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ISOLATION AND SPECTROSCOPIC CHARACTERIZATION OF ASCORBIC ACID FROM *Moringa Oleifera* LEAVES

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Abstract: *Ascorbic acid (Vitamin C) is an essential micronutrient with antioxidant properties known for its sensitivity to heat, light and is usually destroyed by oxygen in the air. In this study, Moringa oleifera leaves harvested were analysed for their ascorbic acid content. An aqueous extract was prepared and ascorbic acid content was quantified using iodometric titration, yielding 0.79 mg/g with 56.88% degradation. The functional groups in the isolated sample were identified using Fourier Transform Infrared (FTIR) spectroscopy, revealing hydroxyl groups (3000–3500 cm^{-1}), a C=C double bond (1654 cm^{-1}), enol-hydroxyl (1315 cm^{-1}), and an O-H in-plane bending bond (1274 cm^{-1}). Prominent peaks included the carbonyl group (C=O) at 1755 cm^{-1} and ester (C-O) at 1021–1110 cm^{-1}, consistent with theoretical ranges for hydroxyl (3212–3526 cm^{-1}) and carbonyl (1654–1755 cm^{-1}) groups. Specific O-H bands were assigned as C_1-OH (3526 cm^{-1}, free hydroxyl, alcohol), C_2-OH (3406 cm^{-1}, H-bonded alcohol), C_3-OH (3309 cm^{-1}, H-bonded alcohol), and C_4-OH (3212 cm^{-1}, carboxylic acid), alongside a lactone C=O (1755 cm^{-1}) and C=C (1654 cm^{-1}). Ultraviolet-Visible (UV-Vis) spectroscopy (220–340 nm) showed maximum absorption at 241 nm, aligning with standard ascorbic acid. These findings suggest that Moringa oleifera leaves are a rich source of ascorbic acid, potentially contributing to their nutritional and medicinal properties.*
Keywords: Ascorbic acid, *Moringa oleifera*, FTIR, UV-Vis, Iodometric titration.

Introduction

Ascorbic acid, also known as Vitamin C, is a water-soluble essential nutrient with potent antioxidant properties that help to protect biological macromolecules from oxidative stress (Gopalakrishnan et al., 2016). It plays critical physiological roles, including enhancement of immune function, collagen biosynthesis, and prevention of scurvy (Ojukwu & Amaeze, 2017). However, due to its thermolabile and oxidation-prone nature, ensuring its stability and effective isolation from dietary sources remains a research priority (Al-Owaisi et al., 2014).

Moringa oleifera, a plant of the Moringaceae family, is widely recognized for its high nutritional content and medicinal versatility. Its leaves, seeds, flowers, and roots contain significant levels of vitamins, minerals, and bioactive compounds, making it a target for both nutritional and pharmaceutical applications (Gopalakrishnan et al., 2016; Mangala et al., 2014). The leaves in particular are rich in vitamin A, calcium, potassium, and Vitamin C, and have been employed in treating conditions such as diabetes, hypertension, and tuberculosis (Doriya et al., 2016; Natsir et al., 2019).

In Nigeria, *M. oleifera* is cultivated extensively for its affordability, resilience, and health-promoting benefits. Its widespread use in both rural and urban diets justifies scientific efforts to evaluate and validate its phytochemical constituents. Given the increasing interest in natural antioxidants, this study aimed to isolate and spectroscopically characterize ascorbic acid from *M. oleifera* leaves cultivated in Ilaro, Nigeria, using titrimetric, FTIR, and UV-Vis techniques.

Materials and Methods

Sample Collection and Preparation

The fresh leaves were collected in the botanical garden of the Federal Polytechnic, Ilaro in July 2024. All the samples were thoroughly washed with tap water to remove any adhering contaminants. The fresh Moringa leaves were then air-dried for three weeks to remove the water.

Extraction of Moringa oleifera Juice

A 250 g sample of fresh leaves was blended with 500 cm^3 of distilled water, 2 g of oxalic acid (to stabilize ascorbic acid), and 50 mL of 0.05 M sulfuric acid in an electric blender. The mixture was filtered through four layers of muslin cloth to obtain Moringa juice.

Ascorbic acid isolation

The juice was centrifuged at 5000 rpm for 10 minutes, yielding 325 cm^3 of supernatant. The residue was pressed through muslin cloth to recover additional juice. The

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supernatant was diluted with 250 cm^3 of distilled water and left to macerate overnight. The solvent was evaporated at 30°C using a vacuum rotary evaporator. Calcium carbonate and calcium oxide were added with constant stirring until a pH of 8.0–8.5 was achieved, forming a milky solution rich in calcium ascorbate. The process continued by adding 30cm^3 of acetone and it was filtered under a vacuum in a Buchner funnel to yield a yellowish-green precipitate. The analysis showed that Ascorbic acid of the juice remains in solution as calcium ascorbate which was recovered by concentrating the aqueous solution by adding excess calcium chloride. The precipitate was stirred in water and 1N sulphuric acid was gradually added while a process of magnetic stirring was continued until a pH of 2.3-2.8 is attained. The mixture was left to settle for a few minutes and the pH is rechecked.

Determination of Ascorbic Acid

The vitamin C content was estimated by the titration method. It was determined by titrating against 2, 6-dichlorophenol indophenol which is a blue colour dye and gives a pink colour at the endpoint. The dye solution was prepared by dissolving 52 mg of 2, 6-dichlorophenol indophenol in distilled water. Pure ascorbic acid was taken as standard by preparing 1 mg/mL in 4% oxalic acid and was diluted to 100 µg/mL as a working standard. 1–2 g of the fresh sample (crushed) was prepared with 4% oxalic acid. It was diluted to 100 mL with distilled water, filtered and the filtrate was taken as a test solution. 5 mL of the standard solution was taken and 10 mL of 4 % oxalic acid was added to it. It was then titrated against the dye solution that gave pink colour at the endpoint which persisted for a few minutes.

FTIR Spectrophotometric determination

The IR analysis was performed in a Spectrum Two FTIR spectrometer (Perkin Elmer, USA) by using circular KBr cell window, 0.05 mm round Teflon spacers, Spectrum10 software (Perkin Elmer, **USA**), Homogenizer (Trumark, India).

Results

Table 1: Showing the different wave numbers and functional groups present in the *Moringa Oleifera* leaf extract.

S/N	Wave number (cm^{-1})	Functional group	Compound Class
1.	3526	O-H stretching	Alcohol
2.	3406	O-H stretching	Alcohol
3.	3309	O-H stretching	Carboxylic acid
4.	3213	C-H stretching	Alkene
5.	2918	C-H stretching	Alkane
6.	2117	CΞC stretching	Alkyne
7.	1755	C=O stretching	Ester
8.	1654	C=C stretching	Conjugated Alkene
9.	1427	O-H bending	Carboxylic Acid
10.	1386	O-H bending	Alcohol
11.	1315	O-H bending	Phenol
12.	1214	C-O stretching	Vinyl ether
13.	1222	C-O stretching	alkyl aryl ether
14.	1110	C-O stretching	Aliphatic ether
15.	1066	C-O stretching	alkyl aryl ether
16.	1021	C-O stretching	Vinyl ether
17.	987	C=C bending	Alkene
18.	868	C-H bending	1,3-disubstituted
19.	820	C=C bending	Alkene
20.	752	C=C bending	Alkene
21.	715	C=C bending	1,3-disubstituted

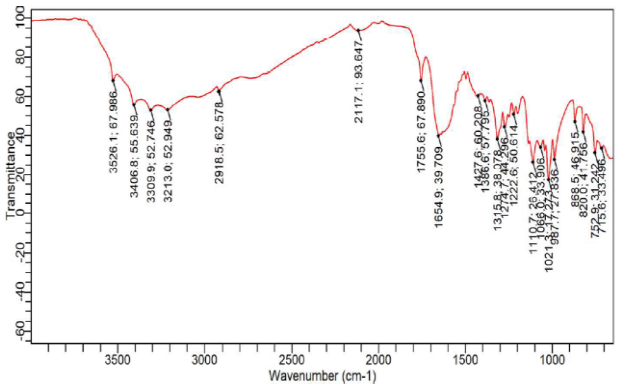

Fig. 1 FTIR Spectrum of *M. oleifera* leave extract

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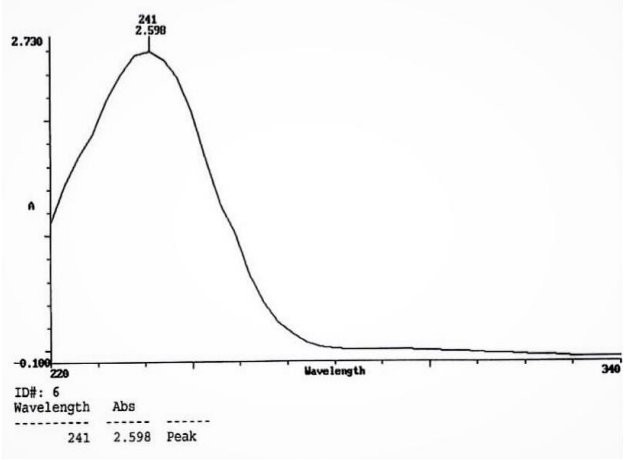

Fig 2: UV-Spectrum of Ascorbic acid in *M. oleifera* leave extract

Discussion

The ascorbic acid content in *Moringa oleifera* leaves was determined to be 0.79 ± 0.02 mg/g (mean ± SD, n=3) via iodometric titration, indicating a significant presence of vitamin C. This value is comparable to reported ascorbic acid contents in other green leafy vegetables (Ojukwu et al., 2017).

FTIR analysis (Table 1) revealed characteristic functional groups of ascorbic acid. The hydroxyl group (O-H stretching) was observed at 3526, 3406, and 3309 cm^{-1}, corresponding to free and hydrogen-bonded alcohols and carboxylic acids, respectively. The carbonyl group (C=O stretching) at 1755 cm^{-1} and the conjugated alkene (C=C stretching) at 1654 cm^{-1} confirmed the lactone structure of ascorbic acid, consistent with Sreeja et al. (2015). These functional groups are critical for ascorbic acid's antioxidant properties, contributing to *Moringa oleifera*'s medicinal potential.

IR spectra of ascorbic acid are presented in Figure 1. Different functional groups can be observed in the FTIR spectrum.

The prominent peaks for the FTIR Spectrum are the carbonyl group for ester (C=O) at 1755 cm^{-1} and ester (C-O) from 1110 to 1021 cm^{-1}. The theoretical value of frequency range cm^{-1} for the hydroxyl group is from 3526 to 3212 cm^{-1}, carbonyl group in the range 1755 to 1654 cm^{-1}. The four O-H bands observed in the isolated Ascorbic acid can be assigned as follows C_1-OH(3526.06, O-H stretch, free hydroxyl (alcohol), C_2-OH (3406.78, O-H stretch, H-bonding (alcohol), C_3-OH (3309.87, O-H stretch, H bonding (alcohol), C_4-OH (3212.96), O-H stretch (carboxylic acid) and the lactone C=O (1755.57 cm^{-1}) and C=C stretch (1654.93 cm^{-1}) band.

The peak observed around 1386 cm^{-1} corresponds to the symmetric bending of CH_3, while the peak observed at 1315 cm^{-1} corresponds to the C-H bending (Bekci et al,2009). The presence of C-H bend which is typical of alkenes was made evident by the band at 868 and 752 cm^{-1} respectively (Marcus et al, 2015).

The UV-Vis spectra of ascorbic acid in distilled water were scanned from 220 to 340 nm which showed maximal absorption at 241nm (Figure 2). This was a little lower than the reported value by Pancham et al, (2018) which says the UV-Vis spectra of ascorbic acid in distilled water were scanned from 200 to 400 nm which showed maximal absorption at 265 nm the UV-Vis absorption of moringa leaves has the maximum UV-Vis absorption at the same wavelength as standard ascorbic acid.

Conclusion

This study successfully isolated and characterized ascorbic acid from Moringa oleifera leaves grown in Ilaro, Nigeria, with a content of 0.79 mg/g determined by iodometric titration. FTIR analysis confirmed the presence of hydroxyl, carbonyl, and alkene functional groups, while UV-Vis spectroscopy showed maximal absorption at 241 nm, consistent with ascorbic acid. These findings underscore the nutritional and medicinal potential of Moringa oleifera as a rich source of vitamin C. Limitations include the use of a single sampling location and reliance on iodometric titration. Future research could explore alternative extraction methods and compare ascorbic acid content across different regions to further validate its applications in combating malnutrition and related health conditions

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