Study on the Qualitative Determination of Amino Acids from the Seeds of *Moringaoleifera*Lam. (Dan-da-lun)

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Abstract

In this research work, the seeds of *Moringaoleifera*Lam. were selected for chemical analysis and the samples were collected from Myit-Nge, Amarapura Township, Mandalay Region. The preliminary phytochemical screening of the seeds of *Moringaoleifera*was performed and the mineral contents were also determined by EDXRF analysis, respectively. In addition, the total amino acids of plant materials were extracted with ethanol for the qualitative test of amino acids. And then, the variety of amino acid constituents from protein acid hydrolysate of meal cake was estimated by Thin-Layer Chromatography, one dimensional Paper Chromatography, colour developer ninhydrin reagent and identified with eighteen standard amino acids.

Keywords: *Moringaoleifera*. Lam, EDXRF, Thin- Layer

Introduction

*Moringaoleifera*Lam, locally known as Dan-da-lun, belongs to familyof Moringaceae. It was found in north-west India and was plentiful on recent aland in or near sandy beds of rivers and steam. It grows in all types of soils,except stiff clays and thrives best under the tropical insular climate of southIndia. It is also distributed in all parts of Myanmar. Dan-da-lun is one of the Myanmar indigenous medicinal plants. All partsof the tree are considered medicinal and used in the treatment of ascites,rheumatism, venomous bites and circulatory stimulants.

In Myanmar, all parts of *Moringaoleifera*Lam, plants are medicinallyused. The seed of *Moringaoleifera*Lam, contains some amino acids, such asalanine, arginine, glutamic acid, glycine, serine, threonine, valine, aspartic acid,trytophan, cystine, other vitamins and minerals. Hence, it was selected forchemical investigation of the rich source of amino acid.In this research work, amino acid constituents from the seed of Dan-dalunwere identified with authentic amino acid in Paper Chromatography andThin layer Chromatography by measuring the $R_f$values for the qualitative estimation (Website 1).

(a) **(b)** **(c)**

Figure 1. (a) Dried Pods
(b) Seed Kernel with Husk
(c) Seed Kernel without Husk

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Materials and Method

General experimental techniques

The seeds of *Moringaoleifera*Lam., (Dan-da-lun) were collected from Myit-Nge, Amarapura Township, Mandalay Region. The air-dried sample was crushed into small pieces and collected in a well-stoppered bottle.This sample was ground into powder in an electric blender and stored in airtight container. Preliminary photochemical constituent was investigated by test tube method. Nutritional values were determined by AOAC method.

Preliminary Phytochemical test

A few grams of dried*Moringa*seeds powder were subjected to the tests of alkaloids, amino acids, glycosides, phenolic compounds, polyphenol, steroids and terpene according to the standard producers (Harborne,1984; M-Tin Wa, 1970;Robinson.,1983;Trease and Evans,1980; Vogel, 1966; Marini-Bettolo,1981).

Determination of Nutritional Values by AOAC method

The moisture content was determined by the oven drying method. The nitrogen content was determined by Kjeldahl digestion method and protein content was calculated by multiplying percent nitrogen by the factor 6.25. The fat content was determined by Soxhlet extraction method using petroleum ether (b.p 60-80˚C) run for 8h. The ash content was determined by placing sample in pre-weighed crucible and placed in muffle furnace at 500˚C for 6h. Carbohydrate percentage was determined by calculation(Mark and Stewart, 1975, Pearson, 1981, Joslyn, 1973, AOAC, 2000, Woodman, 1941, Anderson, 1984; Atwater and Woods, 1907).

Qualitative Tests of Amino Acid
Extraction of Total Amino Acid from the Seeds of *Moringaoleifera* Lam. (Dan-da-lun)

The air dried samples (30 g) were taken place in a conical flask and97 % ethanol (150 mL). Then, the mixture was heated in a water bath for about 30 minutes. It was cooled, filtered and centrifuged. The supernatant wasdecanted in a test tube and the residue was washed for 2-3 times with the samesolvent and the supernatant was collected in the same test tube. The volume ofthe filtrate was reduced to dryness by heating in a water bath at (40-50°C). Thefinal sample can be used for different qualitative experiments forThin Layer and Paper Chromatography of amino acids.

Hydrolysis of Meal Cake

Apparatus Required:1. Test tubes, 2. Electronic Balance, 3. Filter paper Whatman No. 1, 4. Funnels.

Reagents Required:1. 6 N – HCl, 2. 75 % ethanol, 3. 0.2 % ninhydrin reagent solution in acetone.

The defatted Dan-ta-lun seed meal cake (0.5 g) was exactly weighed andintroduced into a test tube and 7 mL of 6 N hydrochloric acid was then added.The test tube was tightly sealed and hydrolysed in a boiling water bath for24 hours.After the hydrolysing period, the contents in the test tube were cooled toroom temperature and then filtered with filter paper. The residue was washed for many times with hot distilled water until the last drop of the filtrate showednegative to ninhydrin test. The

filtrate and washings were put together in aprocelain basin and decolorized with activated charcoal and filtered. Thecharcoal residue was filtered and washed with hot distilled water until the final drop of the washing was not responed to ninhydrin reagent. Then the clear andcolourless filtrate was concentrated by evaporating on a water bath. Theconcentrated hydrolysate was introduced into a glass bottle and the volume wasmade up to 5 mL with 75 % ethanol. It was then stored in a refrigerator when itwas not in use.One drop of filtrate was spotted on a filter paper Whatman No.1. Then it was dried and sprayed with 0.2 % ninhydrin reagent. Then the paper was heated in an oven at 60°C for two minutes. The appearance of purple colour spot on the paper shows the remainder of amino acids.

Separation of Amino Acids from Sample Solution (Acid Hydrolysate of Meal Cake) by Thin Layer Chromatography

Apparatus Required:(1) TLC plate, (2) Capillary tube, (3) TLC tank
Reagents Required:(1)n-Butanol : Acetic acid : Water (4 : 1 : 5 v/v), (2) 0.2 % ninhydrin reagent

The tank to be used for TLC was first cleaned and dried. The solventwas poured into the tank in order to allow the sheet to dip into a depth of 5 mm.20 × 20 cm sheet was applied with sample solution for one-dimension. A baseline was drawn with a pencil along 2 cm from one edge in length on the sheet.On the front side, a line paralled to the base line, was drawn along 0.2 cm fromthe edge so that the solvent front would be reached to 18 cm from the base line.To be spotted on the sheet, was marked on the base line with a pencil.After spotting, the sheet was placed in care into the tank by harding itwith forceps so that the lower edge having origins was inserted into the solventto a depth of approximately 3 mm in all cases. The tank was then covered withthe lids as quickly as possible. When the solvent front was 0.5 cm mark fromthe top of the sheet, the chromatogram was run off and dried with a dryer.After development the sheet was removed from the tank and dried aftermarking the front. Amino acids were detected by spraying the sheet with 0.2 %ninhydrin reagent.

Results and Discussion

The amino acid and mineral content of the seeds of *Moringaoleifera*Lam. (Dan-da-lun) were qualitatively investigated.Firstly, the preliminary phytochemical screening of the seeds of Dan-dalunwere carried out, in which, the seeds of Dan-da-lun contain flavonoid,phenolic, polyphenol, sterol, terpene, amino acid, glycoside and amino acidcompounds.The chemical composition of the sample such as moisture, ash, protein,carbohydrate and fat were determined by the usual methods. The water solublecarbohydrate was estimated by the phenol-sulphuric method and protein byKjeldahl's method. The result obtained was tabulated in Table (1).Furthermore, mineral contents of this sample were determined byapplying Energy Dispersive X-rays Fluorescence Spectroscopy. The seeds ofDan-da-lun consists of K (0.7516 %), S (0.4001 %), P (0.3624 %), Cl(0.1671 %), Ca (0.1313 %), Al (0.1201 %), Fe (0.0106 %), Zn (0.0036 %), Mn(0.0021 %) and Cu (0.0008 %) respectively.The many qualitative tests of amino acid were done. From these results,the seeds of Dan-da-lun contain twelve amino acids such as alanine, arginine,aspartic acid, glycine, serine, threonine, valine, glutamic acid, cystine,tryptophan, tryosine and lycine.Finally, separation and identification of amino acid constituents from theseeds of Dan-da-lun were investigated by one dimension Paper Chromatographyand Thin Layer

Chromatography techniques. The amino acid constituents wereidentified with the R_f values of standard amino acids. These results confirm thetwelve amino acids containing in the seeds of *Moringaoleifera*Lam.(Dan-da-lun).

Table 1. Moisture, Ash, Protein, Carbohydrate and Fat Contents in the Seeds of *Moringaoleifera*Lam.

No.	Contents	Value (%)
1.	Moisture	2.0
2.	Ash	4.2
3.	Protein	19.0
4.	Carbohydrate	16.9
5.	Fat	29.3

All results obtained by three times repeatation were described.The seeds of *Moringaoleifera*Lam., were tested by phytochemicalscreening and the results are shown in Table (2).

Table 2. Results of Preliminary Phytochemical Test for*Moringaoleifera*Lam.

No.	Test	Reagent used	Observation	Result
1.	Alkaloid	Dragendroff's reagent	Yellow ppt	+
2.	Phenolic compound	10 % $FeCl_3$	Purplish color solution	+
3.	Polyphenol	1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6]$	Blue green color solution	+
4.	Steroid	Pet ether, H_2SO_4 (conc:)	Green color solution	+
5.	Terpene	1-2 drops of conc: H_2SO_4, $CHCl_3$, acetic anhydride	Yellow color solution	+
6.	Glycoside	10 % $Pb(COOCH_3)_2$	White ppt	+
7.	Amino acid	Ninhydrin reagent	Purple color solution	+

(+) = Presence, (–) = Absence

Table 3. Mineral Content in the seeds of *Moringaoleifera*Lam. (Dan-da-lun) by EDXRF

No.	Element	Symbols	Concentration (%)
1.	Potassium	K	0.7516
2.	Sulfur	S	0.4001
3.	Phosphorus	P	0.3624
4.	Chlorine	Cl	0.1671
5.	Calcium	Ca	0.1313
6.	Aluminum	Al	0.1201
7.	Iron	Fe	0.0106
8.	Zinc	Zn	0.0036
9.	Manganese	Mn	0.0021
10.	Copper	Cu	0.0008

According to this table, potassium is the highest amount in these sample.

Separation and Identification of Amino Acids Composition in the Seeds of *Moringaoleifera*Lam. by One-Dimensional Thin Layer Chromatography

Figure 2. Separation of Amino Acids from Sample Solution (Ethanol Extract, Crude) by Thin-Layer Chromatography

Solvent - n-Butanol : Acetic acid : Water (4 : 1 : 5 v/v)
Spraying reagent - ninhydrin reagent (0.2 %)

The R_f values of authentic amino acids and sample solution wereidentified. The R_f values from ThinLayer and paper Chromatograms of sample solution and standard amino acids are shownin Table (4) and (5).In occurrence with the identification of the seeds of Dan-da-lun contain twelve amino acids such as Cystine, Lysine, Arginine, Serine, Aspartic acid, Glycine, Glutamic acid, Threonine, Alanine, Tyrosine, Valine and Tryptophan.

Table 4.R_f Values of Individual Standard Amino Acids and Amino Acid in the Seeds of *Moringaoleifera*Lam. by Thin Layer Chromatography

No.	Amino acid	R_f values for standard amino acid	R_f values for sample solution
1.	Cystine	0.363	0.378
2.	Lysine	0.242	0.257
3.	Histidine	0.196	-
4.	Arginine	0.272	0.287
5.	Serine	0.303	0.318
6.	Aspartic acid	0.304	0.333
7.	Glycine	0.287	0.301
8.	Glutamic acid	0.303	0.316
9.	Threonine	0.303	0.318
10.	Alanine	0.287	0.303
11.	Proline	0.348	-
12.	Tyrosine	0.424	0.439
13.	Methionine	0.484	-
14.	Valine	0.333	0.348
15.	Phenylalanine	0.484	-
16.	Tryptophan	0.439	0.454
17.	Isoleucine	0.439	-
18.	Leucine	0.590	-

Separation of Amino Acids from Sample Solution (Meal Cake) by One Dimensional Paper Chromatography

Table 5.R_f Values of Individual Standard Amino Acids and Amino Acid in the Seeds of *Moringaoleifera*Lam. by One Dimensional Paper Chromatography

No.	Amino acid	R_f values for standard amino acid	R_f values for sample solution
1.	Cystine	0.117	0.116
2.	Lysine	0.186	0.178
3.	Histidine	0.181	-
4.	Arginine	0.234	0.221
5.	Serine	0.247	0.246
6.	Aspartic acid	0.236	0.246
7.	Glycine	0.254	0.261
8.	Glutamic acid	0.274	0.272
9.	Threonine	0.319	0.303
10.	Alanine	0.356	0.329
11.	Proline	0.378	-
12.	Tyrosine	0.534	0.565
13.	Methionine	0.544	-
14.	Valine	0.365	0.375
15.	Phenylalanine	0.559	-
16.	Tryptophan	0.476	0.470
17.	Isoleucine	0.606	-
18.	Leucine	0.689	-

Conclusion

The seeds of *Moringaoleifera*Lam., Myanmar name Dan-da-lun wereselected for chemical investigation. Moisture, ash, protein, carbohydrate and fatcontents of *Moringaoleifera*Lam. (Dan-da-lun) were determined and it wasfound that moisture content was (2.0 %), ash content was (2.4 %), protein content was (1.9 %), carbohydrate content was (6.9 %) and fat content was(4.3 %). The seeds of Dan-da-lunconsistof many chemical constituents,minerals such as (K, S, P, Cl, Ca, Al, Fe, Zn, Mn, Cu) and twelve amino acids.Among them, the highest K value (0.7516 %) was observed in these results.This fact support that the seeds of Dan-da-lun respond the lowering bloodpressure and circulatory stimulants. Hence, it should be suitable to eat forhealthy and long life for human. In addition, the seed of Dan-da-lun alsocontains the five essential amino acids such as arginine, lysine, threonine,tryptophan, valine and seven non-essential amino acids. The amino acids infood must be consumed in amounts and proportions that closely approximatethe pattern required by the body. Amino acid is needed for vital processes like the building of proteins and synthesis of hormones and neutrotransmitters. According to the results from present researchwork, the seeds of *Moringaoleifera*Lam. (Dan-da-lun) are edible for the benefits of human health.

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