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Evaluation Of Total Phenol, Flavonoids, Antioxidant Activity And Gc-Ms Of Ethanol Extract Of Moringa Oleifera Whole Leaves

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ABSTRACT

The medicinal plant known as moringa (Moringa oleifera Lam) is also a nutrient source. It contains antioxidant properties and is heavy in protein, fatty acids, minerals, and comparatively high levels of polyphenol chemicals. Finding out about the ethanol extract of Moringa leaves' secondary metabolites, total phenol content, total flavonoids, antioxidant activity, and GC-MS is the goal of this study. The colorimetric method was used to quantify total flavonoids, whereas the Folin Ciocalteu method was used to determine total phenol. The DPPH (2,2-diphenyl-i-picrylhydrazyl) technique was used to measure the antioxidant activity at a wavelength of 520 nm. Flavonoids, phenols, tannins, saponins, alkaloids, and steroids were found in the ethanol extract of Moringa leaves, according to the results. 9.1983 ± 0.00132 GAE/g extract was the total amount of phenol in the ethanol leaf extract. The flavonoid concentration in the extract was 0.3474 ± 0.00195 QE mg/mL. Moringa leaf ethanol extract is categorized as having moderate antioxidant activity due to its IC50 of 27.79 µg/mL. Eleven chemicals were found in the extract according to GC-MS analysis, with three compounds found in each peak. 30.15% methyl (11E)-11-octadecenoate, 19.16% cis-octadecenoic acid or cis-oleic acid, and 17.67% methyl-14-methyl pentadecanoate. Because the majority of the chemicals on the list are bioactive and have therapeutic qualities, the use of the traditional Moringa oleifera plant in the development of new treatments is further supported.

Keywords: *Antioxidant Activity, Flavonoids, GC-MS analysis, Moringa oleifera, Total phenol content*

1. INTRODUCTION

Antioxidant, anti-inflammatory, and anticancer phytochemicals are abundant in Moringa oleifera [1,2]. According to [3]and [4], M. oleifera's high content of nutritionally vital elements, vitamins, and minerals makes it a promising crop for food security and nutrition. According to [2], the leaves in particular showed higher therapeutic efficiency than other plant components, and their potencies were enhanced by extracting them using ethanol and ethyl acetate. Comparative analyses, however, have shown that where ethanol extracts contain more alkaloids and triterpenoids, water extracts of Moringa leaves contain more flavonoids and phenolics [5]. According to research conducted on animals, these extracts have positive effects on lipid metabolism, hyperglycemia, and liver histopathology [6]. More thorough clinical trials are necessary to verify its efficacy in evidence-based medicine, even if it has been widely supported by traditional use. Additionally, large dosages should be avoided due to potential toxicity [7]. Flavonoids are polyphenolic chemicals that have a variety of medicinal uses and natural bioactivities. Their anti-inflammatory, anti-cancer, and antioxidant qualities make them promising for treating a variety of ailments, including skin cancer [8]. Via the release of cytokines, growth factors, and signaling pathways, flavonoids influence inflammation, angiogenesis, and re-epithelization to aid in wound healing [9]. The purpose and extent Because of its preventative qualities and health advantages, phenolic compounds are gaining more and more attention. They exhibit anti-osteosarcoma properties [10] as well as immunomodulatory, antiviral, anticancer, and antibacterial properties [11]. However, solid lipid nanoparticles can carry phenolic chemicals increasing their pharmacokinetics features, making them potential oral administration vehicles [12]. These compounds, which are biopharmaceutical prospects for microalgae-derived phenolics, have been discovered more quickly thanks to recently developed extraction and characterisation methodologies [11] The bioactivities of phenolic glycosides vary depending on the aglycone and glycone moieties they contain. Although bioavailability may be enhanced by glycosylation, aglycone form biological activity is frequently higher [13]. There is great diagnostic potential for stomach cancer biomarkers using gas chromatography followed by mass spectrometry (GC-MS), a sensitive and reliable technique for identifying chemicals in biological materials. Additionally, according to some earlier research, GC-MS can identify between patients with malignant pleural mesothelioma and at-risk asbestos-exposed individuals with 97% accuracy [14]. Up to 139% of malathion was detected in aqueous samples and over 134% in solid.

samples using GC-MS in conjunction with solid-phase extraction procedures in pesticide analysis applications [15]

The phytochemical makeup and antioxidant capacity of Moringa oleifera leaves are being investigated in this study, which contributes to the increasing body of information demonstrating the plant's ability to enhance health and wellness and its application in traditional medicine.

2. SIGNIFICANCE OF THIS STUDY

The current investigation aims to provide empirical proof of the phytochemical abundance and antioxidant qualities of Moringa oleifera leaves. The objective of this investigation is to ascertain the antioxidant activity of Moringa extract in addition to its total phenolic and flavonoid content. It seeks to investigate if Moringa can be utilized as a dietary strategy in disorders linked to oxidative stress. Only then, especially in regions where it grows easily, may its promise as a functional food rich in bioactive chemicals that support nutrition and health be fulfilled.

3. METHOD AND MATERIALS

Materials

Moringa leaf samples, distilled water, ammonia, chloroform, hydrochloric acid, dragendorff and Mayer reagents, sulfuric acid, acetic acid anhydride, 96% ethanol, methanol, magnesium powder, amyl alcohol, quercetin, 1,1-diphenyl-2 picrylhydrazyl (DPPH), gallic acid, $FeCl_3$, ether, $AlCl_3$, Folin-Ciocâlteu reagent, and Na_2CO_3 were among the materials used in this investigation. All materials, with the exception of the maceration solvents, were analytical grades that were purchased from Merck.

While the typical glassware, macerator, electronic balance, aluminum foil, Buchi R-114 rotary evaporator, porcelain exchange, drip plate, Bunsen burner, stirrer, spatula, filter paper, hot plate, ultrasonic, spray dryer, and UV-vis spectrophotometer were among the equipment utilized.

Methods

Sample Preparation

Following washing, moringa leaves were allowed to air dry at room temperature. Once the sample had dried, it was blended and mashed to create a powder (simplicia).

Phytochemical Screening

Phytochemical screening was used to identify the secondary metabolite content of Moringa leaf powder. Screening assays for phytochemicals included alkaloids, tannins and phenolics, flavonoids, saponins, and steroids,terpenoids. Moringa leaf ethanol extract was also subjected to phytochemical screening [16].

Ethanol Extract Preparation

Three hundred grams of powdered moringa leaves were macerated using a 96% ethanol solvent. Every 24 hours, a fresh solvent was added until the solvent was comparatively clear. It went through filtering after maceration. A thick ethanol extract was then produced by evaporating the filtrate using a rotary evaporator.

Isolation & Extraction: Spry Dryer and Ultrasonication

The ultrasonic bath (Hielscher Ultrasonics) used to extract Moringa oleifera leaves was set up with a fixed frequency of 35 kHz and a power intensity of 160 W. A 1:40 (g/mL) ratio was used to combine the sample (5 g) with 200 mL of 70% ethanol in the flask. For every temperature (30, 40, and 50 °C), the ultrasound-assisted extraction was carried out for 10, 20, and 30 minutes.

The spray drying was done according to the method described

which uses two fluid nozzles with a standard 0.5 mm jet, the spray drying was completed. The spray dryer's parameters were set to 4.3 m/s for air speed exhaust, 485 mL/h for liquid flow rate, medium for deblocked, and two bars for compressor. 120°C, 140°C, and 160°C were the inlet temperatures used for the microencapsulation process. Throughout the process of spray drying, the liquid was constantly mixed. Ultimately, the desiccated sample was packaged and kept for additional examination in a desiccator.

Determination of Total Phenolic Content (TPC)

Getting the Standard Gallic Acid Ready for the Calibration Curve

To ascertain the total phenolic content (TPC) of moringa leaf extracts, the Folin-Ciocalteu colorimetric technique was employed. To create the Folin-Ciocalteu Reagent, dilute 2.5 mL of the substance in a beaker with distilled water ten times, resulting in a cylinder with a maximum capacity of 20 mL. After that, fill a 100mL conical flask with 7.5g of sodium carbonate dissolved in 10mL of distilled water to create a 7.5% sodium carbonate solution. Then, think of a solution of 4 mL

sodium carbonate, 1 methanol, and 5 mL FCR. To make a standard gallic acid solution, mix 10 mgs of gallic acid with 10 mL's of methanol (1 mg / mL). In methanol, gallic acid solutions were made at different concentrations from the reference solution (20, 40, 60, and 100 µg/mL). Add 5 mL of Folin-Ciocalteu reagent (FCR) and 5 mL of 7.5% Na2CO3 for each concentration, resulting in a final volume of 10 mL. Following a 30-minute shaking, the blue liquid was incubated at 40°C in a water bath. A blank was used as a reference to measure the absorbance at 765 nm. Plant extracts containing phenols are oxidized by the FCR reagent, giving them a dark blue hue that may be detected with a UV-visible spectrometer. The calibration curve was made by averaging the absorbance data at different dosages of gallic acid, and all tests were conducted in triplicate.

Preparation of Sample for Total Phenolic Content

There has been use of the moringa extract. After following the conventional gallic acid technique, the absorbance for the number of compounds was calculated. To find the amount of phenol in the extracts, the calibration curve was plotted using the sample's mean absorbance value, which was created in triplicate for the experiment. Use 10 mLs of methanol to dilute 0.5 mLs of moringa extract. Then, add 2.5 cc of distilled water to a test tube. To make 10 mLs, add 2.5 mLs of Folin-Ciocalteu reagent (FCR) and 2 mLs of 7.5% Na2CO3. Following a 30-minute shaking, the blue liquid was incubated at 40 degrees Celsius in a water bath. " A blank control was used to compare the absorbance at 765 nm. Total phenolic content in the extracts was expressed as mgs of gallic acid equivalents (GAE) / gram of dry weight. All samples' total phenolic content was calculated using the following formula:

$$C = c\frac{v}{m}$$

T = total phenolic content mg GAE/g dry extract, V = extract volume in mL, m = extract mass in gram, and c = gallic acid concentration from calibration curve in mg/mL [17].

Total Flavonoid Content Calculation

Preparation of Standard Quercetin for Calibration Curve

Standard Quercetin for Calibration Curve Preparation

The colorimetric technique using aluminum chloride was used to determine the total flavonoid concentration of the extracts. In 1 mL of methanol, dissolve 4 mgs of quercetin to create the stock solution (4 mg/mL). The concentrations of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL were achieved by diluting the standard solution. First, get your reagents together. In order to make 5% NaNO2, dissolve 0.5g of NaNO2 in 2mL of distilled water to make 10mL. 10% AlCl3 can be made by dissolving 1g of AlCl3 in 2mL of distilled water to create a volume of 10mL. 2.5g of NaOH should be dissolved in 63mL of distilled water to create 1M NaOH. Next, make a 10 mL volume with a blank by adding 0.5 mL of NaNO2, 0.5 mL of AlCl3, and 2 mL of 1M NaOH. One mL of each quercetin concentration and four mLs of distilled water were added to the test tube. After five minutes, fill the test tube with 0.3 mL of 5% NaNO2 and then 0.3 mL of 10% AlCl3. 2 mL of 1 mM NaOH should be added to the mixture after 6 minutes. Right away, 4.4 mL of distillation water was added to the mixture to form 10 mL. To test the absorption, a spectrophotometer set to 510 nm was used. The total flavonoid content was calculated by averaging the absorbance values from three separate readings. The linear equation of the standard calibration curve was used to calculate the flavonoid content as quercetin equivalent (mg QE/g) [17].

Preparation of Samples for Total Flavonoid Content

To create the solution, 4 mg/mL extract stock solutions in methanol were made and diluted one at a time. Pour 2 mL of the extract and 4 mL of water into test tubes. Add half a mL of 5% sodium nitrite and half a mL of 10% aluminum chloride after five minutes. Then, six minutes later, add two mLs of the 1M sodium hydroxide solution. Ten mLs of distilled water should then be added and thoroughly mixed. Ten minutes should be spent incubating at room temperature. At 510 nm, the absorbance was measured in relation to a blank sample that contained 10 mL of water, 0.5 mL of 10% aluminum chloride, 2 mL of 1M sodium hydroxide solution, and 0.5 mL of 5% sodium nitrite. The amount of TFC in 100 g of material was measured in mgs of quercetin equivalent (QE). To determine the overall flavonoid content of each sample, a specific formula was employed:

$$C = c\frac{v}{m}$$

where c is the quercetin concentration from the calibration curve in mg/mL, V is the extract volume in mL, m is the extract mass in grams, and C = total flavonoid content in mg QE/g dry extract [18].

Antioxidant activity determination (DPPH assay)

To examine how DPPH radicals are impacted by varying chemical concentrations, the methodology was adjusted. A DPPH solution containing 3.943 mg was prepared in 100 mL of methanol. Next, we prepared a stock by mixing 10 mL of methanol with 10 mg of ascorbic acid. We then took 1 mL of the mixture and mixed it with 10 mL of methanol at various concentrations (0.5, 1, 2, 3, 4, 5 mL). Finally, we added 3 mL of DPPH solution to a 96-well microtiter plate and made a volume of 10 mL

with methanol. The drop in absorbance at 517 nm was measured after the combination had been left at room temperature in the dark for half an hour. As a blank and positive control, ascorbic acid was used as methanol in both the standard and the sample, while DPPH was used as a negative control in both. The following formula was used to calculate the inhibition of the DPPH radical:

% Radical Inhibition = 100 x Ao-A1/Ao

where A0 and A1 stand for the control and sample absorbances, respectively. The IC50 value, which is reported in µl of substance / mL, was determined via linear regression from RSA values. The concentration needed to 50% block the DPPH radical is indicated by this number. A DPPH 3.943 mg solution was prepared in 100 mLs of methanol. Ten mLs of moringa extract and ten mLs of methanol are combined, and one mL is mixed with ten mLs of methanol. Then, at various concentrations (0.5, 1, 2, 3, 4, and five mLs), three mLs of DPPH solution are added to a 96-well microtiter plate, and ten mLs of methanol are made. After allowing the mixture to sit at room temperature in the dark for half an hour, the absorbance at 517 nm at various concentrations (0.5, 1, 2, 3, 4, 5 mL) was measured. Using the formula above, the inhibition of the DPPH radical was ascertained, and the IC50 value was computed. [19].

GCMS analysis of Moringa oleifera

Using GC-MS QP2010 Plus (Shimadzu, Japan), the phytochemicals in Moringa oleifera were characterized. Using a Shimadzu QP2010 gas chromatography system with a Thermal Desorption System, TD 20, and mass spectroscopy, the phytochemicals in the sample were identified. The voltage of ionization was 70 eV. Gas chromatography using a Restek column (0.25 mm, 60 m, XTI-5) was carried out in the temperature programming mode. After a one-minute 80°C initial column temperature, the temperature grew linearly at 70°C min-1 to 220°C, held for three minutes, and then raised linearly at 10°C min-1 to 290°C for ten minutes. The injection port was kept at 290°C, and the GC-MS interface was kept at 290°C. Using an all-glass injector operating in split mode and a helium carrier gas low rate of 1.2 mL min-1, the sample was inserted. Compound identification was achieved by comparing the fragmentation pattern and retention period with the GC-MS mass spectra.

Moringa oleifera Phytocompoments Identification The identification of the extract's active ingredients was done by comparing their mass spectra fragmentation pattern, retention indices, and peak area % with those found in published literature and the National Institute of Standards and Technology's (NIST) digital library. WILEY8.LIB, NIST08.LIB, and other library resources were used to match the components found in the plant material. We determined the compounds' names, molecular weights, formulas, structures, and bioactivities.

4. RESULT

Phytochemical Analysis

Table 1 and Fig. 1 indicate that Moringa leave Extract contains varying amounts of active compounds along with unidentified components.

Sample Name	Phytoconstituents test	Observations	Result
Moringa leave Extract	Bromine water Glycoside	Yellow Precipitated observed	+
	Keller-Hillani Glycoside	Blue colour observed	+
	Terpenoid	Formation of reddish-brown	+
	Phenolic (Iodine test)	Red Precipitated observed	++
	Saponins	Foam observed	+
	Flavonoids	Presence of yellow colour	++
	Alkaloids	Deep reddish-brown colour appear	+
	Tannins	Green to Blank Precipitated appear	+

(+) = positive reaction; (-) = negative reaction; (++) = highly positive reaction

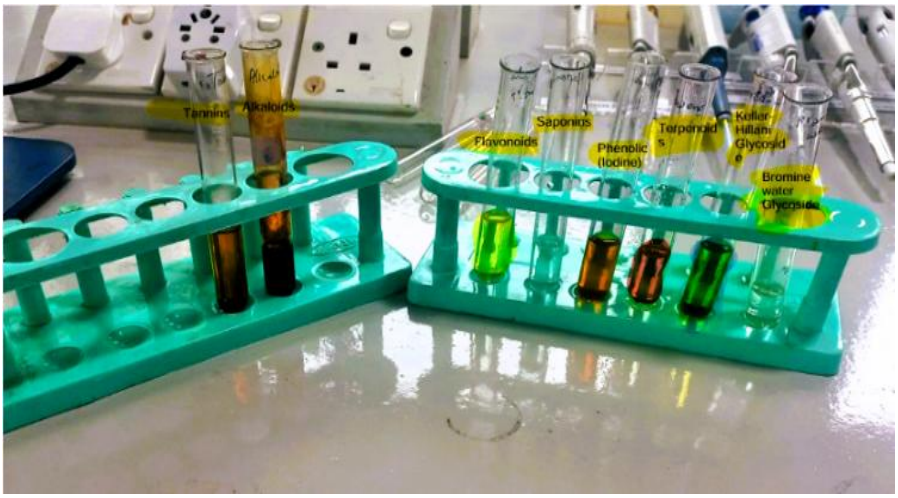
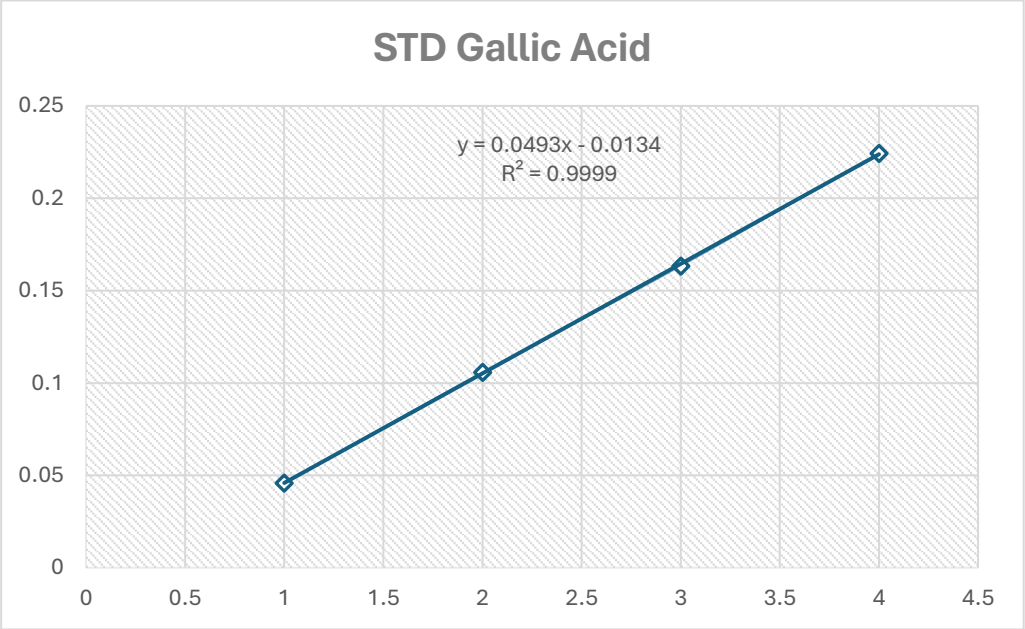


Figure 1: Results of Phytochemical Screening Moringa oleifera

4.2. Estimation of Total Phenolic Content

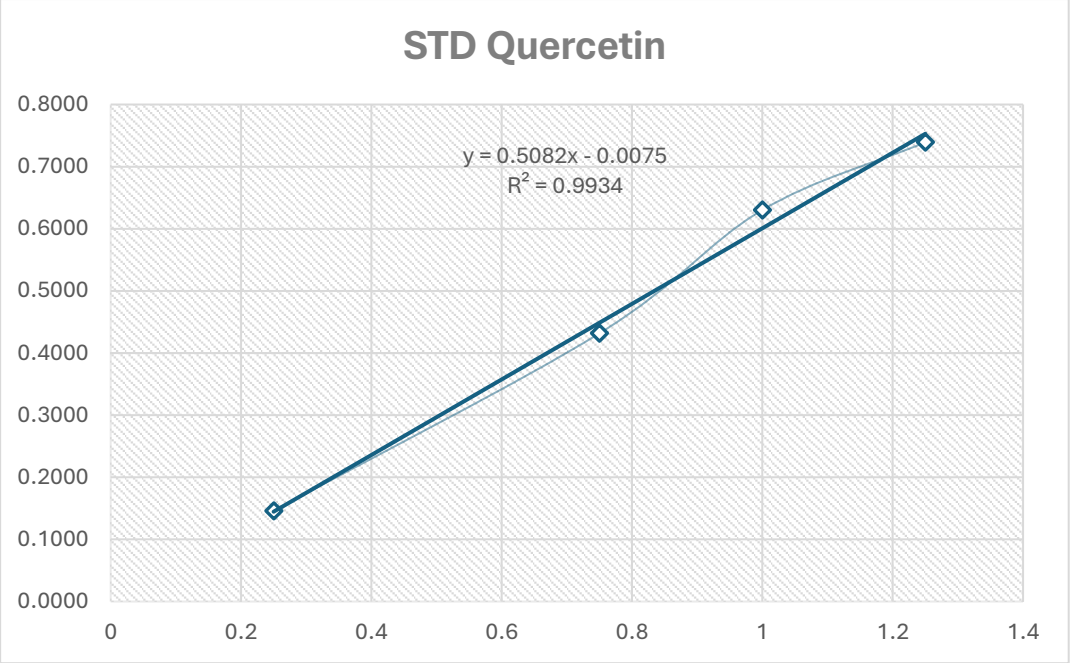


Graph 1: Standard curve of Gallic Acid

Table 2: Total Phenolic Content of Moringa leave Extract

Sample	Total phenolic content GAE mg/mL
Moringa leave Extract	9.1983 ± 0.00132

Estimation of Total Flavonoid Content



Graph 2: Standard graph of quercetin

Table 3: Total Flavonoid Content of Moringa leave Extract

Sample	Flavonoid content Quercetin equivalent mg/mL
Moringa leave Extract	0.3474 ± 0.00195

Assessment of DPPH Free Radical Scavenging Activity

Table 5: DPPH Free Radical search Activity of the Sample

Concentration (µg/mL)	Average Absorbance	FRSA (%)	Standard Deviation (± SD)	Ascorbic Acid (IC50)
5	0.1567	67.61	±0.00021	
10	0.2015	50.78	± 0.00015	
15	0.5678	35.88	± 0.00015	
20	0.7684	27.70	± 0.0002887	
25	0.9864	24.57	± 0.000058	
Negative control	0.2865			
IC50		20.00 µg/mL		27.79 µg/mL

GCMS chromatogram of the ethanolic extract of Moringa oleifera showed eleven peaks which indicated the presence of eleven phytochemicals constituents. shows the mass spectra of ethanolic extract of Moringa oleifera

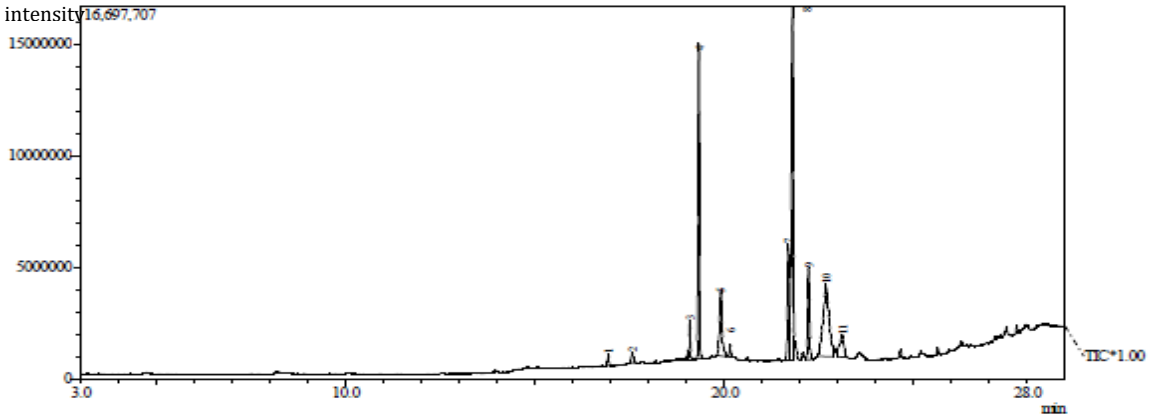


Fig 1 Shows GCMS chromatogram of ethanolic extract of Moringa oleifera

The mass spectra of the phytocomponents in Moringa oleifera were compared with that in the NIST Library database, then the eleven compounds were characterized and identified Figure 2

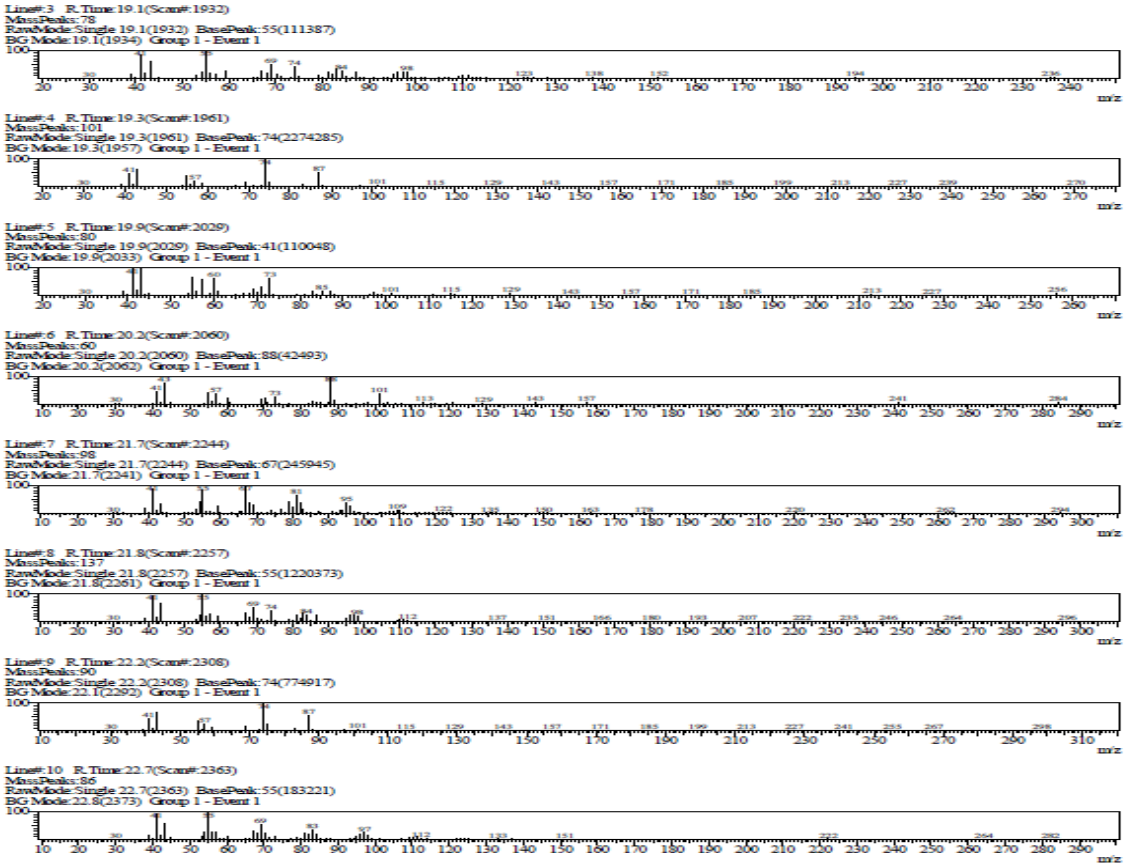


Table 1 lists the active principles together with their retention time (RT) and concentration (Peak area %). Table 1 lists the phytochemicals' molecular weight, formula, structure, and bioactivities that aided in Moringa oleifera's therapeutic effects. The two most prevalent compounds among the eleven that were found were methyl (11E)-11-octadecanoate (30.15%) and cis octadecanoic acid (21.90%). These compounds had antimetabolic syndrome and anticardiovascular risk factor bioactivities. Stearic or octadecanoic acids.

S. No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Bioactivity
1	Methyl undecanoate	14.93	0.57	200.31	C12H24O2	Natural flavoring substances
2	Undecanoic acid	17	0.98	186.29	C11H22O2	Antifungal agent, antiseborrhoeic
3	Methyl tetradeca-8,10,12- trienoate	8.9	2.08	236.34	C15H24	Protective against metabolic syndrome and cardiovascular disease risk factors [9].
4	Methyl -14- methyl pentadecanoate	9.328	17.67	270.45	C17H34O2	Flavoring agent
5	n-Hexadecanoic acid or Palmitic acid	18	7.46	256.42	C16H32O2	Mild antioxidant and anti- atherosclerotic activity [10]
6	Ethyl hexadecanoate or Ethyl palmitate	0.178	0.86	284.47	C18H36O2	Mild antioxidant and anti- atherosclerotic activity [10]
7	Methyl linolelaidate or Methyl trans,trans-9,12-octadecadienoate	21	9.21	294.47	C19H34O2	Protective against metabolic syndrome and cardiovascular disease risk factors [9].
8	Methyl (11E)-11-octadecenoate	20.502	30.15	296.48	C19H36O2	Protective against metabolic syndrome and cardiovascular disease risk factors [9].
9	Methyl hexadecenoate	21	7.64	268.43	C17H32O2	Antioxidant, hypocholesterolemic nematicide, pesticide, flavor, lubricant, antiandrogenic, hemolytic 5-Alpha reductase inhibitor
10	cis-.Octadecenoic acid or cis-Oleic Acid	21.9	19.16	282.46	C18H34O2	Protective against metabolic syndrome and cardiovascular disease risk factors [9].
11	Octadecanoic acid or Stearic acid	22	4.23	284.47	C18H36O2	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic

						Hypocholesterolemic, 5-Alpha reductase inhibitor, anemiagenic
						insectifuge, flavor [11]

5. DISCUSSION

The current study produced significant results and provided a complete assessment of the phytochemical profile and antioxidant properties of Moringa oleifera leaves, which may further our understanding of the plant's health advantages. High quantities of bioactive chemicals that are known to have antioxidant properties and related health benefits are indicated by the total flavonoid content of 0.3474 ± 0.00195 QE mg/mL extract and the total phenolic content of 9.1983 ± 0.00132 GAE/g extract.

The total phenolic content is particularly noteworthy since phenolic compounds are known to scavenge free radicals, thereby lowering oxidative stress, which can play a significant role in the development of numerous chronic illnesses, such as cancer and cardiovascular disorders. When the antioxidant activity of moringa leaf extract was assessed using DPPH, the extract's IC50 value was 27.79 g/mL, indicating moderate antioxidant activity. The possibility that Moringa oleifera is an antioxidant that may save living things from oxidative harm makes this discovery intriguing.

Using Gas Chromatography-Mass Spectrometry (GC-MS) to identify specific substances was an additional addition to them. The two most prevalent of the 11 chemicals found were cis-octadecenoic acid (19.16%) and methyl (11E)-11-octadecenoate (30.15%). These are known to have therapeutic benefits, such as cardioprotective and anti-inflammatory properties. Their presence implies that Moringa may be a source of novel medications, in addition to confirming its traditional usage.

These findings suggest that Moringa oleifera has potential health benefits that extend well beyond the lab. Natural therapies that complement traditional therapy are in high demand as lifestyle disorders become increasingly common. This paper contributes to the body of ethnopharmacology literature that provides scientific validation for the traditional use of moringa for illness prevention and health promotion across cultural boundaries.

Furthermore, in areas where Moringa oleifera is easily accessible, the results support the inclusion of this plant in diets. Moringa is an antioxidant, anti-inflammatory, high-nutrient food that may be regarded as a functional food that promotes health and well-being. Clinical trials in people would now be helpful to verify these findings, determine whether Moringa extracts are effective for a given ailment, and observe the results.

The results of recent studies demonstrate the value of Moringa oleifera in the diet and its numerous health advantages. Furthermore, it requires the identification of any bioactive substances that could still exist. By combining cutting-edge research with globally recognized information, we can optimize the health benefits of this remarkable plant, which was previously exclusively used to soothe the skin. The study's values serve as the foundation for next investigations on Moringa oleifera's medicinal qualities.

6. CONCLUSION

Along with highlighting Moringa oleifera's nutritional and therapeutic benefits, this study highlights the significance of additional research into its bioactive components. We can now better understand this remarkable plant and evaluate its role in health and wellness thanks to the integration of traditional knowledge with cutting-edge scientific techniques. These values provide the basis of future study, with the primary goal being the full medicinal potential of Moringa oleifera.

In this study, the phytochemical makeup and antioxidant potential of Moringa oleifera leaves were investigated. The findings revealed that the extract's total flavonoid concentration was 0.3474 ± 0.00195 QE mg/mL and its total phenolic content was 9.1983 ± 0.00132 GAE/g. The antioxidant activity, represented by an IC 50 value of 27.79 µg/mL, suggests that Moringa can be used as a natural resource to produce health-care compounds.

This research focuses on the remarkable health advantages of Moringa oleifera, which justifies its traditional use as a medication. Regular consumption of this plant has been confirmed to be one way to prevent chronic illnesses.

The scientific evidence of Moringa's nutritional worth and medicinal potential, which may be used to build public health initiatives, has had a huge impact. In order to further understand the health advantages of moringa, future study should focus on investigating the bioavailability of its bioactive ingredients as well as conducting clinical trials that test the extracts' effectiveness in humans.

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