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Biochemical characterization of Drumstick (*Moringa oleifera* L.) germplasm

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Abstract

Drumstick (*Moringa oleifera* L.) also known as miracle tree is one of the most nutritious vegetables all over the world. This multi-function plant cultivated in tropics for high protein, minerals, vitamins and carbohydrate content. This experiment was conducted by following a Completely Randomized Design (CRD) with 15 germplasms of *Moringa oleifera* (Mo-1 to Mo-15). The maximum TSS in leaf (2.43%) & in pod (8.86%) were found in germplasm Mo-2 & Mo-15 respectively. The highest TA content in leaf (14.44%) & in pod (5.53%) were recorded in Mo-2 & Mo-15. The highest vitamin C content of leaf (58.76mg/100g) & in pod (86.19mg/100 g) were recorded in Mo-14 & Mo-2 respectively. The maximum chlorophyll-a content of leaf was observed in germplasm Mo-9 (504.5µg/g) and minimum was in Mo-11 (356.6µg/g). The highest chlorophyll-b content of leaf was found in germplasm Mo-12 (454.5µg/g) while the lowest was found in Mo-11(297.5µg/g). The highest carotenoid content in leaf was noticed in germplasm Mo-12 (457.5µg/g) and the lowest was in Mo-1 (316.1µg/g). The maximum pH of leaf (6.31) & in pod (6.43) both were recorded in Mo-3. From the research it was concluded that Mo-2, 14 and 15 were the superior germplasm in respect of TSS, TA and Vit-C.

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Introduction

Moringa oleifera L., known as *Moringa*, is native to north India but is now found throughout the tropics. It is also known as horseradish tree, drumstick tree. Drumstick is cultivated in almost every districts of Bangladesh. It is extensively cultivated in middle and western region of Bangladesh, especially in the division of Khulna, Rajshahi and Dhaka. It can survive without any type of fertilizer, manures, pesticides even watering and weeding, which is very important for the protection of our environment.

This fast growing, small to medium sized tree is used as animal forage, source of nutrition, medicine, water purification, cosmetics and even as biofuel (Anwar *et al.*, 2007; Rashid *et al.*, 2008 and Fuglie, 2001). Leaves, flowers, fruits and seeds of drumstick are very rich in different vitamins. Leaves are rich in Vitamin A and C (Nautiyal, 1987). Flowers and seeds contain a significant amount of thiamin, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene and alpha-tocopherol. Flowers, and seeds of drumstick contain more of the vitamins (Dahot, 1988). 100g drumstick leaves contains water 78.66 g, vitamin-C 51.7mg, vitamin-A 6.8mg and 100 g drumstick pods contains 88.20g of water, 26 calorie, 4.8 g of fiber, 0.11mg of vitamin-A, 141.0mg of vitamin-C (ascorbic acid) (Anonymous, 2013). One table spoon of leaf powder can provide 14% of protein, 40% of calcium, 23% of iron and most of the vitamin-A needs of a child aged one to three (Stepenson, 2006). In Bangladesh (Akhter *et al.,* 2014); characterized 10 drumstick germplasm based on some chemical properties.

Despite of being very important it isn't cultivated in the low-lying coastal area due to several problems such as water logging condition, in some places soil isn't suitable for drumstick production, there is no systematic production practices, the crop isn't locally available. Moreover, no such step has yet taken to find out the biochemical potentiality. With this circumstance the aim of the present study has been undertaken with the following objectives is to find out the best drumstick germplasm on the basis of biochemical characteristics.

Materials and methods

The research on "Biochemical characterization of drumstick (*Moringa oleifera* L.) germplasm" was carried out during the period of February 2018 to April 2019. In this study, 15 germplasms (Mo-1 to Mo-15) were randomly selected. Data on leaves and pods were collected from selected 15 drumstick plant located at Germplasm Centre and then the collected leaves and pods were brought to the Post Harvest Laboratory, Department of Horticulture, PSTU.

Experimental materials

Fifteen germplasm of drumsticks were selected as the experimental materials for the investigation. All the selected germplasms were six years old tree.

Methods of Studying Parameters

By using the following methods biochemical parameters of the collected drumstick germplasms were studied. The methods for the determination of pH, TSS, TA, Vitamin-C of drumstick leaf & pod extract were followed as described by (Mazumdar and Majumdar, 2003), (Ranganna 1977), and (Saini *et al.*, 2006).

Total soluble solids (TSS)

The TSS of drumstick pulp & leaf were determined by using a digital refractometer (BOECO, Germany). The remaining of the filtrated juice from TA determination was used to measure the TSS of the drumstick pulp & leaf. Before measurement, the refractometer was calibrated with distilled water to give a 0% reading. About 1-2 drops of the filtrate was placed on the prism glass of the refractometer to obtain the% TSS reading. The reading was multiplied by dilution factor to obtain an original% TSS of the pulp & leaf tissues. Since difference in sample temperature could affect the measurement of TSS, each of the reading was standardized to a temperature of 20° C by adding 0.28% to obtain% TSS at 26 ± 1° C.

Determination of titratable acidity (TA)

Titratable acidity (TA) was determined according to the method by (Ranganna, 1977). Ten grams of pulp & leaf tissues were homogenized with 40 ml distilled water using a kitchen blender for two minutes and

filtered through a Whatman filter paper No. 2. Five milliliters of the filtrate were transferred into a 100 ml conical flask and two drops of 1% phenolphthalein solution as an indicator were added. The sample was titrated with 0.1 M sodium hydroxide (NaOH) solution until the color changed to pink and persistent for at least 15 seconds. The titrated volume was recorded and result was expressed as percentage citric acid, which was calculated using the following formula:

$$\text{Citric acid (\%)} = \frac{\text{Titre (mL)} \times \text{NaOH normality (0.1 M)} \times \text{Vol.made up (50 mL)} \times \text{Citric acid eq.weight (64 g)} \times 100}{\text{Volume of sample for titrate (5 mL)} \times \text{Weight of sample taken (10 g)} \times 1000}$$

Ascorbic acid content (Vitamin C)

Ascorbic acid was determined according to the dye method by (Ranganna, 1977). Ten grams of leaf & pod were homogenized with 40 ml of 3% cold metaphosphoric acid (HPO_3) using a blender for two minutes and filtrate through the Whatman filter paper no.2. Five milliliters of aliquot were titrated with 2, 6 dichlorophenol- indophenol dye until the solution used was recorded and ascorbic acid content was calculated using the following formula: Ascorbic acid (mg 100 g^{-1})

$$= \frac{Titre\ (mL) \times dye\ factor \times Vol.made\ up \times 100}{Aliquot\ used\ estimation\ (5\ mL) \times sample\ Weight}$$

To standardize the dye, 5 ml of standard ascorbic acid solution was added to 5 ml of 3% cold HPO_3. The mixture was titrated with the dye solution to a pink color, which persisted for 15 seconds. The dye factor was calculated as follows:

$$\text{Dye factor} = \frac{0.05}{Titre\ value}$$

Determination of chlorophyll-a

Accurately weighted 0.5g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of different extractant solvent. Homogenized sample mixture was centrifuge for 10,000 rpm for 15 min at 4 ^oC. The supernatant was separated and 0.5ml of it is mixed with 4.5ml of the respective solvent. The solution mixture was analyzed for Chlorophyll-a content in spectrophotometer (Parkin). The equation used for the quantification of Chlorophyll-a by acetone extractant solvents are given below:

Ch-a=12.25A663.2 – 279A646.8

Determination of chlorophyll-b

Accurately weighted 0.5g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of different extractant solvent. Homogenized sample mixture was centrifuge for 10,000 rpm for 15 min at 4^oC. The supernatant was separated and 0.5ml of it is mixed with 4.5 ml of the respective solvent. The solution mixture was analyzed for Chlorophyll-b content in spectrophotometer (Parkin). The equation used for the quantification of Chlorophyll-b by acetone extractant solvents are given below:

Ch-b=21.5A646.8 – 5.1A663.2

Determination of carotenoid

Accurately weighted 0.5g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of different extractant solvent. Homogenized sample mixture was centrifuged at 10,000 rpm for 15min at 4^oC. The supernatant was separated and 0.5ml of it is mixed with 4.5ml of the respective solvent. The solution mixture was analyzed for carotenoid content in spectrophotometer (Parkin). The equation used for the quantification of carotenoid content is given below:

C x+c = (1000A470 – 1.82Ca – 85.02Cb)/198

Experimental Design and Analysis

The laboratory experiment was laid out in Completely Randomized Design (CRD). After collection of some drumstick they were kept in ambient temperature for the study of biochemical characteristics.

Statistical analysis

Statistical analysis: Analysis of variance will be done with the help of MSTAT-C computer package program. The mean differences among the treatments will be calculated with the help of Duncan"s Multiple Range Test (DMRT) at 1% and 5% levels of probability.

Results and discussion

A wide range of variability was observed among fifteen germplasm under the study respect of biochemical characteristics of leaves and fruits. The results obtained in the present investigation entitled

"Biochemical characterization of drumstick (*Moringa oleifera* L.) germplasm" in table for discussion, comprehension and understanding. The results and discussion of the study are presented under the following sub-headings.

Biochemical characteristics of drumstick leaf & pod

Total soluble solids of drumstick leaf & pod

Total soluble solids of drumstick leaf ranged between 1.03% and 2.42% with an overall mean of 1.74% and in case of pod it ranges between 5.46% and 8.86% with an overall mean of 6.35%. The best germplasm observed for total soluble solids of drumstick leaf were germplasm Mo-2 (2.42%) followed by germplasm Mo-14 (2.25%) which was statistically similar to germplasm Mo-2, whereas the lowest total soluble solids was noted in case of germplasm Mo-15 (1.03%) (Table 1). (Akther *et al*., 2014); observed the percentage of TSS ranged from 1.00% & 1.67%.

The maximum pod TSS was found in germplasm Mo-15 (8.86%) followed by germplasm Mo-2 (8.76%) and Mo-14 (8.68%) which were statistically similar. The minimum TSS was found in germplasm Mo-5 (5.46%) (Table 2). (Akther *et al*., 2014); observed the percentage of TSS ranged from 5.33% to 9.33%.

Titratable acidity of drumstick leaf & pod

Total Titrable acidity of drumstick leaf ranged between 14.44% and 4.44% with an overall mean 9.33% and in case of pod it ranges between 1.23% and 5.53% with an overall mean of 3.00%. The highest percentage of titratable acid content in leaf (14.44%) was recorded from germplasm Mo-2 followed by Mo-15 (14.26%), Mo-14 (14.15%) and lowest percentage in (4.44%) was recorded from Mo-13 (Table 1). (Akther *et al*., 2014); observed the percentage of TA ranged from 6.40% to 21.33%.

The maximum TA in pod was found in germplasm Mo-15 (5.53%) followed by germplasm Mo-12 (5.13%) and Mo-4 (4.1%), while the minimum TA was found in germplasm Mo-1 (1.23%) (Table 2). (Akther *et al*., 2014); observed the percentage of TA ranged from 0.121% to 13%.

Vitamin C (ascorbic acid) content of drumstick leaf & pod

There was a significant variation among the 15 germplasm in respect of vitamin C content of drumstick leaf & pod. Vitamin C of drumstick leaf ranged between 58.76mg and 18.76mg with an overall mean 37.14mg & in pod 86.19mg to 30.08mg with an overall mean 48.95mg. The highest vitamin C content of leaf (58.76mg/100 g) was found in Mo-14 & lowest amount in (18.76mg/100 g) Mo-7 (Table 1). (Dogra *et al*., 1975); stated that leaves juice of *Moringa* are rich in vitamin C content and it varied from 126.41 to 132.17mg/100g.

The highest vitamin C content of pod (86.19mg/100 g) was found in germplasm Mo-2 followed by Mo-15 (81.25mg/100 g). The lowest amount of ascorbic acid content (30.08mg/100 g) was observed in germplasm Mo-7 followed by Mo-4 (30.15mg/100 g) (Table 2). Dogra *et al*. (1975) stated that pods juice of *Moringa* are rich in vitamin C content and it varied from 126.41 to 132.17mg/100g. (Dogra *et al*., 1975); stated that pods juice of *Moringa* are rich in vitamin C content and it varied from 126.41 to 132.17mg/100g.

Chlorophyll-a content of drumstick leaf

Significant variation among the 15 germplasms in respect of chlorophyll-a content of drumstick leaf was noted. The highest chlorophyll-a content of leaf (504.5µg/g) was found in germplasm Mo-9 which was statistically similar to germplasm Mo-12 (504.4µg/g) whereas the lowest amount of chlorophyll-a content of leaf (356.6µg/g) was observed in germplasm Mo-11 (Table 1). Average chlorophyll-a content of leaf was recorded 483.01µg/g (Table 1) (Mbaiguinam Mbailao *et al*., 2014); found that the Chlorophyll-a content in leaf ranged from 97.88µg/g to 100.23µg/g.

Chlorophyll-b content of drumstick leaf

Significant variation among the 15 germplasms in respect of chlorophyll-b content of drumstick leaf was noted. The highest chlorophyll-b content of leaf (454.5µg/g) was found in Mo-12, whereas the lowest amount of chlorophyll-b content (297.00µg/g) was observed in Mo-11 (Table 1). Average chlorophyll-b content of leaf was 426.42µg/g (Table 1). (Mbaiguinam Mbailao *et al*., 2014); found that the Chlorophyll-b content in leaf ranged from 43.65µg/g to 44.53µg/g.

Carotene content of drumstick leaf

There was a significant variation among the 15 germplasms in respect of carotene content of drumstick leaf. The highest carotenoid content (457.5µg/g) was found in Mo-12 followed by germplasm Mo-14 (454.6µg/g) and Mo-3 (453.0µg/g), whereas the lowest amount of carotene content (316.1µg/g) was observed in Mo-1(Table 1). Average carotene content of leaf was 434.74µg/g (Table 1). (Akther *et al.*, 2014); stated that carotenoid content in leaves ranged from 0.01mg /100 g to 0.09mg /100 g.

pH of drumstick leaf extract

There was a significant variation among the 15 germplasms in respect of pH of drumstick leaf & pod. The pH ranges between 6.31 and 5.86 with an overall mean 6.05 and in pod 6.83 and 5.84 with an overall mean 6.27. The highest pH of drumstick leaf (6.31) was found in germplasm Mo-3 followed by Mo-11 (6.25) & Mo-7 (6.25), whereas the lowest pH content (5.86) was observed in Mo-6 (Table 1). (Akther *et al.*, 2014); found that pH content in leaves varied from 4.23 to 5.53.

Table 1. Mean performance of 15 germplasm for different biochemical characteristics of drumstick leaves.

Germplasm	TSS (%)	TA (%)	Vit-C (mg/100g)	Chloro-phyll (a) (µg/g)	Chloro-Phyll(b) (µg/g)	Carotene (µg/g)	pH
Mo-1	1.82bc	9.715e	32.5h	358.5h	299.9i	316.1f	6.13bcd
Mo-2	2.42a	14.44a	53.76b	501.3f	444.8ef	452.1cd	5.88fg
Mo-3	1.63cd	9.16f	33.76g	501.9de	443.4h	453.0c	6.31a
Mo-4	1.66cd	8.19i	35.22f	502.8c	444.3fg	452.6cd	6.1bcd
Mo-5	1.70cd	8.54h	28.79i	500.7g	443.7gh	451.9cd	6.13bcd
Mo-6	1.80bc	6.27m	45.08d	501.5ef	445.0e	451.2d	5.86g
Mo-7	1.67cd	6.85l	18.76l	502.3cd	443.9gh	452.7cd	6.25ab
Mo-8	1.7cd	7.23k	27.59j	503.6b	444.3fg	452.7cd	6.05cde
Mo-9	1.65cd	8.57h	21.21k	504.5a	445.0e	452.3cd	6.17abc
Mo-10	1.71bcd	8.78g	35.08f	502.4cd	443.9gh	453.0c	5.9fg
Mo-11	1.93b	7.96j	40.08e	356.6i	297.5j	317.8e	6.25ab
Mo-12	1.53d	11.48d	33.79g	504.4a	454.5a	457.5a	6.02cdef
Mo-13	1.67cd	4.44n	45.08d	502.8c	451.3b	451.9cd	5.97defg
No-14	2.25a	14.15c	58.76a	500.6g	446.5d	454.6b	5.9efg
Mo-15	1.03e	14.26b	47.75c	501.5ef	448.5c	452.5cd	5.92efg
Mean	1.74	9.33	37.14	483.01	426.42	434.74	6.05
CV%	1.03	0.44	0.33	0.07	0.09	0.21	1.53
LSD (0.05)	0.20	0.06	0.17	0.50	0.10	1.31	0.13
Level of significance	*	*	*	*	*	*	*

Common letter(s) within the same column do not differ significantly at 5% level of significance analyzed by DMRT.

*=Significant at 5% level of probability ($p \leq 5\%$)

Table 2. Mean performance of 15 germplasm for different biochemical characteristics of drumstick pods.

Germplasm	TSS (%)	TA (%)	Vit-C (mg/100g)	pH
Mo-1	5.49fg	1.23l	47.26e	6.15c
Mo-2	8.76a	2.68g	86.19a	6.15c
Mo-3	6.23b	3.23e	42.5h	6.43a
Mo-4	5.66ef	4.1c	30.15m	6.41ab
Mo-5	5.46g	3.7d	36.31j	6.42a
Mo-6	5.73cde	1.68k	35.05k	6.38ab
Mo-7	5.71de	2.15hi	30.08m	6.34abc
Mo-8	5.66ef	1.93j	45.0g	6.39ab
Mo-9	5.91c	3.1f	40.08i	6.31abc
Mo-10	5.68de	2.25a	56.29d	6.42a
Mo-11	5.86cd	3.08f	32.58l	6.19bc
Mo-12	5.86cd	5.13b	46.29f	6.43a
Mo-13	5.71de	2.08i	45.15g	6.38ab
No-14	8.68a	3.23e	80.15c	5.88d
Mo-15	8.86a	5.53h	81.25b	5.84d
Mean	6.35	3.00	48.95	6.273
CV%	1.87	2.6	0.22	2.22
LSD (0.05)	0.16	0.11	0.15	0.19
Level of significance	*	*	*	*

Common letter (s) within the same column do not differ significantly at 5% level of significance analyzed by DMRT

*= Significant at 5% level of probability ($p \leq 5\%$)

The highest pH of drumstick pod (6.43) was found in Mo-3 followed by Mo-12 (6.42) and Mo-5 (6.42) which was statistically similar. The lowest pH content of drumstick pod (5.84) was observed in Mo-15 (Table 2). (Akther *et al*., 2014); found that pH content in pod varied from 4.7 to 5.

Conclusions

The present study was undertaken to explore the biochemical qualities of drumsticks. Total 15 germplasms were evaluated with respect to leaf and pod biochemical characteristics. Overall, the germplasm Mo-2, Mo-12, Mo-14 and Mo-15 were found promising. Among these Mo-2 was superior in leaf TSS, pod TA and pod vit-C. Where's Mo-12 was superior in chlorophyll-a, chlorophyll-b and leaf carotene content. Mo-15 was superior in respect of pod TSS, pod TA whereas Mo-14 was the best in relation to content of leaf vit-C. Hence, these germplasms may be used for further characterization at molecular level to efficiently determination of the genetic diversity of drumstick.

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Abbreviation

Mo.= *Moringa oleifera* L., RCBD = Randomized Complete Block Design, TSS = Total soluble solids, TA= Titratable Acidity

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