ISSN: 0972-0626
Volume 28, Issue 12, December, 2024

Antioxidants and Phytochemical Analysis of Drumstick Leaves (*Moringa oleifera*)

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ABSTRACT— Drumstick Leaves (*Moringa oleifera*) are one of the most useful tropical plants. It leaves a valuable source of nutrition for people of all ages. Nutritional analysis indicates that Drumstick leaves contain essential, disease-preventing nutrients. This research was aimed at analyzing phytochemical and non-enzymic antioxidants in Drumstick Leaves. Methods: The photochemical analysis of the leaves was done through phytochemical screening and the non-enzymic antioxidant activity of ascorbic acid in Drumstick leaves was analyzed using Ultraviolet-Visible spectrophotometry (UV-VIS) and Fourier Transformation Infrared Spectroscopy (FTIR). Drumstick Leaves analysis showed the presence of phytochemicals; alkaloids, tannins, saponins, anthraquinones, anthocyanosides, and flavonoids. Ascorbic acid in Drumstick leaves was analyzed and the absorbance of the ascorbic acid increased with the increase in the polarity index of solvents. Water extract having the highest polarity had the highest mass of ascorbic acid extraction of 0.07344g/10g which was closer to the working standard of 0.1M. In addition, 0.04411g/10g and 0.03131g/10g for methanol and chloroform extract. Whereas, petroleum ether and hexane had the lowest polarity hence masses of 0.02292g/10g and 0.02276g/10g were analyzed, which were closer. This research work conclusively demonstrates that *M. oleifera* is a good source of various phytochemicals like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, and flavonoids.

KEYWORDS: *Moringa oleifera*, Non-Enzymic Antioxidants, Phytochemical, Ascorbic Acid.

DOI:
12.1334/Rjce.29.12.2024.01

1. INTRODUCTION

The *Moringa oleifera* plant is widely distributed and utilized in tropical and sub-tropical regions of the world and is often called the drumstick tree. The *M. oleifera* plant, which is also known as "Marengue" in Jamaica, is one of the 14 species in the Moringa. Moringa has been reported to provide human, livestock, and many crop nutritional benefits [1]. Drumstick Leaves have been shown to anti-inflammatory, antioxidant, antimicrobial, cardiovascular, antihyperlipidaemic, CNS depressant, antifertility, anticancer, antihepatotoxic properties [2]. The leaves of *M. oleifera* have been reported to be a source of valuable phytochemicals and act as a good source of natural antioxidants [4].

Phytochemicals are naturally present in plants and show biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes through antimicrobial inhibition or killing mechanisms. Phytochemicals are present in virtually all plant tissues of *M. oleifera* e.g. leaves, roots, stem, and fruits [4]. Research studies stated that the medicinal value of *M. oleifera* plants lies in some chemical substances that produce a definite physiological action on the human body due to the presence of bioactive compounds like alkaloids, flavonoids, tannins, and phenolic compounds [5].

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exist both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in the presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. A few examples of non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione [6].

Ascorbic Acid (AA) is a natural water-soluble vitamin (Vitamin C). Ascorbic acid is a potent reducing and antioxidant agent that functions in fighting bacterial infections, detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries [7]. AA plays crucial roles in electron transport, hydroxylation reactions, and oxidative catabolism of aromatic compounds in animal metabolism [8]. Vitamin C is also required for the synthesis of dopamine and adrenaline in the nervous system or in the adrenal glands [9]. Antioxidants such as ascorbic acid and glutathione, which are found at high concentrations in moringa chloroplasts and other cellular compartments, are crucial for plant defense against oxidative stress [10]. The important components of Moringa like calcium and vitamin C are found to be exploited for exploitation by many developing regions of the world where malnutrition is a major concern, especially for children [11].

The specific aim of this research is to analyze the phytochemical and non-enzymic antioxidants analysis in Drumstick Leaves (*M. oleifera*). The photochemical analysis of the leaves was done through phytochemical screening, whereby chemical tests were done for alkaloids, tannins, saponins, anthraquinones, anthocyanosides, flavonoids, and reducing sugars. In addition, the non-enzymic antioxidant activity of ascorbic acid in *M. oleifera* leaves was analyzed using Ultraviolet-Visible spectrophotometry (UV-VIS) at the specific wavelength >340nm and Fourier Transformation Infrared Spectroscopy (FTIR) used to identify peaks of interest.

2. STUDY DESIGN

Plant Leaf Collection

Drumstick Leaves were collected from the Suva area in Fiji. The leaves were washed under running tap water to remove dust and impurities. The leaves were used for the analysis of phytochemical and non-enzymic antioxidant activity.

Phytochemical Analysis of M. oleifera Leave Sample Preparation

Approximately 20g of Drumstick leaves were prepared by boiling (100°C) 20 g of the fresh plant in 350ml distilled water and 85% ethanol. The solution was filtered using vacuum filtration. The filtration obtained was then used for the phytochemical antioxidant analysis.

Test for Alkaloids

For alkaloids, 1 ml of filtrate was transferred in a test tube and then mixed with 2 ml of Wagner's reagent (2 g: 6 g) iodine to potassium iodide in 100 ml distilled water. A reddish-brown colored precipitate indicates the presence of alkaloids.

Test for Tannins

The test was performed by following a standard procedure of Maxson and Rooney16. That is 1 ml of the filtrate was mixed with 2 m1 of FeCl. A dark green color indicated a positive test for the tannins.

Test for Saponins

1 mL of sample leave filtrate was diluted with 2 mL of distilled water; the mixture was vigorously shaken and left to stand for 10 min. during which time, the development of foam on the surface of the mixture lasting for more than 10min, indicates the presence of saponins.

Test for Anthraquinones

A Borntranger test17 was performed whereby 1 ml of the plant filtrate was shaken with 10 mL of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia was added, shaken, and observed. A pinkish solution indicates a positive test.

Test for Anthocyanosides

1 mL of the sample filtrate was mixed with 5 ml of dilute HCI; a pale pink color indicates a positive test.

Test for Flavonoids

1 mL of sample filtrate was mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 mL of the plant filtrate was mixed with 2 mL of dilute NaOH. a golden yellow color indicated the presence of flavonoids.

Test for Reducing Sugars

The reducing sugar in the sample was determined by adding 1 mL of the sample filtrate into which Fehling A and Fehling B were separately added; a brown color with Fehling B and a green color with Fehling A indicates the presence of reducing sugars.

Non-Enzymic Antioxidants Activity Analysis of M. oleifera Leaves Solvent Extraction

1 kg of leaves was washed thoroughly and dried at room temperature and was produced into fine powdered from using the blender. This blended powder was kept in an airtight bottle for analysis. 10 g of the dry powdered sample was treated with the solvent water, methanol, chloroform, petroleum ether, and hexane. The aqueous sample solution was extracted with treated soxhlet followed for 30 minutes before conducting vacuum filtration. After extraction, the extract solution was concentrated using the rotary evaporator at 60°C in a water bath.

M. oleifera leaves Analysis for Ascorbic acid.

1 mL of the sample extract was made up to 2.0 mL with 4% (Trichloroacetic acid) TCA. 0.5ml of (2,4-Dinitrophenylhydrazine) DNPH reagent was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5 mL of 85% (Sulphuric acid) H_2SO_4, in cold. Working standards of ascorbate solution were prepared from 0.2-0.1 M at 0.2 M intervals ranging within 0.2 0.4, 0.6, 0.8, and 1.0 M and obtained absorbance readings under Ultraviolet Spectroscopy. Immediately after incubation for half an hour at room temperature,

the absorbance was read spectrophotometrically at >340nm.

Ultraviolet-Visible Spectrophotometry (UV-Vis)

UV-VIS, (PerkinElmer Lambda 365) was used to obtain the absorbance reading at a four-fold dilution. The Tungsten-halogen was used to scan the entire UV-VIS band in the wavelength range of 190nm to 1100 nm. Each of the prepared light samples was placed in to cuvette of 1 cm in length. The peaks of ascorbic acid absorbance were identified at the range of >340nm wavelength after scanning the samples.

Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR (Spectrum 100 Instrument Serial Number 76563 was used to determine the absorbance spectra for ascorbic acid. The scanning was done in the range of 500 cm-1 to 4000 cm-1. The molecular analysis of the individual analysis was studied using the transmittance value. Drop of the liquid filtrate was placed onto the face of a highly polished salt plate then place a second plate on top of the first plate to spread the liquid in a thin layer between the plates, and clamp the plates together. Finally, wipe the liquid out of the edge of the plate. The sandwich plate was placed into an FTIR spectrometer and the force garage of 70 was applied. The peaks of interest were identified as 1674 cm -1 and 1322 cm -1, respectively.

3. Results and Discussion

Phytochemicals are naturally present in plants and show biological significance by playing an essential role in the plants. Phytochemicals are present in virtually all plant tissues of *M. oleifera* e.g. leaves, roots, stem, and fruits [12]. The medicinal value of *M. oleifera* plants lies in some chemical substances that produce a definite physiologic action on the human body due to the presence of bioactive compounds like alkaloids, flavonoids, tannins, and phenolic compounds.

Table No. 1 Qualitative Phytochemical Screening Test for the Presence of Various Biochemical.

Phytochemical Test	Alkaloid	Tannins	Saponins	Anthraquinones	Anthocyanosides	Flavonoids	Reducing sugars
Observations	Reddish brown colour	Dark green color	Foam on surface	Pinkish solution.	Pale pink color	Golden yellow color	Brown colour Fehling B Green colour Fehling A

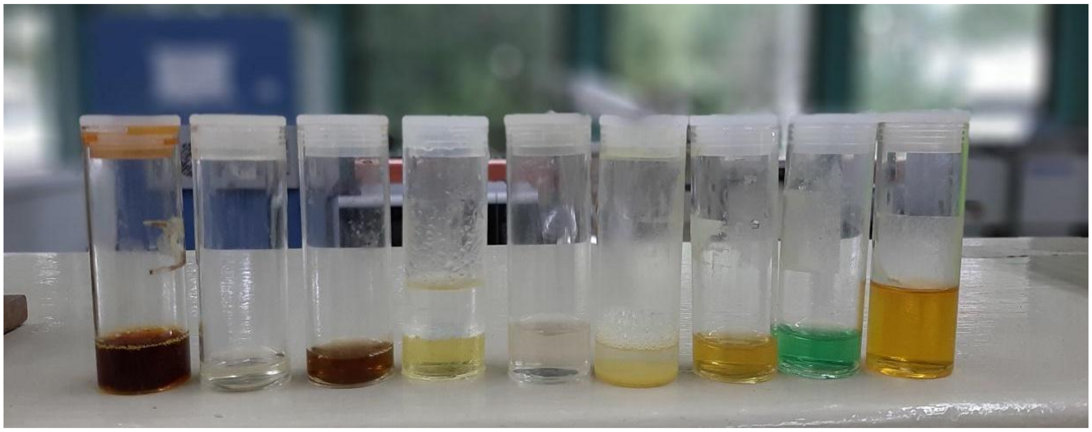

Figure 1 Qualitative Phytochemical Screening test for Leaf Extract of *M. oleifera*.

ISSN: 0972-0626
Volume 28, Issue 12, December, 2024

M. oleifera plant leaves were analyzed to show the presence of phytochemical constituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, flavonoids, reducing sugars in different solvent extracts as shown in Table 1.0 and Figure 1.

Table No. 2 Qualitative Phytochemical Screening of Ethanol and Aqueous Leaf Extract of *M. oleifera*

SOLVENT EXTRACTION	Alkaloid	Tannins	Saponins	Anthraquinones	Anthocyanosides	Flavonoids	Reducing sugars
Water	+	+	+	+	+	+	_
Ethanol	+	-	+	+	+	+	_

Phytochemical analysis showed the presence of the chemicals in plant leaf for water and ethanol solvent extraction as shown in Table 2. The results indicate the presence of compounds in the aqueous polar extracts. Alkaloids turned out to be present in both extracts. Plants having alkaloids are used in medicines for reducing headaches and fever, as they are attributed to their antibacterial and analgesic properties [13]. Tannins were detected in water leaf extract but not in ethanol leaf extract. Tannins are astringent medicines for the treatment of intestinal disorders, such as dysentery and diarrhea. They also react with protein to form stable crosslink polymers, which transform animal skin into leather. They are useful in food processing, fruit ripening, manufacture of cocoa and wines [14]. Saponins are present in both the solvent extracts of the leaf. Saponins are used as medicines for the treatment of increased blood cholesterol and are beneficial to patients with arteriosclerosis and hypertension and in the control of post-menopausal syndrome [4]. Anthraquinones were simply present in both solvent extracts. Found in applications as semiconductors in microelectronics, possess antibacterial, antiparasitic, insecticidal, fungicidal, and antiviral properties and are also used as anticancer agents [15]. Anthocyanosides and flavonoids were both found in leaves. Anthocyanosides are part of a class of nutrients called flavonoids that are found abundantly in several purple, blue, and red berries and also in purple-colored vegetables [16]. Flavonoids enhance the effects of Vitamin C and function as antioxidants, they are also known to be biologically active against liver toxins, tumors, viruses, and other microbes [17]. Solvent extraction has the absence of reducing sugars from the photochemical list.

Non-Enzymic Antioxidants Activity: Ascorbic Acid Analysis

Antioxidants can be categorized in multiple ways. Based on their activity, they can be categorized as enzymatic and non-enzymatic antioxidants. Non-enzymatic antioxidants work by interrupting free radical chain reactions. A few examples of non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione [6]. Ascorbic Acid is a natural water-soluble vitamin (Vitamin C). During this research study, the non-enzymic antioxidant activity of ascorbic acid in M. oleifera leaves was analyzed using Ultraviolet-Visible Spectrophotometry and Fourier Transformation Infrared Spectroscopy. Dried powdered plant material was extracted with the solvents water, methanol, chloroform, petroleum ether, and hexane.

UV-VIS Analysis of Ascorbic Acid in M. oleifera

Working standards of ascorbate solutions were prepared having various concentrations of 0.00 – 1.0 M at 0.2 M intervals. The ascorbate solutions data under UV analysis (Figure 2) was done in 4-fold dilution due to having self-absorption. Readings were inconsistent at their original concentrations after running the same sample being run a few times. The peaks appeared at 340 nm in which the peak absorbance readings were placed as y from the linear equation and obtained x (concentration of ascorbate solution).

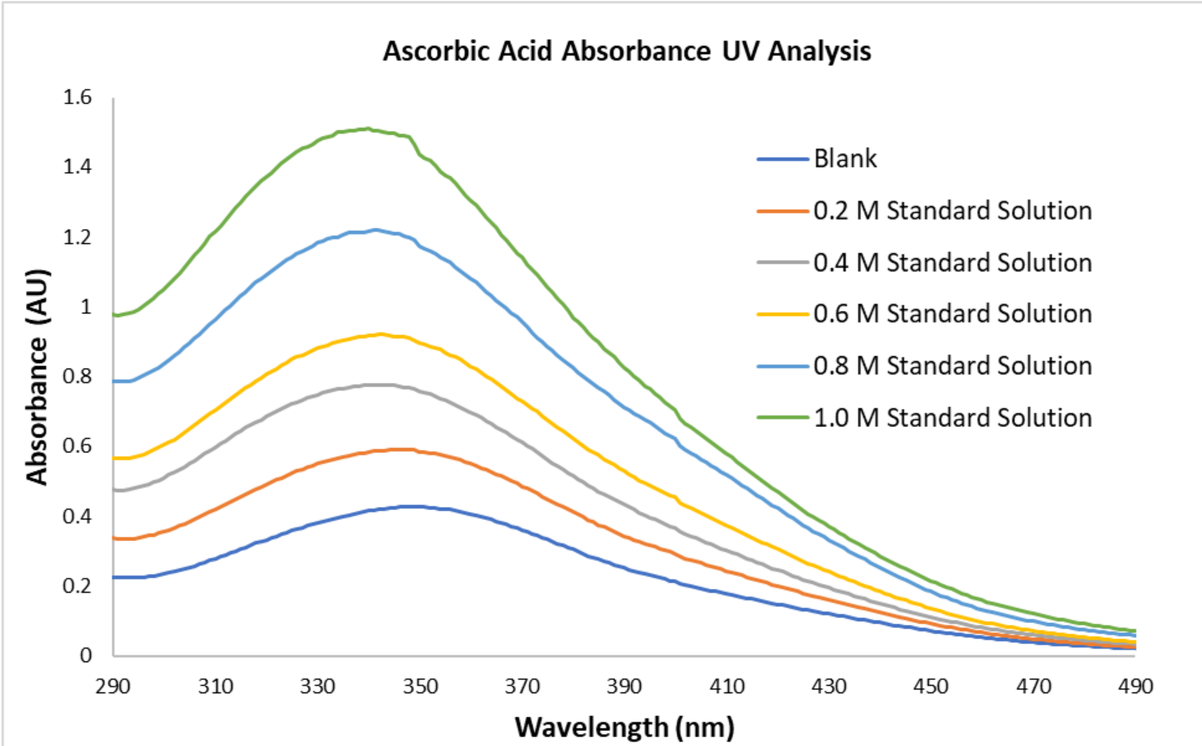

Figure 2 Ultraviolet-visible spectrophotometry (UV-VIS) data on the ascorbate solution after the addition of 4% (Trichloroacetic acid) TCA, (2,4-Dinitrophenylhydrazine) DNPH reagent, 10% thiourea solution, 85% (sulphuric acid) H_2SO_4. Various concentrations of 0.00 – 1.0 M at 0.2 M intervals of ascorbate solutions were prepared.

A standard calibration curve was constructed with the analyzed working standards (Figure 4) in which the equation of the line was to determine the concentration of ascorbate solution from its absorbance. The absorbance data was obtained from the UV spectrometer at the wavelength >340nm. Concentration and absorbance results stated a directly proportional relationship after analysis; increase in concentration the absorbance turned to increase as well. Further, an analysis of ascorbic acid was done on the *M. oleifera* leaf extracts. The dry powdered plant material was extracted with the solvents water, methanol, chloroform, petroleum ether, and hexane as shown in Figure 3.

UV analysis indicated the highest absorbance reading was for water than methanol and chloroform. Petroleum ether and hexane turned out to have lower absorbance readings. Results show that the solvent polarity affects the absorbance of ascorbic acid present in the leaf sample. Polar solvents have an electronegative atom with a lone pair of electrons or nonbonding electrons; this nonbonding electron can form solute-solvent complexes through H-bonding, hence, if the lone pair of electrons is in conjugation with the chromophoric group, the wavelength of maximum absorbance (**λ**max) increases to a higher value [18]. Research work states reveals that non-specific interaction measured by solvent polarity has more influence on absorption and solvent bipolarity contribution is significant in the case of fluorescence [19].

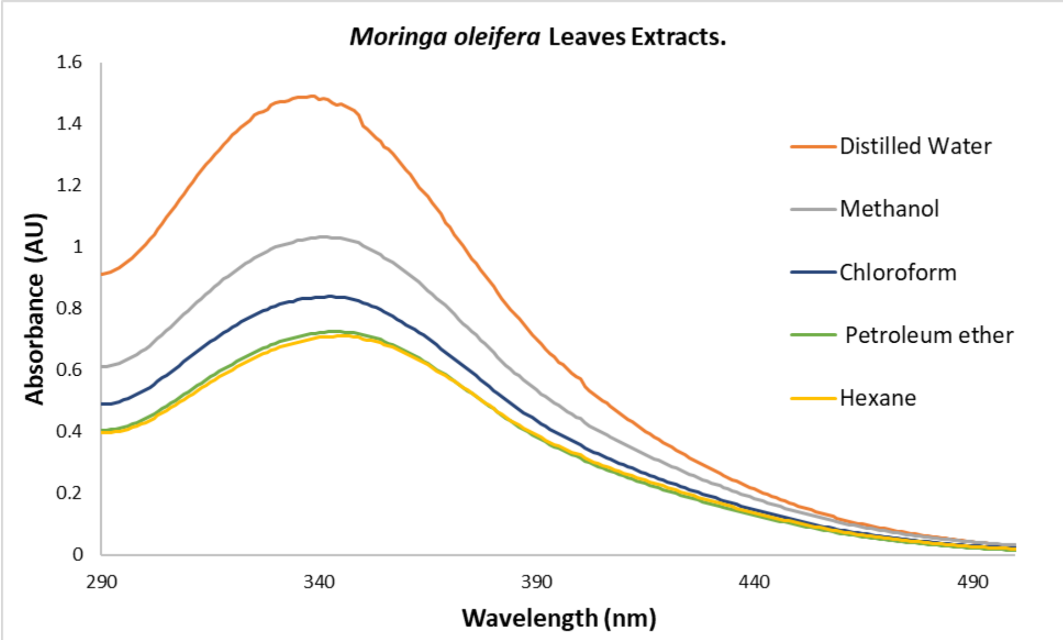

Figure 3 Ultraviolet-visible spectrophotometry (UV-VIS) data on the ascorbate solution after the addition of 4% (Trichloroacetic acid) TCA, (2,4-Dinitrophenylhydrazine) DNPH reagent, 10% thiourea solution, 85% (sulphuric acid) H2SO4. Different solvents water, methanol, chloroform, petroleum ether, and hexane having different polarity were used for extraction.

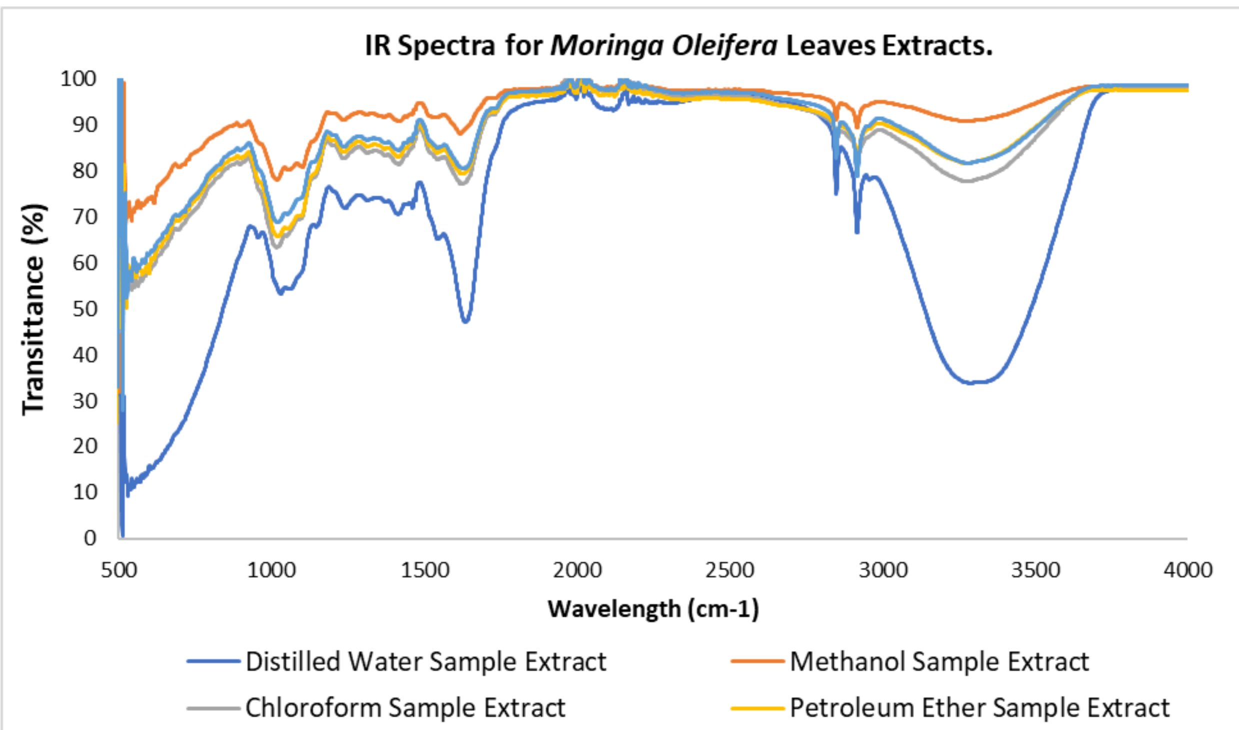

Figure 4 Fourier Transformation Infrared Spectroscopy (FTIR) data *M. oleifera* leave extracts for Ascorbic Acid (AA).

IR Analysis of Ascorbic Acid in M. oleifera

The IR spectra of Ascorbic Acid in *M. oleifera* leaf extracts are represented in Figure 4. The spectrum Ascorbic Acid revealed that the stretching vibration of the C-C double bond and the peak of enol- hydroxyl were observed at 1674 cm^{-1} and 1322 cm^{-1} [20]. This shows the presence of Ascorbic Acid in the leaf extracts. The water extract had a strong peck as combined with the other extracts. There was the presence of an OH peak at 3315 cm^{-1} [20].

Ascorbic Acid Total Mass Analysis in M. oleifera

The ascorbic acid mass was calculated using the absorbance values from the UV analysis at the wavelength of >340nm. Equation (1) explains how it was done. Volume is 0.001L of *M. oleifera* leaf sample used and Mr is of ascorbic acid which is 176.124 $g \cdot mol^{-1}$.

Mass of Ascorbic Acid (g.g)= [1.0 M-final concentration(M) × volume (L) × Mr] / [mass of leaf sample used (g)] ……………………………………………………………………...……………………… 1

Since the solutions were 4-fold dilution, the absorbance values were multiplied by 4 to get back the final concentration using Beer's law of A = εmCl; whereby A=absorbance, εm = molar extinction coefficient, C = concentration, l=path length of 1 cm.

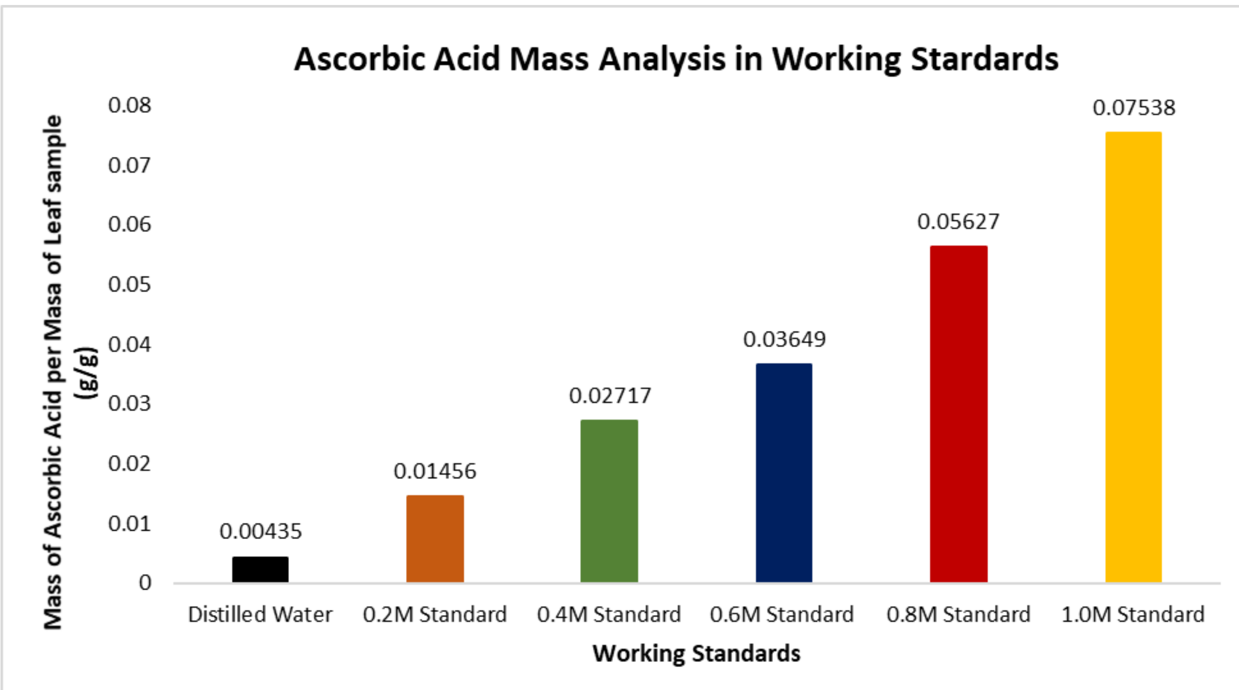

Figure 5 Mass of Ascorbic Acid in Different Concentrations of Working Standards. Various concentrations of 0.0 – 1.0 M at 0.2 M intervals of ascorbate solutions were prepared.

Results in Figure 5 shown, indicated an increase in the concentration of the working standards from 0.0-1.0M, and the mass of ascorbic acid increased. The highest mass was 0.07538g/10g at the 0.1M working standard and the lowest mass was 0.01456g/10g at 0.2M. During the preparation of the working standards, there was no ascorbate solution added to distilled water which was the blank.

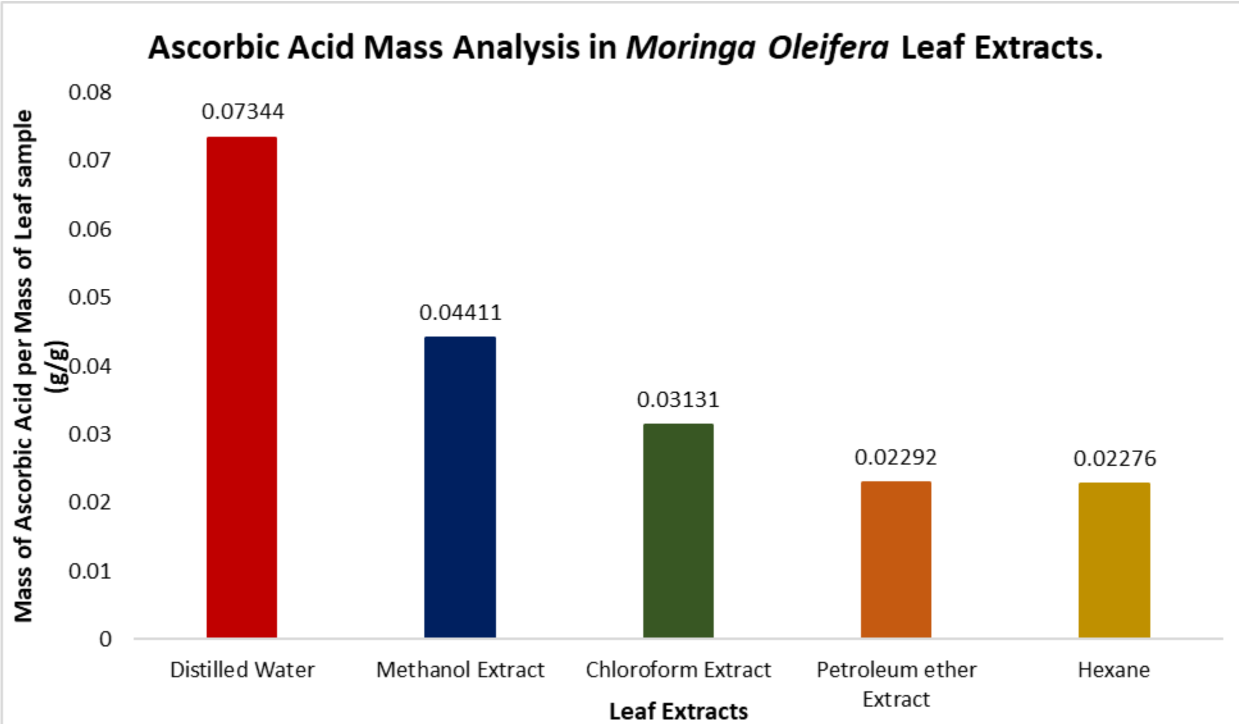

Figure 6 Ascorbic Acid (AA) Mass Analysis Presents in *M. oleifera* Leaf Extract. Different solvents water, methanol, chloroform, petroleum ether, and hexane having different polarity were used for extraction.

The dry powdered plant material was extracted with the solvents water, methanol, chloroform, petroleum ether, and hexane, and the mass of ascorbic acid was calculated from the UV analysis as shown in Figure 8. Results show that water having the highest polarity had the highest mass of ascorbic acid being extracted at 0.07344g/10g which was closer to the working standard of 0.1M from figure 6. In addition, 0.04411g/10g and 0.03131g/10g for methanol and chloroform extract. Whereas, petroleum ether and hexane had the lowest polarity hence masses of 0.02292g/10g and 0.02276g/10g were analyzed, which were closer values. According to US standards minimum of 60 mg of ascorbic acid [21], approximately 0.06g for daily requirement by a human body. Research studies have shown that dried leaves of Moringa were found to contain 0.07- 0.14mg/g of ascorbic acid, which was much lower in comparison to fresh leaves; due to reduction content after drying may be because of oxidative decomposition of vitamin C catalyzed by heat [12]. It indicates that the drying process is adversely affecting the amount of ascorbic acid.

Moringa leaves contain more vitamin A than carrots, more calcium than milk, more potassium than bananas, more iron than spinach, and contain 7 times more vitamin C in comparison to oranges [11]. In addition, other methods of analysis in previous literature available for the determination of Vitamin C are titration, spectrophotometer, and (HPLC) [22]. Considering these properties, the cultivation and extraction of Moringa for ascorbic acid should be encouraged especially in developing countries where people are suffering from malnutrition.

Discussion and Comparability of Variables

M. oleifera is known for its rich phytochemical content. Studies consistently identify compounds such as alkaloids, flavonoids, tannins, saponins, anthraquinones, and phenolic acids in Moringa leaves [23]. The analysis of non-enzymic antioxidant activity, particularly the ascorbic acid in Moringa leaves, is an important area of research that demonstrates its potential to combat oxidative stress and support various physiological functions.

Water has the highest polarity index of 10.2 and the lowest polarity index of 0.1 was for petroleum ether and hexane [19], [20]. These solvents were of different polarity, meaning charge separation due to the presence of atoms having different electronegativity. Polar solvents stabilize the ground state more than the excited state, the change in energy increases with the polarity of the solvent.

Polar solvents have an electronegative atom with a lone pair of electrons or nonbonding electrons; this nonbonding electron can form solute-solvent complexes through H-bonding, hence, if the lone pair of electrons is in conjugation with the chromophoric group, the wavelength of maximum absorbance (λmax) increases to a higher value [18]. Research work states reveal that non-specific interaction measured by solvent polarity has more influence on absorption and solvent bipolarity contribution is significant in the case of fluorescence [19]. Variability can be compared with the environmental influence on the extraction of the specimens of Moringa antioxidants. The leaves used were from the fresh vegetational area which had the ideal environmental factors in moderate amounts, with least exposure to highly charged toxicity materials. Given the scope of this research, it can be inferred that the distilled water-based extraction has proved to be a weak electrolyte which enabled the extraction from the specimen to be obtained. Due to the polarity in the bases of distilled water, methanol, chloroform, petroleum ether, and hexane, the reaction of the extracts was significantly obtained due to the charges interacting within the specimen and the base medium. Water extract was the highest due to being the pure element with no other component within the mix.

This research findings indicate that the water extract with the highest polarity yielded the highest mass of ascorbic acid (0.07344g/10g), aligning with previous studies on the extraction efficiency of antioxidants from Moringa. Ascorbic acid's IR spectral analysis, showing characteristic peaks at 1674 cm^{-1} (C-C double bond stretching) and 1322 cm^{-1} (enol-hydroxyl group), further supports the chemical identity and presence of this important antioxidant. These findings are consistent with research showing that water is a superior solvent for extracting hydrophilic compounds like ascorbic acid, whereas organic solvents like methanol, chloroform, petroleum ether, and hexane, with lower polarities, result in reduced yields of ascorbic acid.

For example, a study demonstrated that Moringa leaves contain significant amounts of vitamin C, along with a variety of other antioxidants and bioactive compounds, which are essential for their anti-inflammatory and immunomodulatory properties [24]. Other studies have also corroborated the finding that the polarity of the solvent plays a critical role in the extraction efficiency of different bioactive compounds. Methanol and chloroform were found to be effective for extracting certain flavonoids and phenolic compounds, while water extracts are better for water-soluble antioxidants like ascorbic acid [25]. Additionally, the presence of low-polarity solvents such as petroleum ether and hexane yielded minimal antioxidant extraction, further emphasizing the relationship between solvent polarity and compound solubility.

The non-enzymatic antioxidant activity of ascorbic acid in Moringa leaves is well-documented. Ascorbic acid is a potent antioxidant that plays a significant role in neutralizing free radicals. The IR spectroscopy analysis you provided, showing characteristic peaks for the C-C double bond and enol-hydroxyl groups, is a typical method for confirming the presence of ascorbic acid, as seen in previous studies [26]. The observation that higher polarity solvents (such as water) extract more ascorbic acid is consistent with the literature, where polar solvents are typically more efficient at extracting water-soluble antioxidants like Vitamin C [27].

The trend of higher ascorbic acid content in the water extract compared to methanol, chloroform, petroleum ether, and hexane is in line with what other studies have reported. Research found that aqueous extracts of

Moringa leaves consistently showed the highest concentrations of Vitamin C compared to other organic solvent extracts [28]. The water extract in this study, yielding 0.07344g/10g of ascorbic acid, is comparable to findings in other studies, where water extraction yields around 0.07–0.1g of Vitamin C per 10g of Moringa leaves [29].

4. Conclusion

M. oleifera leaves are indeed a very useful breakthrough in the demand for alternative natural medicine for the treatment of various disease activities by pathogenic organisms. This research work conclusively demonstrates that *M. oleifera* is a good source of various phytochemicals like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, and flavonoids. This plant has the added advantage of other nutrients also. Non-enzymic antioxidant activity of ascorbic acid in *M. oleifera* leaves was analyzed and the absorbance of the ascorbic acid increased with the increase in the polarity index of solvents. Ascorbic acid revealed that the IR stretching vibration of the C-C double bond and the peak of enol- hydroxyl were observed at 1674 cm^{-1} and 1322 cm^{-1}. Water extract having the highest polarity had the highest mass of ascorbic acid extraction of 0.07344g/10g which was closer to the working standard of 0.1M. In addition, 0.04411g/10g and 0.03131g/10g for methanol and chloroform extract. Whereas, petroleum ether and hexane had the lowest polarity hence mass of 0.02292g/10g and 0.02276g/10g were analyzed, which were closer values. *M. oleifera* leaves can be called a Miracle tree as far as its nutritional importance is concerned.

5. Conflict of interest

No

6. References

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