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Ascorbic acid and mineral content in *Moringa oleifera* leaves: A study of ascorbic acid stability

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Abstract

Aim: *Moringa olieifera* is a plant of medicinal and nutritional food that is used to eradicate malnutrition owing to its potential phytochemicals. The present study aims to estimate the ascorbic acid and minerals content in *Moringa olieifera* leaves. Ascorbic acid and minerals are playing a vital role in many biological functions and prevent many degenerative diseases.

Materials and Methods: Fresh *Moringa oleifera* leaves were collected from the local area of Kuvempu Nagara of Tumakuru Karnataka state, India. The ascorbic acid was estimated by the Dinitrophenylhydrazine (DNPH) method and estimation of minerals by Inductively Coupled Plasma Optical Emission Spectroscopy Method (ICP-OES) method.

Results: Ascorbic acid in fresh *Moringa oleifera* leaves showed 43.26 mg % and also the estimate the concentration of ascorbic acid under different storage conditions such as -20^{o}C, 4^{o}C, room temperature (RT), 40^{o}C for six days storage. The results of minerals in *Moringa oleifera* leaves Calcium (2.4%) , Phosphorus (0.21 %), Magnesium (0.5 %), Sulfur (1.54 %), Potassium (0.48 %), Zinc (30.35 ppm), Cobalt (1.15 ppm), Iron (19.05 ppm), Manganese (34.25 ppm), Copper (14.47 ppm).

Conclusion: The present study results showed a good amount of ascorbic acid in fresh leaves compared to different storage conditions. The results of mineral content in *Moringa oleifera* leaves showed a marginal amount. The consumption of Moringa *oleifera* leaves for maintaining good human health owing to an appreciable amount of vitamins and minerals.

Keywords: Ascorbic acid, Minerals, *Moringa oleifera* leaves, Storage.

INTRODUCTION

Ascorbic acid

Moringa olieifera is a medicinal plant that belongs to the family Moringaceae and commonly called by different names as a miracle tree, horseradish tree, mother's best friend [10, 18, 30, 32]. It is a plant of medical and nutritional food that is used to eradicate malnutrition due to its potential nutrients. The UN Sustainable development goal is to set a target to eradicate different forms of malnutrition in the world by 2030. In India, 190.7 million populations are undernourished, 38.4 % are stunted children under five and 51.4 % are anemic in the reproductive women group. Moringa leaves are recommended for mothers, infants, pregnant women, and breastfeeding mothers to control malnutrition in developing countries [30]. The research results showed that there is a marginal improvement in the protein-energy malnutrition in children after the supplementation of Moringa leaf powder [18, 30, 32]. Moringa leaves are rich sources of water-soluble vitamins (folic acid, ascorbic acid, and pyridoxine), fat-soluble vitamins (carotenoids, vitamin-D, and vitamin-E), minerals (iron, zinc, calcium, copper, magnesium, potassium) and phytochemicals (flavonoids, tannins, sterols, saponins, alkaloids and anthraquinones). It has been shown to treat diabetes where non-production of insulin and insulin resistance resulting in hyperglycemic and showed anti-diabetic agents. It can be used as natural, reliable, safe agents act as anti-cancer, anti-microbial, anti-helminthic, anti-asthmatic activity, antipyretic activity, antioxidant activity, hepatoprotective activity, anti-ulcer, anti-cardiac [2, 5, 7, 11, 15, 16, 19, 25, 28, 33, 35]. In this paper, we have focused on ascorbic acid and minerals content in *Moringa olieifera* leaves which are essential for many biological and pharmacological functions [6]. The chemical nature of ascorbic acid is a white solid, acidic, and sensitive to temperature, heat, light, pH, air, and minerals particularly iron and copper [37]. In humans, ascorbic acid is not able to synthesize due to lack of gluconolactone oxidase is not expressed in animals. The deficiency symptoms of ascorbic acid include weakness & fatigue, bleeding gums, blood vessel, connective tissues, bones, hair, teeth and impair wound healing. It has to be supplemented with external sources to control diseases such as scurvy, hemorrhages, bone, and cartilage degeneration, anemia [3, 8, 31]. Ascorbic acid acts as an antioxidant and strong reducing agent which helps to prevent molecular oxidation leads to cell damage, in turn fend off diseases like cancer, cardiovascular diseases, and muscular degeneration [19, 20]. Due to its chemical potential involved in the synthesis of collagen, carnitine, epinephrine, norepinephrine, serotonin, and iron absorption [37]. Ascorbic acid is metabolized in the liver and kidney. It is a scavenger of free radical through the oxidation of ascorbic acid to dehydroascorbic acid and reduction in reactive oxygen species (Superoxide anion, hydroxyl radical, hydroxyl radicals, singlet oxygen, nitrogen dioxide, and nitric oxide radicals). It is an effective cytoprotective antioxidant activity in protecting macromolecules proteins, lipids, and nucleic acids from the free radicals [37]. The recommended dietary allowance of ascorbic acid daily intake is 60 mg/ day for adult men and women [3, 25]. Experiments were conducted with fruits and vegetables on guinea pigs fed with apple, cabbage, lemon juice, and the results showed preventive characteristics of many diseases [3]. Ascorbic acid is present in both plant and animal sources, some of the plant sources are fresh fruits, green leafy vegetables, some of the sources are amla, lemon,

orange, mango, papaya, cabbage, *Moringa oleifera* leaves, spinach and guava and some of the animal sources are meat, fish, poultry, eggs. The high amount of ascorbic acid is present in fresh raw food when compared to dry food, post-harvesting, and food processing. The loss of nutrients may be observed when the foods were exposed to light, air, peeling, slicing, chopping, heat, and storage. The nutrient density has to be maintained in the food from the food spoilage due to the growth of bacteria, yeast, and mold that can be controlled by the removal of water by drying method. The food dying causes changes in color, aroma, texture, physical shape, and nutritive value. The loss of ascorbic acid in dried food was observed ranging from 30-80 % and this indicates it is less stable during processing and storage [5].The research evidence also showed that processed foods are rich sources of bioactive molecules that may be preventing many chronic diseases [37]. The recent review showed that there is a correlation between ascorbic acid & diabetes and supplementation of ascorbic acid may activate insulin sensitivity in both type-1 & type-2 diabetes [14].

Minerals

Minerals play a vital role in human nutrition including the prevention and treatment of many diseases and /or disorders. In the classification of minerals are macro and micro minerals, macro minerals are required high quantity include calcium, phosphorus, magnesium, sulfur, sodium, and chloride and micro minerals are required minute quantity include iron, copper, cobalt, iodine, zinc, manganese, molybdenum, selenium and chromium to perform many biological functions [25]. Minerals are available in body tissues and fluids to undertake physiochemical progression in both animals and humans. Due to a deficiency of minerals includes anemia, osteoporosis and rickets, stunting of growth and development. To sustain life many metalloenzymes required minerals for its enzyme activity. Calcium is involved in the maintenance of bone density, nerve, and muscle functions. Iron is an essential component of hemoglobin, myoglobin, cytochromes, ribonucleotide reductase, growth, wound healing. The bioavailability of iron depends on the form of sources, whether it is from the plant, animal, chemical, and animal sources are showing high bioavailable compared to plant sources due to nutrient inhibitors [3]. Zinc is an essential element in various enzymes for energy production, protein synthesis, and growth regulation. Manganese is a divalent metal ion it involved in the bones and wound healing by increasing collagen. Phosphorous is essential for bone and teeth formation, energy, protein and repair of cells and tissues, energy carrier nucleotide molecule adenosine triphosphate, acid-base balance. Magnesium is needed in the biochemical reactions, nerve and muscle functions, immune system, bone, activation, and chelate negative ion in the enzymes, regulate blood glucose levels, and aid in the production of energy and protein. Sulfur is for muscle, skin, bone, amino acids for cells and tissue formation, hormones, and antibodies. Potassium acts as an electrolyte, a crucial role in heart, skeleton, smooth muscle contraction. Cobalt is a part of cyanocobalamin to treat megaloblastic anemia, myelin, which protects nerve cells, the formation of hemoglobin, and some infectious diseases. Recent times the imbalance of thyroid hormones due to deficiency of iodine and lifestyle dietary pattern. Therefore, the objectives of the present study are to estimate the concentration of ascorbic acid and mineral content in *Moringa oleifera* leaves and further investigate the stability of ascorbic acid under different storage conditions.

MATERIALS AND METHODS

Fresh *Moringa oleifera* leaves were collected from the local area of Kuvempu Nagara of Tumakuru Karnataka state, India.

Chemicals

Highest purity chemicals were used in the present study, and unless otherwise specified.

Estimation of ascorbic acid

The ascorbic acid content was estimated by the dinitrophenyl hydrazine (DNPH) method [22]. Briefly, Fresh *Moringa oleifera* leaves were selected and washed thoroughly with distilled water. *Moringa oleifera* leaves were homogenized by using a pestle and mortar with 5% Trichloroacetic acid (TCA) solution. The homogenate solution was centrifuged at 10,000 rpm for 10-15 min. the supernatant solution was collected and dilute the sample 1:10 and 1:20 by using 5% TCA solution. 1ml of diluted solution taken in a different test tube, then add 2% DNPH in each test tube kept in boiling water bath for 15-20 min orange-red color appearance and cooled to room temperature. Oxidation of ascorbic acid to dehydroascorbic acid by cuprous ion present in the DNPH reagent and finally to 2, 3 - diketo-gulonic acid reacts with 2, 4 dinitrophenylhydrazine to form red bis-hydrazone. Add 5ml of chilled 85% sulphuric acid, then the colored solution absorbance read at 540 nm in a spectrophotometer. The above procedure repeated for different temperatures -20^{o} C, 4^{o}C, Room temperature (RT), 40^{o}C respectively.

Estimation of minerals

The fresh leaves were collected and clean the leaves by using water, dried at room temperature, later in hot air oven [21]. Followed by muffle furnace for ashing at 600^{o}C for 2h and after ash, the sample was dissolved in nitric acid. The main purpose of sample digestion is to separate the minerals from the organic matter. After the digestion sample filtered through Whatman filter paper and transferred to a flask. Estimation of minerals by Inductively Coupled Plasma Optical Emission Spectroscopy Method (ICP-OES): The analysis of minerals in ICP-OES involves the following steps: Sample digestion and analysis by ICP-OES method and the measurement results are expressed as an average of triplicate [17, 26].

A separate determination of percent solids must be performed. The concentrations determined in the digest are to be reported based on the dry weight of the sample.

Concentration (mg/kg) = C × V× D / W

Where, C- Analyte concentration in the final extract (mg/L), V-Final volume in liter after sample preparation,

D-Dilution factor (Diluted volume by aliquot volume), W-Weight in kg of dried sample.

Statistical analysis

For the calculation of t-value statistical software SPSS version 21 and for the graphical representation origin software version 8.1 was used.

RESULTS

Ascorbic acid

To study ascorbic acid in Moringa leaves for many reasons, Moringa leaves are easily available and ascorbic acid involves in many biological functions. To prevent many deficiency diseases by supplementation of ascorbic acid and to meet the recommended dietary allowance (RDA). In India, many types of food are available to maintain good health. The consumption of ascorbic acid-rich foods every day but recent reports showed that there is 30-80 % ascorbic loss during processing means not able to provide the required quantity of ascorbic acid [5]. In this context, we have collected fresh *Moringa oleifera* leaves from the local area of Kuvempu Nagara, Tumakuru District of Karnataka State, India. The ascorbic acid was estimated by the dinitrophenylhydrazine (DNPH) method. The ascorbic acid estimation in fresh *Moringa oleifera* leaves on the same day to check the concentration of ascorbic acid in fresh leaves and storage for 6 days at different storage conditions like -20^{o}C, 4^{o}C, RT, 40^{o}C. The present results of fresh *Moringa oleifera* leaves showed appreciable amount 43.26 mg % on the first day (**Table 1 and Figure 1**) and the results of second day showed 39.26 mg % (-20^{o}C), 37.45 mg % (4^{o}C), 29.25 mg % (RT) 16.80 mg % (40^{o}C) respectively. Third day results showed 38.42 mg % (-20^{o}C), 36.90 mg % (4^{o}C), 26.04 mg % (RT) and 14.50 mg % (40^{o}C) respectively. Fourth day results showed 34.89 mg % (-20^{o}C), 30.08 mg % (4^{o}C), 23.54 mg %, (RT) and 6.33 mg % (40^{o}C) respectively. Fifth day results showed 33.40 mg % (-20^{o}C), 29.86 mg % (4^{o}C), 20.15 mg %, (RT) and 4.72 mg % (40^{o}C) respectively. Sixth day results showed 30.05 mg % (-20^{o}C), 25.34 mg % (4^{o}C), 19.12 mg %, (RT) respectively. The statistics report showed significant results at -20^{o}C, simple t-test value 0.00; 4^{o}C simple t-test value 0.00; RT simple t-test value 0.00 and 40^{o}C, simple t-test value 0.038 (**Table 1 and Figure 1).**

Table 1: Ascorbic acid in *Moringa oleifera* leaves (mg / 100 g)

Days	Fresh Leaves	-20°C	4°C	RT	40°C
Day 1	43.26				
Day 2		39.26	37.45	29.25	16.80
Day 3		38.42	36.90	26.04	14.50
Day 4		34.89	30.08`	23.54	06.33
Day 5		33.40	29.86	20.15	04.72
Day 6		30.05	25.34	19.12	N D

ND - Not Determined, Simple t-test: t > 0.05

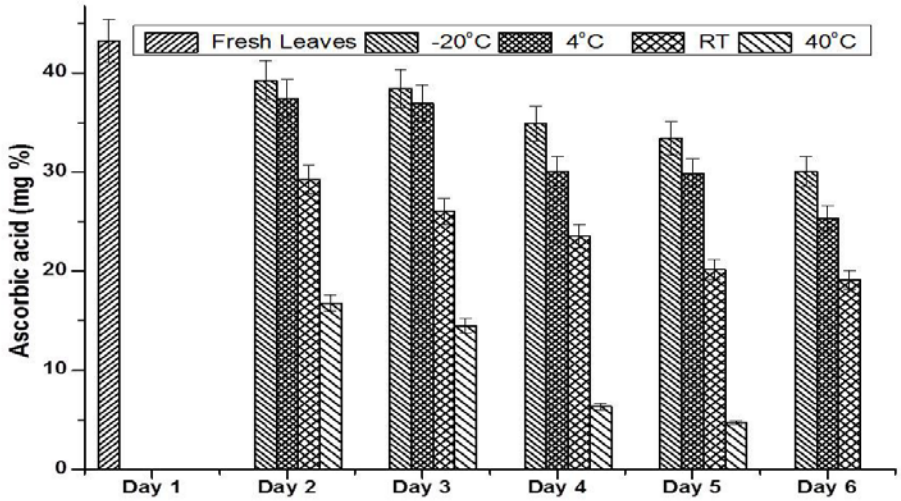

Figure 1: Ascorbic acid in *Moringa oleifera* leaves (mg / 100 g)

Stability of ascorbic acid

The ascorbic acid content was measured to study the stability of ascorbic acid under different conditions in Moringa leaves. The results of ascorbic acid under different conditions showed at - 20^{o}C second day (9.25 mg %), the third day (11.19 mg %), fourth day (19.35 mg %), a fifth day (22.80 mg %), the sixth day (30.54 mg %). The results of ascorbic acid showed at 4^{o}C second day (13.44 mg %), the third day (14.71 mg %), the fourth day (30.47 mg %), fifth day (41.43 mg %). The results showed at room temperature (RT) second day (32.39 mg %), third day (39.81 mg %), fourth day (45.59 mg %), a fifth day (53.43 mg %) and sixth day (55.81 mg %). The results showed at 40^{o} C second day (61.17 mg %), the third day (66.49 mg %), the fourth day (85.37 mg %), and the fifth day (89.09 mg %). The statistics report indicates that it is significant (t > 0.05) under different storage conditions (**Table-2 and Figure 2**).

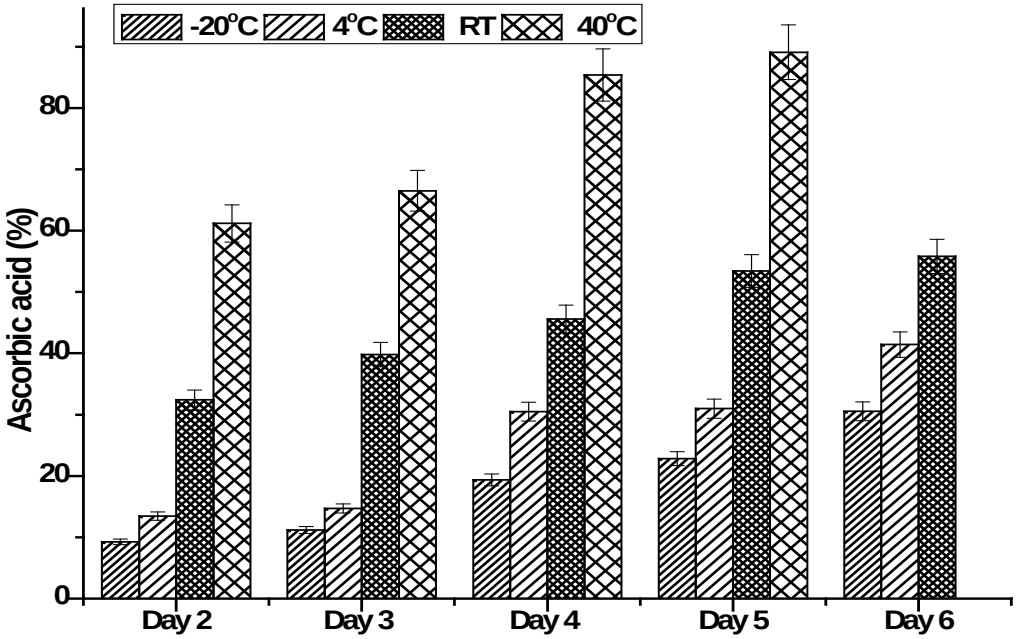

Figure 2: Ascorbic acid content under different storage conditions

Table 2: Ascorbic acid content under different storage conditions (mg / 100g)

Days	-20°C	4°C	RT	40°C
Day 2	09.25	13.44	32.39	61.17
Day 3	11.19	14.71	39.81	66.49
Day 4	19.35	30.47`	45.59	85.37
Day 5	22.80	30.98	53.43	89.09
Day 6	30.54	41.43	55.81	N D

ND - Not Determined, Simple t-test: t > 0.05

Minerals

Minerals are playing a significant role in many biological functions and the prevention & treatment of many diseases [25]. Minerals are very much essential components for metalloenzymes, hemoglobin, myoglobin cytochromes, ribonucleotide reductase, collagen, and deficiency of minerals lead to anemia, osteoporosis, rickets, growth, bone, nerve & muscle and wound healing. Due to the vast biological functions of minerals in the biological system, we have selected Moringa leaves to assess the minerals content. The present study results showed Calcium, 2.40 %; Phosphorus, 0.21 %; Magnesium, 0.50 %; Sulfur, 1.54 %; Potassium, 0.48 %; Zinc, 30.35 ppm; Cobalt, 1.15 ppm; Iron,190.5 ppm; Manganese, 34.25 ppm and Copper, 14.47 ppm in the Moringa leaves **(Table 3 and Figure 3).**

In the present study, the minerals are estimated by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method. In the recent past, minerals estimation in the food with different methods such as conventional and atomic absorption spectroscopy (AAS) but in our study, we have used the ICP-OES method for the mineral estimation in the foods. The results showed that the minerals content in Moringa leaves is marginal to prevent many diseases.

Table 3: **Minerals in *Moringa oleifera* leaves**

Minerals	Leaves powder
Calcium (%)	2.40
Phosphorus (%)	0.21
Magnesium (%)	0.50
Sulfur (%)	1.54
Potassium (%)	0.48
Zinc (ppm)	30.35
Cobalt (ppm)	01.15
Iron (ppm)	190.5
Manganese (ppm)	34.25
Copper (ppm)	14.47

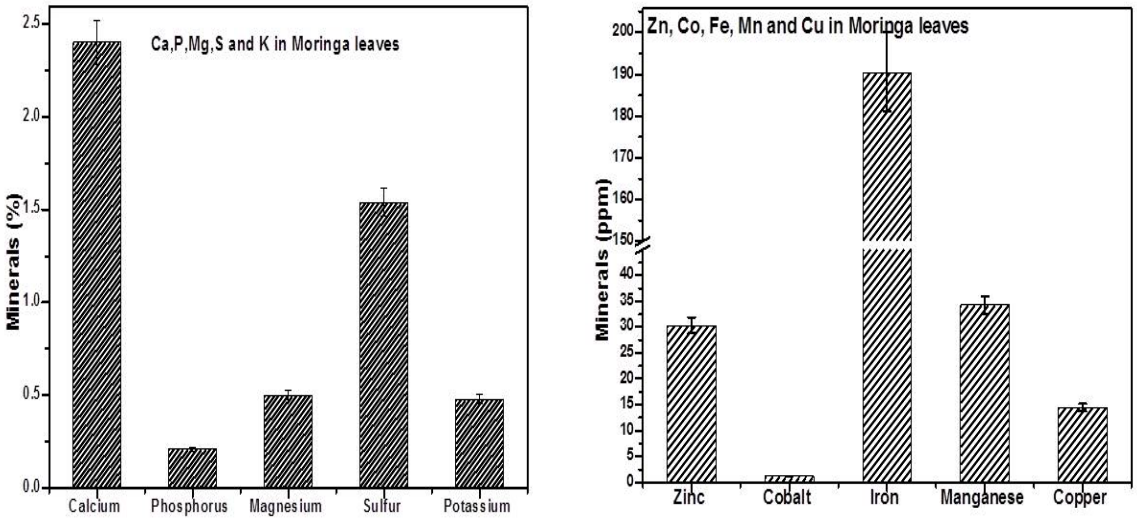

Figure 3: Graphical representation of minerals in percent (%) and parts per million (ppm)

DISCUSSION

Moringa olieifera is a nutritional food due to its rich essential nutrients and phytochemicals to control many degenerative diseases [10, 30] .The supplementation of ascorbic acid and minerals rich foods to prevent many diseases. In this context, we have selected locally available *Moringa olieifera* leaves were consumed by the rural and urban populations. The fresh *Moringa olieifera* leaves were collected from the local area of Kuvempu Nagara, Tumakuru Karnataka state, India The ascorbic acid estimation by dinitro phenylhydrazine (DNPH) method and minerals estimation by estimation of minerals by inductively coupled plasma optical emission spectroscopy (ICP-OES) method. In the present study we have estimated the concentration of ascorbic acid in fresh leaves and at the same time leaves are stored under different conditions (-20^{o}C, 4^{o}C, RT, 40^{o}C). The first day we have used fresh leaves for the ascorbic acid estimation and followed by stored leaves are freshly extracted in the trichloroacetic acid. According to the present investigation results, ascorbic acid concentration gradually decreases under different storage conditions when the storage time increases. The present results revealed the ascorbic acid content in fresh (first day) leaves contain 43.26 mg %. The ascorbic acid content in Moringa *oleifera* leaves under different storage conditions for 6 days and results showed at -20^{o}C the second day (39.26 mg %), the third day (38.42 mg %), fourth day (34.89 mg %), fifth day (33.40 mg %), the sixth day (30.05 mg %). The results revealed at 4^{o}C second day (37.45 mg %), third day (36.90 mg %), fourth day (30.08 mg %), fifth day (29.86 mg %), sixth day (25.34 mg %). The outcome of the results showed at room temperature (RT) second day (29.25 mg %), third day (26.04 mg %), fourth day (23.54 mg %), fifth day (20.15 mg %), sixth day (19.12 mg %). The results showed at 40^{o}C second day (16.80 mg %), third day (14.50 mg %), fourth day (6.33 mg %) and fifth day (4.72 mg %). The scientific reports of Sankhyan Nidhi *et al*., (2013) indicated ascorbic acid in leaves with different methods and showed 0.87 mg / g by spectrophotometer method 0.8 mg /g by the titrimetric method and 0.78 mg / g by HPLC method and showed high ascorbic acid content when compared to the present study 43.26 mg % in the fresh leaves. Ahmed, K. S et.al., (2016) results showed that in different parts of four weeks aged Moringa tender leaves (62.66 to 143.59 mg %), mature leaves (51.23 to 150.16 mg %), flowers 3.96 to 8.27 mg % by HPLC method this is the results of the average of three harvests and in the present results 43.26 mg % compared to 51.23 - 150.16 mg % this indicates the present results showed there is an agreement with Ahmed, K. S et.al., (2016). Kushwaha S (2015) in the review indicates the ascorbic acid (143.6 mg %) and also showed total carotene (27.1 mg %), β-carotene (17.4 mg %) in fresh *Moringa oleifera* leaves. Gopalakrishnan L et al., (2016) results revealed the ascorbic acid content was 220 mg % in fresh leaves. El Sohaimy S A et al., (2015) report showed the ascorbic acid was 245.13 mg %. Witt K A (2013) review showed ascorbic acid (162 mg %). Abbas R K et al., (2018) indicates ascorbic acid was 8.6 mg. Saini R K et al., (2014) showed fresh leaves 271 mg % of ascorbic acid. Ajantha A et al., (2018) showed ascorbic acid in leaf meal was 17.31 mg %. Ahmed, K. S. et al., (2016) showed ascorbic acid in matured leaves was 51.23 to 150.16 mg %. Singh B. N et al., (2009) revealed ascorbic acid in aqueous extract of fresh leaf was 91.22 mg %. Yang, R. Y. et al., (2006) showed ascorbic acid in mature Morigna leaves 249 mg %. Samidha M Pawaskar and Sasangan K C (2017) showed ascorbic acid was 516 .66 mg % in the leaf extract **[Table 4]**.

Present results showed that the ascorbic acid content in fresh leaves 43.26 mg % nearly agreement with 51.23 mg % [3], 87 mg % [25]. Ajantha A et al., 2018 showed ascorbic content was17.31 mg % in leaf meal. Most of the research studies showed high ascorbic acid content in leaves due to different extraction methods and estimation methods that are used for the estimation. Ascorbic acid content depends upon the region of collection, transport, extraction method, estimation methods, and some external factors that are also responsible for the accurate estimation

of ascorbic acid. In the extraction method, many studies have shown different extraction solutions (TCA, Metaphopshoric acid). In the estimation of ascorbic acid content with different detectors were used to detect viz. UV-Vis, fluorescence, electrochemical, evaporating light scattering detector, mass spectrometer, and HPLC & GC. HPLC methods can be used to estimate the reduced form of ascorbic compared to the spectrophotometer method where the DNPH reagent reacts with the oxidized form of ascorbic acid that may provide total ascorbic acid include ascorbic acid and dehydroascorbic acid. The scientific reports revealed that the high quantity of ascorbic acid present in the fresh raw food when compared to dry, post-harvesting, and processing methods due to loss of ascorbic acid. Ascorbic acid is sensitive to light, air, heat, peeling, slicing, chopping, and storage. During processing and storage ascorbic acid is less stable ranging from 30-80 % [5]. The present study conducted on the storage stability of ascorbic acid for six days and presented the results of ascorbic acid under different conditions at - 20^{o}C second day (9.25 %), the third day (11.19 %), fourth day (19.35 %), fifth day (22.80 %), the sixth day (30.54 %). The results of ascorbic acid loss at 4^{o}C second day (13.44 %), the third day (14.71%), fourth day (30.47 %), fifth day (41.43%). The results showed at room temperature (RT) second day (32.39 %), third day (39.81 %), fourth day (45.59 %), fifth day (53.43 %) and sixth day (55.81 %). The results showed ascorbic acid loss at 40^{o} C second day (61.17 %), the third day (66.49 mg %), fourth day (85.37 %), and the fifth day (89.09 %). Clement A *et al*., (2017) reported there is a loss of ascorbic acid due to processing ranging from 30-80 % and the present study results showed 89.09 % loss of ascorbic acid on the fifth day of storage. The ascorbic acid stability showed at -20^{o}C (39.26 to 30.05 % up to the sixth day), at 4^{o}C (37.45 to 25.34 % up to the sixth day), at RT (32.39 to 55.81% up to the sixth day) and 40^{o}C (61.17 to 89.09 % up to the fifth day) **[Table2 and Figure 2]**. The present results suggest that the consumption of fresh Moringa leaves will provide a good amount of ascorbic acid to meet RDA compared to the storage and processed leaves.

Table 4: Ascorbic acid content in *Moringa oleifera* leaves from various authors

Ascorbic acid	Authors	Parts
516.66 mg %	Samidha M Pawaskar, 2017	Leaf extract
249 mg %	Yang, R Y et al., 2006	Mature fresh leaves
91.22 mg %	Singh B N, 2009	Fresh leaves aqueous extracts
51.23 -150.16 mg %	Ahmed K S et al., 2016	Matured leaves
17.31 mg %	Ajantha A et al., 2018	Leaf meal
271 mg %	Saini R K, 2014	Fresh leaves
162 mg %	Witt K A, 2013	Fresh raw leaves
245.13 mg %	El Sohaimy S A, 2015	Leaves
220 mg %	Gopalakrishnan L, 2016	Fresh leaves.
143.6 mg %	Kushwaha S (2015)	Leaves
0.87 mg /g	Sankhyan Nidhi et al., 2013	Leaves Spectrophotometer
0.8 mg /g	Sankhyan Nidhi et al., 2013	Leaves Titrometric method
0.78 mg / g	Sankhyan Nidhi et al., 2013	Leaves HPLC method
43.26 mg %	Present study	Fresh leaves

Minerals

Minerals are required to carry out the physiochemical process of life both animals and humans. The minerals are essential in nerve & muscle, bone, ATP, acid-base balance, cytochromes, hemoglobin, myoglobin, blood clotting, and cofactors [16, 18]. The present results obtained as follows calcium 2.40 %, phosphorus 0.21 %, magnesium 0.50 %, sulfur 1.54 %, potassium 0.48 %, Zinc 30.35 ppm, cobalt 1.15 ppm, iron 190.5 ppm, manganese 34.25 ppm, copper 14.47 ppm **[Table 3 and Figure 3**]. The research results of Sengev A I et *al*., (2013) showed that the Iron (8.3 mg %), Magnesium (244 mg %), Calcium (442.2 mg %), potassium (1320 mg %) copper (3.10 mg %) compared to our study results showed iron (190.5 ppm), magnesium(0.50 %), calcium (2.4 %), potassium (0.48 %), copper (14.47 ppm). Yaméogo, C. W *et al*., (2011) revealed Calcium (2098 mg %), Magnesium (406 mg %), potassium (1922 mg %), iron (28.3 mg %), Zinc (5.4 mg %) phosphorus (351 mg %) present results are showed agreement in calcium 2400 mg %, magnesium 500 mg %, potassium 480 mg %. Moyo B et al., (2011) revealed in dry *Moringa oleifera* leaves calcium (3.65 %), phosphorus (0.3 %), magnesium (0.5 %), potassium (1.5 %), sodium (0.164), sulphur (0.63 %), zinc (31.03 mg

/ kg), copper (8.25 mg / kg), manganese (86.8 mg / kg), iron (490 mg / kg) selenium (363 mg / kg) . Gopalakrishnan L et al., (2016) showed calcium (440 mg %), magnesium (42 mg %), phosphorus (70 mg %), potassium (259 mg %), copper (0.07 mg %), iron (0.85 mg %) in fresh leaves. El Sohaimy S A et al., (2015) revealed sodium (289 mg %), potassium (33.63 mg %), magnesium (25.64 mg %), phosphorus (105.23 mg %), iron (9.45 mg %), (1.63 mg %), copper (0.88 mg %) calcium (486.23 mg %) and manganese (5.21 mg %). Korsor M et al., (2017) showed calcium (1.2%), phosphorus (0.265 %) , potassium (0.578%), magnesium (0.074), sodium (0.656) , copper (15.1 ppm) , iron (177.1 ppm), manganese (0.358 ppm) and zinc (20.5 ppm). Witt K A (2013) in the review showed that the calcium (532 mg %), phosphorus (90-112 mg %), Sodium (16 mg %), potassium (414 mg %), magnesium (26-151 mg %), iron (10.8 mg %), zinc (0.3-1.3 mg %) copper (0.23 mg %). Abbas R K et al., (2018) Calcium (99.1 mg) , iron (1.3 mg) , magnesium (35.1 mg), manganese (0.119 mg) , phosphorus (70.8 mg), potassium (471 m), sodium (70 mg), zinc (0.85 mg). Ajantha A et al.,(2018) revealed in leaf meal calcium (1.6 %) , phosphorus (0.28 %), magnesium (0.43 %), sodium (0.12 %), potassium(1.38 %), iron (285 ppm), zinc (38.02 ppm), copper (5.9 ppm). Glover-Amengor et al.,(2016) showed in dry leaves copper (0.36 mg %), iron (20.26 mg %), manganese (5.8 mg %) zinc (6.79 mg %). Yang R Y et al., (2006) showed in mature Morigna leaves fresh weight iron (9.2 mg %), calcium, (638 mg %). Samidha M Pawaskar and Sasangan K C (2017) calcium (480.96 mg %), phosphorus (14 mg %), iron (34.4 mg %), magnesium (1416 mg %) **[Table 5].**

The present study of iron (19.05 mg %) content was comparing with 17.71 mg % [12], 20.26 mg % [9]. Magnesium (0.5 %) content was agreement with 0.5 % [16], 0.406 % [35], 0.43 % [4]. Calcium (2.4 %) result was compare with the results of Yaméogo C W *et al*., 2012 (2.098 %). Potassium (0.48 %) result was compare with the 0.578 % [12], 0.414 % [34], 0.471 % [1] and 0.397 % (I FC Table, NIN, 2017). Copper (1.447 mg %) result was compared with the results of 0.88 mg % [6], 1.51 mg % [12]. Zinc (3.035 mg %) result was compare with 3.8 mg % [4], 3.103 mg % [16]. Phosphorus (0.21 %) result was compared with 0.28 % [4] 0.265 % [12]. Sulfur (1.54 %) result was compared with 0.63 % [16]. Manganese (3.425 mg %) compared with the results of near to 5.21 mg % [6] and 5.8 mg % [9]. Fresh Moringa leaves are showing an appreciable amount of ascorbic acid, minerals and stored leaves at -20^{o}C, 4^{o}C, RT, 40^{o}C for 6 days showed minimum amount compared to the fresh leaves. According to the results, 43.26 mg % of ascorbic acid is essential to meet RDA levels for controlling anemia through enhances iron bioavailability and degenerative diseases. The present study results may provide information to create awareness and easily available sources of morimga leaves to control ascorbic acid and mineral deficiency in the populations.

Table 5: Minerals content in *Moringa oleifera* leaves from various authors

Fe	Mg	Ca	K	Cu	Zn	P	S	Mn	Co	Authors
8.3mg%	244mg%	442.2mg%	3.1mg%							Sengev A I et al., 2013
28.3mg%	406mg%	2098 mg%	1922mg%		5.4mg %	351mg %				Yaméogo, C. W et al., 2011
490mg/kg	0.50%	3.65%	1.50%	8.25 mg/kg	31 mg/kg	0.30%	0.63 %	86.8 mg/kg		Moyo B et al., , 2011
0.85 mg %	42mg %	440 mg %	259 mg %	0.07 mg %		70 mg %				Gopalakrishnan L et al., 2016
9.45mg%	25.64 mg %	486 mg %	33.6 mg %	0.88 mg %	1.63 mg %	105 mg %		5.21 mg %		El Sohaimy S A et al., 2015
177ppm	0.07%	1.20%	0.58%	15 ppm	20.5 ppm	0.27%		0.36 ppm		Korsor M et al., , 2017
10.8 mg %	26-151mg %	232 mg%	414mg %	0.23 mg %	0.3-1.3 mg %	90-112mg %				Witt K A. , 2013
1.3mg %	35 mg%	99mg%	471 mg%		0.85mg%	70.8mg%				Abbas R K et al., 2018
285 ppm	0.43%	1.60%	1.38	5.9 ppm	38 ppm	0.28%				Ajantha A et al., 2018
20.96mg %				0.36 mg%	6.79 mg %			5.8 mg %		Glover Mary -Amengor et al., 2016
9.2 mg %		638 mg%				14 mg%				Yang, R. Y. et al., 2006
34.4 mg%	1416 mg %	480.96								Samidha M Pawaskar, 2017
4.56 mg %	97.1 mg %	314.mg%	397 mg %	0.44 mg %	0.72 mg %	109 mg %		1.26 mg %		I F C Table, NIN, 2017*
190.5 ppm	**0.50%**	**2.40%**	**0.48%**	**14.47 ppm**	**30.35 ppm**	**0.21%**	**1.54 %**	**34.25ppm**	**1.15ppm**	**Present study**

*I F C Table- Indian Food Composition Table, National Institute of Nutrition (NIN), Hyderabad, 2017[38]

CONCLUSION

Ascorbic acid is a powerful functional food ingredient with numerous health applications. Proper intake helps to maintain health and prevent future diseases. Recent data showed ascorbic acid 60 mg/day required to meet RDA and the present study showed 43.26 mg % of ascorbic acid in Moringa fresh leaves. The ascorbic acid estimation under different storage conditions such as -20^{o}C, 4^{o}C, RT, 40 ^{o}C for 6 days storage, and the results showed there is a loss from day1 to day 6 in all the conditions. Mineral content in *Moringa oleifera* leaves showed a good quantity of calcium, phosphorus, magnesium, sulfur, potassium, zinc, cobalt, iron, manganese, and copper. This information may be useful to create awareness about the nutrients of *Moringa oleifera* leaves and to accomplish the requirements of ascorbic acid and mineral in the target population.

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