Qualitative and Quantitative Investigations of Amino Acids Content from the Flowers of *Moringa olifera* Lam. (Dan-da-lun)

Aye Mon Thida Nyo[1], Arnt Win [2], Thi Thi Khaing [3], Zin Min Htun[4]

Abstract

In the present research work, one of Myanmar well-known medicinal plants, *Moringa olifera* Lam., Myanmar named Dan-da-lun was selected for chemical investigations. Firstly, the preliminary phytochemical screenings of selected sample were carried out. Furthermore, mineral contents of selected sample were determined by Atomic Absorption Spectrometry (AAS) method. Moreover, qualitative tests of amino acids were examined. In addition, the qualitative determination of total amino acid content in selected plant materials were estimated by one and two dimensional Paper Chromatography methods and Thin Layer Chromatography methods by using the color developer ninhydrin reagent and identified with standard amino acids. Finally, the quantitative determination of amino acid content was determined by using two dimensional Paper Chromatography, ninhydrin assay method and UV visible spectrophotometer. The content percentages of some amino acids in flowers of Dan-da-lun were calculated as Arginine 0.8%, Aspartic acid 1.6%, Glycine 2.64%, Lysine 2.0%, Threonin 0.6%, and Valine 0.6% by applying the various concentration absorbance curves of standard six amino acids.

Key words - *Moringa olifera* Lam., Thin Layer Chromatography, one dimensional Paper Chromatography, two dimensional Paper Chromatography, Atomic Absorption Spectrometry (AAS) method

Introduction

Moringa oleifera Lam. of the family Moringaceae, popularly called 'miracle tree' and commonly known as horse radish tree or drumstick tree, is a native of sub Himalayan tracts of Northern India and is widely cultivated in tropical and subtropical regions. Various parts of the plant are used as nutritious food commodity in many countries, such as Pakistan, India, Philippines, Myanmar, and in some parts of Africa (Anwar *et al*, 2005). As a member of the Moringaceae family, *M. oleifera* Lam. is one of the most useful trees currently found throughout the tropics of the world (Jahn, 1988). Flowers are fragrant yellowish white, bisexual born in 10 to 25 cm long axillary, compound inflorescence called panicles. Individual flowers slightly zygomorphic has dimensions of about 1 cm length by 2 cm breadth set in a basal cup of thalamus namely hypanthium. There are two peaks of flowering October-November and April-May (Folkard *et al*., 1999). *Moringa* flowers are used in treating malnutrition in traditional settings (Fuglie, 2001), (Foild *et al*, 2001). They are used astonic, diuretic and considered to be anti-helminthic (Ozumba, 2008) (Fuglie, 1999).

Also, most of the parts of plant like seeds, leaves, flowers and roots are used for treatment of various diseases. Despite the wide claims on the nutritional and medicinal uses of the *Moringa* flowers, studies on the nutritional and bioactive potentials of this plant remain scanty. Almost all parts of this plant, like fruit (pods), gum, root, seed, bark, leaf, flowers and seed oil are used as a nutritional and nutraceutical resources for human and animal diets (Falowo *et al*, 2018). The leaves and flowers are good source of protein and dietary fiber with an adequate profile of amino acids and ash (Gopalakrishnan *et al*, 2016), (Rocchetti *et al*, 2019).

[1] Associate Professor,Dr., Department of Chemistry, University of Mandalay

[2] Associate Professor,Dr., Department of Chemistry, Kyaukse University

[3] Lecturer, Department of Chemistry, Monywa University

[4] Lecturer, Department of Engineering Chemistry, Mandalay Technology University

Research on *Moringa* mainly pivoted around its leaves and seeds because of their immense nutraceutical potential but recently there is a greater interest in flowers too, mostly inspired by the positive outcomes of several pharmacognostical studies on flowers (Fahey, 2005).

Moringa flower is a rich reservoir of bioactive phytochemicals and crude flower extracts showed promising antibacterial, antifungal, antilarval, antioxidant, antiinflammatory and anticancer properties. On the other hand *Moringa* flowers received little attention of researchers in spite of its significant nutritional and traditional healing properties (Inbathamizh and Padmini, 2013).

Moringa plants are widely used in folkloric medicine for the treatment of ailments such as Ulcer, wound, inflammation, heart problem, cancer, stroke, obesity, anemia and liver damage. *Moringa* can prevent up to 300 diseases, and aside from preventative measures, its leaves are capable of curative properties as well (Palada, 1995). Extracts from all parts of the plant show pharmacological properties such as hypocholesterolaemic (Mehta *et al*, 2003), hypotensive, bradycardic (Gilani *et al*, 1994) and anti-ulcerative activity (Pal *et al*, 1995).

In this research work, the amino acids content in flowers of *M. oleifera* Lam. was qualitatively and quantitatively determined by chromatographic and spectroscopic methods.

Aim

The aim of present research work is to determine the qualitative and quantitative determination of amino acid contents in flowers of *Moringa olifera* Lam.

Botanical Description of the *Moringa olifera* Lam.

Figure 1 Plant and flowers of *Moringa olifera* Lam.

Family name	:	Moringaceae
Botanical name	:	*Moringa olifera* Lam.
Myanmar name	:	Dan-da-lun
Genus	:	*Moringa*
Species	:	*olifera*
Part used	:	Flowers
Medicinal uses	:	astonic, di-uretic, anti-helminthic, antibacterial, antifungal, antilarval, antioxidant, anti inflammatory and anticancer (Inbathamizh, and Padmini, 2013), (Ozumba, 2008), (Fuglie, 1999)

EXPERIMENTAL

Sample Collection

The flowers of *Moringa olifera* Lam. (Dan-da-lun) (Figure 1) were collected at Maha Aung Mayay Township, Mandalay, Myanmar. They were cut into small pieces and dried in air at room temperature for use throughout the experiments.

Determination of Phytochemical Constituents from Flowers of *M. olifera* Lam.

Phytochemical Constituents from flowers of *M. olifera* Lam. were determined by test-tube method (Harbone, 1984).

Determination of Mineral Contents from Flowers of *M. olifera* Lam.

The mineral contents in flowers of *M. olifera* Lam. were measured by Atomic Absorption Spectrometry (AAS) method.

Extraction of Amino Acids in Flowers of *M. olifera* Lam.

Preparation of Sample Solution

Flowers of *M. olifera* Lam. (30 g) were added with 97 % ethanol (150 mL) and heated on water-bath for about 30 minutes. Then, it was cooled, filtered and centrifuged. The supernatant was decanted in a test tube and residue was washed with ethanol by two to three times. The volume of filtrate is reduced by heating on water-bath at 40-50°C. The final sample was used for qualitative and quantitative determinations of amino acids (Aparna Buzarbarua, 2000).

Qualitative Tests of Amino Acids in Flowers of *M. olifera* Lam.

(i) Ninhydrin Reaction

1 mL of sample solution was taken in a test tube and added 1 mL of ninhydrin reagent. Purple color was observed, which indicated the presence of amino acids.

(ii) Xanthoproteic Reaction

1 mL of sample solution was mixed with 1 mL of HNO_3 in the ice bath. The sample solution changed to yellow color which indicated the presence of amino acids, (glycine, tyrosine, tryptophan, and arginine).

(iii) Sakaguchi Test

3 mL of sample solution was mixed with 1 mL of 40% NaOH solution, 2 drops of 1% alcoholic α - naphthol and few drops of bromine water solution. Red color was developed, which indicated the presence of arginine.

(iv) Lead Sulphide Test

Sample solution (1 mL) was boiled together with 2mL of 40% NaOH solution for 2 minutes. This solution was cooled down and added 0.5 mL of sodium pulmbate solution in it. Black precipitate was observed. It indicates amino acid cystine in sample solution.

(v) Glyoxylic Reaction

2 mL of sample solution was mixed with glyoxylic acid and then 2 mL of H_2SO_4 was added slowly down by the side of test tube. Violet ring appeared, which indicated the presence of amino acid tryptophan.

Qualitative Estimation of Amino Acids in Flowers of *M. olifera* Lam. by Two Dimensional Paper Chromatography Method

The amino acids content in selected sample was estimated by two dimensional ascending Paper Chromatography method. Whatmann No.1 chromatographic paper was used as the stationary phase for Paper Chromatography. The first solvent system was n-butanol: acetic acid: water, 4:1:5 (v/v). Phenol: water, 4:1 (v/v) was used as second solvent system. Ninhydrin reagent was applied as the color developer (Aparna Buzarbarua, 2000).

Square sheet of Whatmann No.1 chromatographic paper (18x18 cm) was taken and origins were made the lower left hand corner on the intersection of two lines which were 2 cm from the two edges of the applied to the origins by means of based line. The sample solution (ethanolic extract of sample) was spotted on base line by capillary tube and desiccated by using a dryer. The paper was placed in the chromatographic tank and developed in the first dimension by the ascending technique, using n-butanol: acetic acid: water, 4:1:5 (v/v). After seven hours, the chromatographic paper was taken out and dried. The dried paper was turned at right angle and developed in the second dimension by phenol: water, 4:1 (v/v). After running the solvent system, the paper was taken out and left over night to let it dry by airing. The amino acids spots were then located with the ninhydrin spray reagent.

Separation and Identification of Amino Acids Composition by One Dimensional Paper Chromatography Method

The amino acids in flowers of *M. olifera* Lam. were separated by one dimensional paper chromatography method by using the solvent system n-butanol : acetic acid : water, 4: 1: 5 (v/v) with above procedure, and identified with standard amino acids (Aparna Buzarbarua, 2000).

Separation and Identification of Amino Acids Composition by Thin Layer Chromatography Method

The amino acids in flowers of *M. olifera* Lam. were separated by Thin Layer Chromatography method by using aluminium pre-coated silica gel plate (Merk Co. Inc, Kieselgel F_{256}) and solvent system n-butanol: acetic acid: water, 4: 1: 5 (v/v), and identified with standard amino acids.

Quantitative Determination of Amino Acids Content in Flowers of *M. olifera* Lam.

The amino acids content in flowers of *M. olifera* Lam. was quantitatively determined by using two dimensional Paper Chromatography and ninhydrin assay method (Aparna Buzarbarua, 2000).

Sample extract (100 μL) was done by the two dimensional Paper Chromatography using first eluting solvent with n-butanol: acetic acid: water, 4: 1: 5 (v/v) and second solvent system with phenol: water, 4:1(v/v). Then the paper sheet was dried in air and sprayed with 0.2 % ninhydrin reagent. It was then introduced in an oven at 100°C for two minutes. The purple spots of amino acids appeared on the sheet. The R_f values of purple spots were measured and identified with the R_f values of standard amino acids.

Clear six-color spots were cut and eluted with methanol for the measurement of absorbance with UV visible spectrophotometer. The absorbance values of six amino acids in sample solution were measured at 570 nm with ninhydrin reagent and ethylenediamine tetra acetic acid (EDTA) reagent. The standard curves of these six amino acids such as arginine, asparticacid, glycine, lycine, threonine, and valine were constructed by using the absorbance values of these amino acids which measured by the above procedure (Aparna Buzarbarua, 2000). The percentages of amino acids in flowers of *M. olifera* Lam. were calculated by standard amino acids curves.

RESULTS AND DISCUSSION

Phytochemical Test for the Flowers of *M. olifera* Lam.

According to the phytochemical investigation on the flowers of *M. olifera* Lam., it was found that the flowers of *M. olifera* Lam. consist of alkaloid, amino acids, flavonoids, glycosides, reducing sugars, phenolic compounds, polyphenols, steroids and terpene compounds respectively. The results of phytochemical constituents of selected sample are described in Table 1.

Table 1 Results of Phytochemical Constituents in Flowers of *M. olifera* Lam.

No.	Test	Reagents	Observation	Remark
1.	Alkaloid	i)Dragendroff's reagent ii)Wagner's reagent	Orange precipitate Reddish brown ppt	+
2.	Amino acid	Ninhydrin reagent	Purple color solution	+
3.	Flavonoid	Mg pieces, conc: HCl	Pink color solution	+
4.	Glycoside	10% Leadacetate	Yellow precipitate	+
5.	Lipophilic	0.5M KOH	No deep color sol^{n}	-
6.	Reducing sugar	Benedict's solution	Brick red precipitate	+
7.	Phenolic compound	10% $FeCl_3$	Purplish	+
8.	Polyphenol	1% $FeCl_3$, 1%$K_3[Fe(CN)_6]$	Greenish blue	+
9.	Saponin	H_2O, shaking	No frothing	-
10.	Steroid	Petether, H_2SO_4,$(CH_3CO)_2O$	Green color solution	+
11.	Terpene	H_2SO_4,$(CH_3CO)_2O$, $CHCl_3$	Pink color solution	+

(+) = presence of constituent (-) = absence of constituent

Determination of Mineral Contents in Flowers of *M. olifera* Lam.

The mineral contents in flowers of *M. olifera* Lam. were measured by Atomic Absorption Spectrometry (AAS) method and the results are described in Table 2.

Table 2 Mineral Contents in Flowers of *M. olifera* Lam.

No.	Minerals	Measuring Values (ppm)
1.	Potassium	27225
2.	Magnesium	2739
3.	Sodium	666
4.	Calcium	636
5.	Iron	299
6.	Zinc	40
7.	Manganese	27

Qualitative Tests of Amino Acids in Flowers of *M. olifera* Lam.

The qualitative tests of amino acids in flowers of *M. olifera* Lam. were examined and the results are shown in Table 3.

Table 3 Qualitative Tests of Amino Acids in Flowers of *M. olifera* Lam.

No.	Test	Observations	Inference
1.	Ninhydrin Reaction	Purple color solution	Presence of amino acids

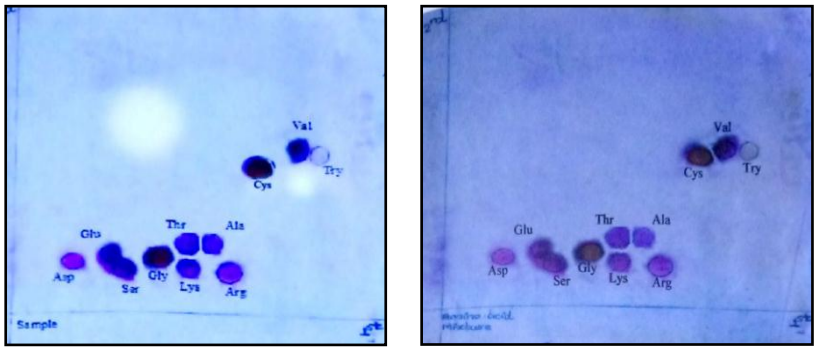
2.	Xanthoproteic Reaction	Yellow color solution	Presence of glycine, tyrosine, and tryptophan
3.	Sakaguchi Test	Red color	Presence of arginine
4.	Lead sulphide Test	Black precipitate	Presence of cystine
5.	Glyoxylic Reaction	Violet ring	Presence of tryptophan

It is explored that, flowers of *M. olifera* Lam. contain various amino acids.

Qualitative Estimation of Amino Acids in Flowers of *M. olifera* Lam.

The amino acids content in flowers of *M. olifera* Lam. was estimated by two dimensional ascending Paper Chromatography methods. As a result, it is clearly seen that ethanol extract of flowers of *M. olifera* Lam. contains eleven amino acids: aspartic acid, glutamic acid, serine, glycine, threonine, lycine, alanine, arginine, cystine, valine and tryptophan respectively. These amino acids were identified with the two dimensional Paper Chromatogram of eleven amino acids mixture.

The results of two dimensional Paper Chromatogram and R_f values are described in Figure 2 and Table 4.



(a) **(b)**

Figure 2 Separation of (a) amino acids in sample solution and (b) amino acids mixture by two dimensional paper chromatography method

Table 4 R_f Values of Standard Amino Acids and Sample Solution of the flowers of *M.Olefera* Lam.

No.	Amino Acids	Standard Amino Acids		Sample Solution	
		Solvent -1	Solvent -2	Solvent -1	Solvent -2
1.	Aspartic acid	0.13	0.15	0.16	0.17
2.	Glutamic acid	0.16	0.25	0.18	0.26
3.	Serine	0.10	0.36	0.13	0.31
4.	Glycine	0.17	0.40	0.17	0.41
5.	Threonine	0.22	0.50	0.22	0.49
6.	Lycine	0.10	0.48	0.14	0.48
7.	Alanine	0.22	0.54	0.22	0.55
8.	Arginine	0.11	0.59	0.12	0.59
9.	Cystine	0.46	0.70	0.48	0.71
10.	Valine	0.47	0.77	0.51	0.78
11.	Tryptophan	0.47	0.83	0.51	0.84

Solvent 1= n-butanol: acetic acid: water, 4: 1: 5 (v/v), solvent 2= phenol: water, 4:1(v/v)

Separation and Identification of Amino Acids Composition by One Dimensional Paper Chromatography Method

Ethanol extract sample solution was performed by one dimensional paper chromatography method and identified with standard amino acid solutions. According to the results, eleven amino acids contain in selected sample. The results of Thin Layer Chromatograms are shown in Figure 3.

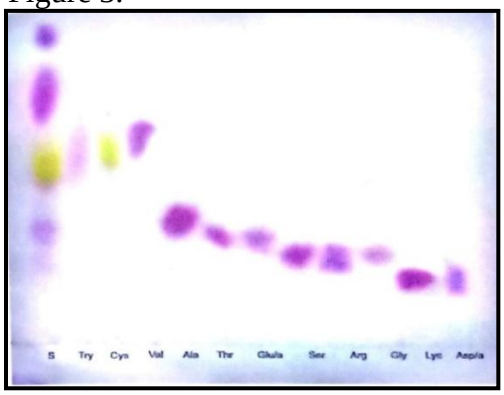


Figure 3 One dimensional paper chromatogram of amino acids compositions in sample solution and standard amino acids

The R_f values of standard amino acids and amino acids in sample solutions of the flowers of *M. Olefera* Lam. are described in Table 5. According to the results of Thin Layer Chromatograms, the flowers of *M. Olefera* Lam. was found to contain eleven amino acids.

Table 5 R_f Values of Standard Amino Acids and Sample Solution of the flowers of *M. Olefera* Lam. by One Dimensional Paper Chromatography Method

No.	Amino Acids	R_f Values of Standard Amino Acids	R_f Values of Amino acids in Sample Solution
1.	Aspartic acid	0.550	0.537
2.	Glutamic acid	0.532	0.523
3.	Serine	0.503	0.510
4.	Glycine	0.346	0.328
5.	Threonine	0.304	0.315
6.	Lycine	0.286	0.287
7.	Alanine	0.253	0.261
8.	Arginine	0.226	0.275
9.	Cystine	0.253	0.295
10.	Valine	0.193	0.201
11.	Tryptophan	0.233	0.241

Separation and Identification of Amino Acids Composition by Thin Layer Chromatography Method

The amino acids in flowers of *M. olifera* Lam. were separated by Thin Layer Chromatography method and identified with eleven standard amino acids. According to results

of Thin Layer Chromatography, eleven amino acids, (aspartic acid, glutamic acid, serine, glycine, threonine, lycine, alanine, arginine, cystine, valine and tryptophan) are found to be present in flowers of *M. olifera* Lam. These results are shown in Figure 4.

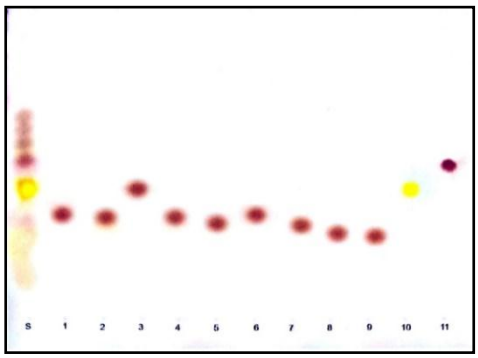


Figure 4 Thin Layer Chromatogrm of amino acids compositions in flowers of *M. olifera* Lam.

Quantitative Determination of Amino Acids Content in Flowers of *Moringa olifera* Lam.

The amino acids content in flowers of *M. olifera* Lam. was determined by Ninhydrin assay method and two dimensional Paper Chromatography method. Among eleven amino acids in flowers of *M. olifera* Lam., the clearly separated six amino acids spots were quantitatively separated and the absorbance values of these six amino acids were measured with UV visible spectrophotometer at 570 nm. The standard curves of these six amino acids were constructed by measuring absorbance values of six standard amino acids. The standard amino acids curves are shown in Figure 5 (a), (b), (c), (d), (e) and (f).The amount of containing amino acids in flowers of *M. olifera* Lam. were calculated based upon the standard amino acids curves. The content percentages of six amino acids in flowers of *M. olifera* Lam. are tabulated in Table 6.

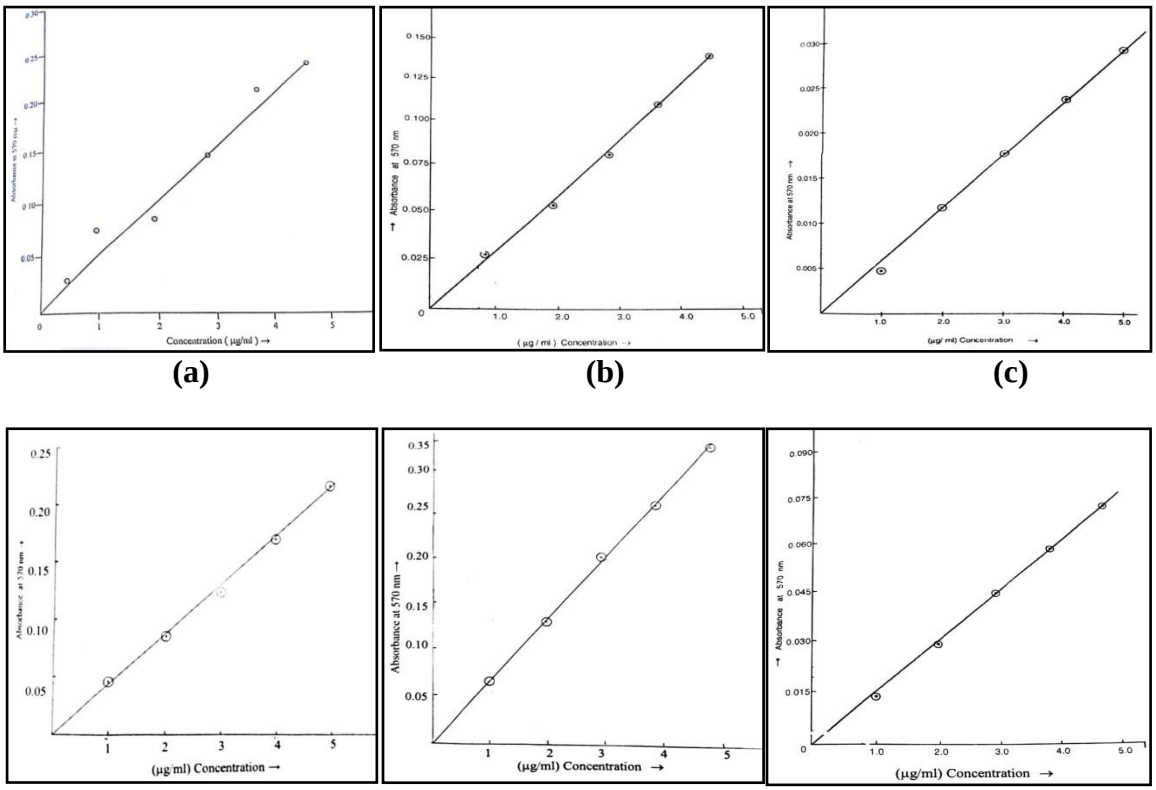


Figure 5 Concentration Vs absorbance curves for standard amino acids (a) arginine (b) threonine (c)glycine (d) lycine (e) aspartic acid and (f) valine solution

Table 6 The Content Percent of Six Amino Acids Present in flowers of *M. olifera* Lam.

No.	Amino Acids	Weight of Amino Acids	Percent (%)
1.	Arginine	0.41	0.80
2.	Aspartic acid	0.80	1.60
3.	Glycine	1.32	2.64
4.	Lycine	1.00	2.00
5.	Threonine	0.30	0.60
6.	Valine	0.30	0.60

According to the results of this research, it can be concluded that flowers of *M. olifera* Lam. consist of eleven amino acids: spartic acid, glutamic acid, serine, glycine, threonine, lycine, alanine, arginine, cystine, valine and tryptophan. Among them, six amino acids were quantitatively determined as arginine 0.80%, aspartic acid 1.60%, glycine 2.64%, lycine 2.00%, threonine 0.60% and valine 0.60% respectively. The result shows that the amount of glycine is the highest in flowers of *M. olifera* Lam.

Conclusion

Based on the findings, it can be concluded that plant serve as vast source for varied phyto-constituents exhibiting varied pharmacological properties. The vital minerals in *Moringa* include Calcium, Iron, Potassium, Magnesium, Manganese and Zinc. The highest potassium value (27225 ppm) could be observed in flowers of *M. olifera* Lam. This fact supported that the flowers of *M. olifera* Lam. respond lowering blood pressure or antihypertensive activity and circulatory stimulants. The flowers of *M. olifera* Lam. contain many essential amino acids and non- essential amino acids. Among them, five essential amino acids arginine, lycine, threonine, tryptophan, and valine are significant amount containing in flowers of *M. olifera* Lam. The effects of processing on the nutritional values of the flowers should be done to ascertain the suitability as a complete nutritional supplement for human.

The amino acids in foods must be consumed in the amount and the proportions that closely approximate the pattern required by the body. The flowers of plant *M oleifera* Lam. possess broad spectrum of phytochemistry. The effective secondary metabolite compounds such as alkaloid, flavonoids, phenolic compounds, polyphenol, reducing sugar, steroid and terpene compounds are also present in flowers of *Moringa oleifera* Lam. The popular dissemination of this plant could constitute an alternative and has ample scope for different medicinal purposes.

Acknowledgement

We are extremely grateful to Dr Yee Yee Myint, Professor, Head of the Department of Chemistry, University of Mandalay, for her permission, understanding and good guidance in doing research and submitting the paper to the University of Mandalay Research Journal, 2020.

References

Anwar F., Ashraf M., and Bhanger M. I., "Interprovenance variation in the composition of Moringa oleifera oilseeds from Pakistan," *Journal of the American Oil Chemists' Society*, (2005), vol. 82, (1), pp. 45–51.

Aparna Buzarbarua, Dr, Professor, Department of Botany, "Practical Plant Chemistry." Cotton College, Guwahati, (Assam), (2000), pp - 45-58.

Fahey W. J., "*Moringa oleifera*: a review of the medical evidence for its nutitional, therapeutic and prophylactic properties," *Trees for Life Journal*, (2005), vol. 1, pp- 1-5

Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J. and Muchenje, V., "Multi-(2018), "Functional Application of *Moringa oleifera* Lam. in Nutrition and Animal Food Products:" *A review. Food Res. Int.* 106, pp - 317–334.

Foild N., Makkar H. P. S., Becker K., Lowell I.N, Fuglie J., (2001), "The Miracle Tree. The Multiple Attributes of *Moringa*." CTA, Wageningen and CWS, New York, Dakar, pp- 52-53

Folkard G., Sutherland J. & Shaw R., (1999), "Water clarification using *Moringa oleifera* seed coagulant." In *Technical Brief* , (No- 60), pp- 109–112. Water and Environmental Health at London and Loughborough (WELL), Loughborough University.

Fuglie L. J., (1999), "The Miracle Tree: *Moringa oleifera*, Natural Nutrition for the Tropics." CWS, New York, Dakar, pp- 4-6.

Fuglie L. J.; (2001), "The Miracle Tree. The Multiple Attributes of Moringa." CTA, Wageningen and CWS, New York, Dakar, pp- 1-172.

Gilani A. H., Aftab K., Suria A., Siddiqui S., Salem R., Siddiqui B. S., (1994), "Pharmacological studies on Hypotensive and spasmolytic activities of pure compounds from *Moringa oleifera*." ,*Phytother Res*., 8 (2), pp- 87-92.

Gopalakrishnan L.; Doriya, K., Kumar, D.S. (2016), "Moringa oleifera: A Review on Nutritive Importance and its Medicinal Application." *Food Sci. Hum. Wellness*, 5, pp- 49–56.

Harborne, J. B., (1984), "Phytochemical methods," London: Chapman & Hall, pp- 49-88.

Inbathamizh L., Padmini E., (2013), "Effect of Geographical Properties on the Phytochemical Composition and Antioxidant Potential of *Moringa oleifera* Flowers." *Bio Med Rx.*, 1, (3), pp- 239 - 47.

Jahn S. A. A. (1988), "Using *Moringa oleifera* seeds as coagulant in developing countries." *Journal – American Water Works Association*, 80 (6), 43–50.

Mehta L. K., Balaraman R., Amin A. H., Bafna P. A., Gulati O. D., (2003), "Effect of fruits of *Moringa oleifera* on the Lipid Profile of Normal and Hypercholesterolaemic Rabbits." *J Ethnopharmacol.*, 86, (2-3),191.

Ozumba N.A., (2008), "*Moringa oleifera*: A Review of Its Medicinal and Other Uses." Institute for Development Studies University of Nigeria, Enugu Campus, Nigeria. ISSN: 1597-9679, pp-1-35.

Pal S. K., Mukherjee P. K., Saha P., (1995), "Studies on the antiulcer activity of Moringa oleifera leaf extract on gastric ulcer models in rats.", *Phytother. Res*., vol. 9 (6), 463

Palada M., (1995), "*Moringa*: A versatile tree crop with horticultural potential in the subtropical United States." *Hort Science*, 31(5), 88.

Rocchetti G., Blasi F., Montesano D., Ghisoni S., Marcotullio M.C., Sabatini S., Cossignani L., Lucini, L., (2019), "Impact of conventional/non-conventional extraction methods on the untargeted phenolic profile of *Moringa oleifera* leaves." *Food Res. Int.*, 115, pp- 319–327.

Shubhashree M., Shantha T., Ramarao V., Reddy M. P., Venkateshwaraul G., (2015), "A review on the Therapeutic Uses of Flowers as Depicted in Classical Texts of Ayurveda and Sidha." *J Res Educ Indian Med*., 21, pp-1-14