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Improving the antioxidant properties of coffee-leaf tea by adding areca nut powder and stevia leaves

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ABSTRACT

In this study, we described ways to improve the antioxidant properties of coffee-leaf tea by adding areca nut powder and stevia leaves. A non-factorial completely randomized design (RALF) was used in this study. We included five treatment formulations with five repetitions per formulation, which included F1 (100% coffee leaves: 0% areca nut: 0% stevia), F2 (95% coffee leaves: 1.5% areca nut: 3.5% stevia), F3 (90% coffee leaves: 3% areca nut: 7% stevia), F4 (85% coffee leaves: 4.5% areca nut: 10.5% stevia), and F5 (80% coffee leaves: 6% areca nut: 14% stevia). The results showed that adding areca nut powder and stevia leaves significantly improved the antioxidant properties of coffee-leaf tea. The IC_{50} value of the tea decreased by 67.96%, and its total phenol content increased by 38.85%. The water content and ash content of coffee-leaf tea produced in this study met the SNI standards. The results of the organoleptic tests showed whether the panelists accepted the color and taste of the samples.

Key words: Antioxidant; areca nut; coffee leaves; stevia leaves; total phenolics.

1 INTRODUCTION

Coffee-leaf tea is a popular and invigorating beverage consumed in Indonesia, particularly within the Minang community of West Sumatra Province. Its history dates back to the Dutch era. Fibrianto et al. (2020) stated that it offers a delightful taste and contains antioxidant compounds while maintaining low caffeine levels. Several studies have elucidated the health benefits of bioactive compounds present in coffee leaves. For example, Campa et al. (2012) reported the presence of the phenolic compound mangiferin in Arabica coffee leaves. Mondolot et al. (2006) and Mazzafera (1999) found that *Coffea canephora* leaves contain caffeoylquinicacid and 21.9 g/kg caffeine. Chen et al. (2018) also found mangiferin, iso-mangiferin, trigonelline, 3-caffeoylquinic acid (3-CQA), and 5-caffeoylquinic acid (5-CQA) in samples dried using certain methods.

Several studies have investigated ways to develop coffee-leaf tea as a functional beverage using two approaches, which include refining the process and adding more ingredients to the formulation. Novita (2018) found that coffee-leaf tea subjected to processing techniques such as oven blower and smoker methods exhibited superior functional properties compared to those processed traditionally. Legowo et al. (2021) stated that factors such as temperature and storage time decreased the content of antioxidant compounds. Besides processing, adding other ingredients to the formulation, specifically natural materials containing antioxidant bioactive compounds, such as Probiotic Beverage, was investigated by Zubaidah et el. (2021). However, that study primarily focused on enhancing the taste, rather than the antioxidant properties. Areca seeds and stevia leaves are natural antioxidant ingredients that may be incorporated into coffee-leaf tea.

Zhang et al. (2009) adding that areca nut powder contains total phenols and flavonoids of 114.14mg/g and 77.36mg/g respectively. Ismet et al. (2010) revealed that stevia leaves extracted using the maceration method have greater total phenol, total flavonoid and antioxidant activity values compared to the Soxhlet extraction process. Putri et al. (2019) added that extracting stevia leaves using maceration had a total phenol value of 65.21mg gallic acid which is equivalent to phenolic compounds. Therefore, the addition of areca nut powder and stevia leaves is expected to enhance the antioxidant properties of coffee-leaf tea and also greatly enhance its taste.

2 MATERIAL AND METHODS

2.1 Tools and Materials

The tools used in this study included an autoclave, cabinet dryer, desiccator, hot plate, laminar airflow (LAF), magnetic stirrer, mortar, analytical balance (Ohaus Pioneer PX223, USA), oven (Memmert S-400, MEMMERT GmbH+Co.KG, Germany), pH meter (Eutech PC-510), 40-mesh sieve (CU Class A high-quality test sieve), and vortex (Digisystem VM-1000, Digisystem Laboratory Instruments Inc., Taiwan). The materials used were young areca seeds from Indonesia Alami MSME, stevia leaf powder from

Gubuk Herbal Indonesia MSME, DPPH (2,2-diphenyl-1-picrylhydrazyl), arabica coffee leaves from Jagadraye coffee Pagaralam MSME collected from the fourth to sixth leaves in one stalk, 95% ethanol (certified for analysis), Folin-Ciocalteu, and methanol (certified for analysis).

2.2 Preparing coffee-leaf tea

The method used for preparing coffee-leaf tea was proposed by Fibrianto et al. (2020) First, the fourth to sixth coffee leaves of a stalk were picked. Then, the samples were washed with running water, dried at room temperature for 20–25 h, and dried in a cabinet dryer at 80 °C for 15 min. Next, the size was reduced, and the fibers of the leaves were separated. Finally, the samples were dried in a cabinet dryer at 80 °C for 7 h.

2.3 Preparation of areca nut powder

Areca nut powder was prepared following the method proposed by Handayani et al. (2016). First, young areca nut fruits were peeled, and the seeds were collected. The seeds were cleaned, cut into pieces, and dried in an oven at 45 °C for 24 h. The dried samples were pulverized using a blender and sieved using a 40-mesh sieve.

2.4 Preparing coffee-leaf tea by adding areca nut powder and stevia leaves

The method used for preparing coffee-leaf tea formulations was proposed by Fibrianto et al. (2020), and modified by us. First, coffee-leaf tea, stevia leaf powder, and areca nut powder were mixed based on the treatment formulation. Then, 3 g of each treatment formulation was placed in tea bags. The tea bags were brewed with 200 mL of hot water at 90 °C for 2 min. This sample was used for analyzing the parameters.

2.5 Study Design

We conducted this study using a non-factorial completely randomized design with five formulation treatments, and each treatment was repeated five times. The formulation treatments are presented in the Table 1.

Table 1: The formulation treatments.

Formulation Treatment	Coffee leaves tea (w/b%)	Areca nut powder (w/b%)	Stevia leaves powder (w/b%)
F1	100	0	0
F2	95	1,5	3,5
F3	90	3	7
F4	85	4,5	10,5
F5	80	6	14

Description: The total weight of the coffee-leaf tea formulation that was added with areca nut powder and stevia leaves was 3 g.

The parameters recorded included water content (AOAC, 2005), ash content (AOAC, 2005), pH of the solution (Kumesan et al., 2017), Total phenol (Marjoni et al., 2015), IC_{50} (AOAC, 2005), and and organoleptic test (Pratama, 2018).

2.6 Water Content

The porcelain cup was dried in an oven at 105 ºC for 1 hour. Place the cup in the desiccator (approximately 15 minutes) and wait until it cools. Weigh the cup until the weight is constant. The cup containing the sample was weighed and then dried using an oven at 105 ºC for 5 hours. Place the cup in the desiccator and wait until it cools and then weigh it again. Calculation of water content: Weight loss (g) = initial sample weight (g) – weight after drying (g). Water content (wet weight) = weight loss (g) x 100% : initial sample weight (g).

2.7 Ash Content

Ash content analysis was carried out using the direct heating method. The sample was weighed at 3-5 g W and put into a porcelain cup of known weight A. The sample was charcoaled until it did not smoke, then put in a furnace at a temperature of 500-600 ºC until it became white ash. The cup containing the ash is cooled in a desiccator and weighed until a fixed weight X is obtained. The ash content is determined by the formula: Ash content = [X-AW] x 100

2.8 Degree of Acidity (pH)

According to Kumesan et al. (2017), the pH value was determined using a modified pH meter as follows: The pH meter must first be calibrated to the sensitivity of the pointer with a pH 7 buffer solution. The sample of about 10 g was weighed and homogenize with 20 mL of distilled water for 1 minute, and pour into a 10 mL beaker. The electrode was immersed in the sample and waited for a while until the pH is stable. The pH value can be directly read on the pH meter scale, after which the electrodes were removed and rinsed with distilled water.

2.9 Total phenol content

The total phenol level was determined based on Marjoni et al. (2015) which have been modified are as follows: 50 g of coffee-leaf tea were weighed, 500 mL of distilled water were added and stirred until homogeneous. 100 mg of the extract are then dissolved to 10 mL with distilled water to obtain a concentration of 10 mg/mL. The concentration of 10 mg/mL was pipetted into 1 mL and diluted to 10 mL with distilled water and the concentration of the extract was 1 mg/mL. Pipette 0.2 mL of extract, add 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, shaken and allowed to stand for 8 minutes. Add 3 mL of 10% Na2 CO3 to the mixture, leave the solution for 2 hours at room temperature. The absorption

was measured using a UV-Vis spectrophotometer with an absorption wavelength of 765 nm. The phenol content was obtained as mg gallic acid equivalent/g sample and created a calibration curve with the regression equation y = ax + b, where X is the concentration and Y is the absorbance.

Preparation of gallic acid calibration curve with FolinCiocalteu. Phenol reagent: 50 mg of gallic acid was weighed, 1 mL of 96% ethanol was added, distilled water was added until the final volume was 50 mL in such a way that a concentration of 1 mg/ mL was obtained as the mother liquor. The mother liquor was pipetted to 1 mL, 1.25 mL, 1.5 mL, 1.75 mL, and 2 mL, respectively and then diluted with distilled water to a final volume of 10 mL at concentrations of 100, 125, 150, 175, 200 ppm gallic acid. Furthermore, a 0.2 mL pipette of each concentration of the gallic acid solution was added, then 15.8 mL distilled water and 1 mL Folin-Ciocalteu reagent are added and the mixture was stirred until homogeneous and allowed to stand for 8 minutes. 3 mL of 10% Na2 CO3 solution was added, shaken homogeneously, and then allowed to stand for 2 hours at room temperature, and the absorption was measured at an absorption wavelength of 765 nm.

2.10 Antioxidant Activity (IC_{50})

The antioxidant activity test was carried out by calculating the IC_{50} value using the DPPH method (1.1 diphenyl 2 picrilhydrazil) according to Association of AOAC (2005), which has been modified: The combined ground coffee-leaf tea sample weighed ± 0.1 g and then dissolved with 100 mL of methanol (1000 ppm). The sample solution was formulated into 5 concentration series, namely 100 ppm, 80 ppm, 60 ppm, 40 ppm, and 20 ppm. A series of 100 ppm dilution was created from 0.5 mL of sample added to 4.5 mL of methanol in a test tube and homogenized. The 80 ppm dilution series was composed from 0.4 mL of the sample, 4.6 mL of methanol was added, placed in a test tube, and homogenized. A series of 60 ppm dilutions were produced from 0.3 mL of sample added to 4.7 mL of methanol in a test tube and homogenized. A 40 ppm dilution series was prepared from 0.2 mL of sample added to 4.8 mL of methanol in a test tube and homogenized. A series of 20 ppm dilutions were prepared from 0.1 mL of sample added to 4.9 mL of methanol in a test tube and homogenized. In addition, 0.2 mL of each concentration was taken and 2 mL of DPPH solution (0.0038 g DPPH plus 50 mL methanol) was added and homogenized with a vortex. The DPPH solution was placed into a cuvette and the absorbance value was measured using a spectrophotometer (wavelength 517 nm) and was recorded as absorbance blank (Ablank). The solution that has been vortexed was left in a dark room for 30 minutes and then placed into a cuvette and the absorbance value was measured using a spectrophotometer (wavelength 517 nm) and recorded as sample absorbance (Asample). Antioxidant capacity (% inhibition) can be calculated using the following formula: Percent Inhibition (%) = (Ablank-ASample)/Ablank × 100%. The value of antioxidant capacity (% inhibition) of each concentration was used to find a linear equation. The linear regression equation (y = ax + b) was obtained to determine the IC50 value. The value of y = 50 in such a way that the value of x can be obtained as the value of the antioxidant activity.

2.11 Organoleptic test

A sensory test was carried out on **c**offee-leaf tea using the hedonic method with semi-trained panelists. A panel of 25 students of the Agricultural Products Technology Study Program, Sriwijaya University who had previously been trained in testing the properties of coffee-leaf tea and had studied plantation plant processing technology courses, especially coffee-leaf tea processing were used in this study. The functioning of the hedonic test is based on (Pratama, 2018), where panelists are asked to provide responses regarding the level of likes or dislikes of the sample presented. Samples are presented one at a time, then the panelists assess the sample based on the level of preference for color, aroma, and taste. Based on available value standards. Score scale: strongly dislike = 1, dislike = 2, like = 3, and like very much = 1).

The data were processed using SAS version 9 for Microsoft Windows. The study was conducted in three stages, including preparing coffee-leaf tea, preparing areca nut powder, and adding areca nut powder and stevia leaves to coffee-leaf tea.

3 RESULTS

3.1 Water Content

The water content of the coffee-leaf tea produced ranged from 5.36% to 6.65%. The F5 formulation had the lowest water content, and the F1 formulation had the highest water content. The average water content in coffee-leaf tea is shown in Figure 1.

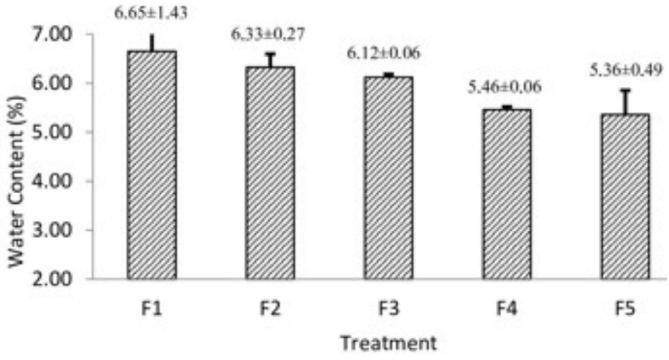
3.2 Ash Content

The coffee-leaf tea produced had an ash content of 7.68–7.91%. The highest ash content was recorded in treatment F5 (7.91%), while the lowest ash content was recorded in treatment F1 (7.68%). The results of the analysis of variance showed that the treatment formulation significantly affected the ash content of the samples. The results of the 5% BNJ test to determine the effect of formulation treatment on the ash content are presented in Table 2.

3.3 Degree of Acidity (pH)

The mean pH of the coffee-leaf tea solution ranged from 6.6 to 6.87. The lowest pH was recorded in the F1 treatment (6.6), while the highest pH was recorded in the F5 treatment

(6.87). The results of the analysis of variance showed that the formulation treatment significantly affected the pH of coffee-leaf tea. The 5% BNJ (Table 2) test results showed that the lower the concentration of coffee-leaf powder and the higher the concentration of areca nut powder and stevia leaves, the higher the pH of coffee-leaf tea, i.e., the tea became alkaline.



Description:
F1 = 100%b/b coffee-leaf tea: 0% w/b areca nut powder: 0% w/b stevia leaf powder.
F2 = 95% w/w coffee-leaf tea: 1.5%b/b areca nut powder: 3.5% w/b stevia leaf powder.
F3 = 90%b/b coffee-leaf tea: 3%b/b areca nut powder: 7%b/b stevia leaf powder.
F4 = 85%b/b coffee-leaf tea: 4.5%b/b areca nut powder: 10.5%b/b stevia leaf powder.
F5 = 80%b/b coffee-leaf tea: 6%b/b areca nut powder: 14%b/b stevia leaf powder.

Figure 1: The average water content in coffee-leaf tea.

Table 2: The results of the BNJ 5% test to determine the effect of formulation treatment on ash content, pH of the solution, total phenol content, and IC_{50} of coffee-leaf tea.

Treatment	Ash content (%)	Degree of Acidity (pH)	Total phenol (ppm)	IC_{50} (ppm)
F1	7.68±0.16a	6.60±0.10a	14.75±0.14a	68.53±0.46a
F2	7.79±0.07ab	6.67±0.06ab	18.15±0.23b	53.21±3.03ab
F3	7.80±0.02ab	6.73±0.12ab	18.90±0.24c	47.79±5.91bc
F4	7.87±0.02ab	6.83±0.12b	21.14±0.24d	40.40±5.91c
F5	7.91±0.03b	6.87±0.06b	24.12±0.27e	38.76±1.36d

Description: Numbers followed by the same letter in the same column are not significantly different (P<5%).

3.4 Total phenol content

The total phenol content of coffee-leaf tea mixed with areca nut powder and stevia leaves ranged from 14.75 to 24.12 mgTAE/mL. The highest content was recorded in treatment F5, while the lowest content was obtained in treatment F1. The results of the analysis of variance showed that the treatment formulation had a significant effect on the total phenolics of the samples. The 5% BNJ test results (Table 2) showed that as the concentration of areca nut powder and stevia leaves increased, the total phenol content in coffee-leaf tea increased.

3.5 Antioxidant Activity (IC_{50})

The antioxidant activity of coffee-leaf tea was measured using the IC_{50} value; a lower IC_{50} indicates a higher antioxidant activity, and vice versa. The IC_{50} value of the coffee-leaf tea produced ranged from 38.76 to 68.53 ppm. The highest value was recorded in the F1 treatment and the lowest value was recorded in the F5 treatment. The IC_{50} of coffee-leaf tea produced was classified in the very strong antioxidant category, as the value was less than 50 ppm. This was consistent with Kurang and Kamengon (2021), who classified IC_{50} values as follows: 0–50 ppm (very strong), 50–100 ppm (strong), 101–150 ppm (moderate), 150–200 ppm (weak), and > 200 ppm (very weak).

3.6 Organoleptic Test

Testing the color, aroma, and taste of coffee-leaf tea using the hedonic test yielded scores of 1.76–3.32, 2.44–2.64, and 1.8–3.2, respectively. Based on these results, the color and taste of coffee-leaf tea were accepted by the panelists with scores above 3. The results of the Friedman-Conover test for the color and taste of coffee-leaf tea are presented in Table 3.

Table 3: The results of the Friedman-Conover test for the color and taste of coffee-leaf tea.

Treatment	Color	Taste
F1	97c	47a
F2	93c	61b
F3	90c	89c
F4	56.5b	97.5d
F5	38.5a	99.5d

Description: Numbers followed by the same letter in the same column are not significantly different (p<5%).

The results (Table 3) of the Friedman-Conover test indicated that increasing the concentration of areca nut powder and stevia leaves powder produced a color after steeping the tea that was distinguishable by the panelists. The steeping color produced in each treatment ranged from dark to light brown. Treatment F5 was the most favorable with a hedonic score of 3.32, and the brew obtained was light brown.

4 DISCUSSION

The results of the analysis of variance showed that the addition of areca nut powder and stevia leaves did not significantly affect the water content of coffee-leaf tea. The water content recorded met the SNI 4342–2014 standard

for green tea with a maximum moisture level of 10% (BSN, 2014). The water content of the sample was lower compared to the values obtained by Novidiyanto and Sutyawan (2022) and Nusa (2020), who used commercially dyed green tea (6.81–8.78%) and Tayu tea (9.15%), as well as, agar wood leaf tea (7.09–8.80%), respectively. Our findings were similar to those of Wickramasinghe, Wickramasinghe and Wijesekara (2020), who found that tea from Moringa leaves had a water content ranging from 4.29% to 7.03%. The coffee-leaf tea produced was not easily damaged, and this matched the findings of Olabode et al. (2015) and Barek et al. (2015), who showed that samples with a water content of < 7% had a longer shelf life.

The results of the 5% BNJ test (Table 2) showed that as the concentration of areca nut powder and stevia leaves increased, the ash content of the samples also increased. This occurred because areca seeds and stevia leaves contain relatively high levels of minerals. Sulaiman, Hashem and Nassar (2022) found that areca seeds contain macro minerals (potassium, calcium, sodium, and magnesium) and micro minerals (manganese, iron, and zinc); among all minerals, calcium has the highest concentration of 1,845.19 mg/100 g. Siagan et al. (2020) showed that the total ash content of fig-leaf tea bags with stevia leaves added as a sweetener ranged from 1.04% to 1.20%.

The ash content of the samples produced was safe for consumption because it met the SNI 4342–2014 standard for green tea, where the maximum value is 8%, and the minimum value is 4%. The values obtained in this study were lower than those recorded in coffee-leaf tea from El Salvador, ranging from 7.81-0.20g/100 g^{-1} (Stegar et al., 2022). Compared to the ash content of green tea leaves (5.31–5.33%) (El-Sayed et al., 2022), the ash content in matcha tea ranges from 4.64% to 4.99% and that in polyherbal dip tea ranges from 6.00% to 6.50% (Mohsina et al., 2022).

The lower the concentration of coffee-leaf powder and the higher the concentration of areca nut powder and stevia leaves, the higher the pH of coffee-leaf tea. This increase in alkalinity is because of stevia leaves that contain alkaloid active compounds, which are alkaline (Howlader et al., 2016). Harismah, Mirzaei and Fuadi (2018) showed that they contained steviol derivation, such as stevioside (4.15%), rebausida A (2–4%), rebausida C (1–2%), and dulkosida A (0.4–0.7%).

The pH of the samples in this study was higher than that reported by Pangestu et al. (2017) and Wijaya, Herlina and Astryani (2021); these researchers prepared tea incorporated with carrageenan, with the pH ranging from 3.55 to 3.79, while robusta coffee-leaf extract had pH ranging from 5.01 to 5.43. The pH recorded in this study was lower than the pH of the fig-leaf tea solution to which different concentrations of stevia leaves were added; the pH ranged from 7.25 to 7.72 (Siagian et al., 2020).

The total phenol content increased with an increase in the concentration of areca nut powder and stevia leaves. This occurred because areca seeds and stevia leaves contain phenolic compounds. Ismail et al. (2012) reported that the total phenol content in areca seeds is around 85.92 mgTAE/mL. Ismet et al. (2010) found that stevia leaves processed through the maceration method had higher total phenols, total flavonoids, and antioxidant activity compared to those that were processed via Soxhlet extraction. The total phenol content obtained via maceration was 65.21 mg gallic acid, which was equivalent to the content of phenol compounds reported in another study (Putri et al., 2019). Fibrianto et al (2020) found that adding stevia leaves increased the total phenol content in Arabica coffee-leaf herbal tea because they had a total phenol content of 80.13 mgGAE/g and polyphenolic compounds of 111.16 mg/g extract (Garcia-Mier et al., 2021).

The total phenol content recorded in this study was similar to that recorded in Arabica coffee-leaf herbal tea (12.95–21.36 mgTAE/mL) after adding stevia leaves Fibrianto et al (2020). Zjahra et al. (2022) showed that the total phenol content of coarse green tea-leaf extract was 20.147 mgGAE/g. Compared to the values obtained by Puspaningrum and Sari (2020), where Arabica coffee cascara tea had a total phenol content of 8.23 mg GAE/100 mL, the values obtained in this study were higher.

The results of the analysis of variance showed that the formulation treatment significantly affected the IC_{50} value of coffee-leaf tea. The 5% BNJ results showed that the higher the concentration of areca nut powder and stevia leaves, the lower the IC_{50} value. Based on these findings, the antioxidant activity of coffee-leaf tea increased with an increase in the concentration of areca nut powder and stevia leaves. Areca nut powder and stevia leaves contain flavonoid secondary metabolites, which act as antioxidants. Cahyanto (2018) showed that they contained 3.7% flavonoids and 8.53% tannins. Stevia leaf extract consist of glycoside sweeteners, such as stevioside, rebaudioside, and dulcoside. Besides providing a sweet taste, it also acts as an antioxidant in the product (Umami; Afifah, 2015).

These results were similar to those reported by Yadav et al. (2020), who found that green and black tea from various plant clones had IC_{50} values of 45.15 and 51.88 ppm, respectively. Wang et al. (2022) and Culas et al. (2021) found that black tea had good scavenging activity on DPPH (IC50 = 9.94 µg/mL) and ABTS (IC50 = 17.26 µg/mL) free radicals. Cinnamon tea was also found to have a strong antioxidant capacity with an IC_{50} of 5.4935 ppm; we reported a higher value in this study.

As shown in Table 3, the results of the Friedman-Conover test indicated that F5 affected other treatments. The F5 treatment had the highest flavor score of 3.2, which indicated that the flavor produced by the treatment was preferred by the panelists. The addition of stevia leaf powder decreased the astringency of areca nut powder and the bitterness of coffee leaves. The astringency of areca nut powder was attributed to the tannin

compounds present, which were polymerized and oxidized due to the heat of the brewing water (Harnowo; Yunianta, 2015). The astringent and bitter taste of coffee leaves might be attributed to their constituent tannin and caffeine compounds.

5 CONCLUSIONS

To summarize, we showed that adding areca nut powder and stevia leaf powder to coffee-leaf tea can improve the antioxidant properties of the tea. The IC_{50} value of the tea decreased by 67.96%, and its total phenol content increased by 38.85%. The water content and ash content of coffee-leaf tea produced in this study met the SNI standards. The results of the organoleptic tests showed whether the panelists accepted the color and taste of the samples.

6 AUTHORS' CONTRIBUTION

PA wrote the manuscript and performed the experiment, LR and ADP supervised the experiment and co-work the manuscript, and BS review and approved the final version of the work, AN conducted all statistical analyses

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