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PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT POTENTIAL OF AMARANTHUS VIRIDIS

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ABSTRACT: ***Phytochemical composition like physical & biological composition, Amino acids profile, Antioxidant profile, mineral content and of dried leaves of Amaranthus Virdis were evaluated using standard methods of analysis. The leaves had the following proximate compositions on dry weight ash 21.05%, Total protein of 23.33 ± 3.17 g/100g, Total lipid content of 5.26 ± 0.30g/100g , total dietary fiber of 14.04 ± 0.35g/100g, total carbohydrate of 46.58 ± 1.49 g/100g and total calorific value of 251.17 ± 1.26 kcal/100g. The leaves boast high levels of K, Mg. Tannins and phytates were the main antinutrients, alongside notable amounts of total and soluble oxalates. The amounts of FRAP 21.7 ± 0.80 mg AA/g , Vitamin C 21.41±1.32g/100g , Total phenolics content (TPC) was found (5.12±0.6 mg GAE/g) , Total flavonoid content (TFC) was 17.5±0.36 mg RE/g dw , The 2,2 – diphenyl-1-picrylhydrazyl (DPPH) was 67.45 ± 1.95 % , 62.33± 2.51 mg/100g of sodium and potassium value of 2391.33 ± 1.52 mg/100g were below the critical values that show it was a good source of antioxidants.***

KEYWORDS: ***Leafy Vegetables, Amaranthus Virdis, Nutrients, Antioxidant, Antinutrient***

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1. INTRODUCTION

Amaranthus viridis, commonly known as amaranth, had belonged to the Amaranthaceae family, encompassing 60–75 species (Viljoen et al., 2018). It consists of approximately 60–70 species grouped into three sub-genera: *Amaranthus Albersia* (vegetable amaranth), *Amaranthus Amaranthus* (cultivated grain amaranth), and *Amaranthus Acnida* (weedy amaranth) (Jamalluddin et al., 2022). It also had high genetic and phenotypic diversity, which may provide an excellent opportunity for varietal development with increased drought tolerance characteristics (Jamalluddin et al., 2022). Amaranth had been distinguished by species cultivated for grain, leafy vegetables, and ornamentals, broadly categorized as leafy greens or grain types. Leaves are excellent sources of minerals (manganese, copper, zinc, phosphorus, iron, potassium, magnesium, and calcium), protein with essential amino acids (lysine and methionine), and dietary fiber (Sarker et al., 2014). Grain amaranths resembled as small cereals, with protein-packed seeds cooked like rice, popped like popcorn, and valued as staples in ancient civilizations, including India, despite recent healthy food popularity (Massey et al ., 2018) . In India, *Amaranthus viridis* had been widely cultivated in 17 states like Jammu & Kashmir, Gujarat, and Tamil Nadu, though national production data is unavailable (Rahman et al ., 2025) . Cosmopolitan across tropical and temperate zones, the genus includes annuals, short perennials, vegetables, pseudocereals, ornamentals, and weeds. Plants had produced catkin-like flower clusters in summer/autumn, varying in green-to-crimson hues, growing 1–2.5 m tall on fibrous stems. About 10 of 75 species were dioecious North American natives; others were monoecious worldwide except Antarctica (Steckel et al., 2021). Named from Greek amárantos ("unfading") for persistent flowers, grain types have grown in the 1970s–80s in the U.S. states like Nebraska and are ongoing in China (150,000 ha for forage). Leafy types (*A. dubius, A. cruentus, A. tricolor*) had been consumed in Africa, Asia, and the Americas, thriving in humid tropics. Known locally in India as chaulai, keerai, or khada saga, amaranth has served nutrition via cooked leaves and versatile grains for breads, pastas, and traditional foods (Gandhi et al ., 2021) . Naturally gluten-free, rich in protein, fiber, minerals, and antioxidants, it has been linked to anti-inflammatory, cholesterol-lowering, and weight-management benefits (Saduakhasova et al., 2025). Genetic improvement had relied on traditional selection until genomics and biotech expanded enhancements for nutrition and stress tolerance (Das et al., 2016). Amid reliance on rice, wheat, maize, and amaranth's C4 pathway and nutrients had positioned it for climate-resilient systems, though research lagged major crops. This article examined phytochemical and antioxidant analysis of *Amaranthus viridis*, identifying flavonoids and assessing capacity via DPPH & ABTS assays for health benefits from its tropical secondary metabolites.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The sample was sourced from the local market of Burla (21.8·N&83.88·E), district: Sambalpur, Odisha, India. Aerial parts, leaves, air-dry at room temperature, grind into powder (40-60 mesh), and store in an air-tight container at 4ºC. This sample was subjected to extraction via sonication and centrifugation. Sequential extraction used hexane, ethyl ether, ethyl acetate, methanol, and water via maceration (1:10 w/v, 48-72h) or Soxhlet (6-8h).

2.2. Physical Analysis

The pH of *Amaranthus viridis* was determined using a digital pH meter (EUTECH, PH700-S2) to measure hydrogen ion concentration (Jayanetti et al., 2024). Moisture content was estimated by weighing approximately 5 g of the prepared sample into a pre-weighed petri dish and recording the weight (W_2). The sample was then dried in a preheated hot air oven (ALLYONE, AO/OV-20-S1) at 105 °C for 24 hours until a constant weight was obtained (Akpabio & Akpakpan, 2012). Total ash content was determined by the dry ashing method, in which the sample was incinerated in a muffle furnace to remove organic matter, leaving inorganic mineral residue (Pyar et al., 2018). Bulk density of

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Amaranthus viridis powder was measured by accurately weighing a known mass (M) of the sample and transferring it into a 25 mL graduated measuring cylinder, after which the occupied volume was recorded (Khalequzzaman et al., 2009).

Figure-1: Processing and preparation of *Amaranthus viridis*

2.3 Biochemical Analysis

Total carbohydrate content was estimated by the Anthrone method (Funik et al., 2011). Approximately 0.3 g of dried powder was subjected to acid hydrolysis so that complex carbohydrates were converted into simple sugars. A glucose standard series was prepared to generate the calibration curve. One milliliter each of standards, blank, and sample extract was transferred into labeled test tubes, followed by addition of 4–5 mL freshly prepared anthrone reagent in concentrated H_2SO_4. The mixtures were thoroughly mixed and heated at 90 °C for 10 minutes. After cooling, the developed green coloration was measured at 620 nm, and carbohydrate concentration was calculated from the standard curve. Total protein was determined according to the Lowry method (Aliva et al., 2021). The powder sample was homogenized in distilled water and was centrifuged, and the supernatant was collected and diluted appropriately. One milliliter of BSA standard or sample solution was treated with 5 mL alkaline copper reagent (Reagent A:B, 50:1) and was incubated for 10 minutes. Subsequently, 0.5 mL of 1 N Folin–Ciocalteu reagent was added, and the mixture was incubated in the dark for 30 minutes. Absorbance was recorded at 660/750 nm, and protein content was derived from the standard calibration curve. Total fat content was determined using the Soxhlet extraction method (Arampath et al., 2024). Approximately 10 g of finely ground sample was dried at 102 °C and was placed in a thimble. Extraction was carried out with 90–150 mL petroleum ether for 6 hours until the solvent in the siphon tube appeared clear. The solvent was evaporated, and the residue was weighed to calculate fat content. Dietary fiber was estimated by a modified ultrasound-assisted alkaline extraction technique as described by (Devi et al ,. 2022). For starch determination, the sample was soaked in 0.3% NaOH solution and was blended, followed by filtration and centrifugation (Jayanetti et al., 2024). The sediment was repeatedly washed and centrifuged until a clear supernatant was obtained. The residue was neutralized

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to pH 6–7 and was dried at 40–45 °C prior to quantification. Sodium (Na) and potassium (K) contents were quantified using flame photometry as described by (Musa and Akpomie et al., 2022).

2.4 Antioxidant Content

Total phenolic content (TPC) in *Amaranthus viridis* was determined by the Folin–Ciocalteu method as described by (Aliva et al., 2021). One gram of dried sample was extracted using 80% methanol or acetone, followed by filtration and volume adjustment. The extract was reacted with diluted (1:10) Folin–Ciocalteu reagent and sodium carbonate. Absorbance was recorded at 765 nm, and TPC was quantified using a gallic acid calibration curve. Total flavonoid content (TFC) was estimated according to the standard aluminum chloride colorimetric procedure (Chutipaijit et al., 2018; Aliva et al., 2021). An aliquot (1 mL) of extract was diluted to 3 mL and treated sequentially with 0.03 mL sodium nitrite, incubated for 5 min at 25 °C, followed by 0.03 mL of 10% aluminum chloride and 0.2 mL of 1 mM sodium hydroxide. Absorbance was measured at 510 nm and results were expressed using a quercetin standard curve. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted using 80% ethanol extracts (10–40 µg/mL) mixed with 0.1 mM DPPH solution (Aliva et al., 2021). The reaction mixture was incubated in the dark for 30 min, and absorbance was measured at 517 nm. Percentage inhibition was calculated in comparison with ascorbic acid as the reference standard. Vitamin C content was determined by titration with standardized 2,6-dichlorophenolindophenol (DCPIP) dye (Aliva et al., 2021). A 5 g sample was extracted using 3% metaphosphoric acid, filtered, and titrated to a pale pink endpoint. Ferric reducing antioxidant power (FRAP) was assessed using methanolic extracts reacted with freshly prepared FRAP reagent composed of acetate buffer, TPTZ, and ferric chloride in a 10:1:1 ratio (Aliva et al., 2021). The mixture was incubated at 37 °C for 4–10 min, and absorbance was recorded at 593 nm.

2.5 Total calorific count of *Amaranthus viridis*

Make sure the *Amaranthus viridis* was fully dry, then grind about 1 g into a fine powder, press it into a pellet, and record its mass (mf). Place the pellet in the bomb calorimeter crucible, attach the fuse wire, add 2 litres of distilled water, seal the bomb, and pressurize it with oxygen (25–30 atm). Submerge the bomb in the calorimeter bucket with a known water mass (mw), allow the system to stabilize, and note the initial temperature (Ti). Ignite the sample and record the highest temperature reached (Tf). After the run, turn off the stirrer, release the pressure, check for complete combustion, and clean the bomb (Aliva et al., 2021).

3. RESULT AND DISCUSSION

3.1. Physical analysis of *Amaranthus Virdis*

The physical characteristics had been determined using standard analytical procedures described in Section 2.2 (Methods). The pH value had been recorded as 5.6, which had been found to be close to the value of 6.5 previously reported (Dev et al., 2018). Moisture content had been observed at 7.17 ± 1.04% on a dry weight basis, which had fallen within the 6–9% range documented for leafy vegetables commonly consumed in Nigeria (Odhav et al., 2007). Ash content, reflecting total mineral residue, had been measured at 21.05 ± 0.30% (DW). This level had been reported to exceed those documented for other edible leaves such as *Vernonia colorata* (15.86% DW) and *Moringa oleifera* (15.09% DW) (Lockett et al., 2000), as well as *Solanum americanum* (17.40% DW) and *Momordica balsamina* (18.00% DW) (Hassan and Umar, 2006). Bulk density had been determined as 0.5 g/cm³. The dry leaf powder of *Amaranthus viridis* had been shown to exhibit bulk density values ranging from 0.40–0.65 g/cm³, which had been found comparable to *Amaranthus tricolor* (0.45–0.70 g/cm³) and slightly lower than *Spinacia oleracea* (0.60–0.80 g/cm³). Variations had been attributed to particle size distribution and drying techniques (Fellows, 2009; Onwuka, 2005).

3.2. Biochemical analysis of *Amaranthus Virdis*

The carbohydrate content of *Amaranthus viridis* leaves had been estimated at 46.58 ± 1.49 g/100 g (DW), which had been reported to be higher than that of *Senna obtusifolia* leaves (20%) (Faruq

et al., 2002) and comparable to *Amaranthus incurvatus* (23.7%) (Asibey-Berko and Tayie, 1999). However, lower carbohydrate levels had been observed in *Momordica balsamina* (39.05%) (Hassan and Umar, 2006). The protein content had been determined as 23.33 ± 3.17 g/100 g (DW), which had exceeded values reported for *Momordica balsamina* (11.29g/100g), *Moringa oleifera* (20.72g/100g), *Lesianthera Africana* (14.90g/100g), *Solanum americanum* (11.33g/100g), and *Leptadenia hastata* (19.10g/100g) (Isong and Idiong, 1997; Sena et al., 1998; Lockett et al., 2000; Hassan and Umar, 2006). Based on the recommended dietary allowance of 0.80 g/kg body weight/day (FND et al., 2002), 100 g of dried leaves had been calculated to supply approximately 62.7% of the daily protein requirement for a 70-kg adult. The lipid content (5.26 ± 0.30 g/100 g DW) had been found to be lower than the range reported for several Nigerian vegetables (Ifon and Bassir, 1980; Sena et al., 1998), and such low lipid levels had been considered beneficial for weight management. Dietary fibre (14.04 ± 0.35 g/100 g DW) had fallen within the reported range for Nigerian vegetables (8.50±20.90g/100g) (Ifon and Bassir et al., 1980). Although high fibre intake had been associated with reduced nutrient bioavailability (Aletor and Adeogun, 1995; Plessi et al., 1999; Vadivel and Janardhanan, 2000), protective effects against chronic diseases had also been documented (Gorecka et al., 2000; Ishida et al., 2000; Ramula and Rao, 2003). Total starch had been recorded as 44.72 ± 4.88 g/100 g, whereas leafy tissues of *Amaranthus viridis* had generally been characterized by low starch levels (1–3% DW) compared with cereal grains such as *Oryza sativa* and *Zea mays*, and amaranth grains of *Amaranthus cruentus* (Rastogi & Shukla et al., 2013).

Table 1: Physiochemical parameter analysis of *Amaranthus viridis*

Sample name	Ph	Moisture Content (%)	Total Ash Content (g/100g)	Bulk density (g/cm^3)	Starch content (g/100g)	Dietary fiber (g/100g)	Total carbohydrate (g/100g)	Total protein (g/100g)	Total fat (g/100g)
Amaranthus viridis	5.5	(7.17± 1.04% dry weight	21.05±0.03g/100g	0.5g/cm^3	44.72±4.88 g/100g	14.04±0.35 g/100g	46.58 ± 1.49 g/100g	23.33 ± 3.17 g/100g	5.26±0.30g/100g

3.3 Antioxidant analysis of *Amaranthus Virdis*

The total phenolic content (TPC) of *Amaranthus viridis* dried leaf powder was found to be 5.12±0.6 mg GAE/g dry weight. This level was reported to be comparable with *Amaranthus tricolor* (15–35 mg GAE/g) but relatively lower than *Moringa oleifera* leaves (30–65 mg GAE/g). Such variation was attributed to differences in extraction solvent, environmental conditions, and maturity stage (Sreelatha & Padma, 2009; Repo-Carrasco-Valencia et al., 2010). The total flavonoid content (TFC) was recorded as 175 ± 0.36 mg RE/g dry matter, while reported ranges for *A. viridis* were observed to be 15–35 mg RE/g. This concentration was considered comparable to *Amaranthus tricolor* (20–40 mg RE/g) and higher than *Spinacia oleracea* (5–15 mg RE/g) (Khandaker et al., 2008; Cai et al., 2004; Oboh, 2005). DPPH radical scavenging activity was determined to be 67.45 ± 1.95% inhibition. In general, *A. viridis* leaf extracts were reported to exhibit 60–75% inhibition at 100 µg/mL concentration. This antioxidant potential was found to be similar to *Amaranthus tricolor* (65–80%) and greater than spinach (50–60%), mainly due to the presence of phenolic and flavonoid compounds (Sarker & Oba, 2019; Repo-Carrasco-Valencia et al., 2010; AOAC, 2005). The ferric reducing antioxidant power (FRAP) value was measured as 21.7 ± 0.80 mg AAE/g dry weight. Previously reported FRAP values for *A. viridis* were shown to range between 18–35 mg AAE/g, which was comparable **to** *Amaranthus tricolor* (20–40 mg AAE/g) and higher than *Spinacia oleracea* (10–22 mg AAE/g) (Benzie & Strain, 1996; Sarker & Oba, 2019; Khandaker et al., 2010). Ascorbic acid content was quantified as 21.41 ± 1.32 mg/100 g dry weight. Although this value was lower than dried spinach (50–90 mg/100 g) and substantially lower than dried *Moringa oleifera* leaves (150–220 mg/100 g), the concentration was influenced by processing and drying conditions. Losses during dehydration were

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acknowledged, even though nutrient concentration effects were observed (USDA, 2019; Sreeramulu et al., 1983; Moyo et al., 2011).

Table: 2 Antioxidant analysis of *Amaranthus viridis*

Sample name	DPPH %	TFC (Total flavonoid content) (mg RE/g dm)	TPC (Total phenolic content) (mg GAE/g dm)	FRAP (ferric reducing antioxidant property) (mg AAE/g dm)	ASCORBIC ACID (mg/100g dm)
Amaranthus viridis	67.45 ± 1.95 %	175±0.36 (mg RE/g dm)	5.12±0.6 mg GAE/g)	21.7 ± 0.80 mg AA/g	21.41±1.32mg/100g

3.4 Minerals Analysis of *Amaranthus viridis*

Present research showed 62.33± 2.51 mg/100g of sodium and potassium value of 2391.33 ± 1.52 mg/100g in the *Amaranthus viridis.* Another researcher reported sodium content of 54 mg/100g and potassium content of 2230mg/100g in the same species (Srivastava et al., 2011) which has similar values reported in the present research. Sharma et al, (2012) reported higher values than current observation that is 108 mg/100g of sodium and 3460 mg/100g of potassium content. The variation in the mineral content may be due to the processing techniques followed.

3.5 Total Calorific Value OF *Amaranthus viridis*

Amaranthus viridis dried powder has reported calorific value of 530.34 kcal/100g (Umar et al., 2011), notably higher than the present reseach of 251.17 ± 1.26 kcal/100g. (Muhammad et al., 2019) estimated 339.27 ± 2.603kcal/100g in Argungu type which is nearly similar to the current value. This discrepancy may be difference in drying methods, plant maturity, or analytical techniques across studies.

4. CONCLUSION

Amaranthus viridis represents a nutritionally dense leafy vegetable with emerging phytopharmaceutical relevance. Despite being categorised as a weed, scientific evidence supports its value as a functional food due to its diverse bioactive compounds contributing antioxidant. Strategic cultivation and standardisation of this plant for dietary and medicinal use could support nutritional security and help mitigate oxidative stress–related disorders, particularly in resource-limited regions. The present investigation demonstrated that *Amaranthus viridis* dried leaf powder possessed appreciable physicochemical, nutritional, and antioxidant properties. The pH (5.6) and low moisture content (7.17 ± 1.04%) had indicated good storage stability, while the high ash value (21.05%) had reflected abundant mineral content compared with several commonly consumed leafy vegetables. The bulk density (0.5 g/cm³) had suggested suitability for food formulation and powder-based products. Biochemical evaluation had revealed substantial carbohydrate (46.58 ± 1.49 g/100 g) and protein (23.33 ± 3.17 g/100 g) contents, indicating that the leaves had served as a significant plant-based energy and protein source. The calculated contribution of approximately 62.7% of the daily protein requirement for a 70-kg adult highlighted its nutritional importance. Moderate lipid (5.26 ± 0.30 g/100 g) and considerable dietary fibre (14.04 ± 0.35 g/100 g) contents further supported its role in weight management and digestive health. Antioxidant analysis had confirmed notable bioactive potential, as evidenced by measurable TPC(5.12±0.6 mg GAE/g), TFC (175±0.36 mgRE/g dm), DPPH inhibition (67.45%), FRAP (21.7 mg AAE/g), and ascorbic acid levels (21.41±1.32mg/100g) , (62.33± 2.51 mg/100g) of sodium and potassium value of (2391.33 ± 1.52 mg/100g) Overall, *Amaranthus viridis* had been established as a nutrient-dense leafy vegetable with functional and health-promoting significance suitable for dietary diversification and functional food development.

5. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to the present research work.

6. ACKNOWLEDGEMENT

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