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Nutritional value and bioactive compounds profiling of selected Vietnamese fruits

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ABSTRACT

This study aimed to evaluate the chemical composition of eight fruit species commonly grown in Vietnam, including guava, persimmon, banana, custard apple, longan, lemon, litchi, and mango. The results revealed substantial interspecific variation in nutritional components, amino acids, and minerals. Guava showed exceptionally high vitamin C (280.13 mg 100 g^{-1}) and protein (6.36%), while longan was remarkable for its vitamin B6 (24.42 mg 100 g^{-1}) and potassium (2.66%). Banana contained the highest starch (12.72%) and phosphorus (2.65%), confirming its role as an energy-rich fruit. Custard apple and mango exhibited higher lipid contents (6.32% and 7.98%), whereas litchi and custard apple were notable for cellulose (2.25% and 2.35%). Persimmon was rich in riboflavin (2.01 mg 100 g^{-1}), and lemon, despite its high-water content (92.98%), provided moderate vitamin C (67.32 mg 100 g^{-1}). Mineral analysis further identified guava and mango as valuable sources of calcium and iron, while longan contained the highest zinc (0.161%). These findings highlight the nutritional diversity of tropical fruits in Vietnam, emphasizing their complementary roles in human diets and their potential applications in community nutrition and functional food development.

Key words: Tropical fruits, nutritional composition, amino acids, mineral content.

INTRODUCTION

Fruits are widely recognized as essential components of a healthy diet due to their high contents of vitamins, minerals, dietary fiber, and various bioactive compounds. Regular consumption of fruits has been associated with reduced risks of chronic diseases, including cardiovascular diseases, diabetes, and certain types of cancer (Slavin, 19; Liu, 14). The nutritional diversity among different fruit species reflects their ecological adaptations and genetic backgrounds, offering unique contributions to human health and dietary balance.

Vietnam located in the tropical and subtropical zones, possesses favorable agroecological conditions for cultivating a wide range of fruit species, many of which are economically important and nutritionally valuable. Common fruits such as guava, banana, mango and lemon are consumed daily, while others including longan, litchi, persimmon and custard apple are valued both locally and internationally for their flavor and nutritional qualities. These fruits not only serve as fresh produce but are also increasingly important for the development of functional foods and nutraceuticals (FAO, 6; Lim, 12).

Previous studies have highlighted significant interspecific variation in the chemical composition of tropical fruits. For example, guava has been consistently reported as one of the richest natural sources of vitamin C, surpassing many citrus fruits (Lim, 12). Longan is notable for its high levels of B vitamins, particularly vitamin B6, which plays a central role in amino acid metabolism and nervous system function. Bananas, on the other hand, are well-known as staple energy fruits due to their high starch and carbohydrate content, making them important in food security strategies in developing countries (Robinson, 17). However, despite their importance, comprehensive comparative analyses of the nutritional composition of these fruits grown under Vietnamese conditions remain limited.

Therefore, the present study was conducted to evaluate and compare the chemical composition including water, protein, lipids, carbohydrates, vitamins, amino acids, and mineral contents of eight commonly cultivated fruits in Vietnam. By identifying their nutritional strengths and potential health benefits, the study aims to provide a scientific basis for promoting dietary diversification, developing functional foods, and enhancing the economic value of Vietnamese fruits in both domestic and international markets.

MATERIALS AND METHODS

Eight fruit species commonly cultivated in Vietnam were selected for analysis: guava (*Psidium guajava* L.), persimmon (*Diospyros kaki* Lf.), banana (*Musa paradisiaca* L.), custard apple (*Annona squamosa* L.), longan (*Euphoria longan* Lamk), lemon (*Citrus*

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limonia Osbeck), litchi (*Litchi chinensis* Sonn.), and mango (*Mangifera indica* L.). Fresh, mature, and disease-free fruits were harvested during the peak season from local orchards in Thanh Hoa Province, Vietnam. After collection, fruits were immediately transported to the laboratory, washed with distilled water, peeled where necessary, and homogenized for analysis. The experiments were conducted at Hong Duc University, Institute of Biotechnology and Institute of Chemistry under Vietnam Academy of Science and Technology.

Water content was determined by oven-drying samples at 105 °C to constant weight (Ahamad *et al.,* 1). The content of protein and lipid was determined by Lowry method and soxhlet method respectively (Chau *et al*., 4). The content of reducing sugar and starch was determined using Bertrand method while the content of vitamin C was based on the titration method (Mui, 16). Crude fiber (cellulose) was determined by the Weende method (Van, 22). B vitamins (B1, B2, B3, B6) were analyzed by high-performance liquid chromatography (HPLC) with UV detection following extraction with acidified ethanol (Ekinci, 5).

Free amino acid composition was analyzed using an automatic amino acid analyzer (Hitachi L-8900, Japan) after hydrolysis with 6 N HCl at 110 °C for 24 h under vacuum. Amino acid contents were expressed as mg 100 g^{-1} fresh weight.

Mineral elements (Ca, K, Na, Cu, Mn, S, Mg, Fe, Zn) were determined by atomic absorption spectrophotometry (AAS, PerkinElmer Analyst 400, USA) after wet digestion of fruit samples with nitric-perchloric acid mixture (Ahamad *et al.,* 1). Phosphorus was quantified colorimetrically using the molybdovanadate method. Total nitrogen content (N) of fruits was determined based on Kjeldahl method (San *et al*., 18).

All analyses were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS v.25.0. One-way analysis of variance (ANOVA) was applied to test differences among fruit species, and means were compared using Duncan's multiple range test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of the nutritional composition of eight fruit species revealed clear differences in water content, dry matter, and micronutrients. Regarding water content, lemon exhibited the highest value (92.98%), while longan had the lowest (63.55%). This reflects the structural characteristics of the fruits, in which highly succulent species such as lemon are primarily used as refreshing beverages, whereas fruits with higher dry matter such as longan provide more energy.

Reducing sugar content varied considerably among species, ranging from very low in lemon (0.57%) to remarkably high in litchi (14.24%) and persimmon (13.11%). These fruits are intensely sweet and suitable for fresh consumption as quick energy sources. By contrast, banana was outstanding in starch content (12.72%), highlighting its role as an energy-providing food comparable to staple crops. Other fruits had low starch levels, with sugars mainly present as simple carbohydrates.

Notably, guava (6.36%) and custard apple (7.18%) showed higher protein levels compared to the other fruits, a relatively rare characteristic among fleshy fruits. Meanwhile, mango (7.98%), custard apple (6.32%), and banana (5.00%) exhibited higher lipid contents, thus increasing the energy density of the diet. In terms of dietary fiber, custard apple (2.35%) and litchi (2.25%) were superior, suggesting a role in supporting digestion and preventing constipation.

For the B-vitamin complex, longan was the most outstanding fruit, containing high levels of B1 (0.37 mg 100 g^{-1}), B3 (3.89 mg 100 g^{-1}), and especially B6 (24.42 mg 100 g^{-1}), far exceeding the other species. This indicates that longan could serve as an important dietary source for supporting nervous system function and hematopoiesis. In contrast, persimmon exhibited the highest riboflavin (B2) content (2.01 mg 100 g^{-1}), contributing to energy metabolism.

Vitamin C showed striking interspecific variation. Guava was exceptional (280.13 mg 100 g^{-1}), more than four times higher than lemon (67.32 mg 100 g^{-1}), which is traditionally considered a vitamin C-rich fruit. This confirms guava as one of the most important natural sources of vitamin C, with significant roles in immune support and antioxidant defense. Other species such as mango, persimmon, and longan contained considerably lower levels (Table 1).

Overall, each fruit species exhibited distinct nutritional advantages: guava was remarkable in vitamin C and protein content; longan was rich in B vitamins; banana provided starch as an energy source; custard apple offered a balance of protein, lipids, and fiber; while lemon supplied abundant water and moderate vitamin C, suitable for refreshing beverages. Mango and litchi contributed mainly to energy through their higher sugar and lipid levels. These findings highlight not only the nutritional diversity among fruit species but also the potential for targeted dietary use of each fruit to optimize health benefits.

Table 1: Chemical and nutritional properties of selected fruits grown in Vietnam.

Factors	Guava	Persimmon	Banana	Custard apple	Longan	Lemon	Litchi	Mango
Water content (%)	86.69 ± 1.25	82.54 ± 1.21	75.24 ± 1.37	70.96 ± 1.51	63.55 ± 1.64	92.98 ± 1.19	75.85 ± 1.24	82.60 ± 1.22
Reducing sugar (%)	6.20 ± 0.05	13.11 ± 0.06	4.87 ± 0.08	10.97 ± 0.32	9.29 ± 0.07	0.57 ± 0.06	14.24 ± 0.04	11.00 ± 0.21
Starch (%)	2.20 ± 0.04	2.52 ± 0.05	12.72 ± 0.06	4.82 ± 0.15	0.48 ± 0.01	0.29 ± 0,03	0.74 ± 0.07	3.14 ± 0.09
Protein (%)	6.36 ± 0.16	0.95 ± 0.02	2.53 ± 0.01	7.18 ± 0.17	2.21 ± 0.04	0.87 ± 0.03	3.73 ± 0.08	4.36 ± 0.02
Lipid (%)	3.80 ± 0.09	1.01 ± 0.01	5.00 ± 0.03	6.32 ± 0.04	0.23 ± 0.01	0.32 ± 0.01	3.35 ± 0.05	7.98 ± 0.12
Cellulose (%)	0.85 ± 0.04	0.84 ± 0.02	0.86 ± 0.02	2.35 ± 0.04	1.70 ± 0.05	0.74 ± 0.05	2.25 ± 0.02	1.55 ± 0.06
Vitamin B_1 (mg 100 g^{-1})	0.05 ± 0.03	0.18 ± 0.01	0.03 ± 0.01	0.14 ± 0.02	0.37 ± 0.02	0.04 ± 0.01	0.01 ± 0.01	0.06 ± 0.02
Vitamin B_2 (mg 100 g^{-1})	0.04 ± 0.02	2.01 ± 0.03	0.06 ± 0.02	0.15 ± 0.02	0.10 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.10 ± 0.01
Vitamin B_3 (mg 100 g^{-1})	0.68 ± 0.04	0.08 ± 0.02	0.65 ± 0.05	0.76 ± 0.065	3.89 ± 0.08	0.09 ± 0.01	0.01 ± 0.01	0.08 ± 0.03
Vitamin B_6 (mg 100 g^{-1})	0.15 ± 0.01	0.12 ± 0.01	0.39 ± 0.02	0.23 ± 0.01	24.42 ± 0.21	0.07 ± 0.01	0.07 ± 0.01	0.26 ± 0.05
Vitamin C (mg 100 g^{-1})	280.13 ± 3.35	30.85 ± 0.72	33.00 ± 0.35	36.20 ± 0.21	57.26 ± 0.38	67.32 ± 0.85	58.67 ± 0.25	30.73 ± 0.44

Notes: Values are given as means of three replicates ± SD. (-) Not detected during the time conducting the research.

The results of this study are consistent with previous reports emphasizing the variability of chemical composition among tropical and subtropical fruits. For example, Lim (12) confirmed that guava is one of the richest sources of vitamin C, far exceeding many Citrus species, which is strongly supported by the present data. The exceptionally high level of vitamin B6 in longan is noteworthy, as this vitamin plays a central role in amino acid metabolism, hemoglobin synthesis, and neurotransmitter function. Meanwhile, banana reaffirmed its position as a staple energy fruit, often described as a “food crop fruit” in developing countries (Robinson, 17).

These findings also underscore the specific nutritional values of different fruits. Vitamin C rich fruits such as guava and lemon are particularly valuable for boosting immunity and antioxidant defense, which is increasingly relevant in modern lifestyles characterized by oxidative stress. Longan, with its high B-vitamin content, may support neurological and circulatory health, while custard apple and litchi, with their higher fiber levels, are beneficial for digestive health. Mango and custard apple, with relatively higher lipid and protein contents, contribute to increased dietary energy, while persimmon and litchi are most suitable for fresh consumption due to their high sugar content and pleasant sweetness.

From a practical perspective, identifying the nutritional strengths of each fruit species allows for the design of balanced and targeted dietary strategies. For instance, guava and lemon may be promoted in community nutrition programs to prevent vitamin C deficiency; longan and persimmon could be utilized as natural sources of B vitamins; while banana and custard apple may contribute to food security strategies in regions at risk of energy deficiency. Moreover, these data suggest potential applications in the functional food industry, such as vitamin C extraction from guava or B-vitamin concentrates from longan.

In general, the marked variation in nutritional composition across fruit species not only reflects biodiversity but also reinforces their indispensable role in maintaining and enhancing human health. These findings further support the argument that dietary diversification with multiple fruit types provides more comprehensive nutritional benefits than reliance on only one or a few species.

The amino acid profiles of the investigated fruits showed marked interspecific variation, both in indispensable and non-indispensable amino acids (Table 2). Guava exhibited the highest content of aspartic acid (2.23%) compared with other fruits, suggesting its strong contribution to organic acid metabolism and characteristic flavor development (Ashraf *et al*., 2016). By contrast, banana was particularly rich in glutamic acid (1.10%) and lysine (1.16%), indicating its high nutritional value, since lysine is often limiting in plant-based foods (FAO/WHO/UNU, 7). Longan displayed elevated alanine (1.12%) and tyrosine (0.55%) levels, which may contribute to its distinctive sweet taste and aromatic properties (Zhang *et al*., 24).

Among indispensable amino acids, leucine, isoleucine, valine, and threonine were consistently detected across species, but at varying concentrations. Banana again showed superior contents of leucine (0.72%), isoleucine (0.27%),

Table 2: Comparative amino acid composition of selected Vietnamese fruits (Unit: %).

Amino acid	Guava	Persimmon	Banana	Custard apple	Longan	Lemon	Litchi	Mango
Aspartic acid	2.23 ± 0.06	0.62 ± 0.02	0.79 ± 0.02	0.21 ± 0.02	0.70 ± 0.02	0.33 ± 0.02	0.44 ± 0.004	0.14 ± 0.01
Glutamic acid	0.28 ± 0.01	0.37 ± 0.01	1.10 ± 0.01	0.32 ± 0.03	0.95 ± 0.04	0.81 ± 0.03	0.44 ± 0.002	0.21 ± 0.04
Serine	0.13 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.07 ± 0.01	0.23 ± 0.02	0.51 ± 0.01	0.13 ± 0.003	0.03 ± 0.01
Histidine*	0.09 ± 0.01	0.12 ± 0.02	0.53 ± 0.03	0.02 ± 0.02	0.17 ± 0.01	0.06 ± 0.01	0.02 ± 0.001	0.01 ± 0.01
Arginine	0.12 ± 0.01	0.52 ± 0.02	0.47 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.33 ± 0.02	0.08 ± 0.002	0.23 ± 0.05
Glycine	0.13 ± 0.02	0.28 ± 0.01	0.29 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.19 ± 0.002	0.08 ± 0.02
Threonine*	0.10 ± 0.01	0.22 ± 0.01	0.27 ± 0.01	0.13 ± 0.02	0.25 ± 0.02	0.42 ± 0.02	0.13 ± 0.005	0.06 ± 0.01
Tyrosine	0.06 ± 0.01	0.17 ± 0.01	0.10 ± 0.01	0.04 ± 0.01	0.55 ± 0.02	0.09 ± 0.01	0.05 ± 0.001	0.02 ± 0.01
Alanine	0.23 ± 0.02	0.29 ± 0.01	0.26 ± 0.01	0.15 ± 0.03	1.12 ± 0.07	0.19 ± 0.02	0.44 ± 0.005	0.11 ± 0.02
Valine*	0.15 ± 0.01	0.24 ± 0.02	0.47 ± 0.03	0.16 ± 0.02	0.23 ± 0.01	0.11 ± 0.01	0.17 ± 0.002	0.10 ± 0.02
Methionine*	0.03 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.01 ± 0.01	0.02 ± 0.001	0.01 ± 0.01
Phenylalanine*	0.14 ± 0.01	0.30 ± 0.02	0.25 ± 0.01	0.12 ± 0.01	0.17 ± 0.02	0.11 ± 0.02	0.14 ± 0.002	0.08 ± 0.01
Isoleucine*	0.12 ± 0.01	0.22 ± 0.01	0.27 ± 0.02	0.10 ± 0.01	0.18 ± 0.02	0.08 ± 0.01	0.10 ± 0.001	0.07 ± 0.01
Leucine*	0.22 ± 0.03	0.31 ± 0.02	0.72 ± 0.04	0.22 ± 0.02	0.29 ± 0.01	0.13 ± 0.01	0.22 ± 0.002	0.13 ± 0.02
Lysine*	0.21 ± 0.02	0.21 ± 0.01	1.16 ± 0.10	0.12 ± 0.01	0.31 ± 0.02	0.12 ± 0.01	0.06 ± 0.001	0.11 ± 0.02
Proline	0.07 ± 0.01	0.24 ± 0.02	-	0.17 ± 0.02	0.12 ± 0.01	0.24 ± 0.02	0.10 ± 0.001	0.11 ± 0.01
Cysteine	0.07 ± 0.01	0.04 ± 0.01	-	0.03 ± 0.01	-	-	0.11 ± 0.001	0.02 ± 0.01
Tryptophan*	-	0.62 ± 0.01	0.79 ± 0.03	-	0.70 ± 0.04	0.33 ± 0.02	-	-

Notes: Values are given as means of three replicates ± SD. (*) indispensable amino acids. (-) Not detected during the time conducting the research.

Table 3: Comparative mineral composition of selected Vietnamese fruits (Unit: %).

Mineral elements	Guava	Persimmon	Banana	Custard apple	Longan	Lemon	Litchi	Mango
Calcium	0.102 ± 0.004	0.058 ± 0.002	0.013 ± 0.001	0.050 ± 0.001	-	0.029 ± 0.001	0.025 ± 0.001	0.063 ± 0.002
Copper	-	-	-	-	0.052 ± 0.001	0.002 ± 0.001	-	-
Iron	0.005 ± 0.001	0.021 ± 0.001	0.001 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	-	0.004 ± 0.001	0.034 ± 0.002
Potassium	0.204 ± 0.005	1.010 ± 0.002	0.302 ± 0.004	0.078 ± 0.002	2.660 ± 0.003	0.066 ± 0.002	0.087 ± 0.003	-
Magnesium	0.113 ± 0.001	0.031 ± 0.001	0.013 ± 0.001	0.098 ± 0.002	0.092 ± 0.002	0.011 ± 0.001	0.027 ± 0.002	0.075 ± 0.002
Nitrogen	1.195 ± 0.006	0.450 ± 0.005	0.606 ± 0.005	0.010 ± 0.001	0.731 ± 0.004	0.245 ± 0.003	0.435 ± 0.006	0.007 ± 0.001
Sodium	0.037 ± 0.001	-	0.001 ± 0.001	0.005 ± 0.001	-	-	0.002 ± 0.001	0.082 ± 0.002
Phosphorus	0.065 ± 0.005	0.089 ± 0.001	2.650 ± 0.005	0.099 ± 0.005	0.316 ± 0.002	0.019 ± 0.002	0.098 ± 0.004	0.065 ± 0.004
Zinc	0.037 ± 0.003	0.007 ± 0.001	0.001 ± 0.001	-	0.161 ± 0.002	0.001 ± 0.001	-	0.037 ± 0.003
Manganese	0.011 ± 0.001	-	-	0.042 ± 0.003	-	-	0.026 ± 0.001	0.011 ± 0.001
Sulfur	0.050 ± 0.002	-	-	0.045 ± 0.002	-	-	0.096 ± 0.005	0.050 ± 0.005

Notes: Values are given as means of three replicates ± SD. (-) Not detected during the time conducting the research.

and valine (0.47%), aligning with reports that Musa species are relatively richer in branched-chain amino acids compared to other tropical fruits (Bugaud *et al*., 3). Persimmon stood out with the highest tryptophan content (0.62%), which is notable given the rarity of this amino acid in many fruits and its role as a precursor of serotonin, potentially contributing to health-promoting properties (Khattab *et al*., 11).

In terms of sulfur-containing amino acids, methionine and cysteine were generally present at low levels, with guava and litchi providing slightly higher cysteine concentrations (0.07% and 0.11%, respectively). Proline was detected in moderate amounts in persimmon and lemon (0.24%), where it may play a role in stress tolerance and fruit texture (Kavi *et al*., 10).

Overall, the results emphasize that each fruit species exhibits a unique amino acid signature. Guava and banana can be highlighted as valuable sources of essential amino acids such as lysine and leucine, while persimmon is exceptional in its tryptophan content. The observed variability may be influenced by genetic background, maturity stage, and environmental conditions during growth (Hoffman, 9). These findings underline the nutritional diversity of tropical and subtropical fruits, supporting their complementary role in dietary amino acid supply.

The considerable variation in mineral composition among the studied fruit species (Table 3). Potassium (K) emerged as the predominant mineral across most fruits, in line with its well-known role in osmotic regulation and fruit quality (Wills *et al*., 23). Notably, longan exhibited the highest K content (2.660%), surpassing other fruits by a wide margin, while persimmon (1.010%) and banana (0.302%) also contained appreciable amounts. In contrast, guava, custard apple, lemon, and litchi contained relatively low K levels (<0.1%), and mango showed undetectable values under the current analytical conditions.

Phosphorus (P) content displayed a particularly striking pattern, with banana recording an exceptionally high concentration (2.650%), suggesting its potential as a rich dietary source of this element. Moderate levels were found in longan (0.316%) and persimmon (0.089%), whereas other fruits contained relatively lower amounts (<0.1%). Similarly, nitrogen (N) was abundant in guava (1.195%) and banana (0.606%), reflecting their high protein-associated nutrient reserves, whereas mango presented a notably low level (0.007%) (Marriott *et al*., 15).

With respect to calcium (Ca), guava exhibited the highest content (0.102%), followed by mango (0.063%) and persimmon (0.058%), whereas banana showed minimal levels (0.013%). Among micronutrients, iron (Fe) content peaked in mango (0.034%) and persimmon (0.021%), while other fruits contained only trace amounts. Zinc (Zn) was highest in longan (0.161%) and guava (0.037%), highlighting their nutritional importance in human diets (Guevara *et al*., 2019). Interestingly, copper (Cu) was only detected in longan (0.052%) and lemon (0.002%), suggesting species-specific accumulation mechanisms.

Sulfur (S) and manganese (Mn) were present in variable amounts, with litchi showing the highest S concentration (0.096%), and custard apple the highest Mn level (0.042%). Sodium (Na) was generally low across all fruits, except mango (0.082%), which contained a comparatively higher amount.

Overall, the findings demonstrate clear interspecific differences in mineral accumulation patterns. Longan and banana emerged as particularly rich sources of K and P, respectively, while guava provided higher levels of Ca and N. The variation observed may be attributed to genetic factors, soil–plant interactions, and fruit developmental physiology (Suresh *et al*., 21; Srivastava, 20). These results underscore the nutritional diversity among tropical and subtropical fruits, highlighting their complementary role in a balanced diet.

This study highlights the substantial interspecific variation in the chemical composition of eight fruit species grown in Vietnam. Guava was remarkable for its very high vitamin C content, together with elevated protein and amino acids, confirming its role as a functional fruit. Longan exhibited exceptionally high vitamin B6 and potassium, indicating benefits for neurological and circulatory health. Banana provided the highest starch and phosphorus, reinforcing its role as an energy-rich staple. Custard apple and mango showed relatively higher protein and lipid levels, contributing to dietary energy density, while litchi and custard apple were good sources of cellulose. Persimmon was notable for its high riboflavin, and lemon, despite its high-water content, supplied moderate vitamin C. The mineral profiles confirmed guava and mango as valuable sources of calcium and iron, while longan had the highest zinc. Overall, these results emphasize the nutritional diversity and complementary values of tropical fruits, supporting their wider use in balanced diets and functional food development.

AUTHOR'S CONTRIBUTION

Conceived the idea and designed the experiments (L.V.T.); Conducted the experiments (L.V.T. and

N.T.V.); Analyzed the research data (L.V.T. and L.T.T.H.); Wrote the manuscript (L.V.T. and L.T.T.H.). All authors read and agree to the submission of the manuscript to the journal.

DECLARATION

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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