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RESEARCH ARTICLE

Laurus nobilis L. essential oil enhances nutrient retention of *Amaranthus viridis* L. for longer storage duration

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Abstract

Amaranthus viridis L. (Family: Amaranthaceae) is a nutritious leafy vegetable known for its rich carbohydrates, proteins, moisture, fat and dietary fiber content. However, due to their high-water content and ongoing biological activity, this leafy vegetable is prone to post-harvest deterioration caused by mechanical damage, biochemical changes, aging and microbial contamination. Proper preservation techniques are therefore crucial to maintain their freshness, nutritional value and marketability. Drying is a widely used method that reduces the moisture content and delays the spoilage of vegetables. In recent years, essential oils (EO) have emerged as effective natural preservatives due to their antimicrobial and antioxidant properties. For instance, EO from *Laurus nobilis* L. (bay leaf) has demonstrated antimicrobial activity against bacteria that cause food spoilage, thereby promoting food safety and sustainable, health-conscious consumption. However, the specific effect of *L. nobilis* EO (LnEO) on the retention of the nutritional composition and antioxidant activities of leafy vegetables, including *A. viridis*, has not yet been reported. Hence, in the present study, the influences of different concentrations (1 %, 1.5 %, 2 %, 2.5 % and 3 %) of *L. nobilis* LnEO on oven-dried *A. viridis* were evaluated in terms of nutritional and antioxidant activities over a storage period of up to two months (60 days). Results indicated that the 3 % LnEO treatment was the most effective, maintaining the highest levels of crude fiber, nitrogen, protein, fat, carbohydrates, antioxidants and minerals even after 60 days. These findings suggest that using 3 % EO in oven-dried *A. viridis* significantly enhances post-harvest nutrient retention. This will provide valuable information for researchers, development partners and farmers engaged in both subsistence and commercial agriculture to support food security and easier market access in rural communities.

Keywords: anti-oxidant activity; EDS; essential oil; leafy vegetable; preservation; proximate

Introduction

Leafy vegetables are important for their nutritional richness, contributing significantly to a balanced diet. They use to provide water, dietary fiber, protein, carbohydrates, essential amino acids, vitamins, minerals, antioxidants and other bioactive compounds that support and protect against diseases (1, 2). Green leafy vegetables are rich in natural antioxidants, which help in neutralizing free radicals and promoting cellular health (3). Their consumption dates back to ancient times, primarily due to their high nutritional content. One such leafy vegetable is *Amaranthus viridis* (Family: Amaranthaceae), also known as slender amaranth or green amaranth and the genus *Amaranthus* includes 60-70 species. *A. viridis* is known for its adaptability to diverse environmental conditions, ranging from tropical to temperate regions. Its leaves are rich in carbohydrates, proteins, moisture, fat and dietary fiber, making it a highly nutritious option (4). Some of the critical environmental factors, like temperature and humidity, are responsible for accelerating spoilage (5). Once harvested, the quality of leafy vegetables cannot be improved, only maintained. Hence, effective preservation becomes essential to reduce wastage and to extend shelf life. Preservation techniques aim to retain the freshness, nutritional value and marketability of leafy vegetables. Drying reduces moisture content and delays spoilage and hence is considered one of the primary preservation methods for leafy vegetables. Traditional drying techniques like sun drying, shade drying, wind drying and solar drying are cost-effective but time-consuming. On the other hand, artificial drying methods-such as cabinet drying, vacuum drying, oven drying and chemical drying-allow for faster and more controlled processing with better quality retention (6). Recently, interest has increased in using natural preservation techniques and preservatives that align with consumer preferences for clean-label and chemical-free food products. Plant-based essential oil (EO) has emerged as a promising natural additive due to its natural antimicrobial and antioxidant properties. These plant-based oils are effective in enhancing the shelf life of food without the adverse effects linked to synthetic preservatives (7). *Laurus nobilis* (bay leaf), a small evergreen tree of the Lauraceae family, produces EO with proven antimicrobial effects against food spoilage bacteria (8). Such natural methods of preservation not only support food safety but also promote sustainable and health-conscious consumption. Although the application of EO as a natural preservative with inhibitory effects against spoiling microbes is well-established, the use of *L. nobilis* essential oil (LnEO) to preserve the

nutritional content and antioxidant activities of *A. viridis* during storage has not been reported earlier. As the rich nutritional and antioxidant value of *A. viridis* has been reported earlier, investigating the potential of applying LnEO as a natural preservative for retention of nutrient composition and antioxidant activities of this leafy vegetable presents novel scientific insights. To find the best LnEO concentration for preservation and storage conditions for ensuring the maximum retention of proximate and antioxidant activity of aerial edible parts of *A. viridis,* leaves and tender stems were subjected to one widely and commercially used drying treatment, i.e. oven drying. The dried-powdered parts were then stored in glass jars for varying lengths of time. The best percentage of LnEO for maximum preservation duration was determined based on optimum retention of nutritional parameters, including proximate composition and antioxidant activity. The findings of this study will help researchers, development partners, local communities and individual farmers engaged in both the subsistence and commercial agriculture systems to create suitable strategies for enhancing food security and income in rural communities through enhanced marketability of *A. viridis*.

Materials and Methods

Collection of *L. nobilis* leaves and extraction of EO

L. nobilis leaves were first collected from the local market of Dharapur, Guwahati, Assam (26°08′14″ N, 91°37′40″ E), at their middle growth stage (between February-March, 2024) and were authenticated in GUBH herbarium. Leaves were then washed thoroughly under running water, followed by distilled water to remove surface impurities and then chopped into small pieces. For hydro-distillation, approximately 2/3 of the round-bottom flask of the Clevenger apparatus were filled with the chopped leaves and distilled water and the extraction was carried out at a controlled temperature of 40 °C, ensuring that the process remained gentle to prevent degradation of heat-sensitive compounds (9). The distillation process was performed continuously for 5 hr, during which the volatile oil vapours were condensed and collected in a graduated side arm of the apparatus. The essential oil obtained (LnEO) was carefully separated from the aqueous phase and stored in an airtight container (amber colour) at 4 °C until further use to prevent oxidation and loss of volatile constituents.

Collection *of A. viridis* and sample preparation

A. viridis leaves were collected from the local market in Lankeswar, Guwahati, Assam, India (26.1461° N, 91.6458° E). The leaves were thoroughly washed, then shade-dried for 12 hr, followed by oven drying at 60 °C for an additional 12 hr. The dried samples were then pounded using a grinder to obtain fine powder. After that, 20 g of the powder were placed into each of six separate glass bottles followed by treating them with different concentrations of LnEO, viz. 1 % (200 µL), 1.5 % (300 µL), 2 % (400 µL), 2.5 % (500 µL) and 3 % (600 µL), respectively by following previous literature (10) and stored for three different time intervals: zero month (before bottling), one month and two months. One portion of the leaf powder was stored as the control with no LnEO treatment (Fig. 1).

Proximate analysis

Using a set of standard methods, the proximate compositions of the LnEO-treated *A. viridis* samples were determined along with the control sample. Each experiment was done in three replicates and the average was determined.

Moisture

About 1 g of each sample was weighed and dried in a hot air oven at 105 °C up to a constant final weight. The percentage loss on drying was calculated by using the following formula (11).

$$\text{Moisture \%} = \frac{W1 - W2}{W1} \times 100 \qquad \text{(Eqn. 1)}$$

Where,

W1 = weight (g) of the sample before drying

W2 = weight (g) of the sample after drying

Ash

About 0.5 g of each sample was taken in a crucible and placed inside the muffle furnace for 4 hr at 450 °C to obtain grey ash. The experiment was done in three replications and the average was taken. The ash content was calculated by the following formula (11).

$$\text{Ash \%} = \frac{W1 - W0}{W1} \times 100 \qquad \text{(Eqn. 2)}$$

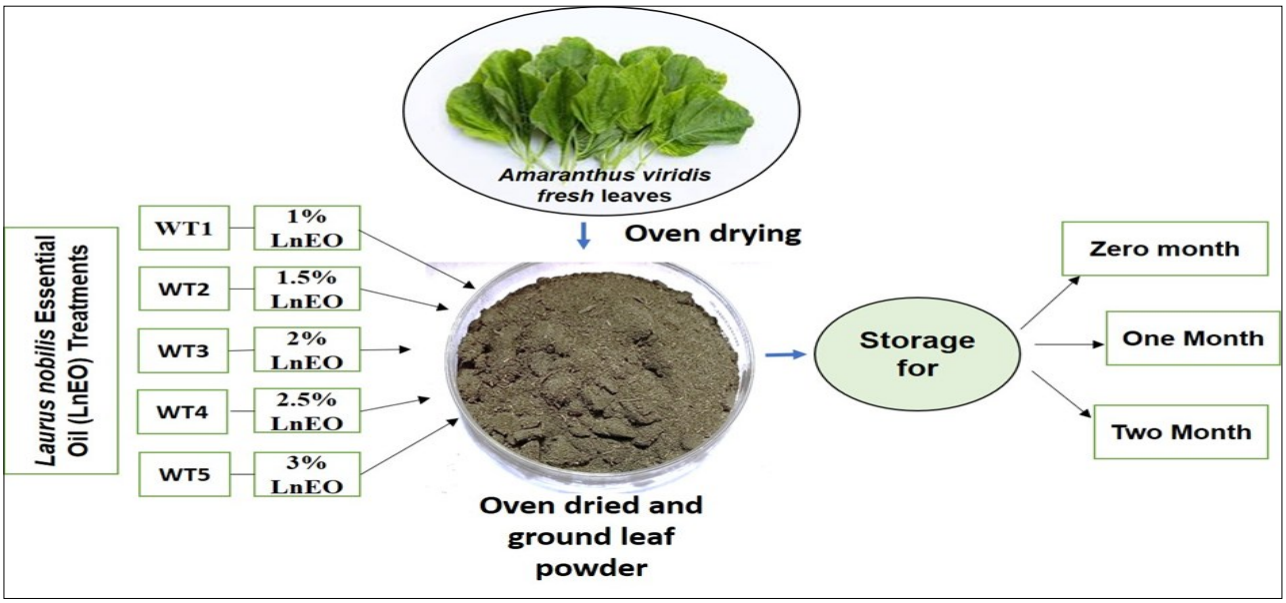

Fig. 1. Schematic representation of sample preparation with different concentrations of LnEO for various durations of storage.

Where , W = sample weight (g)

W0 = weight of the empty crucible (g)

W1 = weight of the crucible with ash (g)

Fat

The fat content was estimated by the Soxhlet method as described in a previous reported study (11). A filter paper thimble with 1 g of each sample was hung in the middle of the extraction chamber and the round-bottom flask was filled with petroleum ether up to 2/3rd of its volume. The round-bottom flask was then placed on a heating mantle and the temperature was set at 40 °C, causing the solvent to vaporize. The solvent penetrated the sample in the thimble, extracting fat which was accumulated in the flask. The flask was allowed to cool down. The thimble was dried at room temperature and reweighed along with the plant material.

$$\text{Fat \%} = \frac{W1 - W2}{W1} \times 100 \qquad \text{(Eqn. 3)}$$

Where ,

W1 = weight (g) of the flask with extracted fat

W2 = weight (g) of the empty flask

W = weight (g) of the sample taken initially

Crude fiber

About 1 g of each sample was transferred to a beaker. A volume of 200 mL of 2.5 % sulphuric acid (H_2SO_4) was added and the mixture was boiled for 30 min at 90 °C in a hot water bath. Then the mixture was filtered through a muslin cloth and the residue was washed using hot water to make it acid-free. After that, 200 mL of 2.5 % sodium hydroxide (NaOH) solution was added to the acid-free sample and the content was again refluxed for 30 min. The residue was washed with dilute HCl and then with hot water to remove the alkali. Then the alkali-free residue was taken in a crucible and dried in a hot air oven until a constant final weight was recorded. The residue was then ignited for 5 hr at 450 °C in a muffle furnace and the fiber content was estimated using the following formula (11).

$$\text{Crude fibre \%} = \frac{W1 - W2}{W1} \times 100 \qquad \text{(Eqn. 4)}$$

Where,

W1 = weight of the sample (g)

W2 = weight of the crucible + residue

W3 = weight of the crucible + ashed residue

Nitrogen

Nitrogen content was determined by the Kjeldahl method (11). A known weight of 0.25 g of the sample was weighed and it was mixed with a digestion mixture consisting of 3 g of K_2SO_4 and 0.4 g of $CuSO_4$. After that, it was dropped at the bottom of a conical flask and 10 mL of concentrated H_2SO_4 was carefully added to each flask. The flask was gently heated in a mantle heater until the appearance of a clear green color of the sample indicated full digestion. After that, the digested sample was distilled and titrated against 0.1 N H_2SO_4.

$$\text{Nitrogen \%} = \frac{(14.01 \times v - 0.1 \times 100)}{0.2 \times 1000} \qquad \text{(Eqn. 5)}$$

Where V = difference between initial and final reading

Protein

Protein percentage was calculated from the nitrogen content of the samples, multiplied by a conversion factor of 6.25, according to previously described methodology (11).

$$\text{Protein \%} = \text{Nitrogen} \times 6.25 \qquad \text{(Eqn.6)}$$

Carbohydrate

Percentage of carbohydrate was obtained by subtracting the total value of moisture, ash, crude fiber, fat and crude protein percentages of the sample from 100, following standard procedure (11).

$$\text{Carbohydrate \%} = 100 - (\text{\% Moisture} + \text{\% Ash} + \text{\% Crude fiber} + \text{\% Fat} + \text{\% Protein}) \qquad \text{(Eqn. 7)}$$

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The antioxidant activity was calculated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, following the standard protocol as described in a previously reported study (12). Accordingly, each powdered plant sample was soaked in methanol and aliquots of 0.3 mL, 0.05 mL and 0.07 mL were taken in test tubes and the volume of each tube was brought up to 1 mL with methanol. Then, 3 mL of 0.1 mM DPPH solution (prepared in methanol) was added to each test tube. A control was prepared by mixing 3 mL of 0.1 mM DPPH with 1 mL of methanol without the sample extract. After a 30 min incubation period, the absorbances of the reaction mixtures were measured at 517 nm wavelength in a UV-vis spectrophotometer (Agilent Cary 60 UV-Vis) and the percentage inhibition of DPPH radical was calculated by using the following formula.

$$\text{\% Inhibition} = \frac{Ac - As}{Ac} \times 100 \qquad \text{(Eqn. 8)}$$

Where,

Ac = Absorbance of the control

As = Absorbance of the sample extract

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was carried out using the procedure used in the previous study (13). A 7 mM ABTS solution was prepared by dissolving 0.360 g of ABTS in 100 mL of distilled water. In comparison, a 2.45 mM potassium persulfate solution was obtained by dissolving 0.066 g of potassium persulfate in 100 mL of distilled water. Equal volumes, i.e. 10 mL of the two solutions, were mixed thoroughly and kept in the dark for 12 hr to generate the $ABTS^{\bullet+}$ radical. The resulting solution was then diluted (1:1) with ethanol to adjust the absorbance to 0.700 ± 0.02 at 734 nm using a UV–Vis spectrophotometer.

$$\text{\% Inhibition} = \frac{Ac - As}{Ac} \times 100 \qquad \text{(Eqn. 9)}$$

Where,

Ac = Absorbance of the control (ABTS solution without sample or standard)

As = Absorbance of the sample or standard

Determination of IC_{50} value

The IC_{50} values (concentration required to inhibit 50 % of the DPPH and ABTS radicals) of the samples were determined by setting Y = 50 in the regression equations generated from the plots (13).

Energy Dispersive X-ray Spectroscopy (EDS) analysis

EDS analyses of dried leaf samples were done according to previously described methodology (14). Leaf samples were first coated with a thin layer of gold using a sputter coater and carefully mounted and placed into the Scanning Electron Microscope (SEM) chamber (Model: SIGMA VP FESEM, ZEISS). The areas of interest on the leaf samples were identified and selected for EDS analyses. The elemental compositions of the leaf samples were determined by analyzing the characteristic X-rays emitted from the samples when bombarded by the electron beam. The spectra were processed using EDS software to quantify the elements present in the samples.

Data analysis

All the analyses were performed in triplicate and the mean values along with standard deviation (± SD) were calculated using Microsoft Excel. Significance tests were done among the variables at probability level $p = 0.05$ by using one-way ANOVA with the help of "Analyse-it" software. Graphical work and Pearson Correlation Coefficient analysis were carried out in MS-Excel.

Results

Yield of LnEO

The yield of LnEO obtained through hydrodistillation was recorded as 0.26 % (v/w). Although the yield percentage appears relatively low, the economic advantage lies in its high value due to the antimicrobial, antioxidant and preservative properties, even in very low concentrations. Even at a very small yield, the concentrated bioactive compounds offer significant functional potential, making the incorporation of LnEO in food preservation a cost-effective and non-synthetic strategy. The obtained LnEO was of a typical pale-yellow colour with a strong aroma. According to previous reports, LnEO is composed of major bioactive constituents such as 1,8-cineole, eugenol, linalool and methyl eugenol, which are responsible for its potent antimicrobial, antioxidant and preservative activities (15). The oil also exhibits good solubility in organic solvents, high volatility and thermal stability under processing conditions, which enhances its applicability in food preservation. These properties, combined with their functional activity even at low concentrations, provide a significant economic advantage, as a small yield capable of exerting a pronounced effect in extending the shelf life and maintaining the nutritional quality of *A. viridis* during storage.

Proximate composition

Moisture

In the present study, *A. viridis* samples treated with 1 % LnEO (WT1) exhibited the highest initial moisture content, while the control sample (WT0), which did not receive any LnEO treatment, showed the lowest (Fig. 2A). The moisture (%) of both the 1st and 2nd month samples showed a significant difference ($p < 0.05$) compared with the before-bottling samples across all the preservation treatments (WT0, WT1, WT2, WT3, WT4 and WT5). This finding is consistent with previous research on moisture retention in potato tubers treated with coriander EO, where EO application was found to mitigate moisture loss (16). Over the storage period, the moisture level decreased in both the control and LnEO-treated *A. viridis* samples. However, the decline in moisture content was significantly less pronounced in the WT1 sample, suggesting a preservative effect of the LnEO treatment.

Ash

In this study, ash content decreased in both control and LnEO-treated *A. viridis* samples over the storage period. However, the highest ash content was found in the samples treated with 3 % LnEO (WT5), while the lowest was found in the untreated control sample (WT0) (Fig. 2B). Regarding ash content (%), WT0, WT1 and WT2 samples were significantly different ($p < 0.05$) from the before bottling sample up to 60 days of storage. In contrast, WT3 and WT4 samples showed no significant difference ($p > 0.05$) within the same period, whereas WT5 samples exhibited a significant difference ($p < 0.05$) with others.

Fat

A gradual decrease in fat content was observed in both the control and LnEO-treated *A. viridis* samples throughout the storage period. Among the treatments, the sample treated with 3 % LnEO (WT5) consistently exhibited the highest fat content, whereas the control sample (WT0), showed the lowest (Fig. 2C). The Fat % of both 1st and 2nd month samples were significantly different ($p < 0.05$) with before bottling sample, across all the preservation treatments (WT0, WT1, WT2, WT3, WT4 and WT5). This suggests that LnEO application may contribute to the retention of lipid components in *A. viridis* during storage, possibly due to its protective effect against oxidative degradation. The observed trend underscores the potential role of LnEO in preserving the nutritional quality of leafy vegetables.

Crude fiber

In the present study, the highest crude fiber content was observed in *A. viridis* samples treated with 3 % LnEO (WT5), whereas the lowest was recorded in the control sample (WT0), (Fig. 2D). No significant difference ($p > 0.05$) in crude fibre (%), was observed between the before-bottling samples and those stored for one month. However, after the 2nd month, the crude fibre (%) of all LnEO-treated samples showed significant differences ($p < 0.05$) compared with the samples before bottling. This finding agrees with previous studies on plum ready-to-serve (RTS) drinks treated with cardamom (*Amomum sublatum Roxb.*) and black pepper (*Piper nigrum*) EO, which also reported enhanced fiber retention (17).

Nitrogen and protein

Among all the treatments, the highest nitrogen and protein contents were recorded in samples treated with 3 % LnEO (WT5), whereas the lowest levels were observed in the 1 % and 1.5 % LnEO-treated samples (WT1 and WT2) (Fig. 2E and Fig. 2F). Both the nitrogen (%) and protein (%) were significantly different ($p < 0.05$) in the 1st and 2nd month samples compared with the before-bottling samples across all the treatments (WT0, WT1, WT2, WT3, WT4 and WT5). These findings are consistent with previous studies on fresh rainbow trout and smoked *Sardinella maderensis* treated with sage EO, which demonstrated improved protein preservation due to the antimicrobial properties of the EO (18).

Carbohydrate

In this study, carbohydrate content increased over the storage period in both control and LnEO-treated *A. viridis* samples (Fig. 2G). The carbohydrate (%) of the 1st and 2nd month samples showed significant differences ($p < 0.05$) compared with the before-bottling

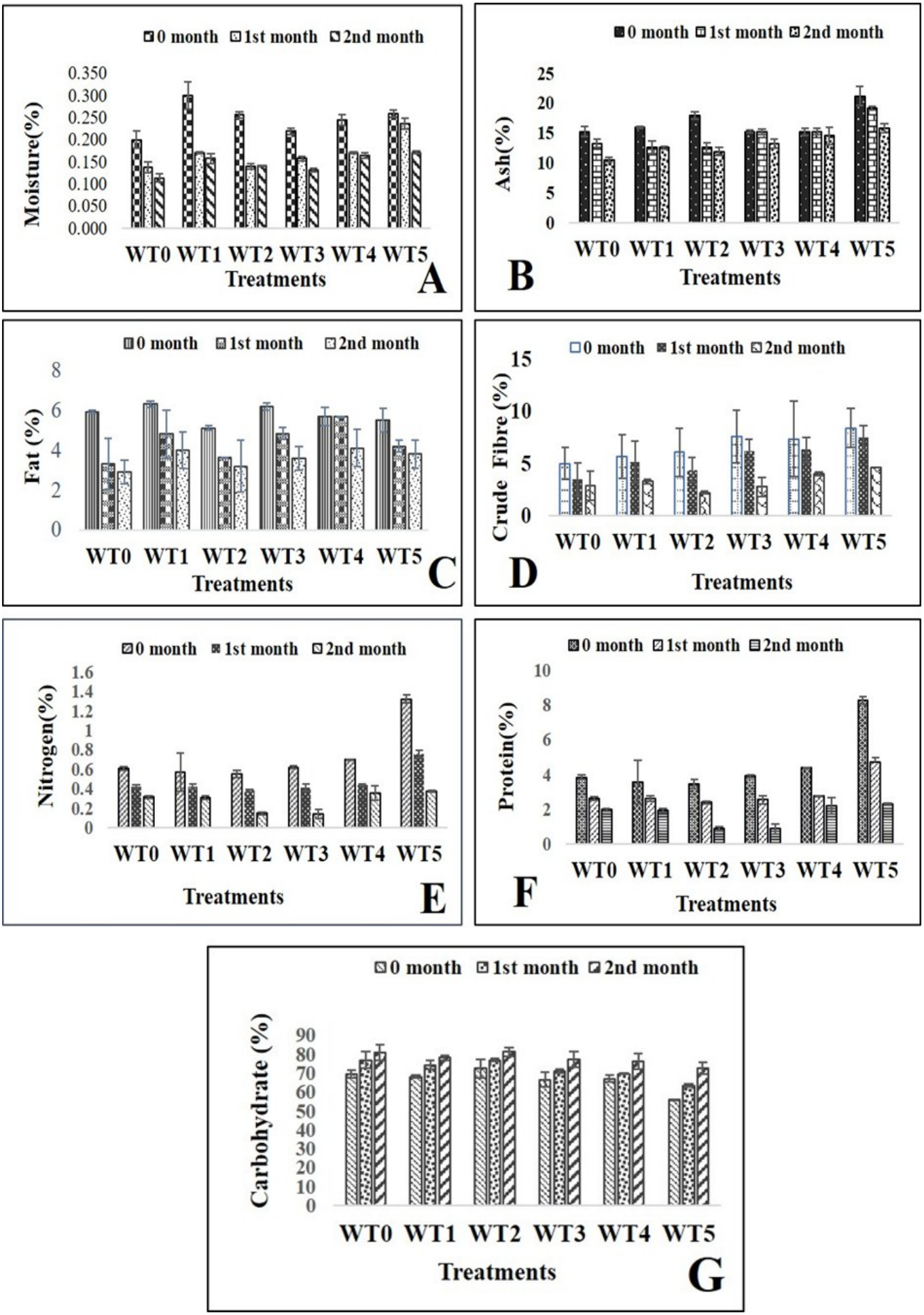

Fig. 2. Proximate composition in *A. viridis* treated with different concentrations of LnEO during 60 days of storage. (A) Moisture %; (B) Ash %; (C) Fat %; (D) Crude fibre; (E) Nitrogen %; (F) Protein %; (G) Carbohydrate %.

samples in all the treatments (WT0, WT1, WT2, WT3, WT4 and WT5). This trend aligns with previous research on preserved fruits and vegetables, where an increase in sugar and carbohydrate levels has been attributed to a decrease in respiration rate during storage (19). Interestingly, the control sample (WT0), which did not receive any LnEO treatment, exhibited the highest carbohydrate content, while the sample treated with 3 % LnEO (WT5) showed the lowest.

Antioxidant activity

In the present study, the *A. viridis* sample treated with 3 % LnEO (WT5) exhibited the lowest IC_{50} value against DPPH and ABTS radicals, indicating the highest antioxidant activity (Fig. 3). Conversely, the control sample (WT0) showed the highest IC_{50} value against both DPPH and ABTS radicals, signifying lower antioxidant potential. These results corroborate the findings on *L. nobilis* EO, which have been recognized as a potent EO having robust antioxidant activity, suggesting that LnEO could be used as a natural antioxidant to counteract oxidation activity effectively (20). The antioxidant activity, measured by both DPPH radical scavenging (%) and ABTS radical scavenging (%), was significantly different ($p < 0.05$) across all the treatments (WT0, WT1, WT2, WT3, WT4 and WT5) with the before bottling samples stored up to two months.

Mineral elements

In this study, both the control and LnEO-treated *A. viridis* samples exhibited high levels of carbon (C), oxygen (O), magnesium (Mg), calcium (Ca) and potassium (K), with low levels of sodium (Na) (Table 1, Table 2 and Table 3). In this study, both the control and LnEO-treated *A. viridis* samples showed a clear dominance of C and O, reflecting the structural role in organic compounds and cellular biomolecules. Among all the mineral elements, Ca, Mg and K were consistently present at high levels, indicating the nutritional potential of the leaf powder (along with the LnEO). Magnesium, as the central atom in chlorophyll, indicates pigment retention, while Ca and K are associated with structural stability, enzyme activity and osmotic regulation. In contrast, Na was recorded at relatively low

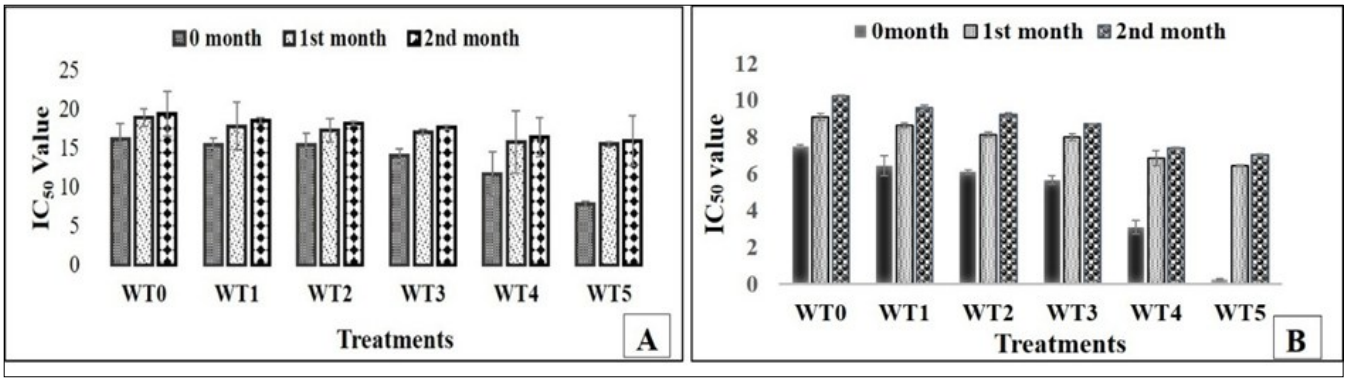

Fig. 3. Antioxidant activity (IC_{50} value) of *A. viridis* with different concentrations of LnEO-treatments against DPPH and ABTS radicals.

Table 1. Mineral elements of *A. viridis* with different concentrations of EO-treatment in 0/zero-month storage

Treatments	C	O	Na	Mg	P	K	Ca	Cl	N
WT0	37.23	42.75	0.96	3.83	1.20	1.51	0.01	0.45	8.33
WT1	41.76	40.30	0.75	2.22	1.02	4.26	1.66	0.56	0.01
WT2	32.53	46.08	0.51	3.77	1.21	2.86	1.77	0.58	4.31
WT3	31.50	41.60	0.42	2.31	1.30	5.45	0.96	1.37	8.55
WT4	44.19	37.97	0.02	2.47	1.17	5.65	0.71	1.53	0.01
WT5	40.50	38.00	0.54	2.10	0.01	5.68	0.93	1.58	2.18

All the values are means of triplicate.

Table 2. Mineral elements of *A. viridis* with different concentrations of EO-treatment after 1st month of storage

Treatments	C	O	Na	Mg	P	K	Ca	Cl	N
WT0	18.71	48.59	0.33	3.17	0.55	8.09	6.04	3.48	3.36
WT1	22.60	46.71	0.37	3.68	2.17	10.38	3.80	2.11	0.01
WT2	24.99	47.35	0.31	2.75	1.44	10.32	0.64	1.94	4.45
WT3	27.97	47.74	0.44	3.17	0.85	5.63	0.45	1.66	5.80
WT4	35.32	48.10	0.56	3.45	1.67	6.26	1.33	0.82	0.01
WT5	23.92	48.65	0.44	3.87	0.01	7.18	2.09	0.98	6.29

All the values are means of triplicate.

Table 3. Mineral elements of *A. viridis* with different concentrations of EO-treatment after the 2nd month of storage

Treatments	C	O	Na	Mg	P	K	Ca	Cl	N
WT0	23.07	41.38	0.01	2.88	0.81	12.40	2.29	5.84	2.00
WT1	27.06	45.01	0.02	4.41	2.29	8.74	1.21	1.69	0.01
WT2	24.11	47.50	0.06	3.32	1.38	9.70	2.87	1.36	3.18
WT3	26.18	45.74	0.49	3.29	2.34	6.20	1.46	1.13	7.85
WT4	27.20	47.49	0.57	4.05	1.76	7.16	1.97	1.01	0.01
WT5	27.80	48.11	0.32	3.69	0.01	6.79	1.87	0.97	7.31

All the values are means of triplicate.

concentrations across all treatments, which is favourable for developing health-supportive food products. These elemental trends are consistent with earlier research on thyme EO-treated *Trifolium resupinatum* (Persian clover) sprouts, which also reported similar elemental compositions (21). Such similarities suggest that LnEO treatments do not disrupt the inherent mineral composition of leafy vegetables but may contribute to upholding their elemental stability during processing and preservation.

Correlation among parameters

Pearson's correlation coefficients among different nutritional parameters and antioxidant activities were calculated to analyze the positive and negative correlations among the parameters (Table 4). Proximate parameters, including moisture, ash, fat, crude fibre, nitrogen and protein, showed positive correlation with each other, which corroborated with previous findings where it was stated that there was a positive correlation of moisture with protein and crude fibre in *Trigonella foenum-graecum* L. (22). However, the carbohydrate percentage had a negative correlation with all other proximate parameters, which also substantiated previous results establishing negative correlations of protein, crude fibre and fat with carbohydrate in *T. foenum-graecum* L. (22). Interestingly, IC_{50} values of both DPPH and ABTS radical scavenging activities showed a negative correlation with almost all the proximate parameters (moisture, ash, fat, crude fibre, nitrogen and protein) except carbohydrate. This implied that the antioxidant activities of all the treated and untreated samples had positive correlation with all the proximate parameters except the carbohydrate percentage, which aligns with earlier reports where it was found that fat and crude fibre was individually correlated with the antioxidant parameters in three indigenous varieties of mulberry namely, *Morus nigra* L., *Morus alba* L. and *Morus rubra* L. (23). Elevated carbohydrate content reduced the antioxidant activity in all the samples in the present study.

Discussion

The moisture content of a food raw material is a key determinant of its quality. Despite some moisture loss, the LnEO-treated samples maintained higher moisture levels compared to the untreated control, highlighting the potential of LnEO in maintaining the physicochemical qualities of the leafy vegetable in long-term storage. Although the sample treated with 1 % LnEO exhibited an increase in moisture content compared to the control, a gradual decrease was noticed with higher concentrations, except at the two extreme levels, WT4 (2.5 %) and WT5 (3 %). This behaviour may be attributed to the proportionally greater amount of EO applied at higher concentrations. Essential oils are volatile in nature, which can facilitate moisture absorption in treated samples relative to untreated controls, whereas moisture itself is non-volatile. Hence, a reduction in moisture content along with the LnEO application is generally considered beneficial, as it limits microbial growth, as suggested in previous studies (24), which reported reduced moisture levels and improved microbial stability in carp fillets treated with EO (0.5 % carvacrol + 0.5 % thymol).

Ash content, which reflects the total mineral content of a food sample, is an important indicator of the nutritional quality of leafy vegetables such as *A. viridis*. It represents the amount of inorganic component remaining after the removal of organic matter and water through incineration. The increase in ash content in LnEO-treated samples relative to the control can be attributed to the presence of trace minerals in the LnEO, which may contribute to the overall mineral profile of the food. These findings are consistent with previous studies demonstrating that EO applications can enhance the mineral content of stored plant-based foods (10). Crude fiber refers to the indigestible portion of plant material that remains after food is subjected to acid and alkaline digestion and it serves as an important indicator of dietary fiber content in plant-based foods. Over the storage period, a gradual decline in crude fiber content was noted in both preserved and control samples, indicating the impact of prolonged storage on fiber degradation. The results highlight the potential of LnEO treatments in preserving dietary fiber content during storage, as the highest crude fiber content was observed in *A. viridis* samples treated with the highest level of LnEO (WT5). This corroborates previous findings, where a meta-analysis was done to evaluate the effect of EO as additives during fermentation of feed products (25).

Determining the nitrogen content in food is essential for calculating protein levels, as proteins are the primary nitrogen-containing compounds and serve as a critical indicator of nutritional quality. In this study, both nitrogen and protein content decreased over the storage period in *A. viridis* samples, regardless of whether

Table 4. Correlation analysis among proximate parameters and antioxidant activities of all the treated samples up to two months of storage duration

	Moisture %	Ash %	Fat %	Crude Fibre %	Nitrogen %	Protein %	Carbohydrate %	IC_{50} DPPH	IC_{50} ABTS
Moisture%	1								
Ash %	0.637339	1							
Fat %	0.438654	0.093885	1						
Crude Fibre %	0.634108	0.888321	0.511064	1					
Nitrogen %	0.627053	0.911672	0.055276	0.76561	1				
Protein %	0.621963	0.911186	0.049069	0.763376	0.999952	1			
Carbohydrate %	-0.64111	-0.93188	-0.36901	-0.94765	-0.90849	-0.90627	1		
IC_{50} DPPH	-0.64215	-0.92956	-0.33725	-0.96142	-0.81546	-0.81531	0.911438	1	
IC_{50} ABTS	-0.63965	-0.91899	-0.31942	-0.94545	-0.81065	-0.81091	0.891912	0.998222	1

they were treated with LnEO or not. The observed retention of protein content in higher-concentration LnEO treatments may be attributed to the LnEO's inhibitory effect on microbial growth, thereby slowing down protein degradation. Overall, the determination of protein content remains a fundamental parameter in evaluating the nutritional value and quality of food products. This finding corroborates the results of a previous study, where an increment of protein levels was reported in carp fillets treated with EO (0.5 % carvacrol + 0.5 % thymol) (24). Carbohydrates, which are one of the primary sources of energy in food, can vary in content depending on storage conditions and treatment methods. The comparatively lower carbohydrate content in LnEO-treated samples may be due to the oil's influence on metabolic activity, potentially slowing down starch degradation or sugar accumulation. These findings highlight the dynamic nature of carbohydrate levels in response to storage and treatment conditions. This corroborates with previous findings, where it was established that the carbohydrate level increased in carp fillets along with the EO (0.5 % carvacrol + 0.5 % thymol) (24). The higher carbohydrate levels observed in untreated *A. viridis* samples as compared to the lower levels of LnEO-treated ones may be attributed to several factors. In untreated samples, microbial proliferation and enzymatic breakdown of complex polysaccharides likely released more simple sugars, while greater moisture loss during storage could have concentrated the carbohydrate fraction on a dry-weight basis. Conversely, the antimicrobial and antioxidant properties of LnEO might inhibit microbial activity, suppress carbohydrate-degrading enzymes and preserve tissue integrity, thereby minimizing polysaccharide hydrolysis and reducing apparent carbohydrate accumulation.

On the other hand, plants protect themselves from oxidative damage caused by reactive oxygen species (ROS) by enhancing the activity of antioxidant compounds, which contribute significantly to overall plant health and productivity. In this context, the DPPH and ABTS radical scavenging assays are commonly performed to evaluate antioxidant activity, where the IC_{50} value represents the concentration of an antioxidant required to neutralize 50 % of DPPH or ABTS radicals, respectively. In principle, A lower IC_{50} value reflects stronger antioxidant potential. The enhanced antioxidant activity in LnEO-treated *A. viridis* samples in the present study suggests the effectiveness of LnEO in preserving the biochemical integrity of leafy vegetables during storage. This, however, contradicts previous results (26), where antioxidant capacity was reduced with the application of EO (carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde and linalool) in raspberry.

EDS is a modern and sophisticated analytical tool used to determine the elemental composition of materials, including plant-based materials. EDS analysis typically provides a spectrum and a table listing the detected elements along with their corresponding weight and atomic percentages. It has been noted that EO contains volatile organic compounds primarily composed of C-based molecules with O-containing functional groups, which may contribute to the observed elemental profiles in treated samples (27). EDS serves as a valuable tool to provide elemental profiling of food samples, particularly for minerals such as Ca, Mg, K, Na, etc. that are essential to nutritional evaluation. Mineral concentration can influence the concentration of anti-nutrients and this interaction indirectly affects nutrient availability on the other side. For instance, Ca, Fe and Zn often form insoluble complexes with anti-nutrients like oxalate, phytates and tannins, which reduces the free concentration of these anti-nutrients in food matrices. This binding not only lowers the anti-nutrient activity but also impacts nutrient bioavailability (28). Conversely, moderate mineral-anti-nutrient interactions can reduce the negative effects of anti-nutrients, thereby indirectly supporting better retention and utilization of nutrients. Thus, the balance among mineral elements, nutrients and anti-nutrients plays a crucial role in determining the overall nutritional quality and bioavailability of essential nutrients in foods.

Conclusion

Low moisture level with higher proximate nutrients, especially protein, fibre and ash contents, as well, are the desirable criteria for choosing an optimal preservation method to retain nutritional value in an edible vegetable. In this study, moisture levels in the nutrient-rich green leafy vegetable *A. viridis* were found to be much lower than the 9.30 % range that is advised for preserved dry vegetables, even after two months of storage. Although the amount of moisture determines whether food will remain safe from spoiling microbes, using plant-based EO is another technique to keep food products safe from microbes. In the present study, significant differences in the proximate and antioxidant properties were observed, with LnEO as a good agent for the preservation of nutrient contents and antioxidant activities of nutritious leafy vegetable *A. viridis*. Regarding proximate nutrients, the best was found in 3 % LnEO-treated *A. viridis* sample as it retained the highest fiber, fat, ash, nitrogen and protein percentage even up to two months of storage. This makes it an economically viable and organic option for small-scale and resource-poor farmers, promoting sustainable agriculture and reducing dependency on healthy post-harvest inputs. However, follow-up studies on the efficacy of specific percentages of LnEO employed in this study to check spoiling microbes in terms of yeast and mold count, bacterial count and total colony count are essential to substantiate the practical implications of the present investigation in the fight against hunger and malnutrition. Again, future studies supported by field data or socio-economic analysis to establish the economic viability of such preservation approaches will further clarify the feasibility scenario.

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Authors' contributions

RM was involved in conceptualization, methodology, plant material collection, treatments, sample preparation, proximate and ant-nutrient analyses on the bench, as well as in writing the original draft of the manuscript. JB was involved in data collection, analysis, writing the review and editing of the draft manuscript. NB was involved in visualization, experimental design and coordination, data analyses and interpretation, as well as performing the statistical analyses, supervision and final compilation of the manuscript. All authors read and approved the final manuscript

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

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