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Soil properties and phytochemical analysis of spleen amaranth (*Amaranthus dubius* Mart. Ex Thell.) from Ankole and Teso sub-regions of Uganda: Implications for management and prevention of hyperglycemia

Caroline Asekenye[1,2] | Paul E. Alele[3] | Patrick E. Ogwang[1] | Eunice A. Olet[4]

[1]Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda

[2]Department of Pharmacy, Faculty of Health Sciences, Victoria University, Kampala, Uganda

[3]Department of Pharmacology and Therapeutics, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda

[4]Department of Biology, Faculty of Science, Mbarara University of Science and Technology, Mbarara, Uganda

Correspondence
Caroline Asekenye, Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda.
Email: 2019phd003@std.must.ac.ug

Funding information
Pharm-Bio Technology and Traditional Medicine Centre, Mbarara University of Science and Technology

ABSTRACT

Background: The current authors reported a remarkably higher prevalence of hyperglycemia in Ankole than in the Teso sub-region of Uganda, and *Amaranthus dubius* was documented among the frequently eaten leafy vegetables in both sub-regions. In an attempt to investigate this remarkable variance in the prevalence of hyperglycemia and find alternative therapies for hyperglycemia, we assessed the influence of soil properties on phytochemical quantity in spleen amaranth (*A. dubius*) from the two sub-regions. The soil properties and vegetable phytochemicals were quantified using spectrophotometric methods.

Results: Soil pH, organic matter (OM), and nitrogen (N) were higher in soil samples from the Teso sub-region than those from the Ankole sub-region. The Teso sub-region had sandy loam soils that were relatively low in exchangeable cations, whereas Ankole had clay loam soils. Total tannin content (TTC) and total saponin content (TSC) were significantly higher in *A. dubius* samples from the Teso sub-region, and total alkaloid content (TAC) was higher in vegetable samples from Ankole. The Pearson's correlation results showed a significant relationship between pH and TTC, N, and TAC. Total flavonoid content (TFC) was correlated with exchangeable cations.

Conclusion: High soil pH, N, cations, and sand percentage found in soil samples from the Teso sub-region supported the biosynthesis of polyphenolic compounds in the vegetable samples. By implication, this consequently benefited its consumers by reducing blood glucose levels ultimately reducing the prevalence of hyperglycemia in the region.

KEYWORDS
Amaranthus dubius, phytochemicals, soil properties, Uganda

INTRODUCTION

The relentlessly increasing prevalence of hyperglycemia is dependent on factors like the geographical region of residence and diet.[1,2] This trend is similar in Uganda, as reported by Asekenye et al. and Bahendeka et al.,[3,4] where a remarkably higher prevalence of hyperglycemia was found in Ankol than in the Teso sub-region. Additionally, Asekenye et al. and Kabwama et al.[3,5] also documented higher consumption of vegetables in the Teso sub-region than in the Ankole sub-region. In an attempt to further investigate this remarkable

variance in the prevalence of hyperglycemia and find alternative therapies, we designed this study as part of an ongoing larger study to assess the influence of soil properties on *Amaranthus dubius* phytochemicals, since it was eaten in both sub-regions. Our working hypothesis was that since the soil properties influence phytochemicals in plants and since sub-regions in Uganda have significant differences in climatic conditions and soil properties,[6,7] the different samples of *A. dubius* (a widely eaten vegetable in Uganda) could have significantly different phytochemical quantities, thereby helping to explain the significant difference in the prevalence of hyperglycemia in these study sub-regions. To test this hypothesis, we studied the phytochemical quantity and soil properties in the vegetable and soil samples, respectively, from these two study sub-regions. The results aided in establishing a correlation between soil properties and phytochemicals, shedding more light on the remarkable variance in prevalence of disease like hyperglycemia between the studied sub-regions. This, in turn, suggests alternative strategies for reducing hyperglycemia. Furthermore, these results can be used to maximize vegetable production for medicinal purposes.

Phytochemicals (plant bio-actives or secondary metabolites (SMs)) are defined as non-nutrient compounds in plants responsible for protecting them against unfavorable conditions (nutrient insufficiency, harsh environment, and plant pathogens) and attraction of pollinators.[8,9] These unfavorable conditions induce the activation of genes involved in the biosynthesis of SMs.[10,11] There are large and diverse classes of SMs (alkaloids, flavonoids, saponins, tannins, phenolics, and terpenoids) that accumulate in plant organs like fruits, leaves, flowers, and roots.

Amaranthus dubius is a cultivar that grows voluntarily and/or is cultivated mostly for its leaves in Africa, Southeast Asia, and Central America[12] for food, since it yields significantly within the shortest period.[13] Recently, it was adopted in the global progress in reducing nutrient-related challenges, especially in developing countries,[12,14] due to the macro- and micronutrients, minerals, vitamins, dietary fiber, low carbohydrate, and calorie elements it contains.[15–17] Some studies have found higher nutrient content in *Amaranthus* species than in some cruciferous species (cabbage and lettuce).[18,19] The popular antioxidant health benefit of the genus *Amaranthus* is credited to its phytochemicals like polyphenols (flavonoids, phenolic acids, and tannins) and terpenes (squalene), where some isolations from the matrix have been done using various solvents (20–22).[20–22] The biosynthesis of these phytochemicals is influenced by a vast array of factors, like genotype/species and climatic conditions (23–25).[23–25] Ankole and Teso sub-regions of Uganda do have completely different climatic conditions that may result in different soil properties and ultimately differently influence the induction of genes responsible for the phytochemicals in the vegetable species.

Soil physical properties, including pH, organic matter (OM), and texture, and chemical properties, such as nitrogen, phosphorus, potassium, calcium, sodium, and iron, were looked at in this study. These properties influence the production of phytochemicals in plants by determining the biological activity, solubility of nutrients, and oxidation and reduction processes in the soil and hence affect the form of interaction between the soil and the plants.[26–28] OM is the summation of plant and animal residues in the soil at various stages of decomposition. Its percentage has been found to affect the soil nutrients in the soil and hence the phytochemicals synthesized in the plant. A study on lettuce showed that the vegetables grown under organic manure increased certain phenolic compounds, and increased organic fertilizer application tended to have a negative effect on its phytochemical content.[29] A decrease of flavonoid and phenolic acid concentrations has been observed in leaves of organically grown barley as a result of increasing fertilization rates using farmyard manure or cattle slurry.[30]

Soil texture is the relative proportions of sand, clay, and silt particles in the soil. When Lata and Winska grew Kale (*Brassica oleracea* acephala group) in lessive soil, the plants were found to contain more anthocyanins, glutathione, and ascorbate compared to those grown in muddy soil.[31] Addition of OM to the soil improved its texture and structure, and when plants were grown in humus-rich soil, they produced more quantities of phenolic compounds compared to those grown in less humus-rich soil.[32] Sandy clay textured soils do not support phytochemical accumulation, especially the terpenes found in essential oils, whereas luvisolic soils, characterized by high clay content, were reported to support the accumulation of phenolic compounds in Iris species.[33] pH is the potential of hydrogen, a measure of the acidity or alkalinity of a soil solution. It is an important factor for solubility, breakdown, and formation of nutrients in the soil. Isothiocyanates are usually produced in plants grown in a neutral to acidic pH range.[34,35] Acidic soils promote nitrification and carbon substrate utilization, which is characterized by vegetation that produces terpenic and polyphenolic phytochemicals.[36]

Supplementary soil nutrition is a common agronomical practice to enhance soil fertility by the addition of either organic/inorganic macroelements (nitrogen, phosphorus, and/or potassium) and/or trace-elements (calcium, magnesium, sodium, etc.). This does not only stimulate plant growth in general, but also influences SMs production. Many studies have concluded that soil fertility levels of these nutrients do play a role in influencing the phytochemical accumulation in plants[37–39] although it has been noted that the application of these fertilizers singly, in pairs, or as a compound does lead to inconsistent results.[40] Nitrogen deficiency that can inhibit primary metabolism (poor growth and reduced protein synthesis) has been proposed to affect the nitrogen balance in plants, which may instead favor secondary metabolism and the accumulation of several phenolic compounds and glucosinolates.[41,42] Stewart et al.[43] noted a significant inverse correlation between the availability of nitrogen and phosphorus in the soil and of flavonol content in Arabidopsis and tomato seedling tissues. However, the concentration of quercetin and kaempferol was noted to increase in response to either nitrogen or phosphorus alone in the same plants. According to the phenylpropanoid metabolic pathway, it has long been reported that phenolic compounds in plants may accumulate due to a deficiency in nitrogen, phosphate, and iron in the soil.[44,45]

Alkaloids are nitrogen-based phytochemicals, and generally, their synthesis and accumulation is in response to increased soil nitrogen fertilization. Barlog et al. applied magnesium and nitrogenous fertilizers on *Lupinus angustifolius*, and it influenced an increase in alkaloid

concentration by 9%–17%.[46] In other studies, alkaloid production (in *Lapinus albus*) was only in response to various amounts of nitrogen supplementation.[47] For example, Sangral is a commercial compound fertilizer; when applied to *Datura innoxia* plants at a rate of 600 kg ha^{-1}, the alkaloids peaked and then decreased at 800 kg ha^{-1}.[48] In the case of terpenic compounds (e.g., monoterpene), although they are carbon-based phytochemicals, their concentration in a plant did respond to nitrogen fertilization.[49] For instance, the application of nitrogen fertilizer increased the concentration of monoterpenes in *Thuja plicata* during its active growth stage, and when the plant growth began to subside, continuous fertilizer application generated even higher amounts of monoterpenes.[50]

Soil cations are introduced to the soil via mineral fertilizers like sodium chloride, calcium carbonates, etc., and they bind to the soil particles. Depending on the exchange capacity in the soil, these cations can be released into the soil and become available to the plant.[51,52]

Saline soil can cause oxidative stress to the plant due to the production of reactive species.[53] Plants respond to this kind of stress by producing SMs that can scavenge and/or detoxify these reactive species.[54] For example, when *Aegiceras corniculatum* was treated with 250 mM of sodium chloride (NaCl), the polyphenolic content increased more than double compared to the controls.[55] The accumulation of polyphenols in a Tunisian seaweed (*Cakile maritima*) was due to the treatment with different concentrations of NaCl salt. Salinity also influences the accumulation of some terpenic compounds in the plants. Cineole and camphor in *Rosmarinus officinalis* were induced by treatment with 100 mM NaCl; however, there was a slight decrease in borneol, α-terpineol, nopol, and camphene monoterpenes.[56]

In the light of these previous studies, it is clear that despite the vegetable species, environmental conditions (climate and soil properties) that are "harsh"/"abnormal" to a plant, favor the phytochemical synthesis in it. We, therefore, designed this study to assess the influence of soil properties on the phytochemical content in *A. dubius* from Ankole and Teso sub-regions of Uganda. Specifically, the phytochemical classes in the aqueous leaf extracts and soil properties were quantified. Data from these variables were correlated to find the relationship between them.

MATERIALS AND METHODS

Materials

A flame photometer PFP7 (JENWAY, United Kingdom) and an atomic absorption spectrophotometer AAS990, AAS932+ (PG Instruments, United Kingdom) were used due to their selective detection of the studied soil nutrients. An ultra–violet/visible (UV–Vis) 6705 spectrophotometer (JENWAY, United Kingdom) was better fitted for quantifying the colored solutions of the vegetable leaf extracts, within the UV range. More materials including a pH meter (Mettler Toledo, JENWAY, United Kingdom), a porcelain mortar and pestle, a vortex machine, and an electric high speed shaker were used for homogenization of soil suspension. Most of these equipment were acquired from Bruker, United States of America. The chemical standards used were quercetin, gallic acid, diosgenin, and atropine. More chemicals and reagents were Folin-Ciocalteu, aluminum chloride, sodium acetate, vanillin, sulfuric acid, hydrochloric acid, bromocresol green, and pure methanol. Distilled water was from a Milli-Q purification system (Millipore, India). The standards and some of these chemicals were purchased from Sigma-Aldrich, Germany, and were of analytical grade.

Methods

Study site characteristics

The Ankole sub-region is located in the South-Western part of Uganda, with geographical coordinates of Latitude: 00 29′ 59.99″ N and Longitude: 00 29′ 59.99′ E. Its average elevation is 394 m, and the study sampled districts (Ibanda, Kiruhura, Mbarara, Rubirizi, and Ntugamo) lie at about 1806 m above sea level. This region experiences a relatively higher average annual rainfall of 1018 mm, and a lower average annual temperature of 17.2°C, making it generally cooler (https://en.wikipedia.org/wiki/Ankole_sub-region).

Teso sub-region is in the eastern part of Uganda with coordinates of Latitude: 1.71590 N and Longitudes: 33.61110 E. Its study districts (Soroti, Ngora, Amuria, Kaberamaido, and Katakwi) lie about 1129 m above sea level, with an average elevation of 1081 m. It has an average annual rainfall of about 1000 mm, and the average annual temperature is about 25°C, making it semi-arid (https://en.wikipedia.org/wiki/Teso_sub-region). Figure 1 shows the map showing the sampling points in the Ankole and Teso sub-regions of Uganda.

Soil and vegetable sample collection

Vegetable gardens (from the sampled districts) were subdivided into relatively uniform sampling units. Within each unit, about 200 g of soil samples were randomly collected 12 inches from the surface, from several different locations, and mixed into one composite sample. From each garden, a portion of the composite sample was transferred to a soil sample bag, which was well labeled with date, name of place, sample, and garden number, and GPS coordinates.[57–59] The soil samples were then taken to the laboratory (Soil, Plant and Water Analytical Laboratory, College of Agricultural and Environmental Sciences, Makerere University, Kampala City, Uganda) for analysis. The samples were air dried at 25°C for 5 days to eliminate moisture. Afterward, they were ground using a porcelain pestle and mortar and then sieved through a 2 mm sieve to remove debris and other non-soil materials.[60,61] The sieved soil samples were analyzed for their physical and chemical properties, and they included soil pH, soil OM, nitrogen (N), available phosphorus (av. P), exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), iron (Fe), and soil texture (the percentage proportions of sand, clay, and silt). N, P, and K are generally the most limiting macronutrients regulating plant growth and are tightly linked to secondary metabolism in terrestrial ecosystems.[62,63] For example, P uptake is promoted under low N conditions.[64] Phenolic and glucosinolate compounds are synthesized

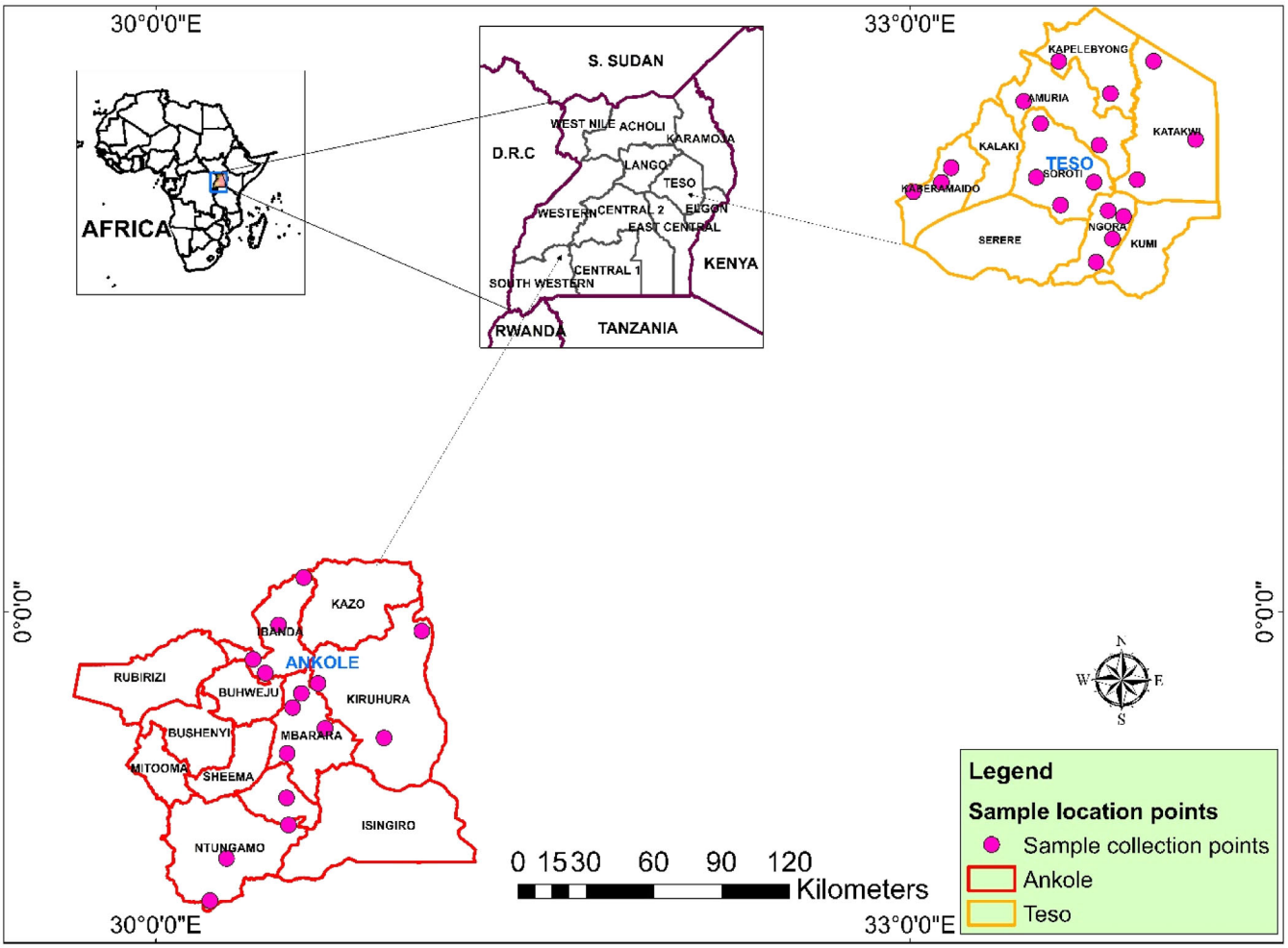

FIGURE 1 Map showing the sampling points in the Ankole and Teso sub-regions of Uganda (drawn from GPS coordinates from November 2023 using Arc GIS version 10.5 on 10/12/2023).

depending on the demand for N nutrients.[65] Terpenic and alkaloids are synthesized based on the trade-off of carbon from the carbon–nitrogen balance in the plant. Soil physical properties are necessary for retention, solubility, and availability of soil chemicals to the plant (66).[66]

At the same time, samples of *A. dubius* leaves were collected (at the end of the vegetative stage (when they are fully mature, and before the leaf nutrients and phytochemicals are used up for flowering) from the same garden points where the soil samples were collected. It was taken for identification and was given a voucher number (51176) by a botanist. The leaves were put in plastic bags with similar labels to the soil samples,[67] and taken to the Pharmaceutical Sciences Laboratory, Mbarara University of Science and Technology (Mbarara City, Uganda) for phytochemical analysis.

Laboratory analysis of soil physical and chemical properties

Soil pH

Soil pH was measured by a pH meter using soil/solution suspension.[68–70]

Organic matter (OM)

OM was determined following wet oxidation using concentrated sulfuric acid and potassium dichromate.[71–73] Soil, potassium dichromate, and concentrated sulfuric acid digest was titrated with ferrous ammonium sulfate solution. The amount of organic carbon in the soil is a measure of the used potassium dichromate, which is the difference between the added and the residual. The organic carbon was calculated using Formula 1

$$\text{Organic Carbon}\,(\%) = \left(\frac{T \times 0.3 \times 0.2 \times 100}{\text{Sample weight}}\right) \times 75\% \qquad (1)$$

where T is the difference in the titer, 0.3 is the indicator volume, 0.2 is the concentration of ferrous ammonium sulfate, and 75% is the oxidation completion factor.

The organic carbon is 58% of OM, shown in Formula 2.

$$\text{Organic matter} = (\text{O.C} \times 100)/58 \qquad (2)$$

Soil particle size (soil texture) analysis

Soil texture was analyzed using the hydrometer/Bouyoucos method.[74–76]

The hydrometer and temperature readings were taken from a thoroughly mixed soil suspension allowed to stand undisturbed for 2 h. The percentage of sand content (after 40 seconds): the hydrometer reading, which reflects the grams of silt and clay in the soil suspension. This value was subtracted from the original soil sample weight to get the amount of sand and was converted to a percentage form. The percentage clay: the hydrometer reading (after 2 h) reflects the clay content of the original suspension and is converted to a percentage. Each hydrometer reading was taken after temperature correction. Silt content was calculated by subtracting the sum of clay and sand contents from 100%.

Analysis of soil N, Fe, available P, and exchangeable cations (Na^+, Ca^{2+}, K^+, and Mg^{2+})

Mg^{2+} and Fe were analyzed using an atomic absorption spectrophotometer (AAS) on Mehlich 1 extracts. K^+, Ca^{2+}, and Na^+ were analyzed using a flame photometer on the same extract.[77,78] Available P content was determined using a spectrophotometer at 882 nm wavelength after the extracts' reaction with ammonium molybdate in the presence of ascorbic acid.[79]

The standard working solutions were measured to calibrate the instrument. The concentration of the above ions was calculated using Formula 3

$$((a-b)\times V\times f)/W \quad (3)$$

where a is the concentration of K, Ca, Na, and Mg in the soil extract, b is the concentration of the element in the blank extract, *V* is the volume of extract solution, *W* is the weight of the soil sample, and f is the dilution factor.

N was determined calorimetrically at a wavelength of 655 nm on the complexed digestion mixtures using N1 and N2 reagents.[80] Reagents in N1: sodium salicylate, sodium citrate, sodium tartrate, and sodium nitroprusside. Reagents in N2: sodium hydroxide and sodium hypochlorite (JIK), mixed in the stipulated proportions by Okalebo et al.[81]

Quantification of phytochemicals in *A. dubius* vegetable samples

A. dubius aqueous leaf extracts (AdALEs) were prepared by boiling 100 g of ground leaves in 1000 mL of distilled water for 15 min.[82] After cooling, the mixture was filtered, and the filtrate was dried in a freeze dryer.

Total flavonoid content (TFC)

TFC in AdALEs was determined using a modified aluminum chloride (AlCl3) colorimetric method by Baba et al., and Khan et al[83,84] To I mL (1 mg/mL) of each AdALEs, 3 mL of methanol was added and agitated, followed by the addition of 0.2 mL of 10% $AlCl_3$ solution and 0.2 mL of 1 M sodium acetate. Thereafter, the solutions were incubated for 30 min at room temperature in the dark. The absorbance of the resultant solutions was then read at 420 nm using a UV–vis spectrophotometer. Quercetin was used as the standard compound. The concentration of TFC in AdALEs was determined using the equation $y = 0.0158x - 0.3077$ and $R^2 = 0.9173$ obtained from a standard quercetin curve. The TFC was expressed as microgram quercetin equivalent of flavonoids (QEF) per milligram of vegetable dry weight (d. wt).

Total saponin content (TSC)

TSC in AdALEs was determined using a vanillin–sulfuric acid assay.[85,86] To 1 mL (1 mg/ML) of each AdALEs was added 0.5 mL of 8% (w/v) vanillin solution, followed by addition of 5 mL of 72% (v/v) sulfuric acid, and thoroughly mixed. The resultant mixtures were then incubated at 60°C in a shaking water bath for 15 min and then cooled in ice-cold water for 5 min. The absorbance of the resultant solutions was then read at 550 nm using a UV–vis spectrophotometer. Diosgenin was the standard chemical used. The concentration of TS compound in the AdALEs was determined using the equation $y = 0.0049x + 0.0504$ and $R^2 = 0.9917$ obtained from a standard diosgenin curve. The TSC was expressed as microgram diosgenin equivalents of saponin (DES) per milligram of vegetable dry weight (d. wt).

Total phenolic content (TPC)

The TPC in AdALEs was determined using a modified Folin-Ciocalteu method.[87,88] To 1 mL (1 mg/mL) of AdALEs was added 2 mL of 10% Folin-Ciocalteu reagent, followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate solution, and incubated at 40°C for 30 min. The absorbance of the resultant solution was then read at 760 nm. Working solutions of gallic acid were used to prepare a calibration curve for the standard. The concentration of TP compound in the AdALEs was determined using the equation $y = 0.0207x + 0.0368$ and $R^2 = 0.945$ obtained from a standard gallic acid curve. The TPC was expressed as microgram gallic acid equivalent per milligram (GAE) of *A. dubius* dry weight.

Total tannin content (TTC)

TTC was determined using a modified vanillin-hydrochloric acid assay method.[89,90] To 1 mL (1 mg/mL) of AdALEs was added 1500 µL of vanillin/methanol (4%) solution and 750 µL of concentrated HCl, and they were allowed to react at room temperature for 1 h. The absorbance at 300 nm was measured against a blank. Gallic acid was used as the standard. The concentration of the TTC compound in AdALEs was determined using the equation $y = 0.011x + 0.0353$ and $R^2 = 0.9358$ obtained from a standard gallic acid curve. The concentration of condensed TT was expressed in micrograms of gallic acid equivalents (GAE) per milligram dry weight.

Total alkaloid content (TAC)

TAC was determined following a reaction with bromocresol green (BCG).[91–93] AdALEs were dissolved in 2 N HCl and then filtered. 1 mL

(1 mg/mL) of each solution was washed (three times) with 10 mL of chloroform in a separating funnel. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Thereafter, 5 mL of BCG solution and 5 mL of phosphate buffer were added to these solutions. The mixtures were shaken and complexes extracted with 1, 2, 3, and 4 mL of chloroform by vigorous shaking; the extracts were then collected in a 10 mL volumetric flask and made up to 10 mL with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The concentration of TAC in the AdALEs was determined using the equation $y = 0.0023x + 0.0389$ and $R^2 = 0.954$ obtained from a standard atropine curve. The total concentration of alkaloid was expressed in micrograms of atropine equivalent per milligram of vegetable dry sample.

All extraction procedures were performed in triplicate ($n = 3$) for each AdALE from the vegetable samples.

Statistical analysis

Analysis of variance (One way) was performed to determine any significant difference between the soil properties, phytochemical quantities in *A. dubius*, from the sampled districts and study sub-regions. Where necessary, Tukey's test ($p < 0.05$) to find out where the significant difference is was performed. A two-sample *t*-test was also performed to find out whether the means of the soil properties and phytochemical quantities in AdALEs differ significantly between the study sub-regions.

Pearson's correlation analysis between phytochemical quantities in *A. dubius* and the soil properties was performed to see the direction and strength of their relationship. Minitab version 19 statistical software was used for the analysis of data.

RESULTS

Physical and chemical properties of soil samples from Ankole and Teso sub-regions of Uganda

The soil pH, N, OM, available P, and texture were significantly ($p < 0.05$) different in the study sub-regions. Soil samples from Teso sub-region had a pH of 7, whereas those from Ankole sub-region had a pH of 6.4. OM percentage is higher in soil samples from Teso sub-region, whereas available P is higher in samples from Ankole sub-region. Soils from Ankole sub-region have less sand, higher clay, and silt percentage compared to those from Teso sub-region. The difference in the quantity of soil minerals in soil samples from the study sub-regions was not statistically significant ($p > 0.05$). More of this result is in Table 1.

Physical and chemical properties of soil samples from the study districts

A significantly ($p < 0.05$) higher average pH (7.7) was recorded in the soil samples from Ngora district. OM percentage was significantly ($p < 0.05$) highest in soil samples from Soroti district (51.3%), and the least from Kiruhura and Kaberamaido districts. Available P is significantly higher in samples from Rubirizi district (25.8 mg/kg), whereas soil samples from Katakwi district had significantly the least amount (0.04 mg/kg). It is also noticeable that soil samples from Katakwi district had significantly highest sand, the least clay, and silt percentages among all the study districts (79.7%, 15.5%, and 3.9%, respectively). There was no significant ($p > 0.05$) difference among the exchangeable cations (K^+, Na^+, Ca^{2+}, and Mg^{2+}) and Fe in the soil samples across the study districts; however, soil samples from Ntungamo district contained the highest K^+, Fe, and Mg^{2+} concentrations, whereas Ca and Na were highest in Rubirizi and Kiruhura, respectively. Table 2 has more of this result.

Phytochemical quantities in aqueous leaf extracts of *Amaranthus dubius* from Ankole and Teso sub-regions

The total phenolic content (TPC), TTC, and TSC were higher in *A. dubius* samples from the Teso sub-region, with the latter two phytochemicals being significantly ($p < 0.05$) higher. TFC and TAC were, however, higher in the Ankole sub-region, though not at statistically significant levels ($p > 0.05$), as shown in Table 3.

Phytochemical quantification in aqueous leaf extracts of *Amaranthus dubius* samples from study districts

There are some significant differences in the phytochemical quantity in *A. dubius* across the study districts. Samples from Ngora district had significantly ($p < 0.05$) the highest quantity of TTC (60.2 mg/g d.wt), whereas the least quantity (11.5 mg/g d.wt) was found in the samples from Ibanda district. Vegetable samples from Rubirizi district had significantly the least quantity of TSC (37.9 mg/g d.wt) compared to those from Soroti district, which had significantly the highest quantity (152.5 mg/g d.wt). TPC quantity is significantly ($p < 0.05$) the highest (34.9 mg/g d.wt) in Kiruhura district and significantly the least in Rubirizi district (20.9 mg/g d.wt). *A. dubius* samples from Kaberamaido and Katakwi districts had significantly ($p < 0.05$) the highest (126.5 mg/g d.wt) and the lowest (22.8 mg/g d.wt) quantities of the TAC, respectively. The difference in quantity of TFC is insignificant ($p > 0.05$) in the vegetable samples across the study districts. More of these results are included in Table 4.

Pearson's correlation between soil properties and phytochemical quantities in *A. dubius* samples

According to the results detailed in Table 5, most of the correlations between the phytochemicals in *A. dubius* and soil properties were positive. TTC in *A. dubius* was the only significant ($p < 0.05$) and

TABLE 1 Physical and chemical properties (mean ± standard deviation) of soil samples from the Ankole and Teso sub-regions of Uganda.

Sub-region	pH	N	OM	Av. P	Fe	Sand	Clay	Silt	K^+	Na^+	Ca^{2+}	Mg^{2+}
		Percent (%)		Milligram per kilogram (mg/kg)		Percent (%)			Centimoles per kilogram (Cmol/kg)			
Ankole	6.4 ± 0.51	0.2 ± 0.05	3.6 ± 1.36	25.8 ± 24.5	75.3 ± 18.8	60.6 ± 5.60	33.2 ± 7.12	11.3 ± 4.43	0.6 ± 0.49	0.5 ± 0.29	5.81 ± 1.88	0.7 ± 0.30
Teso	7.0 ± 0.60	0.9 ± 0.49	28.1 ± 25.70	0.1 ± 0.14	78.4 ± 4.03	72.3 ± 6.54	22.5 ± 6.83	5.7 ± 1.75	0.3 ± 0.21	0.5 ± 0.12	6.3 ± 0.62	0.6 ± 0.10
p-value	**0.02***	**0.00***	**0.02***	**0.00***	0.60	**0.00***	**0.00***	**0.00***	0.06	0.48	0.40	0.56

Note: *p*-values less than 0.05 are statistically significant (**bold value***). N: nitrogen, O.M: organic matter, av. P: available phosphorus, Fe: iron, K^+: potassium ions, Na^+: sodium ions, Ca^{2+}: calcium, Mg^{2+}: magnesium.

TABLE 2 Physical and chemical properties (mean ± standard deviation) of soil samples from the sampled districts.

Soil property		Rubirizi	Ibanda	Ntungamo	Kiruhura	Katakwi	Kaberamaido	Soroti	Ngora
	pH	6.6 ± 0.41^{ab}	6.4 ± 0.24^{b}	6.3 ± 0.10^{b}	6.3 ± 1.05^{b}	6.9 ± 0.11^{ab}	6.2 ± 0.03^{b}	7.0 ± 0.01^{ab}	**$7.7 \pm 0.52^{a*}$**
%	OM	4.8 ± 1.39^{cd}	4.1 ± 1.06^{cd}	3.42 ± 0.34^{d}	**$1.9 \pm 0.41^{d*}$**	7.8 ± 0.16^{c}	**$1.8 \pm 0.04^{d*}$**	**$51.3 \pm 3.62^{a*}$**	19.4 ± 0.72^{b}
	N	0.2 ± 0.02^{cd}	0.2 ± 0.03^{cd}	**$0.1 \pm 0.01^{d*}$**	**$0.1 \pm 0.02^{d*}$**	0.4 ± 0.11^{cd}	**$1.6 \pm 0.11^{a*}$**	1.0 ± 0.01^{ab}	0.7 ± 0.53^{bc}
	Sand	56.0 ± 0.00^{d}	60 ± 3.46^{d}	60.7 ± 5.77^{d}	65.7 ± 7.51^{bcd}	**$79.7 \pm 0.57^{a*}$**	62.7 ± 3.06^{cd}	73.7 ± 0.58^{ab}	73.0 ± 1.00^{abc}
	Clay	29.3 ± 2.31^{bc}	30 ± 40^{bc}	30 ± 0.00^{bc}	43.3 ± 7.09^{a}	**$15.7 \pm 0.46^{d*}$**	33.0 ± 3.00^{b}	21.3 ± 1.12^{cd}	19.9 ± 0.02^{d}
	Silt	14.7 ± 2.31^{a}	10.0 ± 5.29^{ab}	9.3 ± 5.77^{ab}	11.0 ± 4.00^{ab}	**$3.9 \pm 0.17^{b*}$**	6.5 ± 2.20^{ab}	5.2 ± 1.089^{b}	7.2 ± 1.06^{ab}
mg/kg	Av. P	**$55.5 \pm 29.60^{a*}$**	2.6 ± 0.02^{b}	9.5 ± 5.73^{b}	31.6 ± 9.08^{ab}	**$0.04 \pm 0.01^{b*}$**	0.3 ± 0.26^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
	Fe	75.0 ± 5.29^{a}	80.3 ± 4.04^{a}	95.0 ± 18.5^{a}	51.0 ± 7.21^{b}	77.0 ± 2.00^{a}	80.3 ± 1.16^{a}	73.7 ± 3.51^{a}	82.7 ± 1.53^{a}
Cmol/kg	K^+	0.8 ± 0.46^{a}	0.3 ± 0.05^{a}	0.8 ± 0.74^{a}	0.6 ± 0.61^{a}	0.2 ± 0.01^{a}	0.1 ± 0.01^{a}	0.6 ± 0.01^{a}	0.35 ± 0.00^{a}
	Na^+	0.5 ± 0.21^{ab}	**$0.3 \pm 0.01^{b*}$**	0.6 ± 0.41^{ab}	**$0.8 \pm 0.14^{a*}$**	0.36 ± 0.03^{ab}	0.5 ± 0.18^{ab}	0.6 ± 0.03^{ab}	0.5 ± 0.01^{ab}
	Ca^{2+}	7.5 ± 2.13^{a}	4.3 ± 0.07^{b}	4.7 ± 1.81^{ab}	6.8 ± 0.58^{ab}	5.8 ± 0.01^{ab}	5.9 ± 0.18^{ab}	7.3 ± 0.21^{a}	6.24 ± 0.23^{ab}
	Mg^{2+}	0.8 ± 0.14^{a}	0.6 ± 0.19^{ab}	0.8 ± 0.35^{a}	0.3 ± 0.21^{b}	0.5 ± 0.03^{ab}	0.6 ± 0.03^{ab}	0.7 ± 0.05^{ab}	0.6 ± 0.03^{ab}

Note: Values in the same row that do not share a letter are significantly different, and **bold value*** is statistically significant = $p < 0.05$.

TABLE 3 Phytochemical quantities (mean ± standard deviation) in aqueous leaf extracts of *Amaranthus dubius* samples from the study Ankole and Teso sub-regions.

Sub-region	Phytochemical quantity, milligram per gram of dry weight of the vegetable sample (mg/g d.wt)				
	Total phenolic content	Total tannin content	Total flavonoid content	Total Saponin content	Total alkaloid content
Ankole	27.6 ± 6.09	20.1 ± 9.28	43.4 ± 6.79	67.0 ± 30.20	79.1 ± 19.60
Teso	28.2 ± 7.16	**34.7 ± 16.60**	40.8 ± 0.62	**133.3 ± 31.90**	78.5 ± 43.30
p-value	0.83	**0.02***	0.20	**0.000***	0.96

Note: *p*-values less than 0.05 are statistically significant (**bold value***).

positively correlated phytochemical with the soil pH. The correlation between the TSC and OM, and N was positive and significant ($p < 0.05$), and this relationship was even stronger and highly significant with N. The relationship between TAC in the vegetable and the soil properties was not significant ($p > 0.05$) except for that with N and the percentage of sand. Most cations (K^+, Na^+, Mg^{2+}) in the soil were positively correlated with the quantity of TFC in *A. dubius*. The strength of the relationship was strong and highly significant. Clay percentage in the soil did not have any significant correlation with the vegetable phytochemical quantity regardless of the direction of the relationship.

DISCUSSION

This study was part of the ongoing larger study to investigate the remarkable difference in the prevalence of hyperglycemia in Ankole and Teso sub-regions of Uganda, and to ultimately find prevention and/or treatment therapies via consumption of appropriate vegetables in the traditional diet. Bahendeka et al., and the current authors,[3,4] have already reported the other factors (economic status, vegetable consumption, and other lifestyle factors) associated with the prevalence of hyperglycemia in the study sub-regions, and based on these previous studies, we hypothesized that the vegetables could be influenced (at the phytochemical level)

TABLE 4 Phytochemical quantities (mean ± standard deviation) in aqueous leaf extracts of *Amaranthus dubius* samples from the study districts.

Phytochemical quantity (mg/g d.wt)	Rubirizi	Ibanda	Ntungamo	Kiruhura	Katakwi	Kaberamaido	Soroti	Ngora
Total phenolic content (TPC)	**20.9 ± 3.71**^{b*}	29.4 ± 2.51^{ab}	25.2 ± 3.09^{ab}	**34.9 ± 3.84**^{a*}	22.9 ± 7.93^{ab}	23.7 ± 2.98^{ab}	31.9 ± 7.12^{ab}	34.3 ± 3.06^{a}
Total tannin content (TTC)	21.1 ± 4.94^{bc}	**11.5 ± 2.94**^{c*}	15.5 ± 7.94^{c}	32.4 ± 2.64^{b}	18.7 ± 0.92^{bc}	29.4 ± 2.10^{b}	30.4 ± 10^{b}	**60.2 ± 8.82**^{a*}
Total flavonoid content (TFC)	42.6 ± 3.16^{a}	41.2 ± 1.84^{a}	48.3 ± 13.80^{a}	41.7 ± 0.14^{a}	40.5 ± 0.28^{a}	41.2 ± 0.28^{a}	41.5 ± 0.1^{a}	40.1 ± 0.24^{a}
Total saponin content (TSC)	**37.9 ± 3.99**^{b*}	59.5 ± 24.50^{b}	80.9 ± 24.40^{ab}	89.6 ± 37.10^{ab}	135.7 ± 29.70^{a}	145.1 ± 18.10^{a}	**152.5 ± 42.40**^{a*}	99.7 ± 11.62^{ab}
Total alkaloid content (TAC)	65.4 ± 18.40^{bc}	68.4 ± 12.35^{bc}	105.1 ± 10.58^{ab}	77.7 ± 6.61^{ab}	**22.8 ± 6.1**^{c*}	**126.5 ± 6.10**^{a*}	83.7 ± 7.54^{ab}	81.0 ± 6.55^{ab}

Note: Values in the same row that do not share a letter are significantly different, **bold value** and * = $p < 0.05$.

TABLE 5 Pearson's correlation coefficients between soil properties and phytochemicals quantities in *Amaranthus dubius* samples.

		%		Mgkg^{-1}	Cmol/kg				Mgkg^{-1}	%		
Phytochemical	**pH**	**OM**	**N**	**Av. P**	**K^+**	**Na**	**Ca^{2+}**	**Mg^{2+}**	**Fe**	**Sand**	**Clay**	**Silt**
Total phenol content	0.18 ns	0.28 ns	−0.06 ns	−0.13 ns	0.07 ns	0.24 ns	0.09 ns	−0.32 ns	−0.32 ns	0.27 ns	0.11 ns	−0.20 ns
Total tannin content	**0.58***	0.25 ns	0.30 ns	−0.14 ns	−0.05 ns	0.26 ns	0.33 ns	−0.17 ns	−0.09 ns	0.31 ns	−0.17 ns	−0.06 ns
Total flavonoid content	−0.18 ns	−0.10 ns	−0.18 ns	0.07 ns	**0.59****	**0.5****	0.09 ns	**0.63****	0.52**	−0.38 ns	0.12 ns	0.43*
Total saponin content	0.22 ns	**0.45***	**0.62****	**−0.49***	−0.31 ns	0.05 ns	0.11 ns	−0.01 ns	0.01 ns	0.52**	−0.30 ns	−0.57**
Total alkaloid content	−0.18 ns	0.01 ns	**0.42***	−0.03 ns	0.11 ns	0.28 ns	−0.01 ns	0.18 ns	0.18 ns	**−0.43***	0.25 ns	0.12 ns

Note: (− values) = negative correlation; (+ values) positive correlation; ns = not significant ($p > 0.05$); **bold value*** = significant ($p < 0.05$); ** = highly significant ($p < 0.05$).

differently, since they grow in different environmental conditions (soil and climate). Therefore, when eaten, there would be a difference in the degree of reduction of blood sugars in the consumers, and hence the difference the in prevalence of hyperglycemia in the two study sub-regions.

Physical and chemical properties of soil samples from Ankole and Teso sub-regions of Uganda and phytochemical composition of *A. dubius*

Biological activities of plants are a reflection of phytochemicals in them, and these phytochemicals are in turn greatly influenced by the environmental conditions in which plants grow. Physical soil properties provide retention, solubility, and availability of the soil chemical nutrients, and once the latter is absorbed by the plant, it is internally traded off to dictate the carbon–nitrogen balance, which also influences the synthesis and the accumulation of phytochemicals.

In this study, the average pH of soil samples from Teso sub-region is neutral and it is in the alkaline-neutral range (6.2–7.7). This range was different from the one reported by Gachimbi and Maritima,[94] which was extremely acidic (4.5–6.5). Soil pH from Ankole sub-region was in an acidic-neutral range (6.3–6.6), and the difference in soil pH from these sub-regions was significant. It has been reported that soil pH is greatly influenced by the acid-forming cations (H^+, Al^{3+}, $F^{2+/3+}$) and the base-forming cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) in the soil. Due to the relatively lower precipitation in Teso sub-region, there is little leaching of the base cations, resulting in a relatively high degree of their saturation and hence the alkaline pH,[95] recorded in soil samples from Teso sub-region. This alkaline pH could be responsible for the solubility of organic carbon in the soil, which was then absorbed by the plant for the synthesis of carbon-based phytochemicals– polyphenolic compounds (TTC and TPC), that were significantly higher in *A. dubius* samples in Teso sub-region. These phytochemicals are reported to lower blood sugar via various mechanisms of action, for example, free radical scavenging,[96–98] hence the lower prevalence of hyperglycemia reported in Teso sub-region.

Our results show that OM percentage is higher in the soil samples from Teso sub-region; however, the organically bound nutrients (N and P) are instead higher in the soil samples from Ankole sub-region. This irony could mean that the soil samples from Ankole sub-region contain OM in the active pool, that is, still being decomposed by microorganisms, thereby releasing the organically bound nutrients. Contrary to the samples from Teso sub-region, although its OM percentage is significantly higher, it is in the slow and passive pool, which is mainly detritus and partially resistant to microbial decomposition, consequently releasing less organically bound nutrients.[99]

Furthermore, naturally, the accumulation of soil OM is inversely related to altitude. Ankole sub-region is at a higher altitude (1806 m) compared to Teso sub-region (1129 m); this justifies the less OM percentage in the soil samples from Ankole sub-region because low temperatures in higher altitudes impair plant growth and therefore result in less plant residue.[100] Consequently, N and P are released from the soil OM for the plant for the synthesis of the nitrogen-based phytochemicals—alkaloids, that were highly quantified in the vegetable samples from Ankole sub-region. This phytochemical class is not as implicated in the reduction of blood sugars as the phenolic class, and therefore, also not as beneficial in the reduction of blood sugar, and it led to a higher prevalence of hyperglycemia in the residents of Ankole sub-region.

Silt, on the other hand, tends to increase with altitude,[101] and this concept concurs with our results that show a higher silt percentage in soil samples from the Ankole sub-region, which is at a relatively higher altitude compared to the Teso sub-region.

According to the soil textural triangle, the soil textural class for soil samples from Ankole sub-region is sandy clay loam, whereas that from Teso sub-region is sandy loam. Epeju and Rukundo, and the Uganda Investment Authority,[102,103] reported the same soil textural class from Teso sub-region. Muzira et al.[104] also confirm our findings on the textural class of the soils in Ankole sub-region. These classes arise from the significantly different percentages of sand, clay, and silt in the soil samples from these sub-regions. The clay component in the soil improves its nutrient retention ability, whereby the latter are available for plant growth and secondary metabolism.

Phytochemical quantity in aqueous extracts of *A. dubius* from the Ankole to Teso sub-region in Uganda

Reports on the health benefits of vegetables in the genus *Amaranthus* indicate that it is rich in anti-oxidant properties due to the phenolic compounds found in it.[22,96] We quantified TPC, TTC, TSC, TFC and TAC in *A. dubius* samples from Ankole and Teso sub-regions, and TTC and TSC were found to be significantly different in the study sub-regions. TFC and TAC were higher in the Ankole sub-region, whereas TPC was higher in the Teso sub-region, though the difference was not significant. The average quantity of TPC in *A. dubius* in both study sub-regions was 28 mg GAE/g d.wt. This quantity was higher than that in other *Amaranthus* species studied by other researchers. For example, *A. spinosus* contained 25.7 µg GAE/100 g fresh weight (fr.wt), and *A. viridis* contained 43.4 µg GAE/100 g fr.wt, as quantified by Sarker and Oba.[105] Likewise, the average TFC we quantified in *A. dubius* samples was less than that in *A. viridis* (43 µg GAE/100 g d. wt) and in *A. spinosus* (177.6 and 176.1 µg GAE/100 g fr. wt), respectively. All three species, that is, *A. dubius*, *A. viridis*, and *A. spinosus*, are reported to contain less phytochemical quantities compared to the popular drought-resistant *A. tricolor*, which was reported to contain 184 and 335.5 µg/g fr.wt of TPC and TFC, respectively.[106] The difference is probably due to the influence of the adverse drought conditions on *A. tricolor*'s phytochemical synthesis to protect itself. When we further compared our results from *A. dubius* to those from the other frequently eaten leafy vegetable species (*Hibiscus sabdariffa*, *Solanum nigrium*, and *Vigna unguiculata*) in the same study sub-regions,[107] we recorded higher quantities of TPC, TFC, and TTC, that is, 66, 49, and 112 mg/g, respectively, in *Hibiscus sabdariffa* leaf extract. Tsado et al.[108] also extracted even higher quantities in *H. sabdariffa* with the methanol solvent, that is, 51.9, 102.6, 54.8, 67.5, and 121.5 mg/g of TTC, TSC, TAC, TFC, and TPC, respectively. *Solanum nigrium* also contained higher quantities of all these phytochemicals in the aqueous extract, that is, 50, 172, 71, 119, and 81 mg/g of TPC, TTC, TFC, TAC, and TSC, respectively. In *Vigna unguiculata*, its aqueous extract also contained higher TPC, TTC, TFC, and TSC (64, 103, 66, and 126 mg/g, respectively), but lower TAC (45 mg/g). Conversely, Pioltelli et al.[109] quantified very low (0.77 mg/g) amount of TPC in *V. unguiculata* with the same solvent. These differences in the vegetable phytochemical quantities could be due to the influence of environmental conditions (soil and climate), vegetable species, and extraction conditions. Therefore, residents of sub-regions whose climate and soil properties favor the synthesis of phytochemicals, consume the phytochemically-rich vegetables, thereby benefiting from their biological effects (e.g., reduction in blood sugar levels), and reduced prevalence of hyperglycemia in the sub-region.[107]

Relationship between the phytochemical quantities in *Amaranthus dubius* and physico-chemical properties of soil from the Ankole and Teso sub-regions

Results from our study show a linear relationship (in both directions) between phytochemical quantities in *A. dubius* and soil properties from the study sub-regions, and some of these relationships are significant. The quantity of TPC, TFC, and TAC in the samples of *A. dubius* from Ankole and Teso sub-regions was not significantly different. However, the quantity of TTC and TSC was significantly different in both study sub-regions. TTC and TSC are positively correlated with soil pH, and the relationship with TTC was significant. Acidic soils are reported to promote nitrification and carbon substrate utilization, which is characterized by vegetation that produces polyphenolic phytochemicals.[36] This concept is not in accord with our results, which show increasing TTC and TSC with alkaline soils in the Teso sub-region. The negative correlation between TPC and TFC in *A. dubius*, and N in the soil can be explained by the activation of carbon-based phytochemical genes in the plant to produce phenolic compounds. This concept is supported by the carbon/nitrogen balance (CNB) hypothesis.[42] Stewart et al.[43] observed similar results in tomato seedlings, that is, the concentration of phenolic compounds (quercetin and kaempferol) was decreased in response to an increase in nitrogen fertilizer application. On the other hand, the positive relationship between N in the soil and TAC in the vegetable agrees with CNB theory, which states that increased N nutrients promote the synthesis of N-based phytochemicals like alkaloids. TPC and TFC were also noted to positively correlate with clay percentage in the soil, and higher clay

content in the soil was reported to support the accumulation of phenolic compounds in Iris species.[33] Salinity stress is another soil abiotic factor caused by excessive salt ions (Na^+, Ca^{2+}, Mg^{2+}, K^+) in the soil. This condition leads to the accumulation of ROS in the plant, which then induces the production of antioxidant compounds like flavonoids. TFC was higher in *A. dubius* from the Ankole sub-region, and soil samples from this sub-region contained higher Na^+, K^+, and Mg^{2+} concentrations. This relationship between salinity stress and flavonoid phytochemicals was observed by several researchers[56,105,110] in their studies.

The correlation coefficients in our results were medium, and it could be due to the influence of other environmental factors like temperature, soil water, and altitude on the relationship between the vegetable phytochemicals in *A. dubius* and soil properties. Although we did not specifically evaluate these factors, there is documented evidence of their influence on plant phytochemicals. TTC and TSC are both significantly higher in vegetable samples from the Teso sub-region. Knowing that the average annual temperature in the Teso sub-region is higher (25°C) than that (17.2°C) in the Ankole sub-region, the significant difference in the quantity of TTC and TSC in both study sub-regions is not surprising. The above-reported influence of temperature was also reported by Bezruk et al.[27] who found out that growing *Hedera helix* in a warm climate (average of 22°C) does support the accumulation of phenolic components in the leaves. Drought stress from insufficient water in the soil can impair plant growth and induce phytochemical synthesis. The average annual rainfall in the Teso sub-region (1000 mm) is less than that in the Ankole sub-region (1018 mm). *A. dubius* vegetable samples from the Teso sub-region likely suffered water stress that induced the synthesis of polyphenolic phytochemicals like TTC. This finding is corroborative with those from Sarker et al.'s study on drought-resistant *A. tricolor* species that contained higher quantities of polyphenols.[106]

CONCLUSION

Indeed, the phytochemical quantity in a plant does vary depending on geographical location. In this study, we observed the influence of soil properties from different sub-regions on the *A. dubius* phytochemicals. Soils higher in cations, pH, and sand percentage do support the synthesis of polyphenolic compounds (TTC, TPC, and TSC) in the plants. Also, other climatic conditions like higher temperatures, salinity stress, and low precipitation induce the production of the above phytochemicals. Therefore, to enhance and expedite the production of these phytochemicals in vegetables, the above soil and climatic conditions should be guaranteed.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The study was approved by Mbarara University of Science and Technology Research Ethics Committee (MUST-REC) under Protocol number MUST-2021-52 and registered with the Uganda National Council for Science and Technology (UNCST) under registration number HS1840ES.

ORCID

Caroline Asekenye https://orcid.org/0009-0007-7218-2738

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