Phenolic acid and amino acid composition of Ethiopian Chaya (Cnidoscolus chayamansa)

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ABSTRACT
Chaya is one of the underutilized crops in Ethiopia and may become a key solution to alleviate both food and nutrition insecurity in developing countries. The objective of this study was to investigate the phenolic acid and amino acid composition of chaya (Cnidoscolus chayamansa) leaf and stem samples grown in Dire Dawa, Ethiopia. HPLC with a reverse-phase column was used for the determination of individual phenolic acid and amino acid compositions of all chaya samples. Each group of phenolic acid was used for phenolic acid quantification. Deep green, light green, and stem samples were analyzed for phenolic acid and amino acid profiles. The findings from this study show that chaya contains high amounts of phenolic acids and amino acid compositions. The deep green chaya leaf contained the highest amino acids and the lowest composition was found in the chaya stem. High concentration of essential amino acids such as leucine, lysine, and phenylalanine is found in chaya leaf, whereas leucine, lysine, and cysteine essential amino acids are dominant in chaya steam. Therefore, this study could be very significant for future use of chaya leaf as a rich source of amino acids and essential antioxidants to improve mainly the food, nutrition, and health status of rural peoples in Ethiopia. This research article focuses only on the phenolic and amino acid content and so the researchers recommend for others to do so on other untouched parameters like proximate compositions, mineral contents, and anti-nutritional contents of both chaya leaves and stems.

Introduction

Chaya (Cnidoscolus chayamansa) refers to any group of plants of the genus Cnidoscolus, which is a part of the family Euphorbiaceae.[1] Chaya is a commonly domesticated shrub throughout northern and Central America, including Guatemala, Belize, southeast Mexico, and parts of Honduras.[2] Chaya is the most common type generally used for cultivation because of its special feature of lack of stinging hairs on the leaves, which is an advantage over other varieties because they need gloves while harvesting to get protection from stinging hairs. There are four potential varieties that exist globally: estrella, mansa, plegada, and picud. It is mostly grown up to 3 m in length and 2 m in width. Branches may break easily, so even if the plant can grow up to 6 m high, it is usually pruned to less than 2 m for the ease of collection of leaves. It has succulent stems, so when it is cut, it produces a milky sap. It rarely produces seeds; therefore, stems are usually considered for propagation. The initial growth is slow as it takes time for the roots to develop on the cuttings, so the leaves are harvested only after the second year. It can grow well on a wide range of soils in both hot, rainy climates and areas with...
occasional drought. It grows easily and quickly, especially at higher temperatures, and new leaves grow quickly after harvesting. The amount of leaves per square foot of garden space is impressive. Leaves have lower moisture content than most other green leafy plants like spinach or lettuce (Figure 1).

Chaya leaves are consumed for food and medicine.[2] The leaves are highly nutritious, containing significantly higher amounts of crude protein, fiber, calcium, potassium, iron, vitamin A, phenolic acids, and β-carotene than spinach,[3] and different researches show that the chaya is exceptionally rich in protein that is about three times than ordinary spinach or lettuce.[4] Often chaya leaves and tender stem tips are eaten after boiling them in water with salt, with or without the apical shoots and sometimes with petioles. Likewise, chaya greens are frequently combined with other vegetables and/or meat in soups and stews. Boiled chaya greens, covered with ground roasted pepita seeds (Cucurbita sp.), cooked tomato, and Chile (Capsicum sp.), are eaten as a sort of burrito in a corn tortilla. The most famous chaya dish is probably Dzotobilchay, consisting of diced chaya leaves mixed with nixtamalized corn dough, covered with sauce or vegetables and diced eggs, then wrapped in banana leaves or other chaya leaves, and cooked to make tamale.

In recent years, there has been an increased interest in the use of health-promoting compounds in edible plants such as antioxidants including phenolic acids, flavonoids, beta-carotene, and vitamins.[4] Due to the presence of high antioxidants in chaya, make a blood glucose reducer and serving as a free radical scavenger.[5] In recent years, there has been much interest in the health-promoting properties of phenolic acids, which are known to be antioxidants and thus are believed to be have anti-cancer activity. Therefore, a wide range of phenolics are available in plant products, and chaya leaf is known to be rich in phenolic compounds.

Phenolic compounds are known to be the most abundant and widely distributed secondary metabolites that exist in the plant kingdom. These compounds play many roles in the plant life cycle, especially plant-defense-oriented strategies such as mediating auxin transport, signaling, plant defense, antioxidant activity, and free radical scavenging. Among non-enzymatic antioxidants, phenols and flavonoids contribute significantly as scavenging free radicals for tolerating biotic and abiotic stresses by accumulating in various plant tissues. They are known to be responsible for the
free radical scavenging and antioxidant activities of plants; they possess many biological effects, mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation, and chelating transition metals. Free radicals are reactive oxygen species (ROS), which include hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, singlet oxygen, peroxyl radicals, and superoxide anion.\textsuperscript{[6,7]}

Although chaya is a nutrient-rich crop, there are some amounts of anti-nutritional compounds such as oxalic acid, phytic acid, and hydrogen cyanide and which can be eliminated through appropriate processing such as soaking, drying, and slight cooking for 5 min.\textsuperscript{[5]} Nowadays, underutilized crops have become a key solution to alleviate both food and nutrition insecurity in developing countries. Underutilized crops have an unexploited potential to contribute to food security, nutrition, health, income generation, and environmental services.\textsuperscript{[2-5]} Among the underutilized crops, chaya is drought-tolerant and nutritionally rich and can be a household lifesaving crop in developing countries.

In developing countries like Ethiopia, there is underlined food and nutrition insecurity; it is not only because of lack of food but also because of lack of awareness and research attention to underutilized nutrient-rich food crops like chaya. In Ethiopia, chaya was introduced many years ago; however, due to lack of awareness on its nutritional and health potential, it has become a neglected and unexploited crop. Over the last 5 years, chaya has received little research attention with regard to its potential nutritional, economic, and health importance.\textsuperscript{[8]} Previous researches done on chaya, in Ethiopia, are extremely less and focus mainly on the proximate composition of chaya leaf, but there is no awareness among people about the nutritional quality of chaya leaf especially in terms of amino acid profiles and phenolic acid group contents.

This research was carried out to investigate the amino acid and phenolic acid profile of chaya leaves and their stem grown at Dire Dawa, the eastern part of the country Ethiopia and to bring antioxidant potential of chaya leaves for maximum utilization by the rural peoples as rich sources of different amino acids and health-promoting substances, which have a direct impact on the improvement of the nutrition and food security of the peoples in the area of Ethiopia.

**Materials and methods**

**Sample collection**

Two local chaya varieties (\textit{Cnidoscolus aconitifolius}) namely deep green and light green and their stems were harvested in June 2019 at 2 months of age from Dire Dawa Agricultural Research Station. The study sample was selected and collected using simple random sampling techniques from the chaya grown location and brought to Haramaya University Food Science Laboratory for preparation. Among the chaya crops on the farm, the study leaf samples were collected based on their same age of growing and uniform color. Deep green color leaf samples were collected in one category and the light green leaf samples were collected in other categories. Both categorized leaf samples were oven-dried separately at equal temperature (65°C) time (2 h) and kept for analysis. Regarding the stem samples, the stem of the same crop from which leaf samples were taken was again used and the stem samples were cut into small pieces. The drying steps were similar to those of the leaf samples.

**Sample preparation**

All the dried samples were powdered to about 500 g using a stainless steel hammer mill to a powder particle size of 1.6 mm, packaged in polythene bags, and stored at 4°C until required for analysis. Concerning, the chaya stem samples were collected, cut into small pieces, and oven-dried at 65°C for 2 h, and following this, the dried stem powdered (about 500 g) and stored at 4°C until required for analysis.
Amino acid analysis

Amino acid hydrolysis protocol: The amino acid analysis was carried out according to Babalola and Alabi[9] method. Samples of the leaf and stem were digested in acid and alkaline medium to the complete hydrolysis of the protein fraction. Briefly, 100 mg, of each sample, chaya leaf and stem were digested with 3 ml of 6 N HCl at 200°C in the heated oven for 24 h after sealing tubes with nitrogen gas to prevent oxidation. The digested samples were filtered with Whatman No. 6 and the filtrates were evaporated at 100°C water bath for removing the chlorine gas. Hydrolyzed protein was completely dry with nitrogen gas and re-constituted with 200 μl (0.2 ml) of 0.1 N HCl. For tryptophan, alkaline hydrolysis was used, and 50 mg, of each sample, chaya leaf and stem was suspended in 20 ml of 3 N-NaOH and sealed under N2 gas and hydrolyzed for 3 h at 110°C heating oven. Following hydrolysis, centrifuged for 10 min at 4000 RPM and then the supernatant was taken and diluted with 50-folds water.

Derivatization protocol: Both primary and secondary amino acid derivatization techniques were used to determine the amino acids found in the sample. The final hydrolyzates were filtered (0.2 μm) and injected into UHPLC system using MPA/OPA/FMOC derivatization protocol. Mercaptopropionic acid (MPA) was used as catalyst, and o-phthalaldehyde (OPA) and fluorenlymethyl chloroformate (FMOC) were used as reagents for primary and secondary amines derivatization, respectively. Derivatization was taken automatically by the instrument using OPA for all primary amino acids and FMOC for secondary amino acids (proline and hydroxyproline).

HPLC method development: The amino acid analysis was conducted with the Shimadzu UHPLC system (Shimadzu, Columbia, MD). The UHPLC system consisted of a binary pumping system: pump A (LC-10AD VP) and pump B (LC-10AT VP), a degasser (DGU-14A), an Autosampler (SIL-20AC HT), column heater (Brinkmann, CH-30), and fluorescence detector and system controller (CBM-20A). The mobile phase solvents were prepared according to Table 1, and mobile phase A was a mixture of Na2HPO4 and Na2B4O7 NaN3 while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v) (Table 1).

HPLC conditions: Mobile phases consisting of a 0.02 M aqueous phosphate buffer with pH 2.5 and acetonitrile (MeCN) as organic modifier were used. Solutes of concentration 10 lg/ml were injected individually or together using appropriate mixtures. The analysis was done by reverse-phase HPLC. The chromatographic separation of the hydrolyzates was performed using a reverse-phase Pico-Tag column (3.9 × 300 mm) C18 at 400 C and a UV detector at 254 nm. The solvent system consisted of two eluents: (A) an aqueous buffer and (B) 60% acetonitrile in water. Gradient elution was employed using two pumps, programmed to deliver the mobile-phase eluents A and B. A gradient that was run for the separation consisted of 10% B traversing to 51% B in 10 min using a convex curve.

HPLC separation techniques: The separation was obtained at a flow rate of 2 ml/min with a gradient program that 0.01 min (1% B), 7.4 min (40% B), 10 min (45% B), and 10.1 (100% B). Then, washing at 100% B and calibration at 0% B was performed in a total analysis time of 12.1 min.[10] To quantify amino acids, the mixed standard was used from Asparagine, Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Serine, Tyrosine, Valine, Proline, Tryptophan, Cysteine, Norleucine, and Hydroxyproline prepared and used for easy identification of peaks in the mix as well as their amino acid standards.

| Table 1. Solvent gradient table for amino acid analysis in HPLC. |
|---------------------|---------------------|---------------------|
| Solvent A (%)       | Solvent B (%)       | Time in min         |
| 100                 | 0                   | 0                   |
| 60                  | 40                  | 7.5                 |
| 45                  | 45                  | 10                  |
| 0                   | 100                 | 10.2                |
| 100                 | 0                   | 12                  |
Limit of quantification and detection (LOQ and LOD) control: Before real sample analysis, the UHPLC was tested for linearity, precision, limit of quantification (LOQ), selectivity, and resolution by spiking amino acid standards. The amino acid composition in (%) of the chaya samples was quantified with their relative area compared with the amino acid standard, and the concentration of each amino acid in (g/100 g) was also calculated by multiplying the percentage of each amino acid with their percentage CP content. There were 9 essential amino acids and 12 non-essential amino acids detected in the analysis.

**Phenolic acid (antioxidant) analysis**

Extraction of phenolic acids: Due to the varying chemical status of different phenolic acids in the food matrix, it is important to ensure the complete extraction of each of the different classes of phenolic acids. The majority of free phenolic acids are readily soluble in polar or organic solvents and can be extracted with them in a sample extraction procedure. A simple extraction method was employed by using a modified version of Oritoji et al.\[^{11}\] Chaya samples of 200 mg were extracted with 4 ml of acidified methanol (methanol/water, 80:20 V/V) at room temperature (25°C) for 2 h. The extract was then further analyzed for their phenolic compounds using HPLC-fluorescence detector.

Individual phenol separation method: To quantify individual phenolic compounds, a mixture of seven standards flavonoids, polyphenol, anthocyanin, tannins, alkylresorcinols, phytocins, and banzols was used, and the limit of detection on HPLC was tested for easy identification of peaks in the mixture as well as their phenolic compounds. Food phenolic compounds are large in number and structurally varied, and analytical procedures for the analysis of individual phenolic compounds have been relatively complicated. For a better analysis of individual phenolic compounds in food use of sophisticated instrumentation for separation, quantitation and characterization are recommended. HPLC approaches can provide a rapid response offering both high sensitivity and separation. There are no restrictions on sample volatility and derivitization, and HPLC also provides a wide range of selectivity through many solvent combinations and column packing materials. For simultaneous measurement of several phenolic compounds, either a gradient HPLC method or several isocratic methods using various combinations of mobile phase and/or analytical columns can be employed. The internal standard used was dichloro-hydroxybenzolic acid in 80:20 v/v ethanol/water solution (Figure 2).

The extraction method, with slight modification on solvent-type reversed-phase chromatography, is the most popular mode of analytical liquid chromatography for phenolic compounds. In most cases, the reported system for the separation of phenolic and their glycosides in foods is carried out on RP-HPLC on silica-based C-16 or C-18 columns. A phenolic acid analysis of solvent gradient proportion is presented in Table 2, and the maximum time for phenolic acid determination using Rp-HPLC is about 55 min.

**Statistical analysis**

The amino acid and phenolic acid profile data were analyzed in duplicates. The statistical analysis interpretations were done using the general linear model procedures, and the data were subjected to one-way analysis of variance (ANOVA) Systems software (Version 12 SAS Institute Inc., Cary, USA). P values ≤ .05 were considered as significant, and P values ≥ .05 were considered not significant.

**Results and discussion**

As presented in Table 3, the different classes of phenolic acids in chaya leaf were detected. The class of phenolic acid depends on the extraction method and the extraction in the study was carried out by most organic solvents, and the application of such organic solvent increased the phenolic acid constituents.\[^{12,13}\] The phenolic acids obtained from this study were flavonoids, polyphenols,
anthocyanins, tannins, alkylresorcinols, phytocinols, and banzols. The findings from the study are in agreement with the other findings that reported the different classes of phenolic acid in chaya, such as flavonoid, polyphenols, tannins, glycosides, and alkaloids,\cite{6,13,14} and show that chaya is a high source of aspartic acid, glutamic acids, and glycine.

Studies in Chain also revealed that there are different classes of phenolic acids in chaya leaf, such as coumarin, flavonoids, phenols, tannins, anthraquinones, and flobotanins, in aqueous and alcoholic extracts.\cite{15} Phenolic acid is considered a phytochemical and its antioxidant capacity is responsible for many of its health benefits, specifically in controlling chronic diseases. The chaya leaf shows a general

\section*{Table 2.} Solvent gradient table for phenolic acid analysis in HPLC.

| Solvent A (%) | Solvent B (%) | Time in min |
|---------------|---------------|-------------|
| 0             | 100           | 0           |
| 15            | 85            | 30          |
| 50            | 50            | 50          |

\section*{Table 3.} Types of phenolic acid detected from deep green leaf, light green chaya leaf, and chaya stem.

| Detected phenolic compounds | Classification of the compounds | Retention time in min |
|-----------------------------|---------------------------------|-----------------------|
| Hydroxyl benzoic acid       | Hydroxybenzoic acid             | 25.2                  |
| Vanillic acid               | Hydroxybenzoic acid             | 26.4                  |
| Syringic acid               | Hydroxybenzoic acid             | 27.3                  |
| Caffeic acid                | Hydroxycinnamic acid            | 29.1                  |
| Synaptic acid               | Hydroxycinnamic acid            | 30.4                  |
| Ferulic acid                | Hydroxycinnamic acid            | 36.1                  |
| Coumaric acid               | Hydroxycinnamic acid            | 37.5                  |
| Hydroxycinnamic acid        | Phenolic acid                   | 39.5                  |
| Bicinnamic acids            | Cinnamic acid                   | 40.6                  |
| Methocinnamic acid          | Anthocyanin                     | 43.2                  |
| Carboxylic acid             | Banzols                         | 46.8                  |

\begin{figure}
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\includegraphics[width=\textwidth]{chromatograms.png}
\caption{Chromatograms for mixed phenolic acid used for the study.}
\end{figure}
trend toward the presence of different phenolic groups, such as coumarin, flavonoids, phenols, tannins, anthraquinones, and flobotanins, in aqueous and alcoholic extracts. Researchers have been looking into the antioxidant properties of chaya plant species from different parts of the globe.[7]

Even if chaya is an excellent source of several essential nutrients for health, there is also an anti-nutritional factor, and a toxic substance mainly cyanogenic glycoside.[16] This hydrocyanic glucoside is found in raw chaya leaves; therefore the leaves should be cooked to reduce toxicity. Young chaya leaves are safe when they have been boiled or fried for at least 5 min. Older, tougher leaves should be boiled for 10–15 min.[16,17] These authors tested various thermal treatments to remove this compound from the leaves and reported that 5 min in boiling water is sufficient to remove any residue of cyanide.

In Figure 2, phenolic acid composition of chaya leaf powder is presented in their classification. Two important naturally occurring types of phenolic acids are hydroxybenzoic acids and hydroxycinnamic acids, which are derived from non-phenolic molecules of benzoic and cinnamic acids, respectively.[18] Individual phenolic acids are identified by comparing their retention time to authentic, commercially available phenolic acid standards.[19] Each peak in the chromatogram should be distinct from the other, and separate peaks at the base were considered for phenolic acid identification. Quantification was carried out by determining the ratio to an internal standard added to each sample, and then a calibration curve was constructed. The size of each peak is proportional to the concentration of the analyte (in these cases, phenolic compounds) and thus, the peak areas were considered to calculate the concentrations.

Phenolic compounds are simple phenolic acids that are widely distributed in the cell walls of plants and consequently are significant components of the human diet. They have been studied largely about antioxidant activity, though these have been largely in vitro studies and further work regarding in vivo effects in humans is needed before health benefits can be claimed. Leafy vegetables like spinach and chaya are associated with a high level of total phenolic compounds and recommended for health-promoting purposes.[20] Therefore, the consumption of chaya would be useful in antioxidant-deficient diets.

As summarized in Table 4, the amino acid composition of the analyzed chaya leaf powders and stem samples was quantified by their relative area comparing with amino acid standard and the concentration of each amino acid in %. The current results indicated that the essential amino acid composition in decreasing order is deep green leaf > light green chaya leaf > chaya stem (Table 4). The deep green chaya leaf contained the highest amino acids and the lowest composition was food in the chaya stem. This shows that green leaf chaya is more suitable for consumption due to its high amino acid content. Although a biological evaluation of protein quality was not conducted, the essential amino acid profile (Table 4) is often strongly suggestive of protein quality. The study clearly shows that chaya has a good amount of amino acids and can be used as sources of protein, but it must be cooked.

Leucine, lysine, and phenylalanine were the three dominant amino acids in chaya leaf samples, and leucine, lysine, and cysteine amino acids are dominant in chaya steam. The presence of higher leucine and lysine makes chaya rich source of amino acids that are deficient

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**Table 4. Essential amino acids compositions of chaya leaf powders and stem samples.**

| Essential amino acids (EAA) in % | Treatments                  |
|---------------------------------|-----------------------------|
|                                 | Deep green | Light green | CS oven-dried | Retention time, min |
| Histidine                       | His        | 2.43        | 2.65          | 1.10                | 3.915           |
| Threonine                       | Thr        | 5.36        | 4.61          | 1.21                | 4.274           |
| Valine                          | Val        | 6.81        | 4.99          | 1.31                | 6.821           |
| Methionine                      | Met        | 3.54        | 2.75          | 0.57                | 6.935           |
| Cystine                         | Cyt        | 3.09        | 2.41          | 1.53                | 6.384           |
| Tryptophan                      | Try        | 3.59        | 2.51          | 1.30                | 7.425           |
| Phenylalanine                   | Phe        | 6.78        | 5.72          | 1.08                | 7.648           |
| Isoleucine                      | Iso        | 4.34        | 3.40          | 1.26                | 7.791           |
| Leucine                         | Leu        | 10.26       | 7.34          | 1.92                | 8.56            |
| Lysine                          | Lys        | 7.39        | 5.66          | 1.80                | 9.047           |
in cereals and grains. These findings agree with the work of Oyagbemi et al.,[21] who concluded that chaya is a good source of protein, vitamins, calcium, and iron and is also a rich source of antioxidants such as vitamins C and E as well as flavonoids and has a possible anti-diabetic effect.[22] Other kinds of literature also revealed that chaya leaves are an excellent source of several essential nutrients for a healthy, balanced diet.[22,23] Chaya is more nutritious than many green leafy vegetables such as spinach, Chinese cabbage, and amaranth. The leaves are very high in protein, calcium, iron, and vitamins A and C.[24] The exact nutritional value of chaya depends on the cultivar.[16]

Chaya leaves are commonly eaten as vegetables and serve are good sources of protein of about 17% on a dry matter basis.[20] Chaya is also rich with most of the limiting amino acids than in many other tropical root crops, cereals, and legumes.[25] At present, the nutritional and health values are the main concern when a crop is being considered as a food source. Due to the emphasis placed on both nutritional and health importance of food by consumers, a great need exists for information on the nutritional contents mainly amino acid content of vegetables like chaya is common.[26]

The analysis of chaya leaf nutritional and anti-nutritional composition is an essential part of nutrition studies and important to know the overall nutritional qualities. It is a fact that there are some anti-nutritional factors and should be eliminated through thermal processing methods.[11] In this study, both chaya leaf and stem (CS) grown in Ethiopia were analyzed for amino acid and phenolic acid compositions in addition to their proximate, mineral, and anti-nutritional constituents. However, this article includes only the amino acid and phenolic acid profiles of chaya grown in Ethiopia.

The non-essential amino acid composition of chaya leaf and stem samples is presented in Table 5, and the findings from this study show that the plant is also a good source of non-essential amino acids. The non-essential amino acid composition recorded for this study shows that both chaya leaves are a high source of aspartic acid, glutamic acids, and glycine and chaya steam is an excellent source of aspartic acid, glycine, and arginine. The amino acid L-arginine improves the circulation and oxygen supply of the coronary and peripheral vessels through the release of nitric oxide. When people take arginine, the nitric oxide level in the blood increases. Nitric oxide relaxes the walls of blood vessels and thereby improves circulation throughout the body, including the erectile tissue in the penis. Furthermore, arginine increases the nitric oxide level, which makes the arteries more elastic. This effect can lower blood pressure and improve the ability to have an erection. In addition, nutrients and oxygen can reach the organs quicker through the blood, which on the whole has a positive effect on male potency, stamina, and sexual performance.

### Table 5. Non-essential amino acids compositions of chaya leaf powder and stem samples.  
Non-essential amino acids (NEAA) in % composition

| Full name of NEAs | Abbreviations | Deep green leaf | Light green leaf | Chaya stem | Retention time, min |
|-------------------|---------------|-----------------|-----------------|------------|--------------------|
| Aspartic acid     | Asp           | 9.86            | 8.98            | 4.11       | 1.07               |
| Glutamic acid     | Glu           | 11.51           | 8.80            | 2.08       | 1.30               |
| Asparagines       | Asp           | 7.52            | 5.65            | 1.41       | 1.96               |
| Serine            | Ser           | 4.48            | 5.02            | 1.38       | 3.34               |
| Glutamine         | Glu           | 4.48            | 5.56            | 1.45       | 3.52               |
| Glycine           | Gly           | 5.75            | 5.75            | 4.17       | 4.04               |
| Alanine           | Ala           | 2.45            | 2.04            | 1.29       | 4.91               |
| Arginine          | Arg           | 6.32            | 4.95            | 3.33       | 4.39               |
| Tyrosine          | Tyro          | 4.62            | 4.32            | 1.29       | 5.69               |
| Norleucine        | Nor           | 4.05            | 2.86            | 0.40       | 8.18               |
| Hydroxproline     | Hyd           | 2.48            | 1.97            | 1.29       | 10.80              |
| Proline           | Pro           | 2.79            | 2.06            | 0.40       | 11.01              |
Generally, the amino acid profile of the leaf samples showed a favorable balance of both essential and non-essential amino acids to support the nutrient requirement of humans and animals if mutually mixed with other foods. When the amino acid profiles of leaves and steam of chaya are compared, chaya leaves have good dominance on chaya steam. The values of the present studies were in agreement with the reported findings by Wojdylo et al. and Yuan et al. [27,28]

Conclusion

The current study showed that chaya leaves could have a potential phenolic acid and amino acid for the nutrition and health of consumers. According to epidemiological studies, antioxidants have great power in solving diet-related health illnesses and have significant biological and pharmacological importance. However, it is not recommended eating raw chaya, but cooking for 5 min before consumption is encouraged. Therefore, investigating the antioxidant profile of chaya is timely tested to promote the utilization of chaya in developing countries. Since there is no previous investigation on chaya amino acid and phytochemical composition in Ethiopia and this work is new and baseline finding for further scientific work on chaya.

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