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EVALUATION OF AMINO ACIDS COMPOSITION OF AQUEOUS AND ETHANOL EXTRACT OF *PHYLLANTHUS NIRURI* STEM FROM AGBOR, NIGERIA

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ABSTRACT

Global belief in the use of plants as an alternative form of medicine and food has grown as a result of research on the benefits of plants in treating a variety of diseases and as an inexpensive source of nutrients for both healthy and malnourished people. This study therefore evaluated the amino acid compositions of aqueous and ethanol extract of *Phyllanthus niruri* stem. Homogenization at a 25% w/v concentration was used to extract the *Phyllanthus niruri* stem's aqueous and ethanol extract. The qualitative and amino acid compositions were determined using Agilent 1260 High Performance Liquid Chromatography (HPLC). The result revealed that amino acids were present in both the aqueous and ethanol extracts of the *Phyllanthus niruri* stem. However, the aqueous extract which had 14 amino acids with total amino acids of 16245.41445 Pmol/µl (79%) contained a higher concentration of amino acids than the ethanol extract which had 13 amino acids with total amino acids of 4334.39057 Pmol/µl (21%) with tyrosine having the highest concentration of 13717.18855 Pmol/µl and 3047.75364 Pmol/µl in the aqueous and ethanol extracts respectively. The aqueous extract of *Phyllanthus niruri* stem contained 58% more amino acids than the ethanol extract. Therefore, *P. niruri* stems may be used as a medicinal substitute or as a source of nutrients to improve human health and fight malnutrition.

Keywords: *Phyllanthus niruri,* Amino acids, Aqueous extract, Ethanol extract, Stem, HPLC

INTRODUCTION

A typical conventional medicinal product has been produced and developed using knowledge passed down through generations. Growing research on the advantages of plants in treating a range of diseases has raised public acceptance of the use of plants as components of traditional medicine (Widiadnyani et al., 2021). According to Shanavas et al. (2020), several recent medicinal products that are developed from plants or derivatives of plants, indicating that medicinal plants are significant sources of new drug development. Numerous researchers have documented the nutritional and therapeutic benefits of various plant parts (Habila et al., 2024; Okorie et al., 2024; Achuba, 2018; Aganbi et al., 2017).

Malnutrition is a severe challenge in underdeveloped African countries, affecting about three billion people globally with disastrous effects (Gbashi et al., 2021). The prevention of malnutrition is crucial (Humbwavali et al., 2019). Using naturally occurring native fruits and vegetables and broadening the variety of foods eaten can help people absorb more essential nutrients, enhance food security, and potentially prevent malnutrition (Vogliano et al., 2021). Amino acids are utilized by the body for several metabolic processes, such as phosphorylating proteins, regulating gene expression, and regulating food intake (Kari et al., 2022; Machado et al., 2020). Although the body can synthesize non-essential amino acids, it cannot produce essential amino acids, thus we must obtain them from diet (Akram et al., 2011).

Phyllanthus niruri is a member of the *Phyllanthaceae* family and genus *Phyllanthus*. There are multiple methods for differentiating between *Phyllanthus species*. Regarding *P. niruri*, its stem has an angular form, and its leaves are elliptic-oblong and distichous (Varsha, 2022). According to Meilani et al. (2020), *P. niruri* is a tiny, erect herb that can reach a height of 35-45 cm every year. Its leaves are sessile, alternating in position, and can grow up to 7-12 cm. Growing perennially, *P. niruri* is a herb that has been used locally for several disease conditions, such as genitourinary infections, kidney stones, jaundice, diarrhoea, and dyspepsia (Lee et al., 2016). According to Lee et al. (2016) and Ezzat et al. (2020), it is especially well-known for treating liver and renal conditions in Chinese, Ayurvedic, and Malay medicine. There are many different attributes of *P. niruri*, which are antioxidants, anti-inflammation, antimicrobial, hepatoprotective, anticancer etc (Abu Hassan et al., 2023; Al Zarzour et al., 2017; Maheswari et al., 2021; Sowjanya et al., 2021; Sutrisna et al., 2019; Sabdoningrum et al., 2020). According to Widnyani et al. (2021), there are many naturally occurring metabolites found in *P. niruri*, including flavonoids, saponins, steroids, terpenoids, alkaloids, phenols, tannins, and glycosides. There is currently a lack of published information on the amino acid content of the aqueous and ethanol extracts of *P. niruri*, although the extract is frequently used locally for therapeutic purposes and that several distinct extracts of *P. niruri* have been studied. Therefore this study evaluated the amino acid compositions of aqueous and ethanol extract of *P. niruri* stem.

MATERIALS AND METHODS

Sample collection, identification, and extraction

P. niruri was harvested in Agbor, Delta State and was identified and authenticated in the Department of Botany, Delta State University, Abraka with voucher number DELSUH: 181. The fresh *P. niruri* stems were cleaned with distilled water to remove any remaining debris after the leaves were removed from them.

Aqueous extract

25 g of the stems were sliced and homogenized in 100 ml of distilled water and then filtered with whatman grade 1 filter paper. The filtrate was collected and stored in the refrigerator until it was ready for use.

Ethanol extract

25 g of the stems were sliced and homogenized in 100 ml of ethanol and then filtered with whatman grade 1 filter paper.

The filtrate was collected and stored in the refrigerator until it was ready for use.

HPLC analysis of amino acid in aqueous and ethanol extract of *P. niruri stem.*

A vial containing 0.043 g of air-dried sample was filled with hydrochloric acid of 6 N (1ml) and the thermostat was set at 110°C. The hydrolysis process lasted for 24 hrs. To eliminate the hydrochloric acid, a small portion of the centrifuged hydrolyzed sample was evaporated using a rotary evaporator (Supervac, India). 13 mm, 20 µl of regenerated cellulose membrane filters were used to filter the dry residue after resuspension in distilled water of 0.5 ml. On a rotary evaporator, after evaporating 0.5 ml of centrifuged hydrolysate, the pellet was again suspended in distilled water of 2.5 ml and centrifuged for 10 minutes at 4000 rpm. The vials were filled with the supernatant.

Instrumentation

For primary amino acids, amino acids were derivatized online using ortho-phthaladehyde (OPA); for secondary amino acids, 9-flurenylmethylchloride (FMOC) was used and the Agilent 1260 HPLC was used for the separation process. The LC was controlled by "Chemstation" software, which also gathers, evaluates, and publishes the results. Pre-column derivatization and management of multiple samples are handled by the G1367E autosampler.

A narrow bore Hypersil AA-ODS reverse phase column (2.1 x 200mm, 5 um) was used to elute the derivative amino acids. Solvent A is a 20 mM Na acetate buffer containing 0.018% v/v triethylamine and 0.3% tetrahydrofuran that has been pH-7.2 adjusted with 1% acetic acid. Solvent B is a 20% 100mM Na acetate buffer (pH 7.2) that contains 40% methanol and 40% acetonitrile. Applying gradient programming as follows:

Table 1: Parameter for gradient programming

T(min)	A (%)	B (%)	Flow Rate (ml/min)
0.00	100.00	0.00	0.45
17.00	40.00	60.00	0.45
18.00	0.00	100.00	0.45
18.10	0.00	100.00	0.45
18.50	0.00	100.00	0.80
23.50	0.00	100.00	0.80
23.9	0.00	100.00	0.80
24.00	0.00	100.00	0.45
25.00	100.00	0.00	0.45

The column thermostat was set at 40 degrees Celsius. The excitation (Ex) and emission (Em) wavelengths for the Fluorescence Detector (FLD) were 340 nm and 450 nm, respectively. The settings for the Diode Array Detector (DAD) Signal A = 338/10 nm, Ref = 390/20 nm, and Signal B = 262/16 nm, Ref = 324/8 nm.

RESULTS AND DISCUSSION

The qualitative expression of amino acids in the aqueous and ethanol extract of *P. niruri stem* is shown in Table 2. In the aqueous extract, 14 amino acids were detected while in the ethanol extract 13 amino acids were detected. Glycine, alanine, arginine, tyrosine, valine, methionine, phenylalanine, aspartic acid, lysine, histidine, threonine and cysteine were detected in both extracts. Isoleucine and proline were present in the aqueous extract but were absent in the ethanol extract while aspartate was present in the ethanol extract but was absent in the aqueous extract.

Presented in Tables 3 and 4 are the amino acid compositions of the aqueous and ethanol extracts of *P. niruri* stem respectively. Figures 1 and 2 shows the chromatogram of the aqueous extract of *P. niruri* stem by DAD and FLD respectively, while Figures 3 and 4 show the chromatogram of the ethanol extract of *P. niruri* stem by DAD and FLD respectively. In the aqueous extract, tyrosine (13717.18855 Pmol/µl), methionine (1222.14474 Pmol/µl), and Alanine (399.28355 Pmol/µl) were highest with tyrosine being the most abundant amino acid while leucine (0.00000 Pmol/µl) and proline (4.54886 Pmol/µl) were the lowest. Amino acids in the aqueous extract followed this order: Tyrosine > methionine > alanine > threonine > phenylalanine > lysine > isoleucine > cysteine > glycine > valine > arginine > histidine > proline > proline. In the ethanol extract, tyrosine (3047.75364 Pmol/µl), methionine (434.70598 Pmol/µl), alanine (273.78577 Pmol/µl) were also highest with tyrosine being the most abundant amino acid too while aspartic acid (0.00000 Pmol/µl) and histidine (1.71076 Pmol/µl) were the lowest. The total amino acids as presented in Figure 5, show that the aqueous extract (16245.41445 Pmol/µl) which had 79% amino acids was 58% (11911.02388 Pmol/µl) higher than the ethanol extract (4334.39057 Pmol/µl) which had 21% amino acids.

Table 2: Qualitative expression of amino acids in the aqueous and ethanol extract of *P. niruri* stem

Amino acids	Aqueous extract	Ethanol extract
Glycine	+	+
Alanine	+	+
Arginine	+	+
Tyrosine	+	+
Valine	+	+
Methionine	+	+
Phenylalanine	+	+
Aspartic acid	-	+
Isoleucine	+	-
Lysine	+	+
Histidine	+	+

Threonine	+	+
Cysteine	+	+
Leucine	+	+
Proline	+	-

+ = Presesence, - = Absence

Table 3: Amino acid composition of aqueous extract of *P. niruri* stem

Amino acids	RT (min)	RF	Area	Amount (Pmol/µl) Group
Glycine	5.835	0.56752	93.3511	52.97825
Alanine	8.066	1.56532	255.0818	399.28355
Arginine	8.958	1.19698	34.0002	40.69752
Tyrosine	9.476	136.44631	100.5318	13717.18855
Valine	10.351	0.34045	148.8278	50.66787
Methionine	11.134	38.91244	31.4076	1222.14474
Phenylalanine	12.403	0.67225	359.2907	241.53222
Isoleucine	12.943	1.34201	51.7177	69.40540
Lysine	16.344	0.42052	75.4823	75.4823
Histidine	5.887	0.02339	1531.4413	35.82212
Threonine	8.115	0.05793	4624.7207	267.90809
Cysteine	11.280	1.05092	64.4722	67.75498
Leucine	14.814		169.1556	0.00000
Proline	17.788	0.00602	755.4388	4.54886
Total amino acids				16245.41445

Note: RT = Retention time, RF = Retention factor

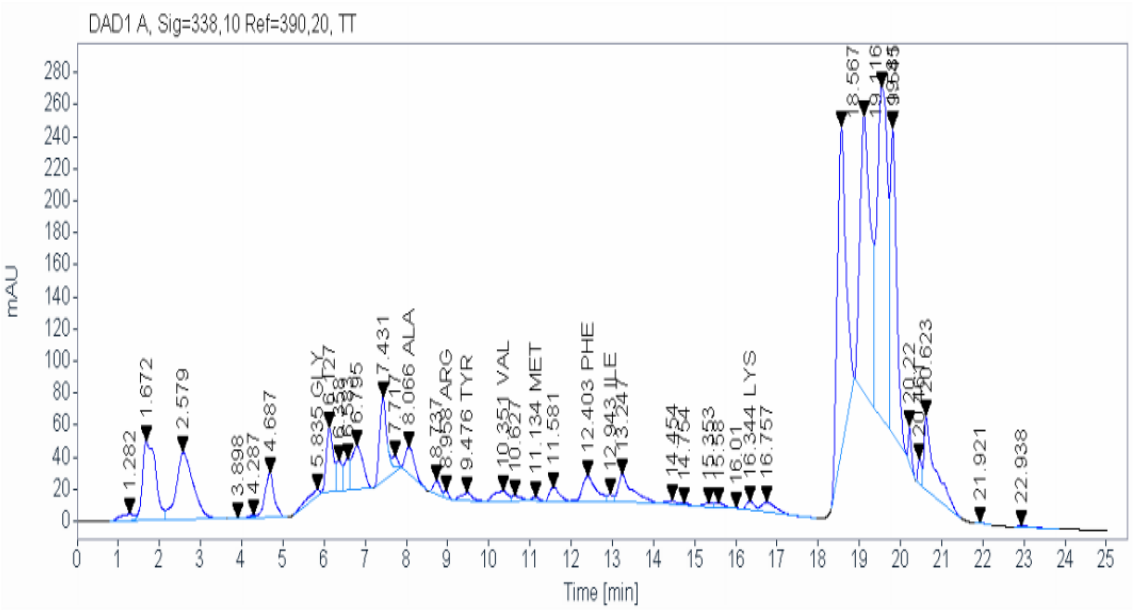

Figure 1: Chromatogram of aqueous extract of *P. niruri* stem by DAD

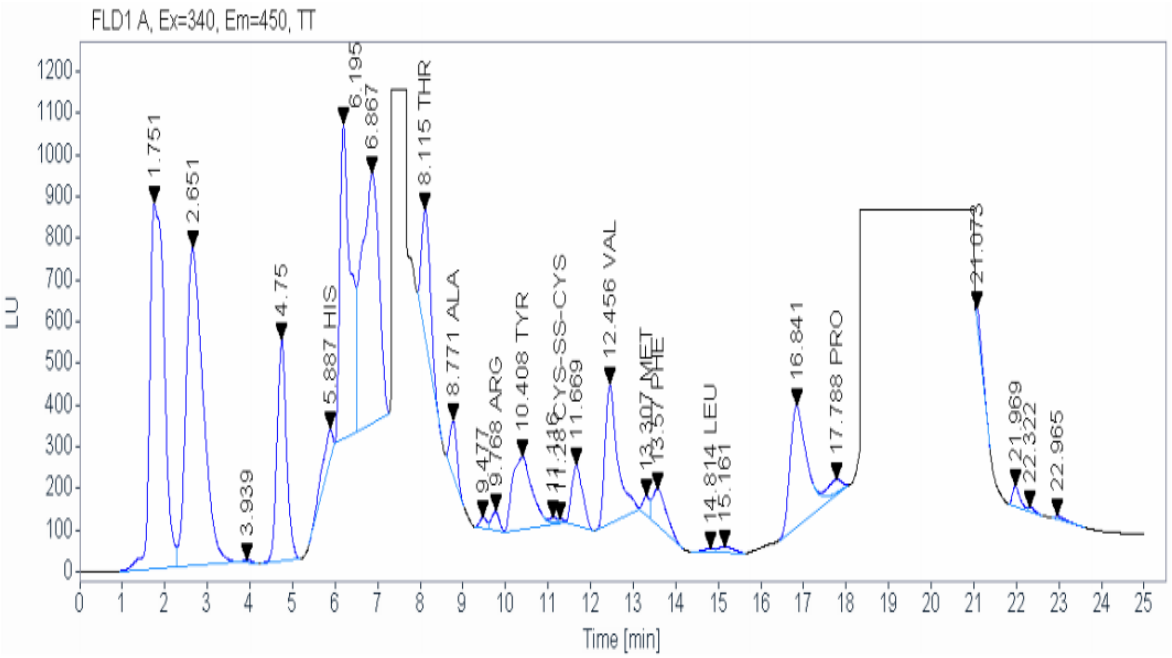

Figure 2: Chromatogram of aqueous extract of *P. niruri* stem by FLD.

Table 4: Amino acid composition of ethanol extract of *P. niruri* stem.

Amino acids	RT (min)	RF	Area	Amount (Pmol/µl) Group
Aspartic acid	1.398		10.5267	0.00000
Glycine	5.825	0.19244	19.0229	3.66072
Alanine	8.061	1.52403	179.6459	273.78577
Arginine	8.721	1.46313	56.2720	82.33358
Tyrosine	9.443	128.16724	23.7795	3047.75364
Valine	10.163	0.18806	38.0831	7.16182
Methionine	11.108	33.34299	13.0374	434.70598
Phenylalanine	12.414	0.50272	53.5455	26.91833
Leucine	13.728	0.60841	32.6089	19.83968
Lysine	16.335	0.68494	107.2142	73.43576
Histidine	5.879	0.00494	346.3201	1.71076
Threonine	8.112	0.05676	2763.9263	156.87385
Cysteine	11.176	1.76779	116.6491	206.21068
Total amino acids				4334.39057

Note: RT = Retention time, RF = Retention factor

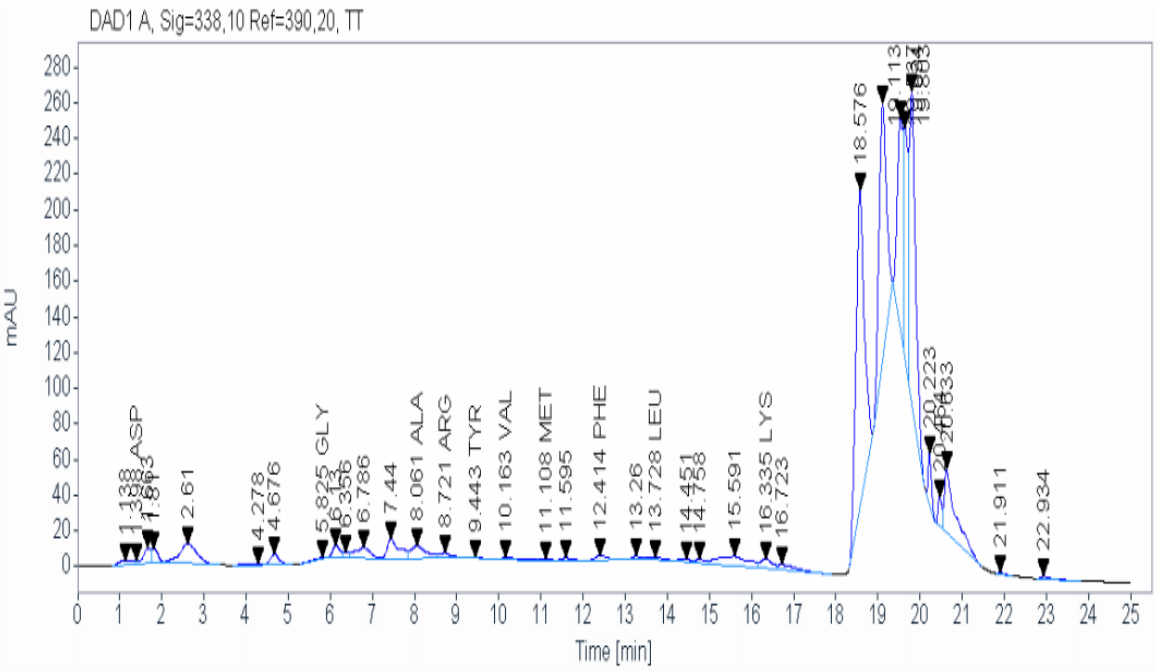

Figure 3: Chromatogram of ethanol extract of *P. niruri* stem by DAD

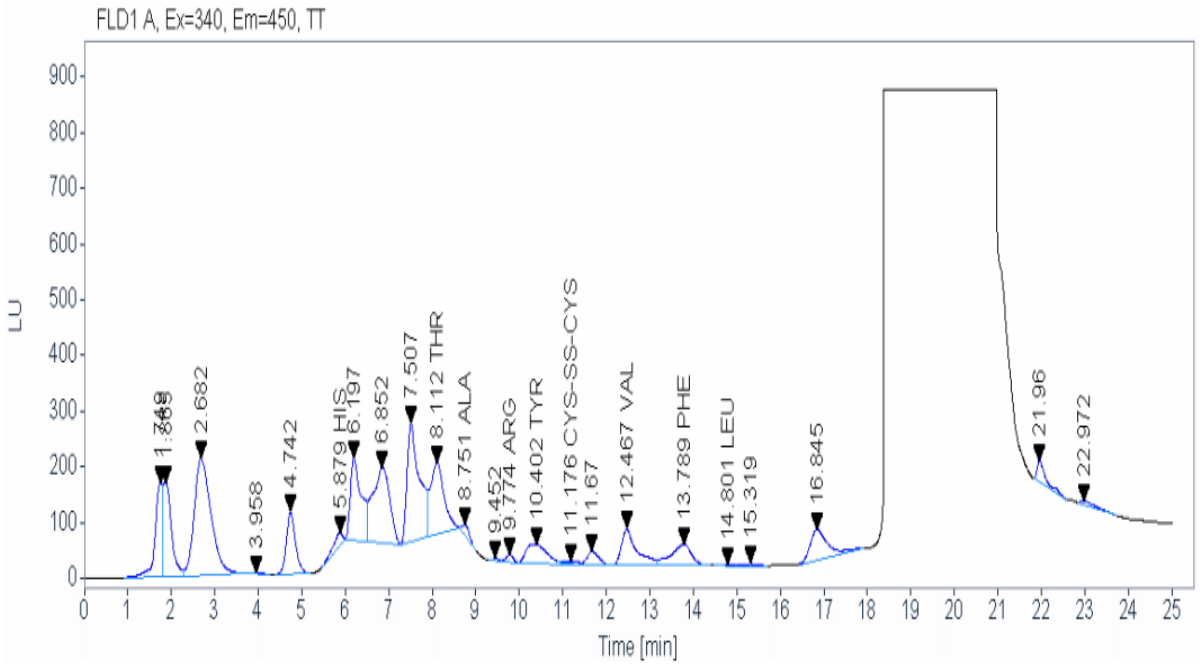

Figure 4: Chromatogram of ethanol extract of *P. niruri* stem by FLD

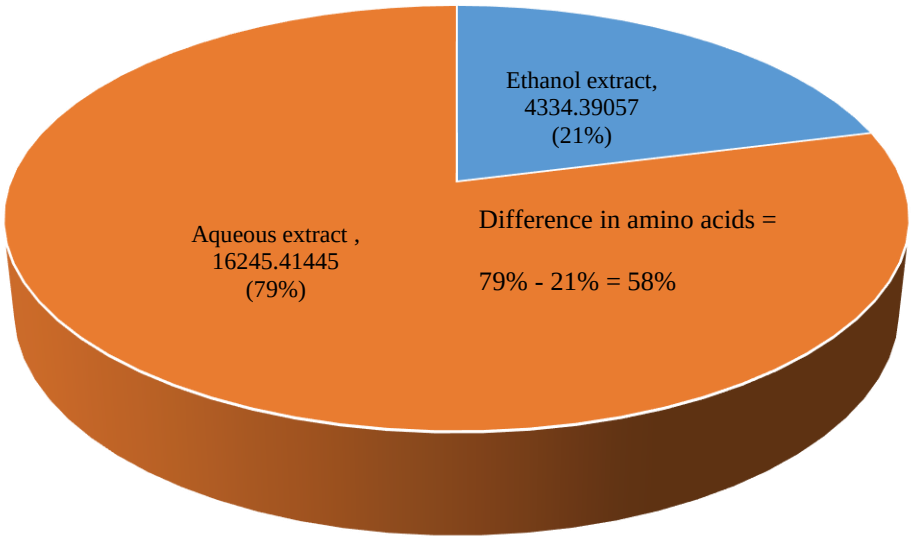

Figure 5: Total amino acid composition of aqueous and ethanol extract of *P. niruri* stem

Discussion

Proteins in the diet are the primary source of essential macromolecules called amino acids. They are the components of proteins and serve as transition points in a variety of metabolic processes (Mohanty et al., 2014). The human body needs amino acids for the synthesis of proteins, which are required for the movement of oxygen, vitamins, carbon dioxide, enzymes, structural proteins, regulation of food intake, gene expression, and protein phosphorylation, among other essential functions (Chalamaiah et al., 2012; Machado et al., 2020). The findings of this investigation demonstrate the abundance of amino acid content in *P. niruri* stem. This concurs with the research conducted by Mediani et al. (2015), which detected amino acids in *P. niruri*. According to Jing et al. (2019), tyrosine and phenylalanine are more important for the central nervous system compared to other amino acids. The use of these supplements in food can help control neurovegetative disorders, which can affect several cognitive functions, such as learning, memory, and reasoning processes (Doma et al., 2021; Colzato et al., 2015). The production of several bioactive compounds known as neurotransmitters, which have an impact on the brain, depends on these amino acids as well (Chen et al., 2021; van de Rest et al., 2017). Methionine has been shown by Zanandrea et al. (2020) to reduce agitation and despair, and by Martínez et al. (2017) to have the power to regulate the body's metabolic functions and disrupt lipid metabolism. The hepatic processes of gluconeogenesis, transamination, and autophagy are mediated by Alanine. Leucine, isoleucine, and valine are necessary substrates. Leucine is a source of psychological energy and controls gene expression, protein turnover (mTOR signaling), and other processes (Dennis-Eboh et al., 2023; Kumar et al., 2021; Cruz et al., 2020; Slobodianiuk et al., 2019). According to Etzel (2004), leucine is the only amino acid that can promote the production of muscle protein. Threonine increases the activity of the central nervous system (Kino, 2018; Iriondo-DeHond et al., 2020; Behl et al., 2022). Research has demonstrated that lysine enhances the body's capacity for growth (Yang et al., 2017). According to Malongane et al. (2017), proline works in concert with gamma-aminobutyric acid to suppress its effects.

CONCLUSION

This work has demonstrated the presence of amino acids in the aqueous and ethanol extracts of *P. niruri* stem. However, *P. niruri* aqueous stem extract has a higher amino acid content than its ethanolic stem extract. Consequently, the stem of *P. niruri* may be used as a nutrient source to improve human health and fight malnutrition, or it may serve as a therapeutic substitute.

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