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Development of a UHPLC-DAD-qTOFMS method for the simultaneous quantification of five water-soluble B-vitamins in *Moringa oleifera*

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ABSTRACT

Moringa oleifera is identified as a good source of water-soluble vitamins. In this study, a method was developed based on simple sample extraction followed by UHPLC-DAD-qTOFMS analysis. *Moringa oleifera* powder samples were extracted by hydrolysis with sodium hydroxide buffered at pH 5.5 with phosphate buffer for 24 hours. Filtered extracts were separated within 15 mins on a Synergi C_{18}, 150 × 4.6, 4 μm column with a gradient program consisting of 0.01% triflouroacetic acid and acetonitrile as solvents. The photodiode array detector was set at wavelengths 254 nm and 300 nm with further confirmation of compounds on mass spectrometry. Instrumental limit of detection (LOD) and limit of quantification (LOQ) for the five water soluble vitamins ranged from 0.20–0.89 μg/mL and 0.60–2.71 μg/mL, respectively with recovery above 97% for all compounds. The developed method was tested to real *Moringa oleifera* samples grown in a greenhouse in clay soil amended with various degree of compost. Quantified water-soluble vitamins concentration ranged from nd to 40.6 ± 1.45 mg/100g depending on the type of water-soluble vitamin and soil amendment. The developed method can easily be applied to check the quality of processed Moringa *oleifera* biomass.

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1. Introduction

Vitamins are essential micronutrients, organic in nature taken in smaller quantities for the proper physiological function of the body, they are key components of a balanced diet (Kennedy 2016). Most of these essential nutrients cannot be naturally synthesized by human beings and are supplied to the body through diet to meet daily requirements (Ofoedu et al. 2021). They are responsible for the optimum function of chemical and physiological processes in the body, and thus maintaining good health (Clifford and Curely, 2020; Abibu et al. 2019, Seal and Chaudhuri, 2017). Vitamins are classified into two major groups, namely fat-soluble and water-soluble vitamins (WSV). As the name suggests, fat-soluble vitamins are soluble in fat, they are mainly absorbed in the fatty filled intestines (Albahrani and Greaves, 2016). Fat soluble vitamins consists of vitamins such as A, D, E, K and some carotenoids with elements of Vitamin A**.** On the contrary, water soluble vitamins are soluble in water and are composed of Vitamin C and eight B complex vitamins (B1 to B6, B9, B12) (Clifford and Curely, 2020). Vitamin B complex functions as a cofactor in many biochemical reactions in the body, each component has a specific role in the growth and development of a healthy body (Lykstad and Sharma, 2019). As a collective, B vitamins are generally involved in the improvement of the body's vitality by being involved in the DNA replication and repair pathways and in energy metabolism pathways (Tamanna et al. 2024).

Vitamin B1 (Thiamine) is a cofactor for enzymes responsible for carbohydrate and amino acid metabolism (Hrubša et al. 2022). Vitamin B1 deficiency may lead to disorders of the nervous system (Fitzpatrick and Chapman, 2020). Vitamin B2 (Riboflavin) on the other hand is responsible for the production of red blood cells and assist in energy release from proteins (Mahabadi et al., 2023). In addition, Vitamin B2 in conjunction with Vitamin B3 (Niacin) and Zinc enables the function of Vitamin B6 (Pyridoxine) which in turn acts as a coenzyme for numerous reactions in the body such as the production of neurotransmitters, metabolism of fats, carbohydrates, and proteins (Stach et al. 2021). Vitamin B9 (Folate) is involved in the synthesis of DNA (Knyazev et al. 2016) and its deficiency leads to various cancers and neurological diseases such as the fetus neural tube defects (Ebara, 2017).

Recommended daily intakes of water soluble vitamins might not be a true reflection of what the body benefits from as bioavailability of these vitamins is affected by a number of factors such as mode of adsorption and gastrointestinal conditions and bioactivity (Yaman et

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al. 2021). Furthermore, the particle size and processing of plant material has a significant impact on the bioavailability of water soluble vitamins not to mention the storage and cooking (Coelho et al. 2022).

The human body cannot produce water soluble vitamins apart from niacin which can be synthesized by the liver from the amino acid tryptophan (Said 2011). In addition, the body is unable to store water soluble vitamins for more than a day due to their nature, they are also easily destroyed during food preparation (Khaneghah et al. 2019). They therefore need to be taken in the form of a diet or supplement to meet the daily body requirements (Kennedy 2016).

Moringa oleifera is a highly nutritive herbal tree native to India which has been naturalized and extensively cultivated across Asia and Africa (Sujatha and Patel, 2017). Among many of its beneficial properties it is identified as a source of water-soluble vitamins (Witt, 2013). The leaves are particularly recommended for their high vitamin content, notably vitamin C (Grosshagauer et al. 2021). Water soluble vitamins were reported to be in impressive amounts in *M. oleifera* leaves (Sultana 2020).

Considering the importance of consuming medicinal plants such as *M. oleifera* to provide a daily supply of vitamins and the drive to implement quality control of nutritional plant products, accurate analysis of vitamins is of paramount importance. Vitamin concentration in plants is largely affected by variation in genetic, environmental, and processing conditions including soil amendments in the form of artificial or organic fertilizers (Lester and Crosby, 2002; Lester 2006; Kolton et al. 2022). Since there are diverse factors that can influence the concentration of water-soluble vitamins in the final plant-based product, several analytical methods such as CE-DAD, electrokinetic chromatography, HPLC-UV, RP- HPLC and LC-MS/MS have been reported (Mazina and Gorbatsova, 2010; Ni et al. 2014; Abibu et al., 2019; Datta et al. 2019; Patle et al. 2022; Seal and Chaudhuri, 2017; Yilmaz et al. 2023).

The quantification of water soluble vitamins in plants is essential in order to have a precise diagnosis of their vitamin status and potential contribution in supplying the vitamin daily requirements in humans (Liu et al. 2022). Moreover, the need to validate the use of plants as nutraceuticals facilitates the development of advanced detection and quantification techniques (Liu et al. 2022). A number of these analytical methods have been reported using either HPLC-DAD/HPLC-UV (Patle et al. 2022; Abibu et al. 2019; Datta et al. 2019) or HPLC-MS (Yilmaz et al. 2023). In addition to that there have been reports on the use of electrokinetic chromatography with UV detection (Ni et al. 2014) or using capillary electrophoresis with DAD detection (Mazina and Gorbatsova, 2010) for the quantification of vitamins. However very there is limited research on the use of LC-DAD-MS on the simultaneous quantification of water-soluble vitamins in leaf biomass. Research conducted by Santos et al. (2012) revealed the use of both LC-MS or LC-DAD, these instruments were however not connected to each other and were thus operated independently. Even less analytical methods are reported for the quantification of water-soluble vitamins in *M. oleifera* (Mbah et al. 2012; Witt 2013; El Sohaimy et al. 2015; Ali et al. 2017; Abibu et al. 2021; González-Burgos et al. 2021). Most of the recorded studies focus on the quantification of vitamin C (Thippeswamy et al. 2020; Ahmed et al. 2016). There is only one publication by Abibu et al. (2021) which details the simultaneous quantification of water-soluble vitamins in *M. oleifera* using HPLC coupled with ultraviolet (UV) detector. While the HPLC-UV is the most commonly used instrument in the analysis of phytochemicals because it is readily available in many laboratories. However, in comparison with LC-MS, the LC-UV has its limitations such as limited sensitivity and requires proper sample preparation such as solid phase extraction (SPE) to minimize the interference of the sample matrix (Abibu et al. 2021).

The current study therefore reports the development of an UHPLC-DAD-MS method for the simultaneous quantification of five water soluble vitamins in *M. oleifera*. The benefits of combining two detection systems are that no rigorous sample cleanup is required.

2. Materials and methods

2.1. Chemicals

The analytical grade vitamin reference standards of B vitamins thiamine (B1; 99%), riboflavin (B2; 99%), nicotinic (B3; 99.5%), pyridoxine hydrochloride (B6; 98%) and folic acid (B9; 97%) (Fig. 1) were purchased from Sigma- Aldrich. HPLC grade and triflouroacetic acid (TFA) were also purchased from Sigma-Aldrich.

2.2. Instrument

The LC system used for the separation and analysis of compounds was the Thermo Scientifc Dionex Ultimate 3000 from ThemoFisher Scientifc (Waltham, MA, USA). The LC was coupled to the diode array detector (DAD) and the compact quadrupole time-of-fight mass spectrometer system (QTOF) from Bruker Daltonics Inc. (Billerica, MA, USA) equipped with Electrospray Ionization (ESI) operating in positive mode. Detection was carried out in a mass range of 50−1300 *m/z* and using a capillary voltage of +4500 V, a dry gas temperature of 220 °C, and a dry gas flow of 9.0 L/min, a nebulizer pressure of 1.8 bar. The accuracy of the mass data for the molecular ions was controlled by the Data Analysis 4.0 software (Bruker Daltonik), which provided a list of possible elemental formulas by using the SmartFormulaTM editor.

The UV detector was used to detect the vitamins and the mass spectrometer was used to further identify the compounds. To attain the mass accuracy necessary to identify the compounds, external instrument calibration was used. The calibrant used was sodium formate clusters consisting of 5 mM sodium hydroxide and water: 2-propanol 1:1 (v/v) with 0.2% formic acid. This calibrant was injected at the beginning of the run using a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL, USA) directly connected to the ion source, equipped with a Hamilton syringe (Reno, NV, USA). The B-vitamins were identified by retention time verified further by the mass spectrometry. Bruker Compass Quant Analysis version 4 was used for collection and processing of quantitative data. Chromatographic separation of the B vitamins was achieved on a *Synergi* C_{18}, 150 × 4.6, 4 μm particle size was from Phenomenex (California, USA). The optimised mobile phase was used of initially, solvent A at 99% with solvent B at 1%. Solvent B then increased to 5, 15 and 45 % in 5, 7 15 minutes respectively. Solvent A was 0.01% trifluoracetic acid and acetonitrile as solvent B with a flow rate of 0.5 mL/min.

2.3. Preparation of standards

The preparation of standards was according to the method by Seal *et al.*, 2018 with few modifications. Briefly, the stock standard solutions of Vitamin B1, B3 and B6 were prepared by dissolving 25 mg of each standard in 1 ml 0.1 M hydrochloric acid (HCL) in 25 ml standard volumetric flask and topped up to mark with double distilled water. Whilst for Vitamin B9 and B2, 25 mg of each standard were dissolved in 1 ml 0.1 M sodium hydroxide (NaOH) in 25 ml standard volumetric flask and made up to mark with double distilled water. The standard solutions were stored in amber glass bottles at 4 °C. The working standards were prepared from the stock standard solutions.

2.4. Linearity

To ascertain the linearity, the stock solution of the standard (1 mg/ml) was diluted to six different concentrations (10, 20, 30, 40, 50 and 60 μg/ml) which were injected and analyzed by the HPLC in triplicate. A calibration curve was then obtained by plotting peak area

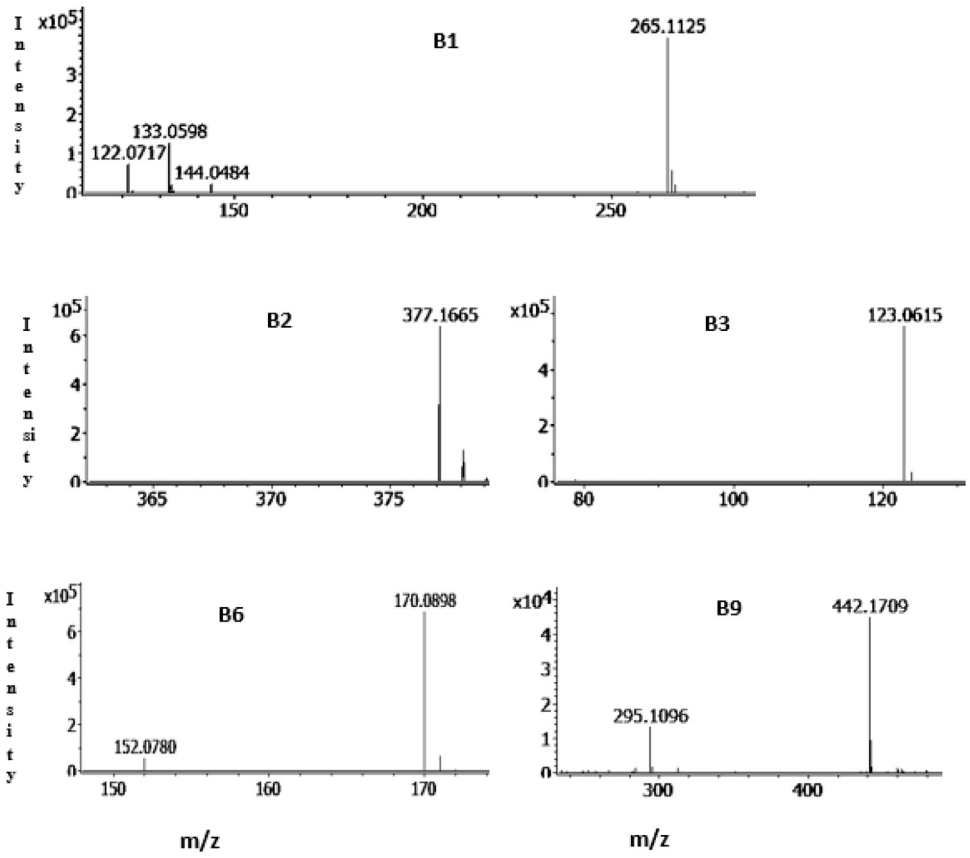

Fig. 1. MS/MS spectra of water-soluble vitamins, indicating the precusor ion and the fragments.

versus concentration for each vitamin standard. The coefficients of determination were used to ascertain linearity, where $R^2 > 0.99$ indicates the degree of linearity.

2.5. Accuracy

The matrix matching method was used to determine accuracy. Two samples of *Moringa oleifera* leaf powder were prepared simultaneously. One sample was spiked with a known standard concentration derived from the standard mixture before sample preparation whilst the other sample was spiked with the known standard concentration after sample preparation. This method considers the matrix effect of the sample.

Recovery was calculated using the following formula by Abibu *et al.*, (2019)

$$\text{Recovery} = \frac{\textit{Concentration in PreSP}}{\textit{Concentration in PoSP}} \times 100$$

Where PreSP and PoSP refer to the sample spiked before sample preparation and after sample preparation respectively.

2.6. Limit of detection (LOD) and quantification (LOQ)

The instrument limit of detection for each vitamin and limit of quantification were calculated using the following formula by Seal and Chaudhuri (2017),

$$\text{LOD} = 3.3 * \frac{\sigma Intercept}{S}$$

$$\text{LOD} = 10 * \frac{\sigma Intercept}{S}$$

Where (σ) = standard deviation of response (peak area) and S = slope of the calibration curve. The % relative standard deviation (RSD) of the retention time and the peak area was used to define precision. Intra and inter day % RSD was calculated for retention time and peak area using 5 replications, the following equation by Abibu *et al.*, (2019) was used: -

$$\%\text{RSD} = \frac{\textit{Standard deviation}}{\textit{Mean}} \times 100$$

2.7. Green house growing of Moringa *oleifera*

2.7.1. Plant and soil material

Moringa oleifera seeds and the clay soil used were obtained from the community *M. oleifera* farm in Hammanskraal, North of Pretoria, South Africa. Topsoil (0–15 cm) was collected using a spade, air-dried, homogenised and sieved to remove gravel, debris and clogs.

2.7.2. Transplanting

The seeds were soaked in water for 24 hours and thereafter planted into germination trays containing potting soil and watered every two days for four weeks. Healthy seedlings were transferred into 20 cm diameter 2 L plastic pots containing clay soil as control and clay soil amended with four different levels (15%, 30%, 45% and 60%) of organic plant-based compost as treatments in four replicates.

2.7.3. Growth

The plants were watered twice a week, plant growth was monitored for six months. The percentages for each compost-soil combination were by volume of the total mixture. The mentioned current percentage compost levels were inspired by the compost levels used by other researchers (Vouillamoz and Milke, 2001; Zheljazkov and Warman, 2004). Futther, compost levels in this study are a norm in

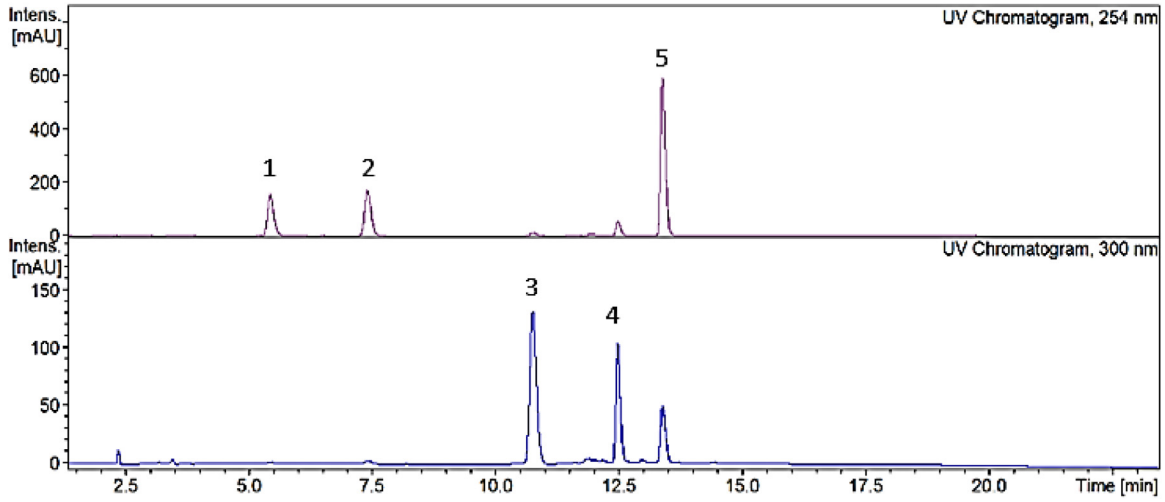

Fig. 2. Chromatogram of the vitamin mixture (1= B3; 2= B1; 3= B6; 4= B9; 5= B2).

greenhouse experiments (Zheljazkov and Warman, 2004). Plants were grown in the greenhouse (under controlled temperature of 25 ° C) at the School of Animal, Plant and Environmental Sciences (APES), University of the Witwatersrand.

2.8. Sample preparation

The method of Seal et al. (2018) was adopted for sample preparation. One g of fnely ground *M. oleifera* leaf powder was soaked in 10 ml water. One ml of 0.1 M NaOH and 10 ml phosphate buffer (1M, pH 5.5) was added to it and the solution was kept in the dark for 24 hours. The solution was first filtered through a Whatman No. 1 filter paper (to remove large particles which can easily clog the micro filters) and the resulting filtrate was taken in a 25 ml volumetric flask and solution was made up to the mark with deionised water. The sample solution was filtered through 0.22 μm membrane filter before injection into LC system.

2.9. Data analysis

Quantification of vitamins was done in triplicates, significant difference between each vitamin was determined by the Kruskall-Wallis test. In cases where normality was assumed, a One-way analysis of variance (ANOVA) was used. Dunn's test was employed at significant level of $P<0.05$. The analysis was done using the R-studio version 4. 1. 2 statistical packages.

3. Results and discussion

3.1. Optimization of chromatographic conditions and recovery of extraction

The optimised gradient elution program was used in the separation of five water soluble vitamins. The solvents used were 0.01% trifluoracetic acid as solvent A and solvent B as acetonitrile. A flow rate of 0.5 mL/min was also maintained throughout the run. The gradient elution program was done by varying solvent A and B as follows: Initially, solvent A was 99% while solvent B was 1%. Solvent B then increased to 5, 15 and 45% in 5, 7 15 minutes respectively. Thereafter, the mobile phase composition returned to the initial composition.

The HPLC chromatogram of the standard mixture of five water soluble vitamins was recorded at wavelengths 254 nm and 300 nm (Fig. 2 a). The MS spectra of each vitamin were also determined in the vitamin standard mixture (2b). The separation of the vitamin's mixture produced good peaks under these wavelengths and were used throughout the study to detect and quantify the vitamins in *M. oleifera* samples. Vitamins B1, B2 and B3 were quantified using the 254 nm wavelength whilst vitamins B6 and B9 were detected by the 300 nm wavelength.

The quality assurance parameters for the developed method are shown in Table 1. The R^2 values recorded were all in the range of $R^2 >$ 0.99 indicating good linearity. The instrumental LODs of the vitamins ranged from 0.2 to 0.89 μg/mL whilst the instrumental LOQs ranged from 0.6 to 2.71 μg/mL for the injection of liquid samples. These values were a bit higher for all the tested vitamins (except for Vitamin B6) than those reported by Seal and Chaudhuri (2017) who used the same solvents for water soluble vitamin quantification. This variation could be due to the instrument used and the vitamin mixture composition. Percentage recovery for all vitamins was above 97.8%. The developed analytical method is much improved when compared to the one by Abibu et al., (2021) on the separation of water-soluble vitamins from M. *oleifera* biomass. In this case, the first peak is well separated from solvent front which makes it easier to identify early eluting vitamins in real samples. Further, in real samples, even though sample preparation does not remove all the matrix, the peaks are easily identified even with PAD detector alone. To our knowledge the current study is the first study to report the

Table 1
Retention time and parameters of calibration curve, instrumental limit of detection, limit of quantification, and percentage recovery study of standard water-soluble vitamins.

Analyte	Retention time(tR)	Regression equation	Correlation coefficient (R^2)	RSD(%) of the retention time	RSD(%) of the peak area	LOD (μg/mL)	LOQ (μg/mL)	Percentage of recovery (%)
B1	5.03	y=270.44x-24.519	0.998	1.80	4.23	0.89	2.71	98.03
B3	6.69	y=174.93x+26.899	0.999	0.90	4.06	0.47	1.43	98.86
B9	12.18	y=97.694x+12.226	0.999	0.16	2.76	0.56	1.71	98.72
B2	13.24	y=218.28x+28.362	0.999	0.16	5.84	0.55	1.70	99.58
B6	7.31	y=162.64x+139.28	0.999	0.55	14.00	0.20	0.60	97.80

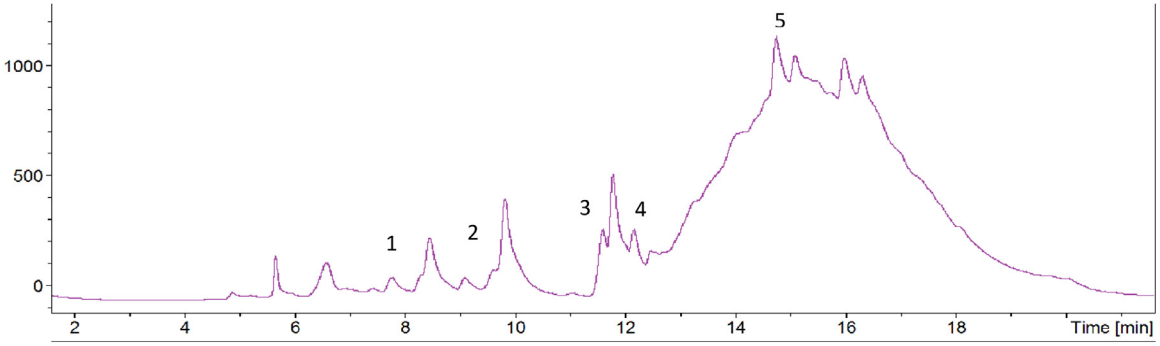

Fig. 3. Chromatogram of M. oleifera leaf powder extract sample on HPLC-DAD (1= B1; = B3; 3= B6; 4= B9; 5= B2).

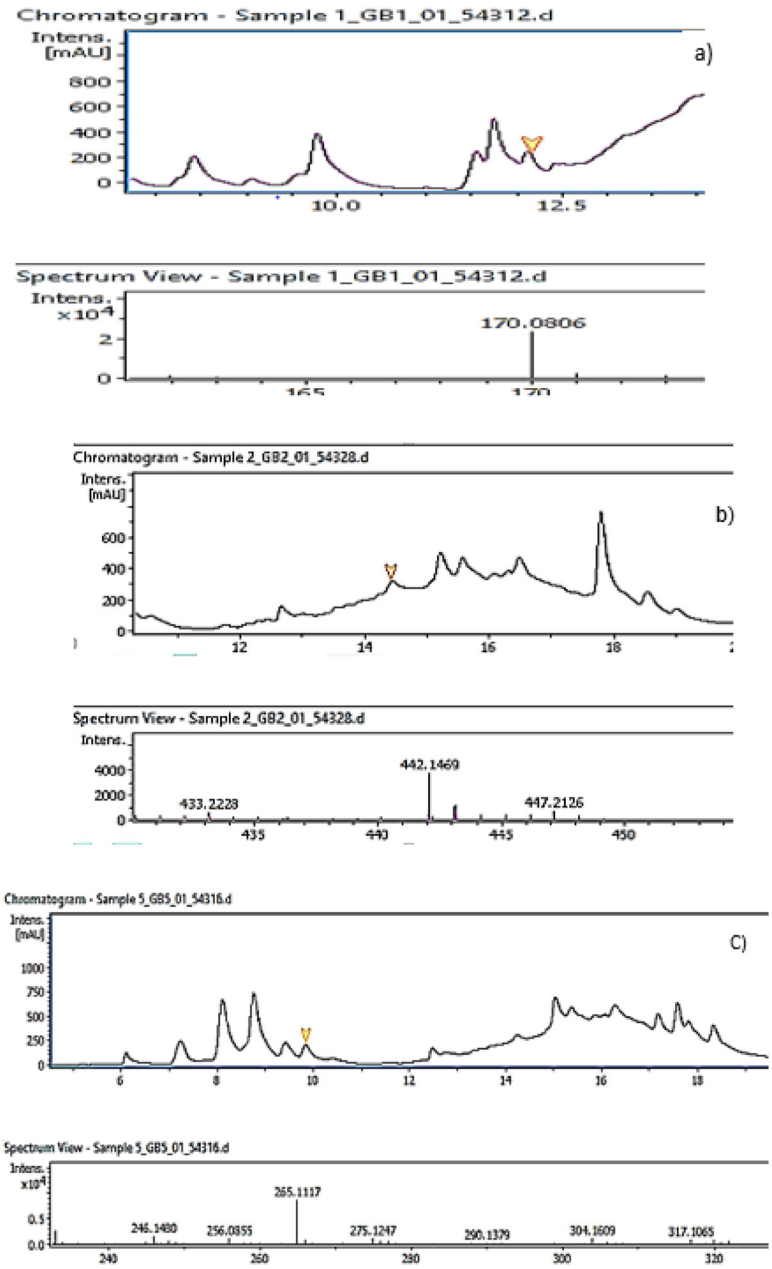

Fig. 4. Identification of Vitamins from the M. oleifera leaf sample using Mass spectrometry in various extract samples (Ms): a= B6, b= B9, c= B1).

Table 2
Concentration of water-soluble (mg/100g, mean ± SD, n = 3) vitamins in *M. oleifera* leaves harvested from clay soil amended with different levels of plant-based organic compost Different letters in each row indicate significant difference ($P < 0.05$).

	Control (clay soil) (mg/100g)	Clay soil + 15% compost (mg/100g)	Clay soil + 30% compost (mg/100g)	Clay soil + 45% compost (mg/100g)	Clay soil + 60% compost (mg/100g)	P-value
Vitamin B1	1.10 ± 0.20	1.67 ± 0.25	8.20 ± 0.46	8.63 ± 0.87	1.40 ± 0.99	0.02
Vitamin B2	25.10 ± 0.70	15.90 ± 0.21	22.00 ± 0.50	13.30 ± 0.50	15.60 ± 0.67	0.01
Vitamin B3	0.00 ± 0.00	13.50 ± 0.60	33.60 ± 0.70	40.60 ± 1.45	6.20 ± 0.42	0.01
Vitamin B6	1.87 ± 0.47	1.87 ± 0.32	2.90 ± 0.20	5.77 ± 0.51	3.83 ± 0.21	0.01
Vitamin B9	3.73 ± 0.57	7.17 ± 0.31	9.77 ± 0.29	1.13 ± 0.45	0.00 ± 0.00	0.01*

simultaneous quantification of B vitamins in Moringa using both LC-DAD and mass spectrometry for determination.

3.2. Preliminary application of the developed method to real samples

Fig. 3 shows a representative 20-minute chromatogram from one of the tested *M. oleifera* samples with all the five vitamins identified. Fig. 4 indicates the identification of the tested water-soluble vitamins in the sample using the MS detector.

Table 2 indicates the individual vitamin concentration in five *M. oleifera* samples grown from different soil compositions altered by the plant-based compost. There is significant difference in the concentrations of vitamins $p < 0.05$ on the plant biomass amended with soil (Table 2). The concentration of Vitamin B1 (Table 2) improved with the addition of plant-based compost to the clay soil. The same trend was reported for Vitamin B6. However, for Vitamins B9 and B2 concentration in *M. oleifera* leaves decreased with the addition of plant-based compost to the soil (Table 2). Other studies have also reported increase in the levels of these water-soluble vitamins upon soil amendments with organic and inorganic fertilizers (Bakpa et al. 2021). Water soluble vitamins such as Vitamin C quantified by indophenol dye redox titration were also noted to increase significantly in organically grown leafy vegetables in comparison with conventionally grown ones (Premamali et al. 2019). In addition, application of organic soil amendment led to higher concentrations of Vitamins B1, B2, B6 and C in another study by Akachukwu et al., (2018) using HPLC analysis. Another study by Kpéra et al. (2019) using the HPLC-UV reported an increase in Vitamin B6 content in organic soil amended for pineapples in comparison with the control.

This increase of vitamins with the addition of compost observed in this study could also be attributed to the increase in soil microorganism upon addition of compost, these organisms increase the availability of plant nutrients essential in the formation of vitamins (Chauhan et al. 2007). However, some studies on other plants (apples, pineapples, and strawberries) revealed that organically grown did not significantly differ in their vitamin concentration from those grown conventionally (Woese et al. 1997; Álvarez et al. 1993). This could explain why some vitamins in this study never increased in concentration with increased plant-based compost additions. Other studies have reported that excessive levels of nitrogen in the soil increase the concentration of nitrates which in turn reduce the synthesis of water-soluble vitamins such as Vitamin C and other essential nutrients in plants and in turn reduce their concentration (Bourn and Prescott, 2002). Organic compost may also not have the same composition in some reported literature studies, and this could explain differing performances (Golijan and Sečanski, 2021).

Table 3
Comparison of the vitamin concentrations in this study with concentrations from other *Moringa oleifera* studies and other related herbal plants.

Analytical method	Sample preparation used	LOD (Range)	LOQ (Range)	Plant type	Concentration of B vitamins reported (mg/100g)					Reference
		μg/mL	μg/mL		B1	B2	B3	B6	B9	
UPHPLC-DAD-QTOFMS	Solid-liquid extraction	0.20–0.89	0.60–2.71	*Moringa oleifera*	1.10 to 8.63	13.30 to 25.10	0.00 to 40.60	1.87 to 5.77	1.13 to 9.77	This study
Fluorometer	Solid-liquid extraction	NA	NA	*Moringa oleifera*	0.33	nd	nd	nd	nd	González-Burgos et al. 2021
RP-HPLC-UV	Sonication	NA	NA	*Moringa oleifera*	14.10	0.30	10.90	14.90	nd	Abibu et al. 2021
Fluorometer	NA	NA	NA	*Moringa oleifera*	6.64 to 11.32	2.60 to 8.83	22.90 to 36.90	nd	nd	Ali et al. 2017
NA	NA	NA	NA	*Moringa oleifera*	0.41 to 0.89	nd	nd	nd	nd	Mbah et al. 2012
HPLC Florouscence Detector (HPLC FLD) for B1 and B2 and Technicon Autoanalyser A II for B3	Dissolution	NA	NA	*Moringa oleifera*	0.8	0.42	220.00	nd	nd	El Sohaimy et al. 2015
Microbiological assays and HPLC	Solid-liquid extraction	NA	NA	*Moringa oleifera*	nd	nd	nd	2.40	nd	Witt 2013
RP-HPLC-UV	Sonication	0.03 – 0.23	0.18 – 0.67	*Sutherlandia frutescens*	6.60	54.30	5.30	2.60	28.90	Abibu et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Ipomoea aquatica*	0.45	0.71	nd	2.42	0.17	Datta et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Achyranthes aspera*	0.13	0.38	0.11	1.23	0.07	Datta et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Aasystasia ganjetica*	0.01	2.51	nd	0.76	0.08	Datta et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Enhydra fluctuans*	0.404	1.043	0.604	1.850	0.050	Datta et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Oldenlandia corymbosa*	0.049	0.472	nd	nd	1.278	Datta et al. 2019
HPLCV-UV	Solid-liquid extraction	NA	NA	*Amaranthus viridis*	0.044	0.012	0.258	1.308	0.038	Datta et al. 2019

*NA: Not available, nd means not determined.

The concentration of the five water-soluble vitamins currently studied were compared to the concentration of the same vitamins in previous *M. oleifera* studies and with some herbal plants. Vitamin B1 content in the current study was in the range of the Vitamin B1 content found in other *M. oleifera* and herbal plants (Table 3). Vitamin B2, B3 and B9 content was higher in the current study for *M. oleifera*, while B3 was also high in other herbal plants (Table 3). Vitamin B6 content in the current study was a bit lower than its concentration in *M. oleifera* leaves studied by Abibu et al. (2021). These differences could be attributed to environmental and climatic conditions. The soil type, its nutrients and its climatic conditions have a pronounced influence on plant nutrients (Kpéra et al., 2019).

4. Conclusion

The combination of HPLC-DAD and MS gives a more reliable method for analysis of water-soluble vitamins in plants. The synergy in detection allows simple extraction of the plant biomass without requiring rigorous clean-up such as SPE. This reduces the number of steps required in sample preparation which is a major source of errors.

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Declaration of competing interest

This is to officially state that authors have no conflict of interest in the work they have performed

CRediT authorship contribution statement

Nkazimulo Ngwenya: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Yannick Nuapia:** Writing – review & editing, Supervision, Data curation. **Ida Risenga:** Writing – review & editing, Supervision, Resources. **Eric Morifi:** Methodology. **Luke Chimuka:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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