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Research Article

Quantitative and Qualitative Analyses of Amino Acids in *Morinda citrifolia* (Rubiaceae)

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ABSTRACT
Background: Amino acids and chemical elements play a lead role in all physiological processes in the human body. The consistency of their composition is one of the most important and critical conditions for normal functioning and development of organisms. The variation in composition of amino acids and elements in the body leads to deterioration in health status. One possibility of timely correction of violations of elemental and amino acid homeostasis in the human body is the use of extracts from medicinal plants. Morinda citrifolia, commonly known as Noni, contains essential amino acids for life. Methods: A total of fourteen essential, conditionally essential and nonessential amino acids were examined by thin-layer chromatography (TLC). Two categories of extraction solutions; aqueous extraction and 1% HCl extraction, were prepared. Total quantity of amino acids in the fruits, leaves and roots of noni were investigated by spectrophotometric method of analysis with glutamic acid solution prepared as a standard. Result: The TLC analysis showed presence and absence of amino acids in the parts investigated. The leaves extracts of noni showed the highest content of amino acids whereas the roots demonstrated the least amino acid content. Methionine, an important ingredient for tissue development and growth were found in the 1% HCl sample extraction analyzed with the leaves and roots of noni. Leucine, a muscle building compound was observed in the fruits of the aqueous sample extraction of noni. Total content of amino acids in alcohol extraction from the roots, leaves and fruits of crude extraction of *Morinda citrifolia* was much lower than in aqueous extractions. Conclusion: This study confirmed the presence of amino acids in noni. Higher total content of amino acids was observed in aqueous medium as compared to ethanol medium. In conclusion, therefore, aqueous extraction of noni presents an optimal amount of amino acids which play crucial antioxidative roles in living organisms.

Keywords: *Morinda citrifolia (Noni), amino acid, glutamic acid, thin layer chromatography(TLC), spectrophotometry.*

INTRODUCTION

Morinda citrifolia Linn (Rubiaceae) as a tropical plant has been known to science since some 2000 years ago[1]. There are several species of this plant, regionally located in Africa, Asia, America and Australia. The Polynesians have been credited for discovering the medicinal importance of Noni[1]. The phytochemical components reported through investigations were alkaloids, vitamins, amino acids and minerals[2]. Dr. Heinicke described in his publication the effect of xeronine on proteins in organisms and its activities of fighting diseases[3]. Amino acids and chemical elements play an important role in all physiological processes in the human body. The consistency of their composition is one of the most important and critical conditions for normal functioning and development of organisms. Leucine, Lysine and Methionine as essential amino acids are critical in health regeneration as they help build muscles, develop tissues and maintain general well-being[4,5]. These acids exert antioxidative effects on healthy subjects. *Morinda citrifolia*, has been investigated to contain amino acids[3.6]. Noni contains essential, conditionally essential and nonessential amino acids which makes it a very potent medicinal ingredient for future drug developments. This development raises the question of which part of *Morinda citrifolia* contains valuable amino acids, and for this, an investigation was conducted. The procedure of investigation and results are made available in this paper.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh roots, leaves and fruits of noni were collected from the south-eastern coastal belt of Ghana in West Africa in 2015. The samples were authenticated at the Centre for Scientific Research into Plant Medicine, Akropong-Akuapem, Ghana, same year.

Preparation of Crude Plant Material

Fresh samples of noni were dried under sunlight within a

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Table 1: Result of TLC performed on noni samples in aqueous extraction.

	Amino Acids	Leaves	Roots	Fruits
1	Ornithine	-	-	-
2	α-Alanine	+	+	+
3	Leucine	+	-	+
4	Lysine	-	-	-
5	Arginine	-	-	-
6	Methionine	+	-	-
7	Glutamic acid	+	-	+
8	Proline	-	-	-
9	Asparagine	-	-	+
10	Cysteine	-	-	-
11	Norleucine	-	-	-
12	Glutamine	+	+	+
13	Serine	-	-	-
14	Tyrosine	-	-	+

+ = presence of amino acid; *- = absence of amino acid*

Table 2: Results of TLC performed on noni samples in acidic extraction (1% HCl).

	Amino acids	Leaves	Roots	Fruits
1	Ornithine	-	-	-
2	α-Alanine	-	-	+
3	Leucine	+	-	-
4	Lysine	-	-	-
5	Arginine	-	-	-
6	Methionine	+	+	-
7	Glutamic acids	+	+	+
8	Proline	-	-	-
9	Asparagine	+	+	+
10	Cysteine	-	-	-
11	Norleucine	+	+	-
12	Glutamine	+	+	+
13	Serine	-	-	-
14	Tyrosine	-	+	+

+ = presence of amino acid; *- = absence of amino acid*

temperature range of 33-40 ^{o}C for two weeks, thereafter packaged and labelled accordingly before being transported to Saint-Petersburg State Chemical Pharmaceutical Academy, Russia and stored at the Department of Industrial Technology of Drug Developments for further investigations. The dried samples were pulverised in the laboratory with an electric rotor, screened through a 2mm sieve and stored in well-labelled containers. One (1) gram sample of each part of noni under investigation was quantified on an electronic scale.

Preparation of Solutions

1 % HCl solution was prepared using the prescribed standards in the 13th edition of the Russian Pharmacopeia, filled into a bottle, sealed and labelled.

Extraction

Extraction for TLC experimentation

Six empty flacons were obtained and labelled appropriately. A gram of dried sample each was introduced into the appropriate flacon. 40 ml of 1% HCl was poured into three of the flacons labelled accordingly, the other

Table 3: Measure of polarity (Rf) in aqueous extracts of noni samples.

	Objects of investigation	Polarity (Rf)	Colors
1	Ornithine	0.08	Brown
2	α-Alanine	0.51	Pink
3	Leucine	0.67	Pink
4	Lysicine	0.06	Light brown
5	Arginine	0.09	Light pink
6	Methionine	0.78	Light pink
7	Glutamic acid	0.51	Deep red
8	Proline	0.39	Light brown
9	Asparagine	0.45	Light pink
10	Cysteine	0.20	Very light brown
11	Norleucine	0.53	Pink
12	Glutamine	0.45	Light pink
13	Serine	0.5	Yellowish pink
14	Tyrosine	0.75	Light pink
15	Leaves	0.3; 0.46; 0.52; 0.71; 0.73; 0.28; 0.55 ;0.74	Light yellow
16	Roots	0.78; 0.63; 0.48; 0.62; 0.63; 0.51	Light yellow
17	Fruits	0.4; 0.54; 0.81; 0.43; 0.48; 0.64; 0.71; 0.55; 0.77	Light yellow

three flacons were filled with 40 ml of distilled water. The flacons were tightly sealed under pressure tap affixed with a tube and a needle. For 180 minutes, the six flacons were vigorously subjected to shaking at a rotational speed of 200 rpm and heating at a temperature of 60 ^{o}C. Then after, each sample was filtered through a 125-micron nylon filter mesh cloth into labelled empty flacons.

Extraction for Spectrophotometry experimentation

2.0 g of pulverised crude sample was placed in a 100 ml conical flask, and 50 ml of purified water added. Then the flask was stoppered, attached to the back of the fridge for 3 hours, heated in a boiling water bath. Next, the flask was cooled to room temperature, the extract was filtered through a paper filter into a flask with a capacity of 100 ml.

TLC Experimentation

Aliquots were drawn and TLC was performed on precoated plates of silica gel 60 F254 (Merck, USA) using the Butanol/acetic acid/Dist. H_2O (4:2:1) as eluent. The plates were dried and sprayed with 0.1 % Ninhydrin solution prepared and sparingly heated in an electric oven at a temperature of 60 ^{o}C for fifteen (15) minutes. The plates were investigated under UV light of 254 nm and 365 nm in a UV chamber. The observed spots were marked. The polarity (reference points, Rf) of each observable point was measured, calculated and recorded.

Spectrophotometry Experimentation

Table 4: Measure of polarity (Rf) in acidic extraction (1% HCl) of noni samples.

	Objects of Investigations	Polarity (Rf)	Colors
1	Ornithine	0.08	Dark brown
2	α-Alanine	0.57	Light pink
3	Leucine	0.76	Pink
4	Lysicine	0.09	Light brown
5	Arginine	0.15	Light pink
6	Methionine	0.63	Pink
7	Glutamic acids	0.59	Brownish red
8	Proline	0.44	Brown
9	Asparagine	0.57	Pink
10	Cysteine	0.29	Brown
11	Norleucine	0.79	Pink
12	Glutamine	0.62	Light pink
13	Serine	0.41	Deep pink
14	Tyrosine	0.75	Light pink
15	Leaves	0.75; 0.56; 0.74; 0.7;0.88; 0.59; 0.81	Yellow
16	Roots	0.73 ;0.93; 0.63; 0.86; 0.75 ;0.56; 0.56; 0.74; 0.94	Yellowish brown
17	Fruits	0.57; 0.49; 0.56; 0.75; 0.58	Light Pink

Table 5: Results of quantitative determination of amino acids in the aqueous extract from roots of *Morinda citrifolia* in terms of glutamic acid.

Series	Optical density (A)	Percentage of amino acids in relation to glutamic acid (X), %	Metrological characteristics
1	0.4112	0.3849	S=0.001696
2	0.4091	0.3829	S_x=0.0006924
3	0.4129	0.3866	t (0.05; n-1) =2.57
4	0.4132	0.3869	Δx=0.001779
5	0.4098	0.3837	ε, %=0.4200
6	0.4101	0.3839	
$\underline{X}$	0.4111	0.3848	

2 ml of extract (fruit, leaves and roots) was placed in three labelled volumetric flasks with a capacity of 50 ml. 1 ml of 0.25% solution of sodium carbonate, 2 ml of 1% Ninhydrin solution in 95% alcohol were added and heated for 10 min in a boiling water bath, cooled, adjusted to the mark with distilled water.

In the aqueous extract from the fruit of Morinda citrifolia, an aliquot of 4 ml was taken and placed in a flask with a capacity of 50 ml, 2 ml of 0.25% solution of sodium carbonate, 4 ml of 1% Ninhydrin solution in 95% alcohol were added and heated for 10 min in a boiling water bath,

Table 6: Results of quantitative determination of amino acids in aqueous extracts from the leaves of *Morinda citrifolia* in terms of glutamic acid.

Series	Optical density (A)	Percentage of amino acids in relation to Glutamic acid (X), %	Metrological characteristics
1	0.3375	0.6742	S=0.002374
2	0.3406	0.6805	S_x=0.0009690
3	0.3402	0.6796	t (0.05; n-1)=2.57
4	0.3397	0.6786	Δx=0.002490
5	0.3401	0.6794	ε, %=0.3500
6	0.3386	0.6764	
$\underline{X}$	0.3394	0.6781	

Table 7: Results of quantitative determination of amino acids in aqueous extracts from the fruit of Morinda citrifolia based on glutamic acid.

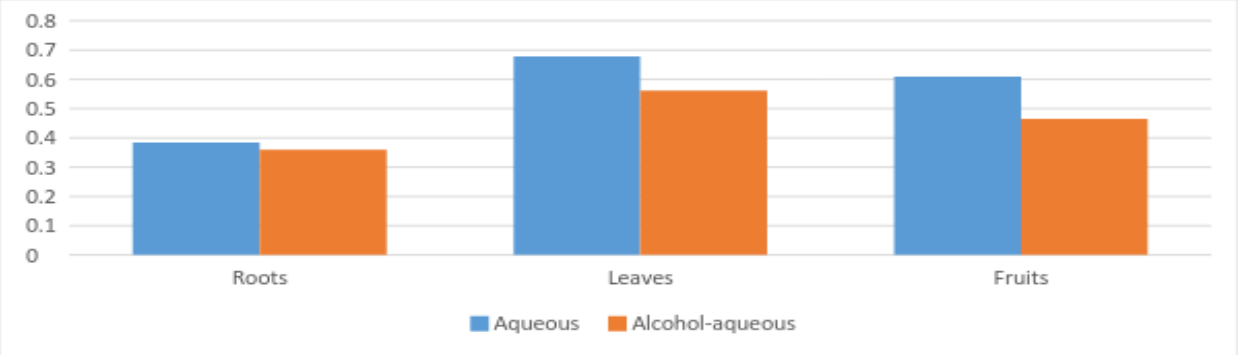
Series	Optical density (A)	Percentage of amino acids in relation to glutamic acid (X), %	Metrological characteristics
1	0.6345	0.6079	S=0.001129
2	0.6370	0.6104	S_x=0.0004612
3	0.6373	0.6107	t (0.05; n-1) =2.57
4	0.6350	0.6085	Δx=0.001185
5	0.6349	0.6084	ε, %=0.1854
6	0.6359	0.6094	
$\underline{X}$	0.6358	0.6093	

Table 8: Results of quantitative determination of amino acids in alcohol extraction from roots of *Morinda citrifolia* in relation to glutamic acid.

Series	Optical density (A)	Percentage of amino acids in relation to glutamic acid (X), %	Metrological characteristics
1	0.1846	0.3627	S=0.001961
2	0.1836	0.3606	S_x=0.000800
3	0.1819	0.3573	t (0.05; n-1) = 2.57
4	0.1826	0.3587	Δx=0.002058
5	0.1839	0.3614	ε, %=0.5448
6	0.1829	0.3592	
$\underline{X}$	0.1832	0.3600	

cooled and adjusted to the mark with distilled water.

In a parallel volumetric flask with a capacity of 50 ml, 1 ml of standard glutamic acid solution was added and the procedure mentioned above repeated. The optical density was then measured by spectrophotometer analyzer at an absorption wavelength of 400±2 nm relative to distilled water. The total content of amino acids in alcohol-water extract was investigated: 1.0 g of pulverised sample was measured into a 100ml conical flask, 50 ml of 70% ethanol was added and the flask attached to a refrigerator and



Graph 1: Total content of amino acids in aqueous extracts from various parts of *Morinda citrifolia*.

Table 9: Results of quantitative determination of amino acids in alcohol extraction from the leaves of *Morinda citrifolia* in relation to glutamic acid.

Series	Optical density(A)	Percentage of amino acids in relation to glutamic acid (X), %	Metrological characteristics
1	0.5588	0.5582	S=0.004188
2	0.5626	0.56205	S_x=0.001709
3	0.5590	0.5584	Δx=0.004394
4	0.5599	0.5593	t (0.05; n-1)
5	0.5699	0.5694	=2.57
6	0.5616	0.5609	ε, %=0.7459
X	0.5619	0.5614	

Table 10: Results of quantitative determination of amino acids in alcohol extraction from the fruit of a *Morinda citrifolia* in relation to glutamic acid.

Series	Optical density (A)	Percentage of amino acids in relation to glutamic acid (X), %	Metrological characteristics
1	0.9661	0.4629	S=0.001993
2	0.9681	0.4638	S_x=0.000813
3	0.9693	0.4644	7
4	0.9773	0.4683	t (0.05; n-1)
5	0.9661	0.4629	=2.57
6	0.9687	0.4642	Δx=0.002091
X	0.9693	0.4644	ε, %=0.4292

heated in a water bath for an hour. Then after, the flask was cooled at room temperature, and 70% alcohol topped up to the mark. 2 ml of extracts from leaves and roots, 4 ml of the extract from the fruit of Morinda citrifolia were drawn and transferred in three different volumetric flasks with a capacity of 50 ml. The procedure of addition of sodium carbonate, Ninhydrin, heating, and topping up with distilled water, was repeated for each extract.

Other preparations

Glutamic acid Standard

0.05g glutamic acid was weighed into a volumetric flask with a capacity of 100 ml. 30 ml of distilled water was added, vigorously shaken to effect complete dissolution, after which distilled water was added to the mark. The preparation was filled into a flacon, sealed, labelled and kept at temperature of 8 ^{o}C for 30 days.

Ninhydrin Standard

1.0g standard Ninhydrin powder was measured into a 100ml volumetric flask, 30 ml of 95% ethyl alcohol was added, gently shaken for complete dissolution and 95% ethyl alcohol was added to the mark. The solution was transferred to a flacon, sealed, labelled and kept in a cool dark place. Due to Ninhydrin coloration, medical gloves were worn throughout the preparation period. Statistical processing of experimental data was calculated in accordance with the requirements prescribed in the 13th edition of the Russian Pharmacopoeia - "Statistical processing of results of chemical experiment"[7].

RESULTS

Qualitative analysis of Amino acids in Noni samples

The comparative analyses of the presence of amino acids in the parts investigated are illustrated in tables 1 and 2. The reference points (Rf)/polarity and the various colorations observed are presented in tables 3 and 4.

Quantitative analysis of Total amino acid content in Noni samples

The total content of free amino acids in the root extracts of *Morinda citrifolia* in percentage (X %) value of sample material relative to glutamic acid was calculated, using the formula:

$$X = \frac{A_x \times m_0 \times K \times 100}{A_0 \times m_x \times (100 - W)}, \qquad (1.0)$$

where X is the optical density of test solution;

A_0 - optical density of the solution of glutamic acid;

K – the dilution factor;

M_x - mass of sample material, g;

M_0 – mass of glutamic acid, g;

W – loss in mass at drying, %.

The amount of amino acids in aqueous and alcohol extracts from roots and leaves of *Morinda citrifolia* was calculated by the formula:

$$X = \frac{A_x \times m_0 \times 50 \times 50 \times 100}{A_0 \times m_x \times \mathbf{2} \times 100 \times 50 \times (100 - W)}, \qquad (1.1)$$

The amount of amino acids in aqueous and alcohol extracts from roots and leaves of *Morinda citrifolia* was calculated by the formula:

$$X = \frac{A_x \times m_0 \times 50 \times 50 \times 100}{A_0 \times m_x \times \mathbf{4} \times 100 \times 50 \times (100 - W)}, \qquad (1.2)$$

The calculation of the quantitative content of the amount of amino acids in the crude samples was done according to

Table 11: Correlation of amino acids in aqueous extraction of *Morinda citrifolia.*

Total Amino acid found, mg	Added quantity of glutamic acid, mg	Expected quantity of amino acid, mg	Obtained quantity of amino acid, mg	Relative error, %
0.3375	0.25	0.5875	0.5902	-0.46
0.3406	0.25	0.5906	0.5890	0.27
0.3402	0.25	0.5902	0.5879	0.39
0.3397	0.25	0.5897	0.5967	-1.19
0.3401	0.5	0.8401	0.8435	-0.40
0.3386	0.5	0.8386	0.8306	0.95
0.3396	0.5	0.8396	0.8379	0.20
0.3409	0.5	0.8409	0.8398	0.13

the formulas (1.1), (1.2). The results are presented in tables 5-11.

$\underline{X}$ – arithmetic average,

S –standard deviation,

S_x – average standard deviation,

P –confidence level,

t – Student's t-distribution

Δx – confidence limit,

$\underline{\varepsilon}$, % - relative error.

Statistical data of the six parallel measurements showed that the quantitative content of amino acids in aqueous extracts from roots, leaves and fruits of *Morinda citrifolia* were (0.3848±0.0018) %, (0.6781±0.0025) %, (0.6093±0.0012) %, respectively. The highest content of free amino acids in terms of glutamic acid was observed in the leaves (see Fig. 5).

Validation of metrological calculations

The relative error of the average result of this method of quantitative determination of amino acids according to the metrological calculations (ε %) was further determined. The result therefore showed a relative error not exceeding 2%. The investigation was carried out using an aqueous extraction of *Morinda citrifolia with addition of* a known amount of glutamic acid (0.25 and 0.5 mg/ml). It can be observed that the relative error of the result of a separate determination does not exceed 2%. Therefore, the method of analysis of amino acids meets the correlation criterion. The result is shown in table 11.

DISCUSSIONS

Thin Layer Chromatography of Noni samples

Varied amino acid groups were present or absent in the aqueous and alcoholic extractions investigated. The polarity of the results defined the density of amino acids identified in the various parts investigated. This gives an ideal presentation of noni being an essential phyto plant with promising activities in the building of proteins and fighting various diseases when incorporated in the development of nutraceuticals and biologically active preparations.

Spectrophotometric analysis of Noni samples

Total content of amino acids in alcohol extraction from the roots, leaves and fruits of crude extraction of *Morinda citrifolia* was much lower than in aqueous extractions (see Tables 5-10). This is due to the fact that amino acids contain multiple charged groups and well soluble in polar solvents[8]. It is well known that the polarity of water as solvent is superior to the polarity of ethanol, so aqueous extract of *Morinda citrifolia* are richer in amino acid content than in alcohol-water mixture. Thus, for the quantitative analysis of free amino acids in various parts of *Morinda citrifolia*, it is highly recommended to rely on the aqueous extraction of Noni for further drug developments.

CONCLUSIONS

Amino acids are present in Noni and their polarity is well established for the development of drug preparations. The total content of amino acid was found to be of good measure, which further guides in drug development from Noni.

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