



Caffeine content and acidity levels of some non-alcoholic water-based beverages sold in Banda zone, Kampala, Uganda

Patrick Onen^{1,2*}, Joel Kissa³, Isaac Odokonyero², Emmanuel Esangu², Boniface Opio^{2,4}, Athony Asiimwe⁵, Tonny Odongo², Basirat Temilola Jogunomi^{1,6}, Augastine Okwir², Eric Niringiyimana⁷

¹University of Kerala, Department of Chemistry, Karyvattom campus, Thiruvananthapuram 695581, Kerala, India,

²Kyambogo University, Department of Chemistry, Kampala, P.O. Box 1, Kyambogo, Uganda, ³Makerere University,

Department of Science and Technical Education, Kampala, P.O. Box 7062, Uganda, ⁴Lira University, Department of

Science and Vocational Education, Lira, P.O. Box 1035, Lira, Uganda, ⁵Mahatma Gandhi University, Department of

Biotechnology, Kottayam, 686560, Kerala, India, ⁶Federal University of Agriculture, Department of Chemistry,

Abeokuta, Ogun State, Nigeria; ⁷Kampala International University, Department of Physical sciences, School of

Natural and Applied sciences, P.O. Box 20000, Kampala, Uganda

***Corresponding Author:** Patrick Onen, Msc., University of Kerala, Department of Chemistry, Karyvattom campus, Thiruvananthapuram 695581, Kerala, India

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ABSTRACT

Background: The consumption of non-alcoholic water-based beverages has gained attention due to its ability to promote acute benefits such as improved attention, cognitive performance, reduction of fatigue, increased wakefulness, and analgesia. However, the result of caffeine consumption is a concern it is important to inform consumers about the caffeine content and acidity levels of all non-alcoholic water-based beverages.

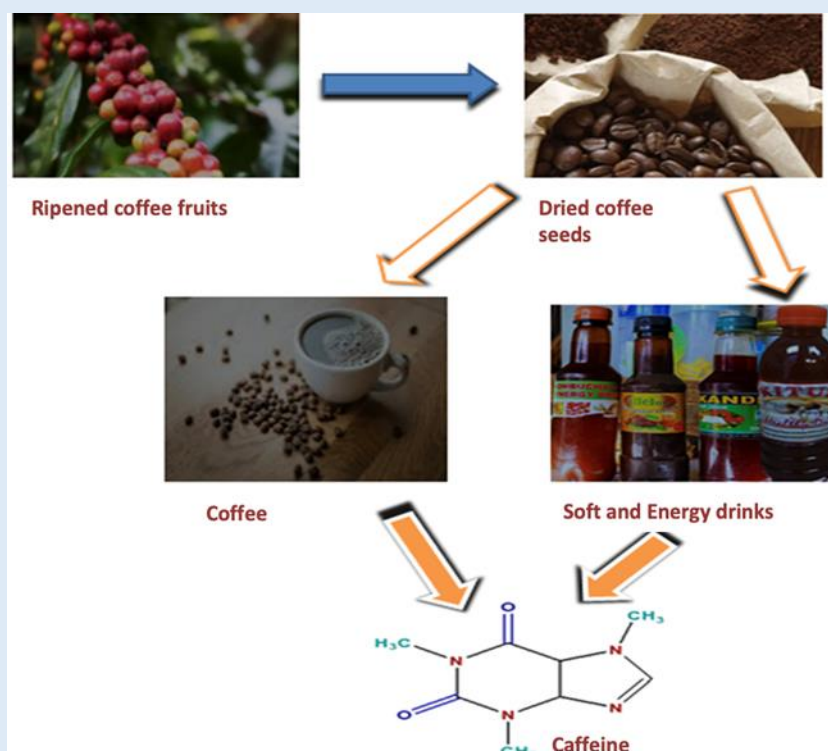
Methods: The samples were sonicated and subjected to analysis by high-performance liquid chromatography-

ultraviolet method and pH meter method for acidity content.

Results: The highest concentration of caffeine was found in energy drink, ED; with 64.01 ± 0.07 mg/L as compared to soft drinks SD1; 20.20 ± 0.02 mg/L, SD2; 23.14 ± 0.08 mg/L, and SD3; 29.88 ± 0.02 mg/L. All obtained concentrations were below the permissible levels for caffeine (200 mg/L) intake by the United States Food and Drug Administration. The study also showed that all the non-alcoholic beverages were acidic, except bottled water, in the sequence $BW < ED < SD$. The categorical range was bottled water; BW2; 8.01 ± 0.03 , BW1; 7.5 ± 0.10 , BW3; 7.5 ± 0.11 , BW4 6.85 ± 1.38 , Energy drink; ED; 3.17 ± 0.01 , and finally, soft drinks; SD2; 2.82 ± 0.02 , SD1; 2.61 ± 0.02 , and SD3; 2.59 ± 0.05 .

Conclusions: The results obtained in this study provide satisfactory information on the caffeine and acidity contents of the commonly consumed non-alcoholic water-based beverages in Banda, Kampala-Uganda. This in the future could pose serious health risks, since some individuals consume multiple servings of the drinks in a short time, which could result in intoxication/overdose.

Keywords: caffeine, soft drinks, energy drinks, public health, Banda



INTRODUCTION

Non-alcoholic water-based beverages have gained popularity in Uganda, as a result of the high consumption of tea and in the nation [1]. The most frequently consumed beverages are grouped into non-alcoholic and alcoholic beverages. Non-alcoholic beverages are sub-divided into soft and energy drinks, and water is the largest ingredient by volume. However, water is not considered to be a beverage. Therefore, in this study, it has been considered a special kind of beverage, as suggested by some authors [2].

Uganda's food and drink consumption has been on a positive growth trajectory since 2021 after the COVID-19 disruptions [1, 3]. It has been forecasted that over the medium term (2021-2024), the spending growth of

non-alcoholic beverages would accelerate positively, as driven by strong demand for fruit and vegetable juices, carbonated drinks, caffeinated and decaffeinated drinks (Fig. 1)[4]. The main ingredients of non-alcoholic water-based beverages are demineralized water (90%), dissolved sugar (7-10%), caffeine, and non-nutritive stimulants [5-7]. Caffeine is one of the natural alkaloids found in plant species like coffee (Fig. 2) [8], which is used as raw material for making beverages or intentionally added as a flavor or enhancer [9]. Caffeine is a semi-addictive drug that stimulates the central nervous system and causes insomnia, nervousness, and irritation when consumed excessively, when mixed with alcohol [10-12], or taken by endurance athletes [13].

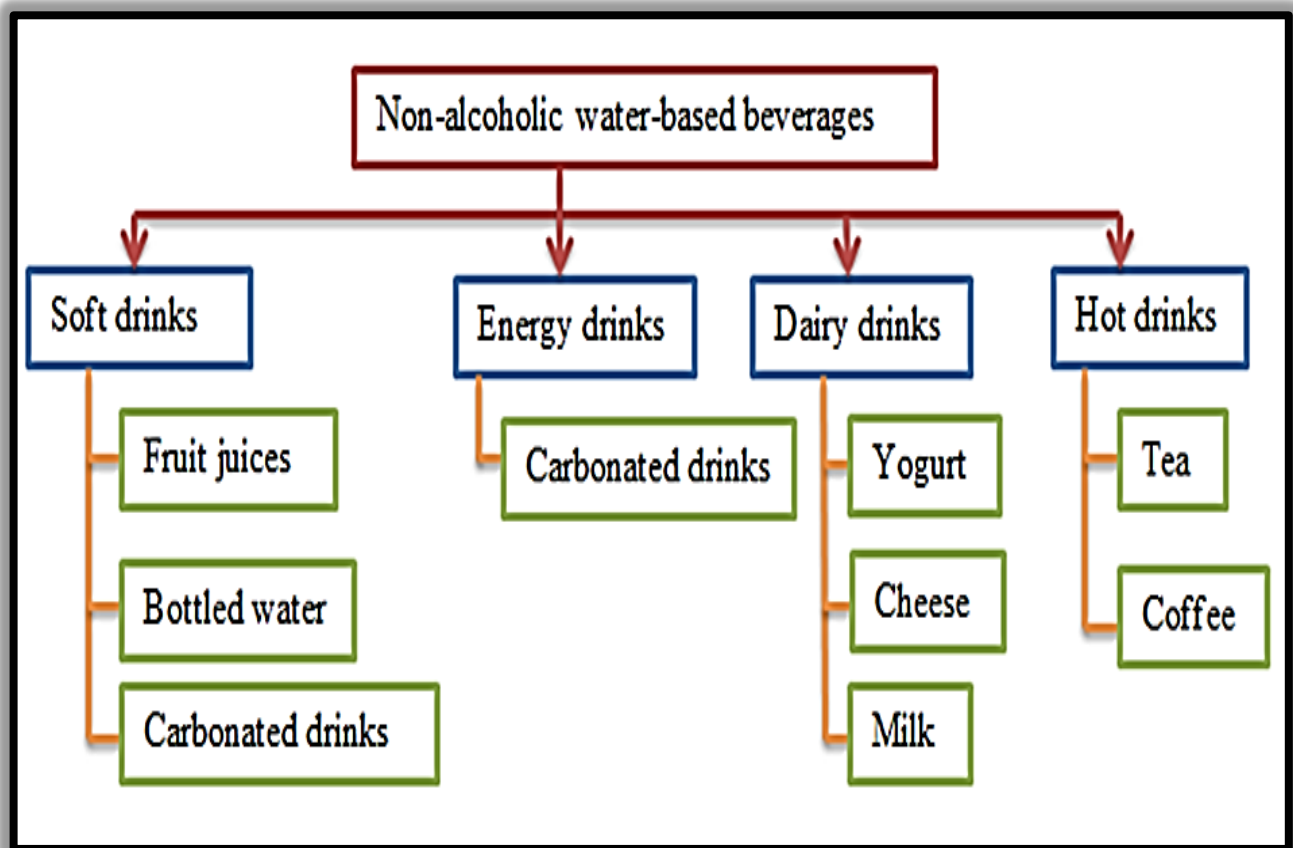


Figure 1: Different non-alcoholic water-based beverages sold in Uganda’s markets.

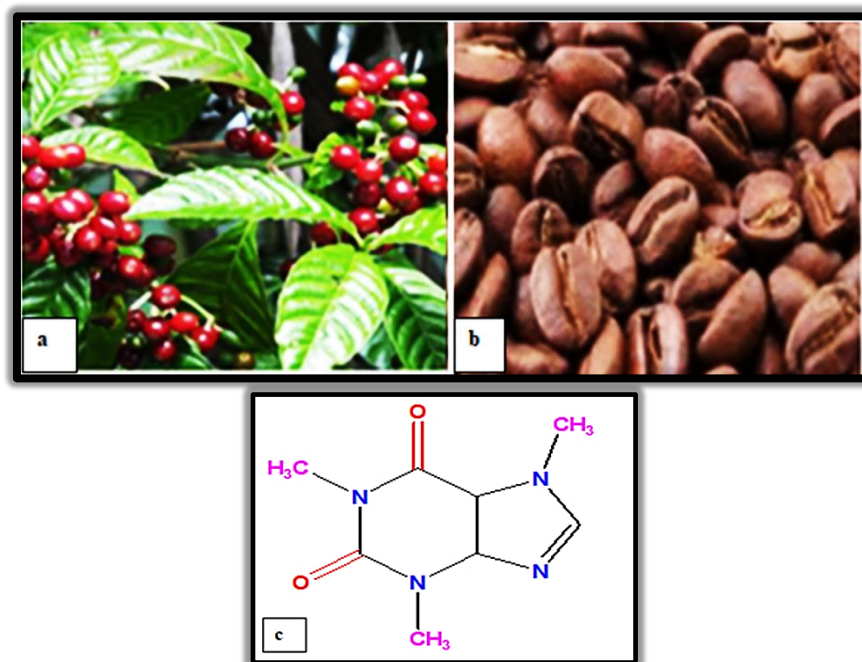


Figure 2: Images as (a) coffee plant, (b) coffee seeds, and (c) chemical structure of caffeine.

Non-alcoholic water-based beverages may be harmful because some are addictive [14]. While soft drinks are killing consumers, the companies that manufacture them go to great lengths to mislead consumers, making it even harder to break the habit of consuming these drinks. They use all marketing techniques necessary to persuade people into consuming these drinks [15, 16]. Epidemiological studies support the beneficial role of moderate coffee intake in reducing the risk of several chronic diseases, but heavy intake is not recommended for pregnant women [17]. Health implications of regular teas and energy drink consumption are inconclusive, but concerns have been raised for caffeinated soft drinks [18-20]. Furthermore, food safety is a major public health concern, and it is important to evaluate the association of foodstuffs consumption and their ingredients. It reveals that unsafe food or drinks can be a significant reason for many chronic and non-chronic diseases including cancer, heart diseases, stomach disorders, birth defects, and various kidney diseases [3, 14, 21]. To understand the information on the

consumption of non-alcoholic beverages with their caffeine levels, acidity levels, and health risks, a literature review was conducted. The consumer's preference for non-alcoholic beverages is based on the organoleptic characteristics of the beverages in terms of aroma, color, and taste, which gives a good reputation to specific producing companies [22]. Considering caffeine and health, the multi-target action of caffeine present in non-alcoholic beverages has been found to have excellent outcomes on brain function, cognitive performance, memory, suppressing body weight, and respiratory function [23]. Excessive consumption of those drinks with high caffeine levels is associated with mental problems, which are increasingly occurring among adolescents [24]. Therefore, the enrichment of non-alcoholic water-based beverages with caffeine and safe, high-quality ingredients with improved functional and sensory properties, requires control and traceability at all various production lines. Many studies have been conducted to determine the caffeine and acidity levels of non-alcoholic beverages from countries other than

Uganda. From the study, there are many methods which were employed to analyze the caffeine contents in coffee products, tea, and non-alcoholic beverages [25]. The method previously used for caffeine determinations was high-performance liquid chromatography [26-28]. Other methods include ion chromatography, capillary electrophoresis, micellar capillary electrophoresis, gas chromatography, and solid-phase microextraction gas chromatography. Of the foregoing analytical methods, HPLC has been the most preferred method [29-31]. This study was undertaken to determine the caffeine content and acidity levels of some non-alcoholic water-based beverages consumed in the Banda zone, Kampala, Uganda.

METHODS

Description of the study area: This study was conducted in the Banda zone. Banda is found east of Kampala's capital city, along Kampala-Jinja Road, latitude $0^{\circ} 21' 10.79''$ N, longitude $32^{\circ} 37' 31.79''$ E. Banda zone was chosen because it is reported to have the highest number of students from different learning institutions in Nakawa division of Kampala capital city, who are the potential consumers of the drinks.

Sample collection: A total of eight (8) samples of non-alcoholic beverages including bottled water were used for the study. Three (3) of these were soft drinks, one (1) was an energy drink and four (4) samples were bottled water bought from shops in the Banda zone.

Preparation of caffeine standards: Caffeine standard powder, which was accurately weighed 100 mg, was transferred into a 100 mL measuring flask followed by the addition of 50 mL of distilled water. The flask was filled up to the 100 mL mark with distilled water to get a final concentration of 1000 $\mu\text{g/mL}$ (stock solution). The caffeine working standard solution was prepared by accurately transferring 25 mL of the stock solution (1000

$\mu\text{g/mL}$) into a 100 mL measuring flask. This was then diluted with distilled water to a final concentration of 250 $\mu\text{g/mL}$. Finally, concentrations of 0, 10, 20, 40, and 80 mg/L caffeine solution were used to construct a calibration curve. The caffeine level of the standard solutions was calculated using the regression equation of the best line of fit.

Quantification of caffeine concentration: In this present study, a pH meter (pH-035) was used to measure the pH levels of the drinks.

Determination of caffeine content was performed by a method previously described by Manwaring *et al.*, [29], with some modifications. The beverage samples were first degassed in an ultrasonic bath for 20 minutes. Thereafter, 50 μL of each sample was diluted to 1000 μL with distilled water in vials. External calibration with peak area integration was used in the quantification of total caffeine concentration in beverage samples. The international standard for the determination of caffeine content in coffee and tea products (ISO 20481:2008) was adopted for the soft and energy drink samples. Briefly, a Shimadzu-LC-2030 plus Model system was used for the HPLC-UV-based quantification of caffeine. A Shimadzu column; Kramosil (250 mm x 4.6 mm), i.d 5 μm particle size was used in isocratic mode at 35 $^{\circ}\text{C}$ column temperature. The rate was 1.5 mL/minute; the injection volume was 20 μL while the chromatographic run lasted for 1.5 times the retention time of caffeine. UV detection was carried out at 254 nm, and additional peak purity measurements were executed at 254 nm to exclude samples containing impurities in the retention window of caffeine.

Statistical analysis: The results were expressed as means \pm standard deviation (SD) of triplicates. The obtained results were analyzed using Minitab statistical software (version 19.1, Minitab Inc., USA).

RESULTS

Table 1 illustrates the beverage type and the pH levels of the different non-alcoholic water-based beverages including bottled water. The pH ranged from 2.59±0.05 to 3.17±0.01 for non-alcoholic beverages and 6.85±1.38 to 8.01±0.03 for bottled water. A summary of these is provided in Table 2.

Table 1: pH of the sampled beverages sampled from Banda zone, Kampala, Uganda

Sample Code	Beverage type	Temp (°C)	Mean pH (±SD)
SD1	SD	24	2.61 ± 0.02
SD2	SD	25	2.82 ± 0.02
SD3	SD	24	2.59 ± 0.05
ED	ED	25	3.17 ± 0.01
BW1	BW	25	7.5 ± 0.10
BW2	BW	25	8.01 ± 0.03
BW3	BW	24	7.5 ± 0.11
BW4	BW	24	6.85 ± 1.38

SD- Soft Drink, ED - Energy Drink, and BW - Bottled Water.

Table 2: Descriptive statistics of the pH of the analyzed beverages from Banda zone, Kampala, Uganda

Beverage type	Number of samples	pH range	Mean pH
SD	3	2.58 - 2.82	2.67 ± 0.03
ED	1	3.18 (23 °C) - 3.16 (27 °C)	3.17 ± 0.01
BW	4	6.50 - 8.10	7.45 ± 0.51

SD - Soft Drinks, ED - Energy Drink, and BW - Bottled Water.

In soft drinks, caffeine content ranged from 20.20±0.02 mg/L to 29.88±0.02 mg/L and in energy drink (ED) ranged from 64.01±0.07 mg/L (Table 3). The performance parameter of HPLC was evaluated by analyzing calibration standards over a range of 0 to 80 mg/L of caffeine concentrations at six-point levels, and the chromatograms were recorded. A calibration curve (Figure 3) was obtained by plotting peak area ratios of caffeine against caffeine

concentrations using a Minitab 19.1, and it illustrated a positive linear relationship between the instrumental signal and the concentration of caffeine standards with the Coefficient of Correlation ($R^2 = 0.999$). The linear equation of the calibration curve for caffeine was $y = -19684 + 19280x$; where y is the intercept and x is the slope of the curve.

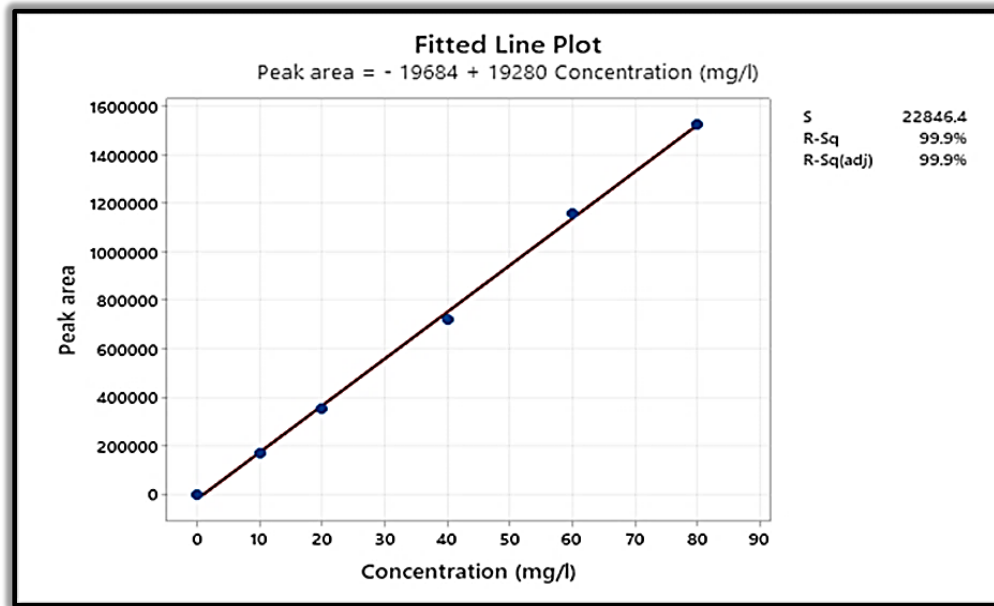


Figure 3: Caffeine calibration curve

Table 3: HPLC-UV method used to quantify the caffeine concentration in non-alcoholic water-based beverages.

Sample code	Weight (mg/mL)	Retention time (min)	Average area	test	Determined caffeine concentration (mg/L)
SD1	1.04	5.163	3589		20.20 ± 0.02
SD2	1.04	5.164	4111		23.14 ± 0.08
SD3	1.04	5.164	5308		29.88 ± 0.02
ED	1.05	5.165	11272		64.01 ± 0.07
STD	-	5.15	1847781		100 ± 0.00

SD1- SD3, and ED; represent the different beverages and STD-standard.

DISCUSSIONS

The results shown in Table 1 indicate that all non-alcoholic beverages were acidic, except bottled water, with Energy drink (pH = 3.17±0.01) being the lowest in acidity and SD3 (pH = 2.59±0.05) being highly acidic. Bottled water is nearly neutral (pH = 6.85±1.38 to 8.01±0.03). These results were found to have some similarities and differences with other published works [32]. The consideration of the pH of the different non-

alcoholic water-based beverages to be the key factor in this study was due to its effects on human body metabolisms [27, 33]. Excess acid in beverages leads to stomach inflammation, and consequently, stomach erosion [34]. It may also interrupt digestion and cause malabsorption or total loss of dental enamel [35-38]. The variation in the obtained pH values was assumed to be linked to the Laboratory methodologies, including temperatures at which the samples were analyzed,

described by Shenoy *et al.* [33]. This is because at high temperatures, the pH of the samples was likely to have lower values than at low temperatures. Differences can also be caused by equipment accuracy [32, 33]. From Table 3; the Caffeine analysis of soft drinks showed low levels of caffeine in the SD1 with the concentration of 20.20 ± 0.02 mg/L and highest in SD3 with the concentration of 29.88 ± 0.02 mg/L. Finally, the energy drink, ED had the highest concentration of caffeine out of all the analyzed non-alcoholic beverages with a concentration of 64.01 ± 0.07 mg/L. The results obtained from both soft drinks and energy drinks were all below the maximum of 200 mg/L as the allowable value by the United State Food and Drug Administration [25, 39]. Low caffeine content enhances the body's performance, alertness, increased activeness, speed of perception, and decision-making [40, 41]. Low caffeine content can also help in weight loss and cancer prevention [19]. However, high doses cause insomnia, rising blood pressure, and anxiety [42] [43-45]. In the previous studies, it was highlighted that the presence of caffeine also stimulates the stomach to excrete large amounts of acid, leading to burning sensations and development of peptic ulcers of the stomach and duodenum, since they play a vital role in the alimentary system [46-48]. Therefore, consumers are more conscious about the demand for non-alcoholic beverages and, at the same time, health-related issues concerning their consumption, not only for the source of energy but also for the excellent delivery of some nutrients and bioactive compounds in the body systems.

CONCLUSIONS

This present study has shown that HPLC–UV is an efficient and reliable method for determining the caffeine contents in beverages. From the findings, we

suggested that if one plans to consume those non-alcoholic water-based beverages, it would be better to select one with a label that quantifies the actual amount of caffeine, given that some products without caffeine listed may contain more than 200 mg/L. Nonetheless, most of the non-alcoholic beverages analyzed were found to be acidic which put human life at risk, especially to children and individuals with stomach ulcers. Even though the consumption rates of non-alcoholic beverages are demanding, forcing the industry to grow faster to meet the demand trends; new evidence and research studies substantiating health effects on both ingredients and product level needs to be done.

Abbreviations: HPLC-UV: High-performance liquid chromatography coupled with an ultraviolet detector, ED: Energy drinks, SD: Soft drinks, BW: Bottled water

Competing interests: The author declared no conflicts of interest.

Author contributions: P.O., J.K., I.O., A.A., T.O., T.B.J., A.O and E.N conceptualization and investigation and wrote the manuscript. P.O and B.O supervised the laboratory experiments. P.O., E.E and E.R conducted the laboratory experiments, edited, read, and reviewed the final manuscript. All authors read and approved the final manuscript.

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