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Characterization of vitamin C, D3, and Magnesium nanocomposites with montmorillonite clay and their quantification in lemon and aronia tea

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Abstract

One of the significant sources of vitamins and minerals is herbal tea, which is most commonly analyzed using HPLC. The study aimed to develop a new, inexpensive, and an innovative method, rather than relying on expensive methods. The rope dyeing method starts with the preparation of two different herbal teas at specific concentrations and the determination of individual and nanocomposite forms of vitamin D3, vitamin C, and magnesium (Mg). After that, macramé strings are dipped in four different ink dyes in the sample. A certain pressure must be applied before pulling the strings, and the reaction on the sketch paper is photographed to proceed with color analysis using the Image J software. While the nanocomposite form of lemon tea has the highest concentration of vitamin C (46.01 ± 0.01 ppm), the individual form of aronia tea has the highest amount of vitamin D3 (49.03 ± 0.01 ppm). Additionally, the highest concentration of Mg was found in the nanocomposite extract of the aronia tea (78.47 ± 0.05 ppm). This consistency between methods reinforces the reliability of the analytical approach, as the HPLC results showed a 96.8% correlation with the rope dyeing method, validating the accuracy and reproducibility of the findings.

Introduction

A variety of herbal tea have been extensively used in traditional medicine by local and indigenous communities to enhance nutrition and address diverse health situations (Fu et al., 2018), and they are usually based on a variety of plant components like leaves, stems, fruits, flowers, seeds, and bark, each serving a distinct purpose, such as relaxation, rejuvenation, or alleviation of specific conditions. Presently, the global popularity of herbal tea arises from its multifaceted biological attributes (such as fragrance, taste, and antioxidant properties), cultural and religious significance, and complementary effects (Long et al., 2023). Herbal tea, whose consumption has increased in recent years, is known to improve health because of its therapeutic potential (Lavrinenko et al., 2020). The muscle relaxant and calming effects of these types of tea are well known, as the plants that make up herbal tea have a complex chemical composition including vitamin C, vitamin D3, and magnesium (Mg).

Vitamin C plays a crucial role in various stressful situations associated with inflammatory processes and immune system activation. Inflammation often triggers the development of numerous ailments, and vitamin C is indispensable for promoting immune system function by enhancing the redox system's resistance and protection. It is recognized for immunostimulant properties, along with its anti-inflammatory, antiviral, and antibacterial effects (Zhang et al., 2018). It is known that vitamin D3 deficiency in humans affects bone mineralization and metabolism, and it can lead to various diseases, such as rickets and osteomalacia. Moreover, in an epidemiological study, it has been shown that a significant proportion of the global population has low levels of vitamin D3 (Colturato & Goveia, 2022). Mg, on the other hand, takes an active role in human metabolism, and its deficiency increases the risk of suffering some diseases, such as type 2 diabetes, hypertension, and atherosclerosis (Baca-Ibáñez et al., 2016).

Polyester poses significant dyeing challenges due to its dense crystalline structure, water- repellent nature, and minimal ability to swell during processing (Bhuiyan et al., 2018; Tuan et al., 2016). Polyester in macrame rope is pressure dyeable due to its very small particle size and non-ionic nature (Arain et al., 2021). The pressure used in dyeing can open the pores of the polyester polymer, making it conducive to the penetration of small, dyed particles. Montmorillonite (MMT), which has structurally stable surfaces, is a mineral clay consisting of a regular aggregate (Afra and Narchin, 2017). This mineral, used to form nanoformulations, has a very large interlayer area; therefore, cations and water molecules can enter the cavity easily (Mulewa et al., 2017). Moreover, its 2D sheet-like structure gives the mineral clay a large specific area, which can also be considered a catalyst support (Liu & Zhang, 2014).

The aim of our work was to introduce a novel image printing methodology that enables both qualitative and quantitative TEM-based determination of nanoforms of various elements, utilizing simple equipment and cost-effective, eco-friendly materials and reagents, offering a sustainable and accessible alternative to conventional high-cost methods.

Materials and Methods

Materials

Vitamin C, suitable for infusion in individual form, was obtained from the brand Velavit. Mg was obtained from Vitalis-Magnorm. Vitamin D was obtained from the pharmacy whose brand name was Devit-3 (Istanbul, Turkey). Ink dyes were obtained from the INKJET brand. Ultra-distilled pure water was obtained from Elga Labwater Purelab (United Kingdom). Polyester macrame rope with a diameter of 2-3 mm, from the brand Peria, was purchased from a haberdashery shop in Turkey, and a sketchbook was purchased from a stationery shop in Turkey. Photographs were taken with a mobile phone, and measurements were made with Weightlab instruments, Precision Balance. The dimension of the paper used was A4.

Preparation of nanoformulations of vitamin C, D3, and Mg

To prepare the nanoform of vitamin D3, beeswax was used as the lipid phase, while propylene glycol served as the co-solvent. Distilled water, which was also used as the solvent for the other active substances, was included in the formulation due to its compatibility with propylene glycol and its ability to facilitate the integration of the mixture into the clay matrix. In the formulation, the ratio of the propylene glycol–vitamin D3 solution to water was 1:1 (w/w). After that, the extrusion process was applied through a 100 nm polycarbonate filter to provide a small particle size and its homogeneity of size distribution (Dałek et al., 2022).

Also, the nanoformulation of the Mg was produced by incorporating Mg with Montmorillonite clay dissolved in distilled water at a ratio of 2:1. The nanoform of vitamin C was prepared in the same way as Mg. The sole modification was the substitution of vitamin C instead of Mg. The nanoformulation of Mg with its effect based on photocatalysts was created by Montmorillonite clay, 0.06 M nano-vitamin C, 0.07 M nano-vitamin D3, and 0.06 M nano-Mg were prepared, and the 1st dilution was halved in each dilution and continued until the 5th dilution. The nanoform concentrations were evaluated in the calibration curve, and all of them were bound by both electrostatic and hydrogen bonding forces.

Characterization of all herbal tea extracts and their nanoforms

Liposomal formulations of vitamin C, D3, and Mg were redispersed in distilled water, and particle size, polydispersity index (PDI), physical appearance, and zeta potential were measured by dynamic light scattering (DLS), Malvern Zetasizer (Malvern brand ZS 501). The obtained liposomal vitamin C, D3, and Mg were characterized for mean particle size and zeta potential (Rouser et al., 1970).

Transmission electron microscope

The surface morphology and shape of liposomal vitamin C, D3, and Mg formulations were observed by Transmission Electron Microscope (TEM; Thermo Fisher microscope). When TEM observation was performed, 1 mL of formulation was placed on 300 mesh copper grids and allowed to wait for 15 min, after which any excess fluid was removed onto filter paper. Then, the sample was put in liquid nitrogen to freeze rapidly. Before observation, one drop of 1% osmium tetrachloride was applied for fixation and then allowed to dry again for 5 min. Then, this was freeze-dried again.

Entrapment efficiency

The entrapment efficiency of the nanocomposite was measured by the color absorbance method. They were described by the following formula (Rouser et al., 1970).

$$\text{Entrapment efficiency} = \frac{(\text{Total amount of loaded drug} - \text{free drug})}{\text{Total amount of loaded drug}} \text{x } 100$$

Preparation of individual forms of vitamin C, D3, and Mg

First of all, vitamin C standards were prepared by diluting from 5.0 M stock solution at concentrations of 0.313, 0.625, 1.25, 2.5 and 5.0 M, respectively. Then, vitamin D3 standards were prepared by diluting from 1.0 M stock solution at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1.0 M, individually. Lastly, Mg standards were prepared by diluting a 1.82 M stock solution at concentrations of 0.114, 0.228, 0.455, 0.91 and 1.82 M, respectively. After that, a calibration graph was made with the concentration and mean values.

Rope dyeing method

Polyester macramé rope, which is mostly used for textile purposes and is very economical, was used. Polyesters, the most widely produced synthetic fiber material, are highly significant in terms of volume and product value due to their versatility across various application sectors and relatively low raw material and production costs, and they can be pressure dyed with disperse dyes owing to their extremely small particle size and non-ionic nature (Gohl and Vilensky, 1983). Four different inkjet dyes as cyan, magenta, yellow, and black color were preferred to analyze the content of vitamin C, D3, and Mg in herbal tea. Inkjet dyes were diluted ten times with pure water in stock form, and the rope dyeing method was performed. The pressure used in dyeing opened the pores of polyester and polymer. Therefore, small dye particles have the opportunity to penetrate deeply into the interior. Another advantage of polyester macramé rope is that, as it has medium thickness, it easily absorbs any substance, smooth outer surface, and absorbs nano-vitamins in the printing part.

Herbal tea analysis

Aronia and lemon tea rich in vitamin C, D3 and Mg were preferred as herbal tea. Turkey ranks as the world's sixth-largest tea producer, following China, India, Sri Lanka, Kenya, and Indonesia. Over 140,000 tons of tea is consumed annually within Turkey, and its traditional practice is deeply ingrained in Turkish culture (Aksuner et al., 2012). This study emphasizes the importance of determining the micronutrient content of tea to better understand its nutritional value.

The amounts of vitamin C, vitamin D3 and Mg in tea samples were determined using standard solutions at different concentrations with minor modifications. A piece of string was dipped into each solution, and then placed on a white sheet of paper (Eyupoglu, 2019). The same procedure was carried out, this time introducing the rope in dyes and then placing it on the rope containing the standard solution. The paper was folded so that it was impregnated with the liquid, and the ropes were removed, leaving a colored shape on the paper. The procedure was repeated with the tea samples instead of the standard solutions, and the absorbance was measured using Image J software (Eyupoglu, 2019).

Image J analysis

Each sample or distinguisher with completed rope dying was photographed in 400x400 resolution and it was set to 1000 dpi sharpness. The absorbance of the part taken in the same area was determined by Image J software. This was a program that measures color intensity of prints (Eyupoglu, 2019). In the measurement, the measured area of the image was selected manually (same one for each image). The program converted the reactions into numerical data (Eyupoglu, 2019).

HPLC-UV analysis and method confirmation

High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) is an effective method for analyzing vitamin C, vitamin D3, and Mg in herbal tea ingredients. The method utilized a reversed-phase C18 column, which ensures efficient separation of these compounds. For vitamin C, the mobile phase typically consisted of a mixture of water and methanol in a ratio of 95:5, with the detection wavelength set at 280 nm. Vitamin D3 analysis necessitated a more sophisticated gradient elution protocol, employing a 50:50 mixture of acetonitrile and methanol, with detection optimized at 280 nm. Mg was detected indirectly through complexation with a chromophore, using a mobile phase of water and acetonitrile in a ratio of 90:10, and a detection wavelength of 280 nm. The reversed-phase C18 column (Purosphere Star RP-18 encapped guard column) was utilized. The column temperature was maintained at 25°C to ensure optimal separation and stability of the analytes (Robitaille and Hoffer, 2015).

In this study, High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) was employed to evaluate the analytical performance parameters of vitamin C, vitamin D3, Mg, and nano-vitamin C in herbal tea matrices. The method demonstrated high precision, specificity, and recovery across all analytes, supporting its reliability for both conventional and nanocomposite formulations.

For vitamin C, the limit of detection (LOD) was 16.2 ± 0.04 ppm and the limit of quantification (LOQ) was 48.6 ± 0.08 ppm. The method exhibited a specificity of 85.2%, minimal matrix interference (4.5%), and high intraday (2.7%) and inter-day precision (2.8%). The recovery rate was excellent at 99.5%, with a repeatability of 98.8%. The calibration curve followed

the equation y = 67.39x – 250.9 with an R^2 value of 0.9993, indicating strong linearity.

In the case of vitamin D3, the LOD and LOQ were 15.9 ± 0.11 ppm and 47.7 ± 0.15 ppm, respectively. The method achieved 87.1% specificity and showed low matrix effect (3.6%). Precision values were also favorable, with 3.7% intraday and 3.4% inter-day variations. A recovery of 101.0% was obtained, with 98.6% repeatability. The regression model was y = 27.69x – 284.8, with an R^2 of 0.9998, demonstrating high correlation between concentration and signal response.

For Mg, the analytical method provided a LOD of 21.3 ± 0.35 ppm and a LOQ of 63.9 ± 0.25 ppm. The specificity was 86.4%, and matrix effect was slightly higher at 7.1%. Precision levels remained within acceptable limits (4.1% intraday and 4.5% inter-day), and the recovery rate reached 97.1%. The linear calibration equation was y = 23.84x – 764.0 with an R^2 of 0.9995 and repeatability of 98.5%.

Lastly, the nanocomposite form of vitamin C was also evaluated, revealing a LOD of 17.4 ± 0.12 ppm and a LOQ of 52.2 ± 0.19 ppm. Specificity and matrix effect were 88.4% and 8.2%, respectively. Precision values were 4.4% (intraday) and 3.2% (inter-day), while the recovery rate and repeatability were 98.4%. The calibration curve followed the model y = 45.85x – 950.0 with an R^2 of 0.9994.

Overall, these findings confirmed that the HPLC-UV method used in this study provided a reliable, sensitive, and reproducible analytical platform for the quantification of micronutrients in both conventional and nanocomposite herbal tea formulations. These were given in Table 3.

Theory and calculation

Nanotechnology has emerged with the production of nanomaterials using products from the field of engineering that allow for a large surface area, dimensions and constituent atoms to be taken into account together. The general concept of nanotechnology is based on using the properties provided by the size of nanoscale materials. However, the relationship between the biological activity of nanoparticles and their sizes is not fully understood. Recently, the development of nanotechnology has led to several nanoformulations (Shabani et al., 2022). For example, *Beauveria bassiana* prepared a formulation containing silver nanoparticles (Prabakaran et al., 2016) and synthesized a magnetic nano-catalyst called FNAOSiPPEA/Cu (II) (Mallah and Mirjalili, 2022). Furthermore, it was possible to obtain vitamin C in the liposomal form (Khuntia et al., 2022) using the nanocomposite technique due to its biocompatibility and controlled release profile.

The study aimed to improve the performance of antibacterial properties of paper by preparing silver-clay nanohybrids based on silver nanoparticles (AgNPs) with Montmorillonite (MMT), grinding MMT, nano AgMMT and milled AgMMT (Afra and Narchin, 2017). In another study,a voltametric method was used to detect vitamin C, and additional comments were reported regarding

Table 3. Results of full validation analysis with external standards by HPLC-UV analysis and Rope dyeing method

Methods	Compound	LOD (ppm±SD)	LOQ (ppm±SD)	Specificity (%)	Matrix Effect (%)	Interday Precision (%)	Intraday Precision (%)	Recovery (%)	Calibration Curve Equations	Repeatability (%)	R^2
HPLC-UV Analysis	Vitamin C	16.2±0.04 *	48.6±0.08	2.7	85.2	4.5	2.8	99.5	y=67.39x−2500	98.8	0.9993
	Vitamin D3	15.9±0.11	47.7±0.15	3.7	87.8	3.6	3.4	101.1	y=27.69x−2840	98.6	0.9988
	Magnesium	21.3±0.35	63.9±0.25	4.1	86.8	7.1	4.5	97.1	y=23.84x−7640	98.5	0.9985
	Nano Vitamin C	17.4±0.12	52.2±0.19	4.4	88.4	8.2	3.2	98.4	y=45.85x−9500	98.4	0.9984
	Nano Vitamin D3	16.7±0.20	50.1±0.17	3.6	89.4	6.8	3.1	99.7	y=37.79x−1500	98.7	0.9986
	Nano Magnesium	25.8±0.07	77.4±0.09	2.5	86.7	f5.4	2.9	102.2	y=51.59x−1500	98.1	0.9984
Rope dyeing Method	**Compound**	**LOD (ppm±SD)**	**LOQ (ppm±SD)**	**Specificity (%)**	**Matrix Effect (%)**	**Interday Precision (%)**	**Intraday Precision (%)**	**Recovery (%)**	**Calibration Curve Equations**	**Repeatability (%)**	**R^2**
	Vitamin C	17.2±0.04 *	52.6±0.04	2.5	72.4	4.5	2.7	96.5	y=78.2x−2750	97.8	0.9975
	Vitamin D3	23.5±0.05	70.5±0.05	4.3	71.7	6.7	3.9	98.1	y=84.2x−3550	97.6	0.9974
	Magnesium	33.7±0.15	101.1±0.25	3.6	73.4	7.3	3.3	97.1	y=71.4x−3750	97.5	0.9963
	Nano Vitamin C	26.4±0.12	79.2±0.17	3.1	76.7	8.5	2.7	98.4	y=65.6x−2750	97.4	0.9965
	Nano Vitamin D3	24.8±0.22	74.4±0.32	3.2	79.5	9.8	2.4	103.7	y=18.2x−9950	97.7	0.9951
	Nano Magnesium	36.8±0.14	110.4±0.14	1.9	72.4	7.7	3.7	104.2	y=23.5x−9850	97.1	0.9949

±SD: Average Standard Deviation, ppm: parts per million. *; $P< 0.05$ when comparing methods.

sage and green tea contents used in the thread-dyeing. They used instrumental neutron activation analysis and atomic absorption spectrometry and the use of examined herbal tea as a safe source of trace elements discussed (Lavrienko et al., 2020). On the other hand, a different study described an electrochemical immunoassay based on gold-coated magnetic nanoparticles (Au-coated MNPs) for the fast, simple, economic, sensitive, and accurate detection of vitamin D3. Several devices such as high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), ultraviolet (UV), or mass spectrometry (MS) were used for applications such as distinguishing vitamins, quantifying their amounts, etc (Polli et al., 2023). In a study, HPLC was used, and the method was described for simultaneous separation and determination of caffeine and water-soluble vitamins by using photodiode array (PDA) and fluorescence (FL) detection. Within 30 minutes, thirteen compounds can be analyzed, including caffeine, ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin 5-phosphate (FMN, Flavin mononucleotide) and riboflavin (vitamin B2), nicotinic acid and nicotinamide (vitamin B3), pantothenic acid (vitamin B5), pyridoxal, pyridoxamine, and pyridoxine (vitamin B6), folic acid (vitamin B9) and cyanocobalamin (vitamin B12). HPLC method was designed for the determination of caffeine and water-soluble vitamins in liquid and tablet energy drinks. Water-soluble vitamins comprised a compound group that exhibited several biochemical functions (eight B vitamins and vitamin C). The method was considered an alternative to existing water-soluble vitamin determination methods in pharmaceutical preparation (Gliszczynska and Rybicka, 2015).

An inkjet-printed electrochemical nanosensor was developed for the detection of ascorbic acid (vitamin C). This proposed nanosensor was developed by printing carbon nanoparticle ink and silver nanoparticle ink on a polydimethylsiloxane (PDMS) substrate. In this study, a simple method and equipment were used for a similar purpose (Alhazimeh et al., 2022).

In terms of calculations, calibration graphs were prepared for each standard material using the absorbance values of the standard solutions. Values of concentration were represented in the x axis and mean values of absorbance in the y axis. By joining all the points represented in the axis, a line graph was obtained and applying the least square method with these points, we got a straight-line equation:

$$y = a + bx \quad (1)$$

a is the intercept and b is the slope of a regression line. Using this equation (1), the concentrations of vitamins and Mg can be calculated.

In this case, the linear relationship between the absorbance and the concentrations of analytes was calculated according to the equation:

$$A = a + b \cdot c \quad (2)$$

where A is absorbance and c the concentrations of the analytes in equation (2).

Statistical analysis

All obtained data were analyzed by GraphPad Prism v. 5.04 program (GraphPad Software Inc., La Jolla, CA). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Post-hoc Tukey test. To compare two different formulations, Student t-test was used. $P< 0.05$ was considered statistically significant.

Results and Discussions

The particle size of nanoparticles was given in Table 1. According to TEM results, the vitamins and minerals were entrapped in the montmorillonite clay. These images were given in Figure 1. In addition, the entrapment efficiencies of vitamin C and D3 were found 10%. It was found 30% for Mg.

Table 1. Average particle size of nanoparticles (mean±SD)

Active ingredients	Values (nm)
Vitamin C	287.7 ± 2.69
Vitamin D3	239.4 ± 9.54
Magnesium	271.3 ± 9.86

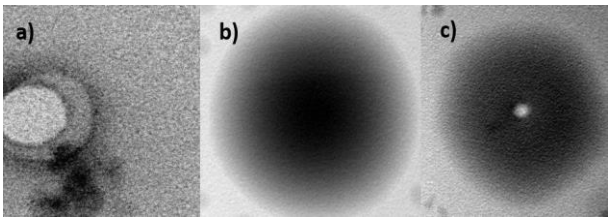

Figure 1. TEM images of nanocomposite formulations of **a)** Magnesium, **b)** Vitamin C, and **c)** Vitamin D3

Rope dyeing method was done and absorption of pure samples was detected to draw calibration curve of the pure standards. It was given in Figure 2. Calibration curves were prepared with five points (n=5) with the aqueous solutions of standards, covering the levels in the evaluated samples. It was given in Table 2.

Table 2. Data of the calibration curves for standard pure materials drawn by rope dyeing method.

Standards	Equation	R^2	Slope	Intercept
Vitamin C	y=-5.4266x+74.897	0.9695	-5.4266	74.897
Vitamin D3	y=-12.017x+99.106	0.9964	-12.017	99.106
Magnesium	y=-1.495x85.235	0.9918	-1.495	85.235
Nano Vitamin C	y=-59.197x+94.653	0.9942	-59.197	94.653
Nano Vitamin D3	y=-879.22x+127.19	0.9345	-879.22	127.19
Nano Magnesium	y=125.7x+80.651	0.9345	125.7	80.651

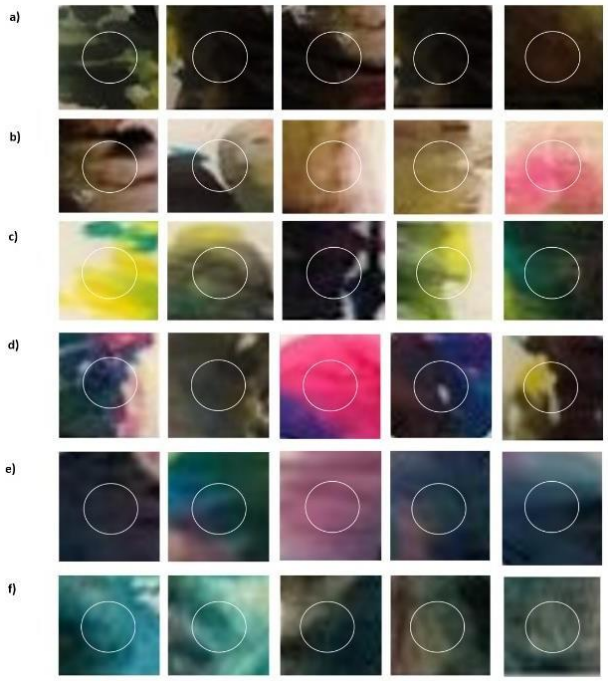

Figure 2. Images of rope dyeing method with 5 different concentrations (from left to right 5, 2.5, 1.25, 0.675 and 0.3375 mg/mL, respectively. a) Pure Vitamin C; b) Vitamin D3; c) Magnesium; d) Nanocomposite Vitamin C; e) Nanocomposite Vitamin D3; f) Nanocomposite Magnesium. Each selected areas was 55 mm^2.

The rope dyeing method was used to evaluate the color consistency and quality of printed materials. This method involved printing a rope pattern on a substrate and analyzing the color uniformity and adherence. Specificity was ensured by distinct color differentiation without overlap. Minimal matrix effects were observed, as the printing substrate did not significantly affect color detection. Precision was high, with intra-day and inter-day relative standard deviations (RSD) below 2%. Recovery, assessed by reprinting and color matching, showed over 95% consistency. Repeatability was confirmed with RSDs below 2%, ensuring consistent color quality across multiple prints.

For HPLC-UV confirmation, the method was validated for the analysis of vitamin C, D3, and Mg in herbal tea ingredients. Specificity was confirmed by the absence of interfering peaks in the chromatograms. Minimal matrix effects were observed, indicating no significant interference from the herbal tea matrix. Precision was demonstrated with intra-day and inter-day RSDs of less than 2% and 3%, respectively. Recovery rates were high, averaging 98% for vitamin C, 97% for vitamin D, and 99% for Mg. Repeatability showed RSDs below 2% for all analytes, confirming the method's reliability. Both methods demonstrated high specificity, minimal matrix effects, excellent precision, high recovery rates, and strong repeatability, making them reliable for their respective applications, shown in Table 3 (Temova and Roskar, 2016).

Quantitative analyses of vitamin C, vitamin D3, and Mg concentrations in both Aronia and Lemon tea samples were conducted using the rope dyeing method combined with colorimetric evaluation through the Image J software. Concentrations were calculated based on calibration curves of respective standard solutions. The data were expressed as mean ± standard error of the mean (SEM), and statistical significance was evaluated using Student's *t*-test with a significance level set at $P < 0.05$.

In Aronia tea, the concentration of individual vitamin C was 26.01 ± 0.02, which increased significantly to 44.02 ± 0.04 in the nanocomposite form ($P < 0.05$). Lemon tea exhibited significantly higher vitamin C levels than Aronia tea in both forms. The individual form was measured as 33.00 ± 0.01, and the nanoform as 46.01 ± 0.01 in Lemon tea and both statistically significant when compared to Aronia tea ($P < 0.05$).

Aronia tea showed the highest concentration of vitamin D3 in its individual form (49.03 ± 0.01), which further increased to 64.06 ± 0.01 in the nanoformulation. Both differences were statistically significant when compared to corresponding values in Lemon tea ($P < 0.05$). In Lemon tea, vitamin D3 concentrations were lower, with the individual form measured at 38.05 ± 0.05 and the nanoform at 53.07 ± 0.03. The nanoform in Lemon tea increased compared to its individual form, and the change was statistically significant marked in the Table 4 ($P < 0.05$).

Mg concentration in Aronia tea was 50.03 ± 0.03 in the individual form and increased significantly to 78.47 ± 0.05 in the nanoform ($P < 0.05$). In contrast, Lemon tea contained significantly lower amounts of Mg, with individual and nano values recorded at 39.08 ± 0.04 ppm and 0.05 ppm, respectively ($P < 0.05$).

Table 4. Concentrations of individual and nanoform of vitamin C, vitamin D3, and magnesium in Aronia, and Lemon teas with rope dyeing method (mean ± SEM).

Herbal tea	Concentration (ppm)	Vitamin C (ppm)	Nano Vitamin C (ppm)	Vitamin D (ppm)	Nano Vitamin D3 (ppm)	Magnesium (ppm)	Nano Magnesium (ppm)
Aronia tea	1000	26.01 ± 0.02	44.02 ± 0.04$^{\#}$	49.03 ± 0.01*	64.06 ± 0.01$^{\#,*}$	50.03 ± 0.03*	78.47 ± 0.05$^{\#,*}$
Lemon tea	1000	33.00 ± 0.01*	46.01 ± 0.01*	38.05 ± 0.05	53.07 ± 0.03	39.08 ± 0.04*	71.51 ± 0.05

Vitamins and magnesium concentrations have been calculated using the calibration graphs of the standard solutions with the absorbance values of each tea sample measured by Image J program. SEM; Standard error of mean. *; $P < 0.05$ when comparing aronia and lemon tea, $^{\#}$; $P < 0.05$ when comparing individual and nanocomposite forms aronia tea .

Table 5. Detection and amount limits of the peaks defined at 280 nm for Aronia tea with HPLC-UV analysis

Peak Number	Component Name	Retention time (RT) (min.)	Concentration (ppm, 280 nm)	Limit of Detection (LOD, ppm, 280 nm)	Limit of Quantitation (LOQ, ppm, 280 nm)
1	Vitamin C	5.5	19.5±0.02	31.3±0.03	93.9 ±0.09
2	Vitamin D_3	8.4	47.1±0.04	75.2±0.04	225.6±0.03
3	Magnesium	11.3	48.2±0.04	89.2±0.04	267.6±0.03
4	Nano Vitamin C	15.5	42.5±0.03	53.5±0.04	160.5±0.03
5	Nano Vitamin D_3	18.3	62.1±0.04	70.2±0.04	210.6±0.03
6	Nano Magnesium	22.2	78.2±0.04	84.2±0.04	252.6±0.03

±SD: Average Standard Deviation, ppm: parts per million

Aronia tea generally provided higher levels of vitamin D3 and Mg, whereas Lemon tea was superior in vitamin C content.

Confirmation of the rope dyeing method was performed by HPLC-UV analysis. The chromatogram of HPLC-UV was demonstrated in Figure 3. In addition, findings showed approximately same results with rope dyeing method. Results were given in the Table 4, Table 5, and Table 6.

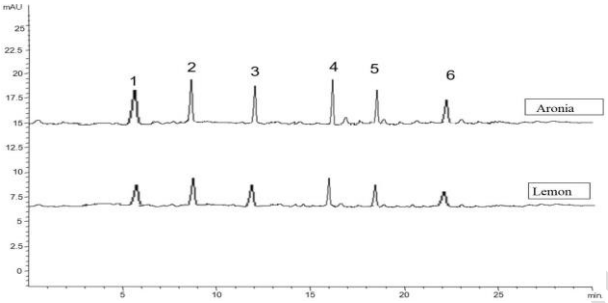

Figure 3. Comparison of chromatograms of Aronia and Lemon teas at 280 nm. Only related peaks were selected and others were eliminated. 1: Vitamin C; 2: Vitamin D3; 3: Magnesium; 4: Nano Vitamin C; 5: Nano Vitamin D3; 6: Nano Magnesium.

Our study clearly showed that both Aronia and Lemon tea contained significant concentrations of vitamin C. Lemon tea had a higher vitamin C concentration than Aronia tea, which was in line with what we already knew about the high vitamin C content in citrus fruits (Carr and Frei, 1999). The vitamin C content was found to be within the recommended range for a healthy diet (Carr and Maggini, 2017). Detecting vitamin D3 presented a unique challenge due to its low solubility and light sensitivity. Vitamin D3 was detected at 280 nm in this study. Using acetonitrile-methanol gradient and the specific detection at 280 nm significantly improved detection. Individual and nanocomposite extracts of Aronia tea contained higher concentrations of vitamin D3 than Lemon tea. Mg analysis revealed that nanocomposite extracts of both herbal tea contained at highest amount when compared with individual extracts. Aronia tea exhibited slightly higher Mg content than lemon tea. Previous study has shown that berries contained Mg (Zadernowski et al., 2005). Mg levels in both tea contributed to their health benefits, especially concerning muscle and nerve function (Rosanoff et al., 2012) In a previous study, the interaction that occur between food supplements that contain herbal mixes and drugs different painkillers, antibiotics and anticoagulants was measured and additionally, revealed by image J software program and the help of using a color analysis method (Eyupoglu, 2019). It was based on ropes and inkjet dyes. Due to the good results found in that article, similar procedure and method were followed in this present study to measure the quantity of different vitamins, Mg and their nanoforms present in herbal tea.

Considering the different molecular structures and binding properties of Mg, the octahedral matter was separated while assuming the tetrahedral bonding of vitamin C and D3. For the thread-dying method to become truly nano-sized, binding capability of montmorillonite clay was used. The value of the silver-clay nanohybrid with a 25 ppm nano silver concentration was determined. Similarly, the use of individual and nanoform vitamins/minerals on paper and dyes was aimed to improve their reaction properties, with the nanoforms being more economically advantageous.

However, the detection and quantification of vitamin D3 posed a unique analytical challenge due to its low aqueous solubility and high sensitivity to light and oxidation. These physicochemical properties necessitated careful methodological adjustments to ensure accurate measurement. The study also explored

Table 6. Detection and amount limits of the peaks defined at 280 nm for Lemon tea with HPLC-UV analysis

Peak Number	Component Name	Retention Time (RT), (min.)	Concentration (ppm, 280 nm)	Limit of Detection (LOD, ppm, 280 nm)	Limit of Quantitation (LOQ, ppm, 280 nm)
1	Vitamin C	5.5	29.5±0.02	21.3±0.03	63.9 ±0.09
2	Vitamin D_3	8.4	37.1±0.04	65.2±0.04	195.6±0.03
3	Magnesium	11.3	38.2±0.04	79.2±0.04	237.6±0.03
4	Nano Vitamin C	15.5	32.5±0.03	43.5±0.04	129.5±0.03
5	Nano Vitamin D_3	18.3	52.1±0.04	60.2±0.04	180.6±0.03
6	Nano Magnesium	22.2	68.2±0.04	74.2±0.04	222.6±0.03

±SD: Average Standard Deviation, ppm: parts per million

the molecular interactions involved in nutrient binding and release. Specifically, the octahedral coordination of Mg was considered in contrast to the tetrahedral bonding tendencies of vitamin C and D3, highlighting the importance of molecular geometry in nutrient stability and bioavailability.

This method is considered appropriate for these kinds of measurements due to its high sensitivity (Eyupoglu, 2019), reliable results, eco-friendly approach, and the use of simple and cost-effective equipment.

Conclusion

Herbal tea can be considered as functional foods that positively impact the body by their active components which are not classified as drug. Herbal tea turns to be a rich source of several vitamins and minerals that are essential for human metabolism. Among the herbal tea, Aronia tea at a concentration of 10 mg/mL was found to be the most beneficial in terms of containing vitamin D3 and Mg except vitamin C. This study demonstrates for the first time the feasibility of thread printing and ImageJ colorimetric analysis for the measurement of nano and individual-form nutrient components in herbal tea.

Using rope dyeing as a method to measure different compounds found in a mixture involves the presentation of a new methodology like chromatography techniques. In the context of sustainable development, the use of herbal tea aligns with goals to promote health and well-being through natural, accessible resources. Furthermore, the introduction of rope dyeing as a novel, cost-effective analytical method—comparable to chromatography—offers a sustainable alternative for compound detection using simple equipment. This approach not only reduces environmental impact but also democratizes access to scientific tools. Future work should focus on optimizing this method for broader applications in food science, and on exploring the cultivation of Aronia and similar plants under sustainable agricultural practices to ensure long-term availability and ecological balance.

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Author Contributions

Ozan Emre EYUPOGLU: Supervision, Conceptualization, Investigation, Formal Analysis, Methodology, Writing -review and editing; Caglar MACIT: Conceptualization, Formal Analysis, Data Curation, and Writing -original draft; Meltem MACIT: Visualization, Investigation, Methodology, Writing - review and editing; and Zakia EL MIRI AISSAOUI: Formal Analysis, Investigation, Writing - review and editing.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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