reaction.

Antioxidant activity (IC_{50}) of Butterfly Pea Flower Steep on The Different Steeping Temperature and Time

As presented in Figure 2, the steeping condition significantly affected the antioxidant activity.



Note: Different superscript indicates significant differences ($p<0.05$)

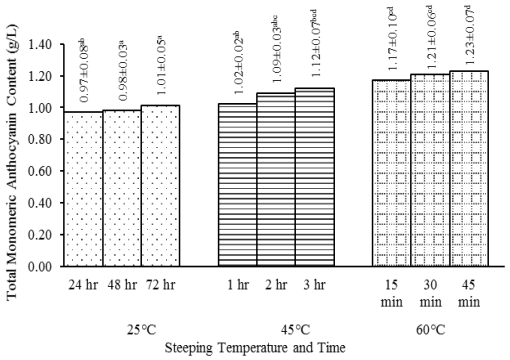
Figure 2. Total phenolic content of butterfly pea flower steep on the different steeping time and temperature

Figure 2 shows that higher temperature and longer time will significantly result in higher

antioxidant activity except at 25°C, which shows that a long time would lower the antioxidant activity. Steeping conditions at 60°C for 45 minutes and 45°C for 3 hours show significantly higher results compared to shorter times at the same temperature. Higher temperatures and longer times give the highest antioxidant activity as more of the phenolic compound is easier to extract at higher temperatures and longer. These results are in good agreement with Figure 1, in which the highest total phenolic content also has the highest antioxidant activity.

Antioxidant activity measured based on the DPPH method could reflect the general antioxidant activity of a sample. Almost all compounds with antioxidant properties will inhibit the 2,2-diphenyl-1-1-picrylhydrazyl. The results also correspond with Rahim *et al*. (2010), in which there was an increment of antioxidant activity as the extraction temperature increased from temperature 40 to 100°C. Furthermore, antioxidant activity has retained better when extracted for 2 hours; however, there was no significant difference between extraction of 1 hour or 2 hours, but with 3-hour extraction, there were significantly different.

Total Monomeric Anthocyanin Content of Butterfly Pea Flower Steep on the Different Steeping Temperature and Time



Note: Different superscript indicates significant differences ($p<0.05$)

Figure 3. Total monomeric anthocyanin content of butterfly pea flower steep on the different steeping time and temperature.

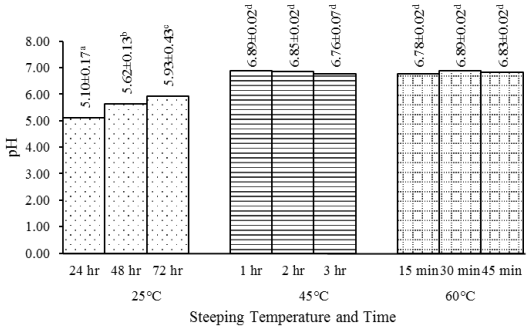
The measurement of total monomeric anthocyanin content was based on the changes in anthocyanin pigment structure when there was a change in its pH. The changes of anthocyanin pigment are reversible, in which they could change back and forth depending on the pH of its environment. Its changes reflected very contrast in different spectrums of absorbance. The pH condition used in this assay was pH 1.0 and pH 4.5, as in pH 1.0, the anthocyanin pigment will show oxonium colour, while at pH 4.5, it will show a colourless hemiketal. The conditions of pH one and 4.5 allow accurate and rapid measurement of total anthocyanin pigment content, even when there are interfering compounds such as a polymer of degraded pigment (Kuspradini *et al*., 2016 and Gan *et al*., 2019).

Several factors have the potential to influence the anthocyanin content during extraction or steeping process, such as temperature and time. The steeping condition significantly affected the total monomeric anthocyanin content, as shown in Figure 3. According to Figure 3, the steeping condition at 60°C for 45 minutes shows anthocyanin content of 1.226 ± 0.073 g/L; however, this result is not significantly different from the steeping condition that is shorter in time (15 and 30 mins) at the same temperature and steeping condition of 45°C for 3 hours.

According to Ruenroengklin *et al.* (2008) and Le *et al*. (2019), extraction of anthocyanin pigment at 45°C and 60°C show higher antioxidant activity compared to 25°C. However, the preservation rate of anthocyanin content at 25°C was better than at 45°C and 60°C (Liu *et al.*, 2018). Pham *et al.* (2019) stated that the extract of butterfly pea flower at 60.6°C exhibits higher pigment anthocyanin content compared to the extraction at lower temperatures or even higher (40°C to 80°C). Thus, these findings follow the result of this research, in which steeping at 60°C exhibits higher total monomeric anthocyanin content.

pH of Butterfly Pea Flower Steep on The Different Steeping Temperature and Time

The pH value of the steep is closely related to colour of butterfly pea flower steep. However, as the flower is used similarly to tea, pH is also essential to show the acidity present in flower. Figure.4 displays the significant effect of steeping conditions on the pH of butterfly pea steep.



Note: Different superscript indicates significant differences ($p<0.05$)

Figure 4. pH of butterfly pea flower steep on the different steeping time and temperature.

The pH of the butterfly pea flower steep obtained from steeping at 25°C is significantly lower than that obtained from steeping at 45°C and 60°C. The deterioration of butterfly pea flower could cause the results during steeping as the steeping period was extended to 3 days and spoiled over time. According to Saptarini *et al*. (2015), the butterfly pea flower extract has a pH value of 5 to 8 and is blue.

°Hue of Butterfly Pea Flower Steep on the Different Steeping Temperature and Time

Butterfly pea flower has a blue colour that is caused by the presence of anthocyanin pigment, which is dependent upon the pH, as pH of the solution can induce the change of anthocyanin structure. There was a significant effect on the steeping time and temperature toward °Hue of butterfly pea flower steep, as shown in Table 3.

As clearly presented in Table 3, the °Hue of butterfly pea flower steep is in quadrant 4, where the colour varies from blue to red. The butterfly pea flower obtained from steeping at 60°C has a significantly lower °Hue value than those obtained from steeping at 45°C and 25°C. Thus, the colour of butterfly pea flower steep at 60°C is blue while steeping at 45°C will result in the colour of steep that is in the brink from blue to purple while steeping at 25°C resulting in steep purple colour.

Table 3. °Hue of butterfly pea flower steep on the different steeping temperature and time

Temperature	Time	°Hue	Colour
25°C	24 hr.	311.09±2.13^{a}	purple
	48 hr.	310.33±2.20^{a}	purple
	72 hr.	312.37±2.02^{a}	purple
45°C	1 hr.	298.44±1.05b	Blue-purple
	2 hr.	295.38±0.90c	Blue-purple
	3 hr.	295.52±1.44^{d}	Blue-purple
60°C	15 min	287.35±2.82^{d}	Blue-purple
	30 min	275.62±2.88^{e}	Blue-purple
	45 min	277.06±2.55^{e}	Blue-purple

Note: Different superscript indicates significant differences ($p<0.05$)

The butterfly pea flower petal steep obtained in this research was comparable to the study by Shruthi and Ramachandra (2019), in which the colour of rosella tea was red with a pH between 2.3 – 2.5. The butterfly pea flower petal steep also exhibited the same color and pH value behaviour with the study conducted by Saptarini *et al.* (2015). They reported that with a pH value of 5, butterfly pea flower extract has a violet colour, while pH values of 6 and 7 has the colour of blue.

CONCLUSION

The steeping process at 60°C for 45 minutes was selected to produce butterfly pea flower steep which showed significantly higher antioxidant activity with IC50 of 251.89 ± 32.02 ppm. In addition, the steep produced also had total phenolic and monomeric anthocyanin content of 138.42 ± 15.88 mg GAE/g sample and 1.23 ± 0.07 mg/L, respectively. Furthermore, the butterfly pea flower steep produced had blue, purple colour with °Hue of 277.06 and pH of 6.83 ± 0.02.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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