

Determination of nutritional composition, enzyme inhibitory activity and antioxidant activity of pretreated, dehydrated *Amaranthus viridis* L. Leaves

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Abstract *Amaranthus viridis* L., belongs to the family *Amaranthaceae* is a perennial edible plant, and traditionally used for medicinal treatments in South Asia. Despite the significant uses of the plant, availability of scientific data on plant's biological efficiency is lacking in the scientific domain. There is a higher potential and demand for dehydrated value-added products from the local and international market. Hence, the objective of the research was to determine the impact of pretreatments and dehydration on nutritional composition, phytochemical profile, antioxidant activity, and enzyme inhibitory activity of *A. viridis* leaves. Magnesium oxide 0.1% (w/v), sodium bicarbonate 0.1% (w/v) and potassium metabisulphite 0.5% (w/v) were selected as the best pretreatment based on the experiments. The pretreated leaves were steam blanched (5 min), followed by dehydration at $60 \pm 1^\circ\text{C}$ for 12 hours. Protein ($21.90 \pm 1.01\%$ db), fat ($3.70 \pm 0.02\%$ db), fiber ($5.90 \pm 0.05\%$ db), ash ($11.30 \pm 0.12\%$ db) and carbohydrate ($49.24 \pm 0.08\%$ db) were determined. Data was statistically analyzed using one-way analysis of variance (ANOVA) test at a 95% confidence level ($p < 0.05$). A qualitative phytochemical analysis was conducted. The total phenolic content, 5.89 ± 0.30 mg in Gallic acid equivalents per 1g was determined in pretreated, dehydrated powder. FRAP, DPPH radical scavenging activity and ferrous ion chelating activity of the methanolic extract were 20.22 ± 0.45 mg Trolox equivalents, 1.68 ± 0.07 mg in Trolox equivalents and 3.83 ± 0.06 mg EDTA equivalents per 1g of pretreated dehydrated leaf powder, respectively. Methanolic extract (500 $\mu\text{g/mL}$) showed inhibitory activity of $25.30 \pm 0.60\%$ on 5-lipoxygenase, $30.80 \pm 0.50\%$ on α -amylase and $25.60 \pm 0.40\%$ on α -glucosidase. Inhibitory activity was not detected against lipase, acetylcholine esterase and butyrylcholinesterase enzymes. In conclusion, pretreated, dehydrated, *A. viridis* leaves are recommended as a health beneficial food ingredient.

Keywords: *Amaranthus viridis*, antioxidant activity, biological activity, dehydration, pretreatment

Introduction

Amaranthus viridis, is a short-lived perennial leafy vegetable belonging to the family *Amaranthaceae*. (Haider et al., 2023; Nighitha & Mathew, 2015; Rehman et al., 2023). The genus is a subfamily of *Amaranthoideae*, and order *Caryophyllales*. There are about seventy varieties of annual herbs reported under Genus *Amaranthus* while seventeen of which are edible (Iamónico, 2015; Peter & Gandhi, 2017). Naturally occurring nutraceuticals in *Amaranthus* species act as a defender against chronic illnesses and the plant has been gaining popularity over the past decade. *Amaranthus* species are extensively grown and consumed in Mexico, Central America,

the Philippines, China, India, Indonesia, Malaysia, and Southern and Eastern Africa (Peter & Gandhi, 2017; Sarker & Oba, 2019b; Sreelathakumary & Peter, 1993; Taylor & Emmambux, 2008). The plant possesses a substantial concentration of health beneficial compounds, phytosterols, polyphenols, squalene, vitamins, minerals and saponin (Ahmed et al., 2013; Ashok Kumar et al., 2012b; Haider et al., 2023; Jin et al., 2013; Olawoye & Gbadamosi, 2017; Popoola, 2022a; Sarker & Oba, 2019b). Further, the plant parts are used in traditional medicine to treat bacterial, helminthic, diabetic, viral, and snake poisoning and act as a source of antioxidant due to presence of plant leaf pigments such as betalain, β -cyanin, β -xanthin, carotenoids,



anthocyanin, and chlorophylls (Ashok Kumar et al., 2010; Sarker et al., 2018a, 2018b; Vardhana, 2011). Plants are more readily accessible, less expensive, and comparatively safer than synthetic counterparts and hence extensively used to prepare of plant-derived medicines (Ahmed et al., 2013; Atanasov et al., 2021; Cragg & Newman, 2013).

Fresh leafy vegetables often experience bacterial spoilage, wilting, oxidation of macromolecules and enzymatic browning at the ambient temperature and relative humidity thus deteriorates the molecular structure and its nutritional content (Jiang et al., 2013). Vegetables are transformed into shelf-stable dehydrated forms to prevent spoilage and wastage. The nutritional values of fruits and vegetables can be effectively maintained in its powder form with appropriate packaging (Jiang et al., 2013; Ying et al., 2021). The vegetable powders are widely used in food product formulation and nutrient preservation. This approach has led to the development of innovative products, in which fibre, antioxidants, vitamins, and other beneficial compounds are enriched at a higher level than the average (Bas-Bellver et al., 2020; Neacsu et al., 2015; Rana et al., 2015; Ying et al., 2021).

Dehydration of perishables plants produce using hot air is one technique for reducing moisture (Huang et al., 2009). However, the hot-air drying process lowers the energy efficiency, extends drying time and results in shrinkage, discoloration, oxidation of essential and functional components, and impaired sensory and nutritional properties. (Orikasa et al., 2018). Blanching is a commonly used pre-treatment to inhibit the polyphenol oxidase and peroxidase enzyme activities. Additionally, blanching can enhance the drying process and drying kinetics and improve various quality parameters of agro-food products. (Deng et al., 2019; Xiao et al., 2017).

Nutritional and phytochemical constituents in fresh *A. viridis* plant have been investigated previously (Ahmed et al., 2013; Bang et al., 2021; Kekere & Totolaye, 2020; Nadeeshani et al., 2018; Nkobole et al., 2021; Popoola, 2022a and Sarker & Oba, 2019a). Further, antioxidant activity of different parts of the plant in terms of total phenolic content, DPPH radical scavenging activity, ferrous ion chelating activity and ferric reducing antioxidant power were reported by Popoola, 2022b and Salvamani et al., 2016). The antidiabetic

activity (Helen & Bency, 2019; Nkobole et al., 2021; Oluwagunwa et al., 2021), lipid lowering activity (Ashok Kumar et al., 2012b; Oluwagunwa et al., 2021), anti-inflammatory activity (Haider et al., 2023; Salvamani et al., 2016, 2016) were reported. However, acetylcholinesterase and butyrylcholinesterase inhibitory activity of *A. viridis* was not reported in recent literature. Nevertheless, there was no evidence of available information on research that has been conducted on the nutritional qualities, antioxidant activity, and enzyme inhibitory activity of pretreated and dehydrated powder of *A. viridis*. Thus, the present study was conducted to determine the effect of pre-treatment followed by dehydration on nutritional composition, phytochemical profile, antioxidant activity, anti-diabetic activity, antilipidemic activity, anti-inflammatory and inhibitory activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in *A. viridis* leaves.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu phenol reagent, aluminium chloride, 2,4,6-Tri(2-pyridyl)-striaizine(TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ethylenediaminetetraacetic acid (EDTA), ,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), arachidonate 5-lipoxygenase, linoleic acid, baicalein, gallic acid, dimethyl sulfoxide, were purchased from Sigma-Aldrich. All chemicals used were analytical grade chemicals.

Sample collection and preparation

The fresh *A. viridis* (10 kg) was purchased from a vegetable supplier from Colombo, Sri Lanka and identified by a plant expert at the Department of Applied Sciences in Sri Lanka Institute of Information technology. The leaves were separated carefully and cleaned, followed by steam blanching (5 min). The leaf samples were treated with different concentrations (0.1%, 0.3%, and 0.5%) of Magnesium oxide, sodium bicarbonate and sodium potassium metabisulphite

The treated leaves were dehydrated at 60°C for 12 hours using an air convection dryer (Memmert GmbH, Germany). The dehydrated leaves were ground (Pulverisette 14, Fritsch, Germany) and sieved by passing through a 0.5 mm sieve.

Preparation of the plant leaf extract

The methanol extract of leaves powder was prepared using 100 g leaves powder with 400 mL of methanol, the mixture was stirred overnight at ambient condition 30 ± 2 °C and centrifuged at 4000 rpm for 20 min. The supernatant was separated and the residue was re-extracted twice using the same conditions. The supernatants were combined and evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (R-114, BüchiLabortechnik AG, Switzerland). The solvent-free extracts were stored in airtight glass containers at -20 °C for further analysis.

Determination of proximate composition

Moisture content (oven drying method), protein content (Kjeldahl method; nitrogen to protein conversion factor 6.25), and ash content were determined of the dried leaf powder using the methods described in AOAC hand book (AOAC 2012). Crude fibre content was determined using the Fibertec hot extractor (M6 1020, FOSS, Sweden) (AOAC 2012, 978.10) and crude fat (Soxhtherm, SOX 416, Gerhardt, Germany) using the method in AOAC 2012, 2003.05. Using a method outlined by Sompong et al., 2011 the total carbohydrate content was calculated by subtraction method and values were expressed as percentages in dry weight basis (% db). Each test was performed in triplicates.

Determination of minerals

Mineral content was determined by microwave acid digestion method using MARS 6 - Microwave

Digestion System. Sample (0.5 g) was weighed into the digestion vessel. 10 mL of ultra-pure HNO₃ was added and gently swirled the mixture and was allowed to stand for approximately 15 minutes before closing the vessel. Samples were heated to 200 °C and were held at that temperature for 20 min in MARS 6 - Microwave Digestion System. Using inductively coupled plasma mass spectrometry, minerals were determined. Each test was performed in duplicates with ICP – MS technique.

Determination of total phenolic content

The Folin-Ciocalteu method (Singleton et al., 1999) was used to determine the total phenolic content (TPC). Methanol extract of *A. viridis* (20 µL) was mixed with 70 µL of sodium carbonate solution (10% w/v) and freshly prepared diluted Folin-Ciocalteu reagent (10 times). The mixture was incubated for 30 minutes at ambient temperature and the absorbance was measured at 765 nm. The standard curve ($y = 0.0532x + 0.0339$; $r^2 = 0.9992$) was plotted using gallic acid, and TPC was determined as mg of gallic acid equivalents (GAE) per 1 g of powder % db on a dry weight basis.

DPPH radical scavenging activity

Free Radical Scavenging Activity was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The disappearance of the DPPH radical absorption at a characteristic wavelength was monitored by the decrease in optical density (Blois, 1958). Methanolic leaf extract of 250 µg/mL was mixed with 0.02% w/v DPPH solution and incubated at RT in the dark for 10 min. Trolox was used as the standard. Changes in absorbance of samples were measured at 517 nm. DPPH radical scavenging activity as percentage inhibition was calculated using the below equation.

$$\% \text{ Free radical scavenging activity} = [(Control OD - Sample OD) / Control OD] \times 100$$

Control OD – Absorbance of the methanol control at 517 nm
Sample OD – Absorbance of the sample at 517 nm

Ferrous ion chelating activity

The method described by Carter, 1971 was used to test the ability to chelate ferrous ions. A mixture of 20 µL of 1mM ferrous sulphate solution, 40 µL of distilled water, and 100 µL of *A. viridis* methanolic extract was combined. Following that, 40 µL of a 1 mM ferrozine solution was added, and the mixture was incubated for 10 minutes at room temperature.

At 562 nm, the absorbance values of the sample (As) and control (Ac) were recorded. As a standard, EDTA was used. The FIC activity as a percentage of chelation was determined using below equation. Results were expressed as mg Trolox equivalents (TE)/1 g dry weight of the *A. viridis* powder. Each test was performed in triplicates.

$$\% \text{ Chelation} = [(Control OD - Sample OD) / Control OD] \times 100$$

Control OD – Absorbance of the methanol control at 562 nm
Sample OD – Absorbance of the sample at 562 nm

Ferric reducing antioxidant power (FRAP)

A modified version of Benzine and Szeto's method (Benzie & Szeto, 1999) was used to determine FRAP. Acetate buffer (pH 3.6), ferric chloride (20 mM), and TPTZ (10 mM) were combined in a 10:1:1 ratio to prepare the FRAP reagent, which was then incubated for 10 minutes at 37°C. An absorbance measurement was taken at 600 nm after 20 µl of *A. viridis* extract, 30 µl of acetate buffer, and 150 µl of freshly prepared FRAP reagent were combined and incubated at room temperature for eight minutes.

To plot the standard curve ($y = 0.17x + 0.15$; $r^2 = 1.00$), trolox was used. Ferric reducing antioxidant power was calculated as mg Trolox equivalents per 1 g of *A. viridis* powder on a dry weight basis. Each test was performed in triplicates.

5-Lipoxygenase Inhibitory Assay

The plant extracts' ability to inhibit lipoxygenase was assessed using a slightly modified spectrometric approach as described in previous literature (Perera et al., 2016; Tappel, 1962). Plant extracts were studied within the 50–1000 µg/mL assay concentration range. In brief, 10 µL of plant extracts dissolved in methanol, 55 µL of 5-lipoxygenase solution, and 110 µL of sodium phosphate buffer (100 mM, pH 8.0) were mixed and incubated for 10 min at 25 °C. Following that, 25 µL of linoleic acid (0.08 mM) solution was added to initiate the reaction. After the formation of hydroperoxy octadeca-9,11-dienoate (9Z,11E)-

(13S)-13, the change in absorbance at 234 nm was observed for 10 minutes at 25°C.

Percentage inhibition of 5-Lipoxygenase was determined by comparison of rates of reaction of samples relative to control using the below formula.

$$\% \text{ Inhibition} = \frac{E - S}{E} \times 100$$

E - Absorbance at 234 nm of enzyme without test sample

S - Absorbance at 234 nm of enzyme with test sample

IC₅₀ values, by which, the inhibitory concentration of the extracts necessary to decrease the enzyme activity by 50% were determined from the plotted graphs of enzyme inhibition (%) against the concentrations of the extracts. Baicalein was used as the reference standard. Each test was performed in triplicates.

Alpha-Amylase Inhibitory Assay

Bernfeld's method was used to study the in vitro amylase inhibition of *A. viridis* extract (Bernfeld, 1955). Briefly, 200 µL of α-amylase enzyme and 100 µL of 2 mM phosphate buffer (pH-6.9) were allowed to react with 100 µL of the test extract. After the incubation period of 20 minutes, 100 µL of 1% starch solution was added. The same procedure was used for the controls, where a buffer was used in place of 200 µL of the enzyme. Dinitrosalicylic acid (500 µL) reagent was added to the control and test samples after five minutes.

Samples were kept in the boiling bath for five minutes. Absorbance at 540 nm was measured, and the below formula was used to determine the % inhibition of the α -amylase enzyme. At the same

time, reagent blank and inhibitor controls were carried out.

$$\% \text{ Inhibition} = [(Control OD - Sample OD) / Control OD] \times 100$$

Control OD – Absorbance of the methanol control at 540 nm

Sample OD – Absorbance of the sample at 540 nm

Plots of percentage inhibition against inhibitor concentration were used to determine the IC_{50} values, which were subsequently calculated using non-linear regression analysis based on the mean inhibitory values. Acarbose was used as the standard drug. Each test was performed in triplicates.

Alpha-glucosidase Inhibitory Assay

The α -glucosidase inhibition was measured by modifying a method described in previous literature (Matsui et al., 1996). The α -glucosidase reaction mixture included 1.0 U/mL α -glucosidase in sodium phosphate buffer (pH 6.9), 2.9 mM p-nitrophenyl- α -glucopyranoside (pNPG), and

varied concentrations (0.5 mg/mL to 2 mg/l) of *A. viridis* extract and the mixtures were incubated for five minutes. Acarbose substituted for the sample extract in positive controls, while DMSO, enzyme, and substrate were the only components in the control samples. Acarbose, sample extract, and enzyme-free mixtures were used as blanks. The reaction mixtures were boiled for two minutes to cease the reaction, which was then incubated for five minutes at 25 °C. Using a spectrophotometer, the absorbance of the resultant p-nitrophenol (pNP) was measured at 405 nm and was found to be directly correlated with the enzyme's activity. Below formula was used to determine the % inhibition of the α -amylase enzyme.

$$\% \text{ Inhibition} = [(Control OD - Sample OD) / Control OD] \times 100$$

Sample OD - Absorbance in the presence of test sample at 405 nm

Control OD - Absorbance of control at 405 nm

Plots of percentage inhibition against inhibitor concentration were used to determine the IC_{50} values, which were subsequently calculated using non-linear regression analysis based on the mean inhibitory values. The reference drug for the alpha glucosidase inhibition experiment was acarbose. Each test was performed in triplicates.

Acetylcholinesterase and Butyrylcholinesterase Inhibitory Assay

The BChE and AChE inhibitory assays were carried out with slight modifications in accordance with (Ellman et al., 1961). The total volume of 200 μ L reaction mixture, included plant extract in appropriate volumes in varied concentrations

ranging from 50–500 μ g/mL, 10 μ L of AChE (0.002 U/mL) or 4 μ L of BChE (0.5 U/mL), and 0.1 M sodium phosphate buffer (pH 8.0). The reaction was pre-incubated for 15 minutes at 25 °C. Next, 10 μ L of either butyrylthiocholine iodide (0.71 mM or 8 mM, respectively) or acetylthiocholine iodide (0.71 mM) and 0.5 mM DNTB were added to 20 μ L of 0.1 M sodium phosphate buffer (pH 7) to initiate the reaction.

The absorbance of the yellow coloured 5-thio-2-nitrobenzoate anion formation was measured at 412 nm in ten minutes. The blank was the incubation mixture without the addition of enzymes and samples. Galathemine was used as the standard drug and all tests were performed in triplicates.

$$\% \text{ Inhibition} = [(Control OD - Sample OD) / Control OD] \times 100$$

Control OD – Absorbance of the methanol control at 412 nm

Sample OD – Absorbance of the sample at 412 nm

Lipid lowering (anti-lipase) assay

Porcine pancreatic lipase (PPL) activity was assessed using p-nitrophenyl butyrate (PNPB) as a substrate. The technique for assessing pancreatic lipase activity was adapted from the one described by Kim et al., 2010. A 0.1 mM potassium phosphate buffer solution with pH 6.0 and PNPB was made by diluting a stock solution with acetonitrile to a final volume of 10 mL. A solution of porcine pancreatic lipase (PPL type II-Sigma) was prepared by dissolving 10 mg of the enzyme in 10 mL of buffer solution (1 mg/mL) by gentle mixing, immediately prior to utilisation. Positive control Orlistat was prepared at different concentrations using DMSO. Similar concentrations were prepared for the leaf extracts of *Amaranthus viridis*. Porcine pancreatic lipase activity was assessed using PNPB as a substrate. PPL stock solutions at a

concentration of 1 mg/mL were made in a 0.1 mM potassium phosphate buffer with a pH of 6.0 and kept at -20 °C.

To evaluate lipase inhibitory action, extracts at different concentrations, or Orlistat at the same concentrations as a positive control, were mixed with PPL and incubated for 1 hour in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before measuring PPL activity. The reaction initiated with the addition of 0.1 µL pNPB substrate in a final volume of 100 µL. The amount of p-nitrophenol produced in the reaction was measured at 405 nm using a UV-Visible spectrophotometer after being kept at 30 °C for 5 minutes. The negative control's activity was determined both with and without an inhibitor. The inhibitory activity was determined using the following formula:

$$\% \text{ Inhibition} = 100 - [(B - b)/(A - a) \times 100]$$

A - Absorbance at 405 nm of the activity without inhibitor,

a - Absorbance at 405 nm of the is the negative control without inhibitor,

B - Absorbance at 405 nm of the activity with inhibitor,

b - Absorbance at 405 nm of the negative control with inhibitor. DMSO was used as a negative control.

Qualitative estimation of phytochemicals

Phytochemical Screening

Extracts were analyzed for the detection of the presence of Alkaloids, Flavonoids, Phenols, Saponins, Tannins and Glycosides according to the previous literature (Bhandary et al., 2012; Harborne, 2005) with slight modifications.

Statistical analysis

Data were statistically analyzed using SPSS Statistics (Version 20) and results were expressed as mean ± standard error (SE). Confidence level of 95% was set as the Statistical significance. One way

analysis of variance (ANOVA) was used to determine the differences among the samples.

Results and Discussion

Pretreatment of the A. viridis leaves

Dipping in alkali solutions facilitates the removal of the wax layer or disrupts the microstructure of the outer wax layer (Bingol et al., 2012; Doymaz & Pala, 2002) for an effective drying process. Furthermore, this improves the permeability of the plant skin to moisture, enables moisture diffusion and improves the dehydration rate (Doymaz & Altiner, 2012). In a prior research evaluating the impact of cabinet drying at 40 °C – 50 °C at 1.1 m/s

drying air velocity, on the nutritive value and dehydration kinetics of fenugreek leaves, the use of chemical pretreatment with magnesium oxide enabled the retention of a higher percentage of nutritious components throughout the drying process (Bishnoi et al., 2020). A different study examining the impact of pre-treating broccoli dehydrated at 50 °C for 8 h, revealed that the use of sodium bicarbonate resulted in improved preservation of both total chlorophyll and colour characteristics in dried broccoli (Kaur et al., 2018). In a similar study conducted with green leafy vegetables dehydrated at 60 °C for 8 - 10 h, it showed that magnesium oxide and sodium bicarbonate had a similar effect on colour retention in the drying process (Gupta & Prakash, 2008). The use of sulfites at low concentration effectively inhibits both enzymatic and non-enzymatic browning as well as microbiological activity (Joslyn & Braverman, 1954). KMS has been used for the pretreatment at a concentration of 6% for a duration of 60 minutes which effectively preserved the quality of dried apricots throughout 12 months of ambient storage and resulted in greater levels of total carotenoids and ascorbic acid, while also reducing non-enzymatic browning (Mir et al., 2009). In the current study magnesium oxide, sodium bicarbonate and potassium metabisulfite in different concentrations varying from 0.1% to 0.5% were used to pretreat the leaves with the objective of improving drying process and overall quality of the leaves. It was optimized to 0.1% magnesium oxide, 0.1% sodium bicarbonate and potassium 0.5% metabisulphite.

Proximate composition

Moisture, protein, crude fat, crude fibre, ash, total carbohydrate and energy contents of pretreated *A. viridis* leafy powder are given in Table 1. The protein concentration of *A. viridis* powder was found to be highest compared to other metrics, suggesting that it might serve as a good protein source. When compared to a previous study conducted by Uwah et al., 2016 on dried *Amaranthus* leaves it was found that protein level of the pretreated *A. viridis* powder (21.9 ± 1.01) was significantly ($P < 0.05$) higher than the non-treated

A. viridis powder (18.54 ± 0.02 %) on dry weight basis. Crude fat was 3.70 ± 0.02 % and was significantly ($P < 0.05$) higher than the non-treated *A. viridis* powder (0.9 ± 0.03 %) on dry weight basis. Ash content, which indicated the mineral content, was 11.30 ± 0.12 % on a dry weight basis. Ash content of non-treated powder was recorded to be 17.19 ± 0.32 % which was comparable (17.84 ± 0.02 %) with a similar previous research (Uwah et al., 2016). Fibre content was 5.9 ± 0.05 % on dry weight basis and lower than the same of non-treated powder as recorded by Uwah et al., 2016. Calculated total carbohydrate content was 49.24 ± 0.08 % on dry weight basis whereas it was reported to be 6.46 ± 0.08 % on fresh weight basis in a similar study conducted by Nadeeshani et al., 2018.

There have been numerous studies on the proximate composition of *Amaranthus* species, particularly in India and Africa (Kekere & Totolaye, 2020; Nadeeshani et al., 2018; Sarker & Oba, 2019b; Sompong et al., 2011; Uwah et al., 2016). According to Sarker & Oba, 2019b, moisture, fat, carbohydrate, ash, dietary fiber and protein contents in *A. viridis* species ranged from 80.35 ± 1.14 % - 81.54 ± 1.18 %, 0.28 ± 0.04 - 0.42 ± 0.03 %, 6.31 ± 0.06 - 8.67 ± 0.07 %, 5.43 ± 0.04 - 6.86 ± 0.02 %, 9.17 ± 0.37 - 9.38 ± 0.35 % and 4.12 ± 0.05 - 4.52 ± 0.04 on fresh weight basis, respectively. In a previous study which was conducted to evaluate the effect of different drying techniques it was found out that air-dried plant materials retained the highest contents of ash (6.87 ± 0.02 %), crude fibre (7.15 ± 0.00 %), lipid content (2.93 ± 0.01 %), crude protein (14.03 ± 0.01 %) and carbohydrate (62.85 ± 0.04 %) on dry weight basis (Kekere & Totolaye, 2020). These fluctuations in the nutritional composition reported in literature may be attributed to soil quality and meteorological factors, including solar radiation, temperature, atmospheric pressure, wind, humidity, and rainfall.

Overall, the proximate composition values were comparable to those found in earlier studies, suggesting that the pretreatment used in this investigation has substantially enhanced the protein and fat levels in the dehydrated *A. viridis* leaves compared to untreated dehydrated leaves.

Table 1: Nutritional composition of the non-pretreated, dehydrated and pretreated, dehydrated *A. viridis* leaves powder

Constituent	Quantity (% db) – non-pretreated, dehydrated and <i>A. viridis</i> leaves powder	Quantity (% db) – pretreated, dehydrated <i>A. viridis</i> leaves powder
Moisture	5.49 ± 0.27	5.46 ± 2.31
Protein	18.25 ± 0.14	21.90 ± 1.01
Fat	0.9 ± 0.03	3.70 ± 0.02
Fibre	5.78 ± 0.17	5.90 ± 0.05
Ash	17.19 ± 0.32	11.30 ± 0.12
Total carbohydrate	52.52 ± 0.05	49.24 ± 0.08

Results are presented as mean ± SE (n=3). Values are presented on dry weight basis.

Mineral composition

Mineral contents of the non-pretreated, dehydrated and pretreated, dehydrated *A. viridis* leaf powder are given in Table 2. There were significant differences ($p < 0.05$) between the mineral contents of the non-pretreated and the pretreated dehydrated leaves powder. Steam blanching of plant leaves at high temperatures has caused a degradation of minerals, leading to a considerable decrease in minerals in the pretreated dehydrated leaves. Blanching food inevitably alters its nutritional characteristics due to the heat it undergoes and causes the loss of some minerals, water-soluble vitamins, and other water-soluble components. Losses mostly result from leaching, heat degradation, and to a lesser degree, oxidation

(Deak, 2013). There were significant differences ($p < 0.05$) among the mineral contents of the pretreated *A. viridis* powder against the non-pretreated powder. The results were comparable with Pradhan et al., (2015) and (Uwah et al., 2016), where it was reported magnesium, calcium and potassium contents were 0.39 ± 0.01 , 0.18 ± 0.03 and 0.14 ± 0.02 mg/g, respectively. The values of the present research were significantly higher than the aforementioned. Mineral content of non-dehydrated, non-pretreated *A. viridis* leaves (Nadeeshani et al., 2018) was significantly lower ($P < 0.05$) than pretreated, dehydrated, leaves powder. It was evident that pretreatment has successfully retained the mineral content such as magnesium, calcium and potassium of the dehydrated leaves powder.

Table 2: Mineral content of the pretreated, dehydrated *A. viridis* powder.

Mineral constituent	Quantity (% db) – Non-	Quantity (% db) – Pretreated,
Fe (mg/kg)	400	67
Ca (mg/g)	19	20
Mg (mg/kg)	1100	384
K (mg/g)	27	8.3

Antioxidant activity

Total phenolic content, DPPH radical scavenging activity, ferrous ion chelating activity and ferric reducing antioxidant power, per 1g of pretreated, dehydrated *A. viridis* is shown in Table 3. Oxidative stress is characterized as a state of imbalance between the presence of pro-oxidants and

antioxidants, leading to harmful effects on molecules and cells. Oxidative stress is a pivotal factor in the progression of age-related illnesses. (Tan et al., 2018). According to the results *A. viridis* powder may be utilized and added as a food ingredient to reduce the detrimental oxidative stress developed in cells of body tissues.

Table 3: Total phenolic content, DPPH radical scavenging activity, ferrous ion chelating activity and ferric reducing antioxidant power, per 1g of the pretreated, dehydrated *A. viridis*

Total phenolic content Gallic acid equivalents /1 g of powder	DPPH radical scavenging activity Trolox equivalent	Ferrous ion chelating activity EDTA equivalents	Ferric reducing antioxidant power Trolox equivalent (to reduce ferric ions)
5.89 ± 0.30 mg	1.68 ± 0.07 mg	3.83 ± 0.06 mg	20.22 ± 0.45mg

Total phenolic content

The total phenolic content (TPC) of the methanolic extract of *A. viridis* was found to be 5.89 ± 0.30 mg in Gallic acid equivalents per 1g of dehydrated, pretreated leafy powder of *A. viridis*. Results are presented as mean ± SE (n = 3) on a dry weight basis. Previous studies have been conducted on the total phenolic content of fresh *A. viridis* leaves. For example, Tatiya et al., 2007 reported that TPC of *A. viridis* were 25.7- 43.4 µg GAE/g on fresh weight basis. According to another study conducted by Bang et al., 2021 the TPC of fresh *A. viridis* leaves ranged from 94.1 mg GAE/g to 111.7 mg GAE/g on fresh weight basis on two different growth years; 2018 and 2019 (Bang et al., 2021). However, the TPC for dehydrated pretreated *A. viridis* has not been conducted previously. TPC generally contributes to antioxidant activity as free radical scavengers, chain breakers, or electron donors (Kainama et al., 2020). Reactive nitrogen species and ROS such as OH⁻, O₂⁻, NO⁻, or OONO⁻ are significantly reduced by polyphenols, protecting biomolecules from damage and reducing the production of new reactive ROS for subsequent chain reactions and damage (Muzolf et al., 2008; Visioli et al., 1998). The observed total phenolic content in pretreatment, dehydrated *A. viridis* suggests that it has the capacity to protect

biomolecules from degradation and decrease the formation of new reactive ROS, which might trigger further chain reactions.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging ability was 1.68 ± 0.07 mg in Trolox equivalents per 1 g of the methanol extract of *A. viridis* powder. Results are presented as mean ± SE (n = 3) on a dry weight basis. De Mello Andrade & Fasolo, 2014 reported that the average DPPH radical scavenging ability varied from 1.1 to 75.2 mg AAE/g on 120 different *Amaranthus* species tested. In accordance with that Ahmed et al. reported that *A. viridis* plant leaves extracted with 100% methanol and 80% methanol showed 50% DPPH inhibition at 14.27 ± 2.2 µg/mL and 83.45a ± 3.87 µg/mL, respectively (Ahmed et al., 2013). According to another study which was comparable with the current study, it was reported that three different genotypes of *A. viridis* leaves extracted with methanol had DPPH radical scavenging ability in terms of Trolox equivalents, which ranged from 21.96 ± 0.14 to 24.65 ± 0.12 µg/g. Hence, the pre-treatment procedure has shown a preventive impact on the deterioration of antioxidants in plant extracts.

Ferrous ion chelating activity

Iron and other transition metal ions play a crucial role as an efficient catalyst for the formation of free radicals during the radical chain reaction's initial stages.

The study evaluated the FIC characteristics in the concentration range of 250–1000 µg/mL. Each extract exhibited ferrous ion chelating capabilities. EDTA equivalents to chelate ferrous ions per 1g of pretreated *A. viridis* powder was 3.83 ± 0.06 mg. The ferrous chelating activity increased from $10.11 \pm 0.64\%$ to $37.23 \pm 0.71\%$ from 250 µg/mL to 1000 µg/mL. In another study conducted on *A. viridis* seeds the ferrous ion chelating activity varied from 2.52 mg to 7.06 mg of EDTA per gram of extract (Popoola, 2022b). Typically, the reactive oxygen species (ROS) produced in humans are eliminated by the presence of antioxidants in the body, resulting in an equilibrium between the created ROS and the available antioxidants. However, due to excessive generation of reactive oxygen species (ROS) or insufficient antioxidant defence, this equilibrium is disrupted, leading to a rise in ROS levels that ultimately result in oxidative stress (Ebrahimzadeh et al., 2008). Hence the addition of dehydrated, pretreated powder to diet would reduce the redox imbalance and provide antioxidant defence against oxidative stress.

Ferric reducing antioxidant power (FRAP)

The antioxidants present in the plant extracts take part in a redox linked colourimetric reaction and donate electrons to the Ferric- TPTZ (Fe (III)-TPTZ) complex, forming blue coloured Ferrous-TPTZ (Fe (II)-TPTZ) complex (Guo et al., 2003). The FRAP 20.22 ± 0.45 mg Trolox equivalents per 1 g of dehydrated pretreated *A. viridis* powder. In a previous study (Alvarez-Jubete et al., 2010) conducted on different parts of *A. viridis* plant, it was shown that seeds, sprouts and breads exhibited 0.55 ± 1.6 mg, 1.22 ± 11.1 mg and 0.60 ± 6.2 mg Trolox equivalents per 1 g on dry weight basis, respectively. Compared with this study, the methanolic leaf extracts showed significantly higher FRAP indicating the antioxidant potential as reducing agents ($p < 0.05$). The effect of thermal processing (autoclaving and blanching) on *A.*

viridis seeds were studied by Olawoye & Gbadamosi, 2017. Ferric reducing antioxidant power (FRAP) of autoclaved flour, germinated flour, fermented seed, blanched flour, whole flour and defatted flour was studied, and values were 0.94 ± 1.32 , 1.63 ± 0.90 , 1.533 ± 0.36 , 1.24 ± 0.74 , 1.45 ± 0.83 and 1.729 ± 0.94 µg/g of ascorbic acid equivalents, respectively. Subsequently it was concluded that thermal processing can significantly reduce the FRAP of the amaranth seeds.

Enzyme Inhibitory Activity

Anti-arachidonate 5-Lipoxygenase activity

The assay measures the plant extracts' inhibitory activity against A5-LOX, a vital enzyme that is essential for the synthesis of inflammatory lipid mediators, including hepoxilins, lipoxins, leukotrienes, and other derivatives of hydroxylated fatty acids (Perera et al., 2016).

Current study on the inhibitory activity of lipoxygenase showed that extracts from *A. viridis* inhibited the enzyme in a manner that was dose dependent. The anti-arachidonate 5-Lipoxygenase activity of the methanolic extracts of dehydrated pretreated *A. viridis* powder is given in figure 1. The test concentrations varied at 50, 100, 500 and 1000 µg/mL. The values were 0.0%, $10.2 \pm 0.8\%$, $25.3 \pm 0.6\%$ and $32.4 \pm 0.8\%$ respectively. The anti-arachidonate 5-Lipoxygenase activity significantly increased ($P < 0.05$) from 50 µg/mL to 1000 µg/mL. In a similar study, the methanolic leaf, seed and stem extracts were tested to determine the anti-arachidonate 5-Lipoxygenase activity. At 500 µg/mL, the leaf extract showed the maximum inhibition, 82%, followed by the seed (60%) and stem (35%). Leaf extract and quercetin had IC_{50} values of 151.59 µg/mL and 98.36 µg/mL, respectively (Salvamani et al., 2016). Therefore, it suggests that the dehydrated powder's thermal treatment may have contributed to the enzyme inhibitory response, reducing the amount of bioactive substance in the plant material leading to decreased inhibitory activity of lipoxygenase. Nevertheless, the investigation of the anti-arachidonate 5-lipoxygenase activity of *A. viridis*'s powder has not been conducted before.

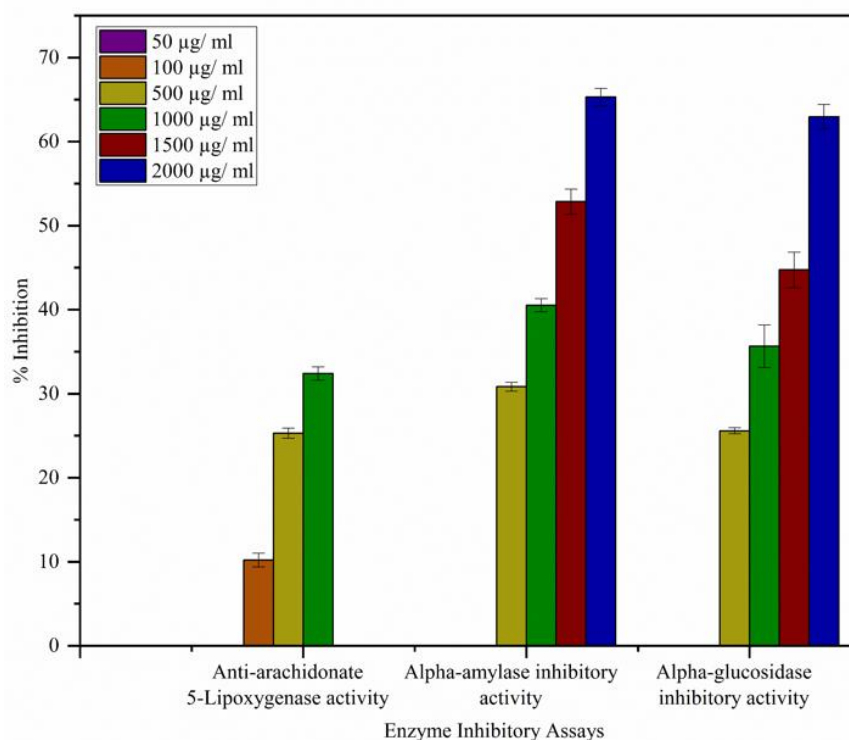


Figure 1: Anti-arachidonate 5-Lipoxygenase activity, alpha-amylase inhibitory activity and alpha-glucosidase inhibitory activity of the methanolic extract of pretreated dehydrated leaves powder of *A. viridis*

Alpha-Amylase Inhibitory Assay

Inhibiting α -glucosidase and α -amylase is one of the therapeutic strategies being used in the management of type 2 diabetes to reduce the intestine's ability to reabsorb glucose (Sim et al., 2010). The pancreas and salivary gland produce alpha-amylase (α -1,4-glucan-4-glucanohydrolases), which is a widely known secretory product that initiates the hydrolysis of complex carbohydrates into a combination of oligosaccharides and disaccharides in the intestinal mucosa (Yamakoshi, 2014). Alpha-glucosidase breaks down these sugars further into monosaccharide (Galasko, 2017). The side effects of the alpha-amylase and glucosidase inhibitors now used in clinical practice, including hypoglycemia, diarrhea, flatulence, and colon bloating, restrict their application in the management of diabetes and its consequences (Oyedemi et al., 2017).

The current study showed that the dehydrated, pretreated *A. viridis* powder in all tested concentrations retained α -amylase inhibitory

activity ranging from $30.83 \pm 0.54\%$ to $65.30 \pm 1.03\%$. The *A. viridis* extract inhibited the α -amylase activity in a dose dependent manner, significantly increasing ($P < 0.05$) the inhibitory activity with increasing concentrations. The results are shown in graph figure 1. However, the IC_{50} value of the leaf extract was $1366 \pm 29 \mu\text{g/mL}$, whereas the IC_{50} value of the standard drug ascarbose was $140.6 \pm 2.4 \mu\text{g/mL}$.

A few studies have been conducted previously on the α -amylase inhibitory activity of *Amaranthus* species. It was shown that the methanolic extract of *A. viridis* leaves had the maximum glucose retention ability 47.05%, at $40.125 \mu\text{g/mL}$ in a dialysis tube, which simulates the human gut (Helen & Bency, 2019). In the same study, it was reported that the water extract of *A. viridis* stem had exhibited the greatest inhibitory impact on α -amylase activity (100%) compared to other extracts, with an IC_{50} value of $(5.058 \pm 0.41 \mu\text{g/mL})$, after 15 minutes of incubation. Oluwagunwa et al., 2021 reported that the aqueous extracts of *A. viridis* showed 68.45 % of α -amylase inhibitory activity at 2.3 mg/mL which was comparable with the current

study's results in spite the difference of the extraction solvent. Even though dehydrated powder's α -amylase inhibitory action has been lowered owing to the loss of biological activity brought on by degradation, it has retained substantial inhibitory activities to act as an α -amylase inhibitor that may work against diabetes mellitus.

Alpha-glucosidase Inhibitory Assay

Complex carbohydrates are converted to a combination of oligosaccharides and disaccharides by the action of alpha-amylase in the intestinal mucosa. Alpha-glucosidase breaks down these sugars further into monosaccharides (Oyedemi et al., 2017). The medicinal plant products could be a source of novel molecules for diabetes treatment, which could result in a more affordable option with less chance of adverse effects (Grover et al., 2002). Herbal supplements with high antioxidant activity have been demonstrated to have positive effects on pancreatic β cells in diabetic patients by avoiding or postponing beta cell dysfunction in response to glucose toxicity (DeFronzo, 2004). The alpha-glucosidase inhibitory activities of the methanolic extracts of dehydrated, pretreated *A. viridis* leaves are given in figure 1. All the test concentrations from 500 $\mu\text{g/mL}$ to 2000 $\mu\text{g/mL}$ were capable of inhibiting the alpha-glucosidase enzyme in a dose dependent manner. IC_{50} value of the leaf extract was $1574 \pm 57 \mu\text{g/mL}$, whereas the standard drug acarbose's IC_{50} value was $140.6 \pm 2.4 \mu\text{g/mL}$.

It was reported that, at the lowest tested concentration of 0.125 mg/mL, wild *A. hybridus* had strong α -glucosidase enzyme activity ($89.92 \pm 0.04\%$), whereas acarbose showed an inhibition of $80.20 \pm 0.13\%$ at the same concentration. Cultivated *A. hybridus* showed inhibitory activities of $72.28 \pm 0.06\%$, while cultivated *A. cruentus* extracts inhibited α -glucosidase enzyme activity ($84.95 \pm 0.04\%$) at the lowest tested concentration (Nkobole et al., 2021). Compared with the aforementioned results it is evident that different varieties of *Amaranthus* plant may possess different α -glucosidase enzyme inhibitory activities. The genomic diversity of different species within the genus *Amaranthus* may be the primary reason to demonstrate different biological properties.

Acetylcholinesterase and Butyrylcholinesterase Inhibitory Assay

When treating Alzheimer's symptoms, inhibition of cholinesterase activity is crucial. Potential inhibitors of the enzyme, which raise acetylcholine levels in cholinergic synapses and enhance neurotransmission, must be included in the patient's diet (Turkiewicz et al., 2019). To study the inhibitory activity against acetylcholinesterase and butyrylcholinesterase, plant extracts were tested at three distinct concentrations: 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$. Prior studies on the inhibitory action of acetylcholinesterase and butyrylcholinesterase of *A. viridis* are very limited. In contrast to the inhibitory activities reported in literature for different kinds of extracts of *Amaranthus* species, the methanol extract of *A. viridis* possessed no inhibitory activity for the tested concentrations. IC_{50} value of the standard drug, galathemine, was $0.58 \pm 0.01 \mu\text{g/mL}$ for acetylcholinesterase and $3.99 \pm 0.25 \mu\text{g/mL}$ for butyrylcholinesterase. A previous study (Hupparage et al., 2020) the IC_{50} value of *A. tricolor* ethanol extract of leaves, was determined to be 193.9 $\mu\text{g/mL}$. Therefore, the dehydrated powder has likely lost its inhibitory activities against acetylcholinesterase and butyrylcholinesterase owing to the heat deterioration that damaged its biological activity.

Lipid lowering (anti-lipase) assay

Lipase inhibitors are useful treatments for obesity and hyperlipidemia due to their ability to lessen the absorption of dietary lipids (Elbashir et al., 2018). In an in vitro study conducted by Oluwagunwa et al., 2021 the aqueous extract of *A. viridis* has shown 50% inhibition against pancreatic lipase at 1.038 mg/mL and has shown inhibitory activity in a dose dependent manner for the tested concentrations. In an in-vivo study it was found that the mice groups administered with methanol extract *A. viridis* exhibited a significant reduction ($P < 0.01$) in total cholesterol levels at a dosage of 400 mg/kg (Girija & Lakshman, 2011). Nevertheless, no inhibitory effect on lipase activity was observed in the methanolic extracts of dried, pretreatment *A. viridis* leaves at doses of 100 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, and 1000 $\mu\text{g/mL}$. The findings align with previous studies, which discovered that the methanolic extracts of *A.*

viridis had anti-lipase activity, but only in much higher doses. Furthermore, a certain proportion of the bioactive compounds likely underwent degradation during thermal processing, resulting in a decrease in anti-lipase action.

Qualitative phytochemical screening

The results are shown in table 4. The presence of phenols, saponins, flavonoids, alkaloids, glycosides, steroids, reducing sugars, carbohydrates and amino acids in the methanolic leaf extract was consistent with findings of a similar studies (Ashok Kumar et al., 2012a; Kumari et al., 2018). Phytochemical compounds, have shown considerable pharmacological benefits, including antiviral, anti-inflammatory, antioxidant, antibacterial, antimutagenic, and chemo-preventive activity, even at lower concentrations (Kumari et al., 2016; Sarma et al., 2016; Sharma et al., 2018).

Table 4: Phytochemical evaluation of pretreated and dehydrated *A. viridis* leaf extracts

Phytochemical constituent	Inference
Phenols	+
Tannins	-
Reducing sugars	+
Terpenoids	-
Saponins	+
Amino acids	+
Carbohydrates (Iodin test)	+
Flavonoids	+
Alkaloids	+
Glycosides (Liebermann's test)	+
Steroids	+

Conclusion

To the best of our knowledge, this is the first study to report nutritional composition, enzyme inhibitory activity and antioxidant activity of dehydrated, pretreated *Amaranthus viridis* L. leaves. Findings of the present study indicate that the developed powder is a good sources of proteins, crude fat as well as minerals (especially calcium) while low in dietary fibre when compared to commonly consumed non-dehydrated leaves of *Amaranthus viridis* L. The nutritional, antioxidant and enzyme inhibitory information obtained from the current

study expands the current knowledge on the nutritional composition and biological activity of the pretreated, dehydrated *A. viridis*, and acts as a useful reference in selecting *A. viridis* for daily consumption for its antioxidant, anti-inflammatory and anti-diabetic activity.

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Author contribution

Madara Jayanetti gave the contribution for the data gathering, application of statistical analysis, investigation process, finding a methodology, and coordination responsibility for writing the original research paper.

Palitha C. Arampath involved in the process of finding the research concept, research planning, supervision and editing the manuscript

Charitha Jayaruk Thambiliyagodage involved in funding acquisition for the research, finding a methodology, supervision of the research study and project administration.

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Conflicts of Interest

Authors declare no conflicts of interest

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