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Evaluation of bioactive compounds in leaves of *Moringa concanensis* accessions

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Abstract
Moringa concanensis Nimmo is a medicinal tree belonging to the family Moringaceae. Leaves, flowers and seeds is used for curing various ailments in humans. Leaves are used to reduce cholesterol and body weight, to increase fertility in women, to reduce fatigue, for constipation and to treat jaundice. Even though its medicinal properties has been known, but there is not much work has been done on the quantitative determination of bioactive compounds in this species. In this present study bioactive compounds in different accessions of *M. concanensis* was analysed. Results revealed that ascorbic acid, total carotenoids, total polyphenol, chlorophyll a, b and total chlorophyll content was highest in accession MC-16 (442.30mg/100g), MC-19 (70.64mg/100g), MC-10 (35.80mg/g), MC-25 (1.812mg/g), MC-19 (0.273mg/g), MC-25 (2.409mg/g) respectively. This study shows that *M. concanensis* is very good source of bioactive compounds which are beneficial to human health. So M. concanensis leaf can be used as functional ingredients in therapeutic food and for the development of nutraceuticals.

Keywords: *Moringa concanensis*, Ascorbic acid, Carotenoids, Chlorophyll, Polyphenol, Bioactive compounds

Introduction
M. concanensis Nimmo is a medicinal tree belongs to family Moringaceae locally known as kattumurungai by tribal people of nilgiri hill region in Tamilnadu. *M. concanensis* occurs in tropical dry forest from south eastern Pakistan to the southern tip of India. This plant as used in *Ayurveda* and *Unani* medicinal system for treatment of several ailments (Ramachandran, *et al*., 1980) [1] including anti-inflammatory, purgative tonic (Kapoor, *et al*.,2001) [2] analgesic, potential antitumor (Hukkeri, *et al*., 2006) [3], anti-fungal (Amelia, *et al*., 1999) [4], antispasmodic and diuretic activity (Armando, *et al*., 1992) [5]. Leaves of *M. concanensis* helps to reduce blood pressure, menstrual pain, jaundices, constipation, skin tumour, diabetes and spleenomegaly. The flower also helps in abortion and leucorrhea (Anbazhakan, *et al*., 2007) [6]. Seeds are used in ophthalmic preparation, venereal affection, in goitre, glycosuria and lipid disorders (Anonymous, 2004) [7]. Even though it's medicinal properties has been known but there is not much work has been done on the quantitative determination of bioactive compounds in different accessions. Hence the present study was conducted to analyze the bioactive compounds in different accessions of *M. concanensis* leaves.

Materials and methods
Plant Material and Analysis
Fresh leaf sample of *Moringa concanensis* accessions were collected in the early hours of morning from the Field Gene Bank of RET (Rare, Endangered, and Threatened) medicinal plants at ICAR- Indian Institute of Horticultural Research (IIHR), Bengaluru. Its geographical position is located between 13^0 58' north latitude, 78^0 east longitude and at an altitude of 890 meters above the Mean Sea Level. Leaves were brought to the laboratory and separated from twigs manually, infected and diseased leaves were discarded. Healthy leaves were taken for analysis of bioactive compounds.

Ascorbic acid

Ascorbic acid content of the leaves was measured titremetrically by 2, 6 dichloroindophenol solution. Dye was prepared by dissolving 50 mg of 2, 6-dichloroindophenol in about 150 ml of water containing 42 mg of sodium bicarbonate and then volume was made upto 200 ml. 1 ml of the sample was taken in a conical flask containing 5ml of 3% metaphosphoric acid and titrated against dye. The end point is marked by appearance pale pink colour, which persists for 30 seconds. The dye solution is standardized by titrating it against freshly prepared ascorbic acid solution (containing 50 mg of ascorbic acid in 50 ml of 3% meta-phosphoric acid solution). The ascorbic acid is calculated by using the following formula.

The result was expressed in mg of ascorbic acid per 100 g of sample.

Ascorbic acid (mg/100g of leaves) = conc. OD std. X volume made up X titrate value of sample X 100
Titer value of std. aliquot taken for estimation X wt. of sample

Carotenoid

Total carotenoid in fresh leaves is quantified as per the method, reported by Ranganna (2000). 0.5 g of leaf sample was weighed and a pinch of magnesium carbonate was added. The carotenoids are extracted with 15 ml of acetone by grinding with pestle and mortar. Extraction is repeated 3 times with acetone and finally with 10- 15 ml of hexane until the entire sample becomes colourless. The aliquot is filtered through cotton in to a conical flask. The filtrate is transferred into a separating funnel containing 10- 15 ml of 10% sodium chloride solution, the mixture is then shaken and allowed for separation in a stand. The carotenoids pigment layer is taken into a 25ml volumetric flask. The volume is made upto 25 ml with hexane kept for 24 hours and optical density was recorded at 470 nm using a Shimadzu-UV-160 spectrophotometer. Hexane was used as blank.

Total phenols

Total phenolic compounds of the leaves were estimated by Folin-Ciocalteau reagent method (singleton *et al*., 1999). Briefly, a known amount of fresh leaf material was subjected to extraction using 99% methanol in HCl for 24 hours, sample was filtered and the filterant was collected for estimation of total phenols. In a series of test tubes, 0.5 ml of the extract was taken and 3.3 ml of distilled water was added, mixed with 0.2 ml of 50% Folin–Ciocalteau reagent and 1 ml of 20% sodium carbonate. After shaking, it was allowed for the reaction for about 30 minute under dark. The absorbance was measured at 650 nm using a Shimadzu-UV160 spectrophotometer. A standard curve was prepared using Gallic acid monohydrate. The linearity obtained was in the range of 1–10 µg/ml. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of leaves.

Chlorophyll

Chlorophylls were estimated spectrophotometrically as per the method described by Arnon (1945) and Witham *et al.* (1971) [10] using Shimadzu-UV-160 spectrophotometer. 0.5 g of leaf sample were weighed and extracted with 20 – 40 ml of 80% acetone by grinding with pestle and mortar. Extraction is repeated 3 times with acetone until the entire sample becomes colourless. The supernatant was filtered through cotton and transferred to a separating funnel and the mixture is shaken and allowed for separation in a stand. The chlorophyll pigment layer is taken into a 25 ml volumetric flask with 4.5 ml of distilled water and then volume was made upto 25 ml with 80% acetone and kept for 24 hours and optical density was recorded at 645 nm (chlorophyll a) and 663 nm (chlorophyll b) using a Shimadzu-UV-160 spectrophotometer.

Results and discussion

Bioactive compounds analyzed in fresh leaves of different accessions of *Moringa concanensis* were given in table 1 and table 2

Vitamin C is (also known as ascorbic acid) abundant in green vegetables, which is a water-soluble vitamin and powerful antioxidant, it plays an important role in bone formation, wound healing and the maintenance of healthy gums, to reduce the effective in lowering the risk of developing cancers and severity of illnesses (Hemila, 1992) [11]. Evaluation of bioactive compounds is revealed that 15 accessions contained ascorbic acid content between 443 and 303 mg/100g and 13 accessions were ranged between 302 to 135 mg/100g of sample. Highest was found in accession MC-16 (442.308).

In plants, Carotenoids contribute to the photosynthetic machinery and protect them against photo-damage. carotenoids have a positive effect on several diseases such as certain type of cancers, cardiovascular diseases and prevention of chronic diseases and especially age related macular degeneration (Rao, 2007). Among the accessions analyzed 14 accessions contained carotenoids from 70 to 50 mg/100g and 14 accessions were contain from 51 to 25 mg/100g of sample, highest carotenoids was in the accession MC-19(70.64 mg/100g).

Phenolic compounds are secondary metabolites in the plants present in natural food. It reduced the risk of developing many diseases because of their antioxidant properties (Ozcan, *et al*.) Total polyphenols content (mg/g of leaves) of methanolic extract of fresh leaves was ranged between 36 to 17 mgGAE/g and Highest in accession MC-10(35.80mgGAE/g) and lowest in accession MC-32 (17.05mgGAE/g).

Chlorophyll is a green pigment found in plants. Greens are important sources of protective food which are highly beneficial for maintenance of good health and prevention of diseases (Rajalakshmi and Banu, 2013) [14]. In this study total chlorophyll content varied from 0.1 to 2.5 mg/g in different accession of M. concanensis. The highest total chlorophyll (a + b) content was found in accession MC-25(2.409mg/g), chlorophyll a is highest in accession MC25(1.812) and chlorophyll b is highest in accession MC-19(0.273 mg/g).

Table 1: Distribution of different types of bioactive compounds in leaves of different accessions of *Moringa concanensis.*

Accessions	Total phenols mgGAE/g (mean±SD)	T.carotenoids mg/100g (mean±SD)	Ascorbic acid mg/100g (mean±SD)
MC-1	37.48±2.88	50.29±3.67	403.84± 69.33
MC-2	45.80±0.90	45.54±8.25	409.17±22.02
MC-3	41.87±2.64	50.50±5.74	261.02±6.109
MC-4	18.33±5.13	48.26±5.43	192.30±9.615
MC-5	30.33±4.67	61.61±3.67	220.64±24.65
MC-6	42.65±2.79	43.66±7.64	194.54±32.87
MC-9	57.37±4.68	45.90±7.06	384.48±98.32
MC-10	75.33±5.77	51.80±2.22	154.21±10.87
MC-11	37.60±8.63	51.87±4.54	217.94±22.20
MC-12	35.80±0.53	62.19±3.94	222.22±11.69
MC-14	51.71±2.63	40.64±4.83	366.47±60.01
MC-15	40.90±3.68	31.68±4.02	374.26±40.51
MC-16	45.57±4.58	28.27±2.44	442.30±33.30
MC-17	47.14±1.01	44.32±3.02	358.97±27.66
MC-18	28.16±1.00	28.71±4.47	179.95±46.45
MC-19	29.04±0.56	70.64±2.78	134.96±12.27
MC-20	45.27±0.69	46.97±4.81	322.34±12.69
MC-21	33.88±3.30	25.93±1.28	344.32±22.88
MC-22	37.79±2.47	58.15±3.22	314.10±29.38
MC-23	26.01±4.16	57.25±5.81	307.69±38.46
MC-24	34.39±1.38	65.94±4.61	151.32±14.17
MC-25	27.84±0.10	60.34±5.46	286.92±52.70
MC-26	38.57±1.97	50.55±2.96	241.75±21.97
MC-28	26.39±0.69	55.79±2.16	368.29±26.47
MC-29	27.84±0.10	48.29±4.83	307.69±21.97
MC-30	16.86±2.79	36.21±0.60	303.03±16.14
MC-31	38.18±8.38	48.60±4.19	329.67±43.95
MC-32	17.05±5.13	66.48±1.21	261.07±86.87

Note: values are means of three replications with standard deviation.

Table 2: Distribution of chlorophyll in different accessions of *Moringa concanensis*.

Accessions	Chlorophyll a mg/g (mean±SD)	Chlorophyll b mg/g (mean±SD)	Total chlorophyll mg (mean±SD)/g
MC-1	0.29±0.06	0.002±0.01	0.298± 2.90
MC-2	0.73±0.10	0.159±0.22	0.895±0.85
MC-3	0.47±0.12	0.176±0.01	0.655±2.54
MC-4	0.63±0.05	0.270±0.03	0.900±5.09
MC-5	0.92±0.12	0.276±0.03	1.196±4.69
MC-6	1.06±0.16	0.277±0.04	1.344±2.89
MC-9	0.23±0.11	0.041±0.03	0.277±4.78
MC-10	1.17±0.13	0.328±0.04	1.503±5.88
MC-11	0.53±0.01	0.128±0.08	0.658±8.64
MC-12	1.18±0.06	0.244±0.08	1.424±0.51
MC-14	0.73±0.00	0.154±0.02	0.889±2.64
MC-15	0.24±0.07	0.234±0.09	0.477±3.76
MC-16	0.25±0.07	0.055±0.01	0.310±4.64
MC-17	0.78±0.04	0.300±0.08	1.081±1.00
MC-18	0.98±0.07	0.156±0.18	1.141±0.94
MC-19	0.99±0.05	1.440±0.15	2.438±0.54
MC-20	1.08±0.02	0.265±0.15	1.346±0.71
MC-21	0.45±0.06	0.168±0.11	0.625±3.34
MC-22	1.09±0.17	0.273±0.05	1.368±2.59
MC-23	1.08±0.15	0.073±0.41	1.163±4.30
MC-24	1.19±0.98	0.589±0.29	1.782±2.36
MC-25	1.81±0.36	0.597±0.05	2.409±0.45
MC-26	0.20±0.12	0.038±0.04	0.244±2.03
MC-28	0.75±0.04	0.244±0.01	1.001±0.72
MC-29	0.74±0.07	0.214±0.06	0.957±0.10
MC- 30	0.27±0.06	0.186±0.04	0.464±2.86
MC-31	0.69±0.10	0.158±0.16	0.849±8.33
MC-32	1.07±0.07	0.494±0.15	1.568±5.08

Note: values are means of three replications with standard deviation.

Conclusion

It can be concluded from the study that the accession of *M.concanensis* leaves contain a good amount of ascorbic acid, carotenoids, polyphenols, chlorophylls. The presence of these bioactive compounds in the leaves shows that *M. concanensis* is a very good source of bioactive compounds which are beneficial to human health. So M. concanensis leaf can be used as functional ingredients in therapeutic foods and for the

development of nutraceuticals.

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