

Evaluation Of Cytotoxicity And Antioxidant Properties Of Ethanolic Extract From Dried Moringa Oleifera Flowers

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Abstract

The aim of this study was to investigate the antioxidant & cytotoxic properties of the ethanolic extract obtained from the dried flower powder of *Moringa oleifera*, a well-known plant celebrated for its medicinal properties. The ethanolic extract of the flower contains a variety of phytochemicals, such as alkaloids, quinones, cardiac glycosides, flavonoids, phenols, saponins, amino acids, tannins, terpenoids, coumarins, & triterpenoids. To assess the antioxidant effects of different concentrations of the ethanol extract, three assay techniques were employed - DPPH radical scavenging assay & Ferric Reducing Ability Power (FRAP) assay. The FRAP assay showed increased absorbance at 700 nm with higher extract concentrations, indicating an effective reduction of Fe^{3+} to Fe^{2+} . At 100 μ g of extract, the maximum absorbance recorded was 0.15, compared to 0.1 for gallic acid, the positive control, at the same concentration. The researchers employed FTIR analysis to identify the chemical bonds present in the extract. Their findings indicate that the flower extract of *Moringa oleifera* demonstrates significant antioxidant & cytotoxic activities. This study provides support for some traditional claims about the therapeutic benefits of the flower extract, & suggests its potential for higher apoptotic activity compared to the ethanolic extract of the leaves. The identification & characterization of specific compounds within the extract could potentially contribute to the development of new anticancer drugs.

Keywords: *Moringa oleifera*, ethanolic extract, antioxidant activity, cytotoxic properties, phytochemicals

INTRODUCTION

Moringa oleifera (MO), drumstick tree" or "horseradish tree" is a versatile plant highly regarded for its nutritional and medicinal benefits. It originates from the sub-Himalayan regions. It has expanded to tropical and subtropical areas globally, owing to its resilience and adaptability to varied climates. This fast-growing, deciduous tree typically reaches heights of 5 to 10 meters, featuring drooping branches, compound leaves, and distinctive drumstick-shaped pods. The leaves are edible and highly nutritious, commonly used in various cuisines for their rich vitamin (A, C, E), mineral (calcium, potassium, iron), and amino acid content.(Al Baloushi et al. 2024)

Moringa oleifera (MO) is celebrated in traditional medicinal practices such as Ayurveda and Unani for its broad spectrum of therapeutic benefits. It is esteemed for its ability to combat ulcers, diabetes, liver damage, and high cholesterol levels. (Metri et al. 2024). Studies have shown that extracts from *Moringa oleifera* (MO) can protect against gastric ulcers, regulate blood sugar levels, improve insulin sensitivity, and lower cholesterol, thus reducing the risk of cardiovascular diseases. Its high antioxidant content, including flavonoids, phenolics, and carotenoids, contributes to its anti-inflammatory properties, supporting overall health and immune function. Additionally, MO has been traditionally used as a diuretic to promote kidney function and eliminate excess fluids from the body.(Adams and Camp 1966) The *Moringa oleifera* (MO) tree offers a variety of edible parts that are commonly used in cooking to enhance flavor and provide essential nutrients. Notably, the seeds of MO are rich in oil, making them ideal for cooking purposes and as a key ingredient in cosmetic items. Additionally, these seeds are

equipped with proteins that have the ability to cleanse water by attaching to particles and bacteria, thus proving to be beneficial in water purification procedures.(Al Baloushi et al. 2024)

Recent studies have explored the potential of *Moringa oleifera* (MO) in the prevention and treatment of cancer, thanks to its bioactive compounds such as glucosinolates and isothiocyanates (Gad et al. 2024). In experimental conditions, these compounds have shown encouraging effectiveness in stopping the proliferation of cancer cells and triggering programmed cell death (apoptosis). Although additional clinical research is needed to confirm these results, MO shows potential as a natural reservoir of anticancer substances.(Al Saiqali, Kaiser Jamil, and Reddy 2024)

Moringa oleifera not only has health benefits, but also plays a vital role in sustainable agriculture and environmental conservation. Its deep roots contribute to improving soil structure and fertility, making it valuable in agroforestry systems and efforts to combat soil degradation. Cultivating *Moringa oleifera* offers economic opportunities for farmers in rural communities, contributing to food security and livelihoods. Its ability to thrive in diverse climatic conditions also enhances resilience against climate variability, supporting agricultural productivity and sustainability. (Al Saiqali et al. 2024)

Research has indicated that extracts derived from various components of the *Moringa oleifera* plant exhibit notable antioxidant properties. Notably, studies employing methodologies such as DPPH radical scavenging and FRAP (Ferric Reducing Ability Power) assays have illustrated the efficacy of *Moringa oleifera* extracts in effectively counteracting free radicals and enhancing cellular antioxidant capacity. This antioxidative effect is linked to specific phytochemicals present in MO, which not only scavenge free radicals but also bolster the body's natural antioxidant defenses. As a result, incorporating *Moringa oleifera* into the diet or using its extracts in medicinal preparations may provide substantial health benefits by combating oxidative stress and supporting overall well-being.(Al Saiqali et al. 2024; Al-Shalabi et al. 2024)

Moringa oleifera has been the subject of thorough research regarding its cytotoxic properties, particularly its ability to trigger apoptosis in cancer cells without harming normal cells. Various studies have investigated the impact of *Moringa oleifera* extracts on different types of cancer cells, showing promising outcomes in terms of halting cancer cell growth and promoting programmed cell death (apoptosis). It is believed that the bioactive components found in *Moringa oleifera*, such as phenolic acids, flavonoids, and glucosinolates, play a significant role in these effects by targeting specific pathways essential for cancer cell survival and proliferation.

Recent research indicates that components extracted from *Moringa oleifera* (MO) have the ability to induce cytotoxic effects through a variety of pathways, such as disrupting cell cycle progression, causing DNA damage, influencing apoptosis-related signaling pathways, and hindering angiogenesis, all of which contribute to tumor development. This investigation highlights the potential of *Moringa oleifera* as a natural source of cytotoxic substances that could have significant implications for the treatment and prevention of cancer. It is essential to conduct further studies to fully understand the specific mechanisms of action and to assess the safety and effectiveness of compounds obtained from *Moringa oleifera* as potential complementary or alternative treatments for cancer.(Al-Shalabi et al. 2024)

Moringa oleifera is an exceptional plant that provides a wide range of benefits for human health, nutrition, and environmental sustainability. Its rich nutritional content, medicinal properties, and ability to thrive in different environments highlight its importance in addressing global health challenges and promoting sustainable development objectives. As ongoing scientific studies reveal its potential, *Moringa oleifera* emerges as a versatile resource with substantial implications for both traditional and modern medicine, providing solutions to improve health and environmental conservation.

MATERIALS & METHODS:

Plant Materials and Chemical reagents

The study utilized solely analytical-grade chemical reagents purchased from Loba Chemicals in India. Fresh *Moringa oleifera* flowers were collected in and around Kanchipuram, Tamil Nadu, India.

Preparation of Ethanol Extract

During a regulated extraction procedure, 200 milliliters of ethanol were obtained from 10 grams of dried flower powder through the utilization of a Soxhlet apparatus over the course of 18 cycles. The ethanol that was extracted underwent a concentration process in a hot air oven, which was kept at a temperature range of 60°C to 80°C, for a duration of three hours. Following this, the concentrated extract was dried

using vacuum desiccators and subsequently stored at a temperature of 4°C (Metri et al. 2024; Nassar et al. 2024).

Preliminary Screening

A comprehensive analysis of the phytochemical composition of *Moringa oleifera* was conducted to identify a diverse array of bioactive compounds. The presence of alkaloids was confirmed through the use of Mayer's, Wagner's, and Dragendorff's tests, which resulted in characteristic precipitate formations. Flavonoids were detected using Shinoda's test, leading to a noticeable pink or red color change, as well as the alkaline reagent test, which initially produced a yellow color that turned colorless upon acid addition. Tannins were revealed through the Ferric chloride test, displaying a blue-black or greenish-black coloration, and the potassium dichromate test, which formed a brownish precipitate. Terpenoids were identified using the Salkowski test, resulting in a reddish-brown interface. Polyphenols were determined through colorimetric analysis with the Folin-Ciocalteu reagent.(Barik et al. 2008)

The Ninhydrin test verified the presence of proteins, which changed color to blue or purple when heated. Saponins were detected using the Froth test, showing consistent froth formation, and the Lead acetate test, leading to a white precipitate. Furthermore, standard methods confirmed the existence of anthraquinones, glycosides, carbohydrates, and phytosterols. This thorough examination highlights the diverse and abundant phytochemical profile of *Moringa oleifera*, providing a solid basis for exploring its pharmacological potential and therapeutic applications.(Al-Shalabi et al. 2024)

Quantitative Analysis

Estimation of Flavonoids

The flavonoid content was determined using a standard solution of quercetin. Initially, 0.2 mL of the homogenized sample were mixed with 3 mL of 95% ethanol. Following this, 0.1 mL of hexahydrate aluminum chloride solution and 0.1 mL of 1M potassium acetate were added to the mixture.(UC and NAIR 2013). After the incubation period, the mixture underwent a 40-minute reaction at room temperature to promote the formation of a complex between flavonoids and aluminum chloride. Later on, the absorbance of the solution at 430 nm was gauged using a spectrophotometer. The quantification of the flavonoid content in the sample was established by utilizing a calibration curve, which was created with known concentrations of quercetin. The flavonoid content is denoted as micrograms of quercetin equivalents per gram or milliliter of the sample.(Barzan et al. 2024).

Estimation of Total Phenolic Compounds

The Folin-Ciocalteu method was utilized to measure the overall phenolic content. This process entailed mixing the Folin-Ciocalteu reagent with a sample extract, then allowing the reaction to take place. Next, an alkaline setting was achieved by adding sodium carbonate (Na₂CO₃) to the solution. (Xu et al. 2023). The complex with a blue hue produced by the reduction of the Folin-Ciocalteu reagent by phenolic compounds was examined through spectrophotometry at a wavelength of 760 nm. The total phenolic compounds in the sample were quantified by establishing a standard curve using known concentrations of gallic acid. The findings were expressed as milligrams of gallic acid equivalents per gram or milliliter of the sample. (Bharali, Tabassum, and Azad 2003).

S.NO	PHYTOCHEMICALS	ETHANOL EXTRACT
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrate	+
4	Saponin	+
5	Tannins	+
6	Terpenoids	+
7	Proteins	+
8	Anthraquinone	-
9	Polyphenol	+
10	Glycosides	+

Table 1: Preliminary Phytochemical constituents of *Moringa oleifera* flower extracts

Extract	TPC	TFC
Ethanol Flower Extract	28.92	13.46

Table 2: Total Phenolic and Flavonoid Content

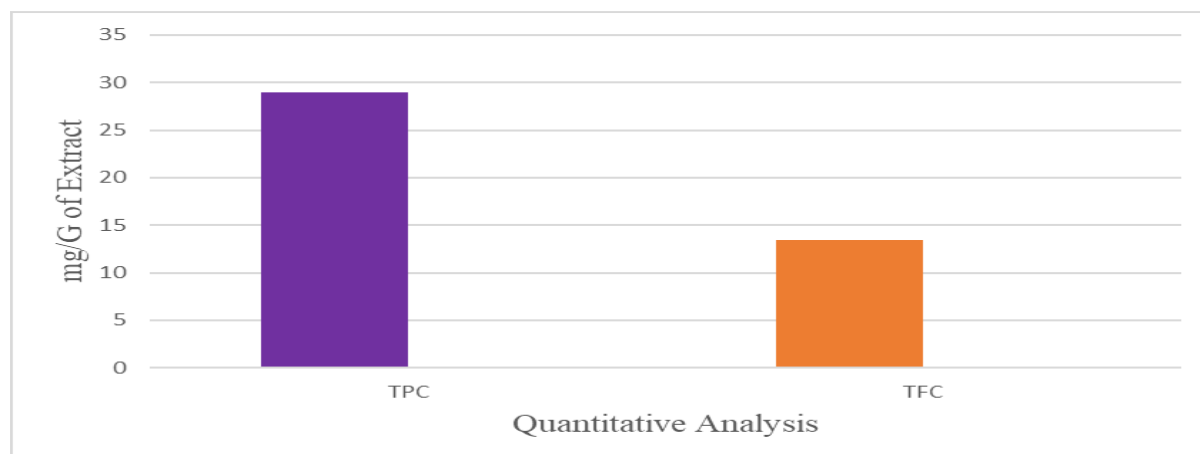


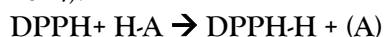
Fig 1: Total phenolic and flavonoid content

Antioxidant analysis:

Evaluation of antioxidants involves a variety of tests created to determine the effectiveness of compounds in fighting oxidative stress and eliminating free radicals. The DPPH assay, carried out through spectrophotometry at 517 nm, gauges antioxidant capacity by measuring the reduction of the stable DPPH radical. Similarly, the ABTS assay assesses the ability of antioxidants to inhibit the ABTS radical cation detected at 734 nm. The FRAP assay, analyzed at 593 nm, evaluates the capacity of antioxidants to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Lastly, the ORAC assay quantifies antioxidant activity against peroxy radicals produced from the breakdown of AAPH. These examinations provide valuable information on the antioxidant properties of a substance, which are crucial for comprehending its potential uses in managing and preventing conditions linked to oxidative stress. (Bhattarai et al. 2024)

Free Radical Scavenging Activity

The antioxidant potential of the plant extract was assessed through its ability to counteract the stable 1,1'-Diphenyl-2-picrylhydrazyl (DPPH) free radical. Using a spectrophotometer, the extent of color alteration, indicating the efficacy of the antioxidant components in combating reactive oxygen species, was determined at 517 nm while the reaction between DPPH and the antioxidant took place. (Bhattarai et al. 2024).



Yellow Purple

% inhibition was computed with the following formula:

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

where the optical density of DPPH with methanol serves as the control.

Conc in $\mu\text{g/ml}$	Standard Ascorbic Acid	Ethanol Flower Extract
5	20.00%	15.38%
10	38.46%	29.23%
25	55.38%	36.92%
50	72.31%	44.62%
100	90.77%	53.85%

Table 3: Free Radical scavenging activity of DPPH

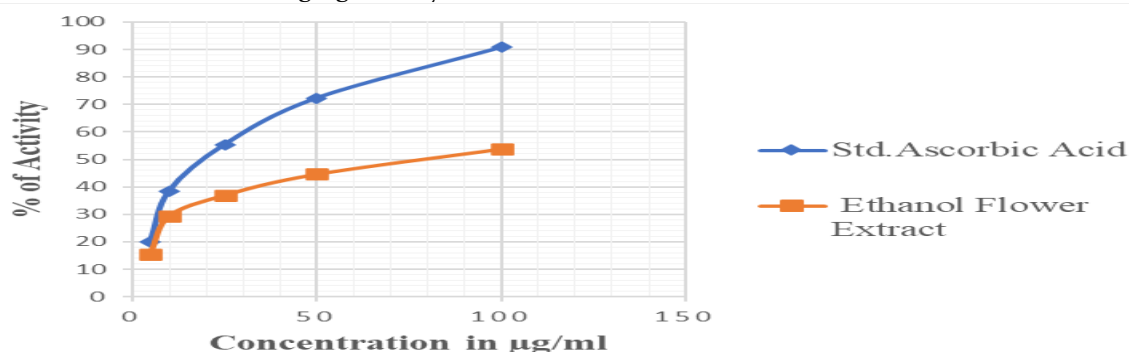


Fig 3: Free Radical scavenging activity of DPPH

Determination of Ferric Reducing Antioxidant Power (FRAP)

According to Oyaizu's method (1986), the reducing power of the plant extract was evaluated. More specifically, 10–100 µg of the extract was mixed with 2.5 mL of 1% potassium ferricyanide in 1 mL of distilled water. After a 20-minute incubation at 37°C, 2.5 mL of 10% trichloroacetic acid was added, and the resulting mixture was centrifuged at 1800 rpm for 10 minutes. The absorbance at 700 nm was measured using a spectrophotometer after combining the supernatant with 0.1% FeCl₃ and distilled water. The percentage of inhibition was used to calculate the reducing activity based on the specified formula.

$$\% \text{ Inhibition} = ((A_o - A_e) / A_o) \times 100$$

A_o = Absorbance without extract

A_e = Absorbance with extract/standard

As a common antioxidant, ascorbic acid was employed.

Conc in µg/ml	Std. Ascorbic Acid	Ethanol Flower Extract
5	0.03	0.02
10	0.06	0.03
25	0.09	0.06
50	0.12	0.07
100	0.15	0.1

Table 4: Determination of Ferric Reducing Ability power Moringa oleifera

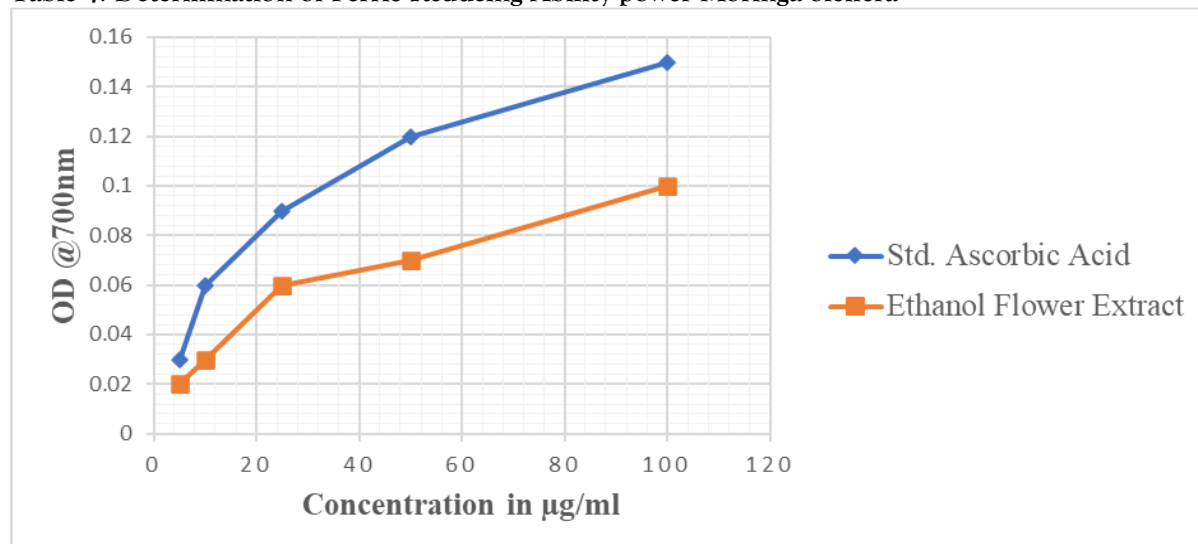


Fig 6: Determination of Ferric Reducing Ability power Moringa oleifera

FTIR Analysis:

A salt disc is created by mixing dried and powdered Moringa oleifera plant material with potassium bromide (KBr) for Fourier-transform infrared (FTIR) analysis. Spectra are then captured within the 500 to 4000 cm⁻¹ range. This approach helps in identifying chemical bonds and functional groups, contributing to the characterization of its phytochemical profile and potential bioactive compounds. The analysis offers valuable insights into the medicinal, nutritional, and agricultural uses of Moringa oleifera, thereby supporting quality control and research advancement (Bouttier-Figueroa et al. 2024).

Peak number	Compound	Peak value	Functional group
1	Alkane	2973.32	C-H Stretching
2	Alkane	2920.28	C-H Stretching
3	Aldehydes	2848.91	C-H Stretching
4	Ester	1765.10	C=O Stretching
5	Alkene	1454.35	C-H Bending
6	sulphate	1415.78	S=O stretching
7	Aliphatic Ether	1086.91	C-O Stretching

Table 5: FTIR Analysis of flower extracts of Moringa oleifera

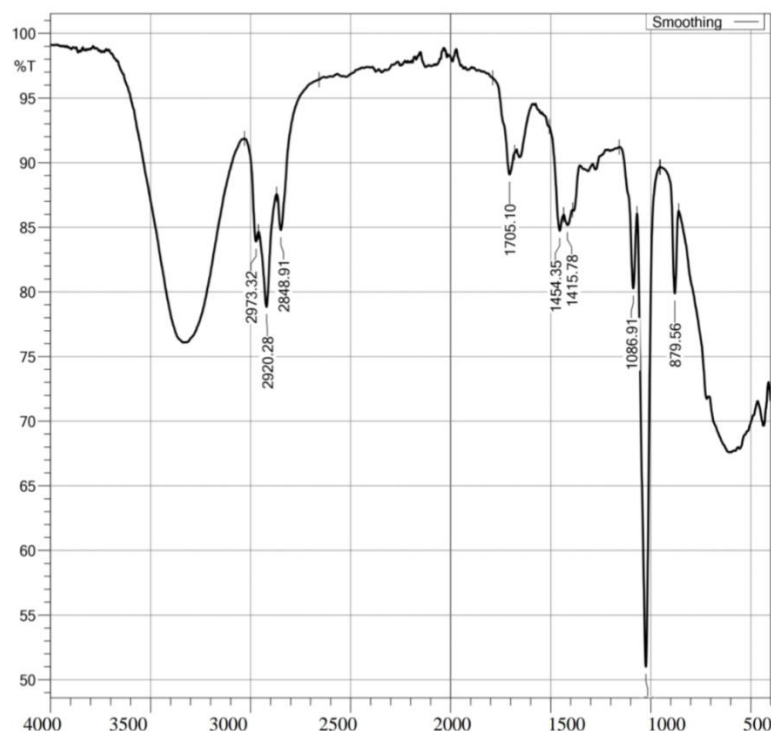
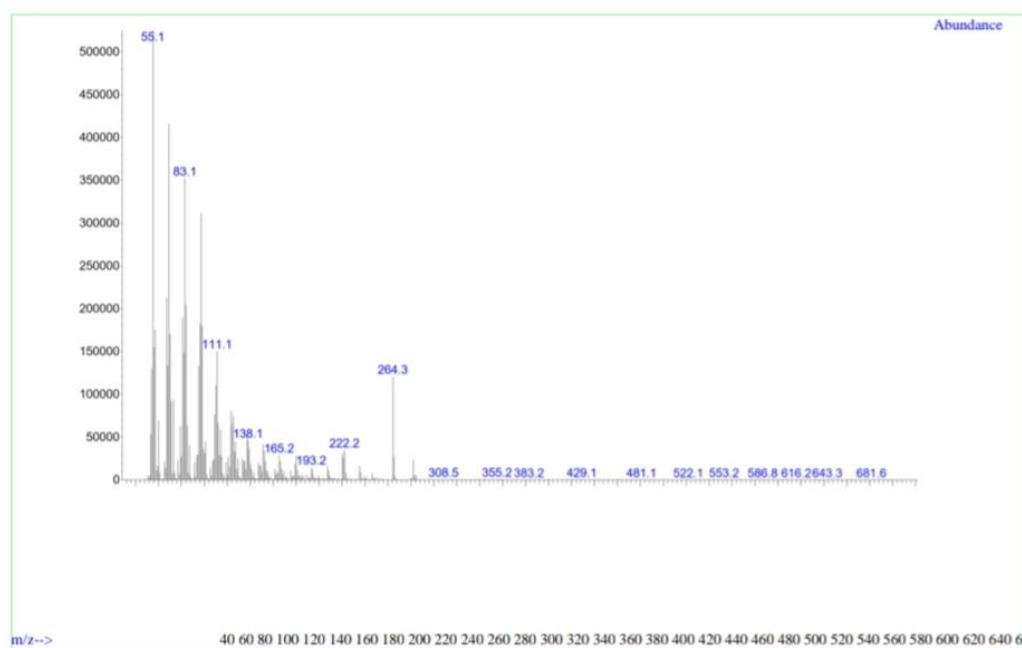


Fig 7: FTIR Analysis of flower extracts of *Moringa oleifera*

GCMS Analysis:

The helium carrier gas was consistently flowed at a rate of 1 mL/min through a fused silica column packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane) to separate components. The column had dimensions of 30 m × 0.25 mm ID × 250 μm df, and the analysis was carried out using the Clarus 680 GC system. The injector temperature was set at 260°C, and a 1 μL extract sample was injected. The oven temperature program was as follows: initially held at 60°C for two minutes, then ramped to 300°C at a rate of 10°C per minute, and maintained at 300°C for six minutes. The mass detector operated with a transfer line temperature of 230°C, an ion source temperature of 230°C, and an electron impact ionization mode at 70 eV. Scans were performed every 0.2 seconds with a 0.1-second interval between scans, covering the mass range of 40–600 Da. Component spectra were compared against the GC-MS NIST (2008) library of known spectra (Bouttier-Figueroa et al. 2024).



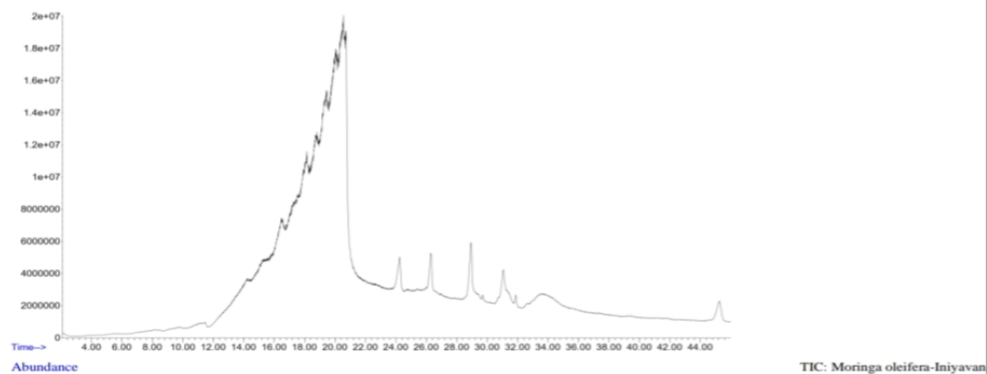


Fig 8: GCMS Analysis of flower extracts of *Moringa oleifera*

S. No.	Compound name	RT	Molecular weight	Molecular formula	Probability	Biological chemical structure
1.	cis-Vaccenic acid	14.241	282	C ₁₈ H ₃₄ O ₂	16.3%	
2.	cis-13-Octadecenoic acid	16.028	282	C ₁₈ H ₃₄ O ₂	16.7%	
3.	6-Octadecenoic acid	16.458	282	C ₁₈ H ₃₄ O ₂	7.85%	
4.	Oleic Acid	18.137	282	C ₁₈ H ₃₄ O ₂	6.36%	
5.	6-Octadecenoic acid	18.808	282	C ₁₈ H ₃₄ O ₂	3.63%	
6.	cis-Vaccenic acid	19.918	282	C ₁₈ H ₃₄ O ₂	23.7%	
7.	11-Eicosenoic acid, methyl ester	20.546	282	C ₁₈ H ₃₄ O ₂	18.5%	
8.	11-Eicosenoic acid, methyl ester	24.243	324	C ₂₁ H ₄₀ O ₂	22.8%	
9.	Eicosanoic acid, methyl ester	26.286	326	C ₂₁ H ₄₂ O ₂	59.4%	
10.	: cis-11-Eicosenoic acid, methyl ester	28.929	324	C ₂₁ H ₄₀ O ₂	21.3%	
11.	19-methyl-eicosanoate	31.053	340	C ₂₂ H ₄₄ O ₂	81.3%	
12.	: Docosanoic acid, methyl ester	45.258	351	C ₂₃ H ₄₆ O ₂	62.0%	

TABLE 8: GCMS Analysis OF *Moringa oleifera*

Treatment of Cancer cell line with Plant extract:

The cell cultures were treated with crude extracts of *Calotropis gigantea*. The plant material was dissolved in water and then filtered using a Whatman syringe filter with a pore size of 0.2 μm . After filtration, the extracts were mixed with 1 mL of MEM that contained sodium pyruvate and 10% FCS, creating a 1 mL stock solution of the crude extracts.(Shahbaz et al. 2024). One hundred microliters of T. Populnea aqueous extracts that were diluted were added to the wells of 96-well plates. Following this, the plates were placed in a CO₂-regulated incubator at 36°C for a period of 72 hours. (Brown and Rice-Evans 1998; Cáceres et al. 1992).

Concentration in $\mu\text{g/mL}$	Doxorubicin	EMO
50	26.81	15.89
100	65.17	24.71
150	78.86	29.90
200	86.38	39.12
250	96.23	43.39

Table 9: Anti-Cancer Activity of *Moringa oleifera*

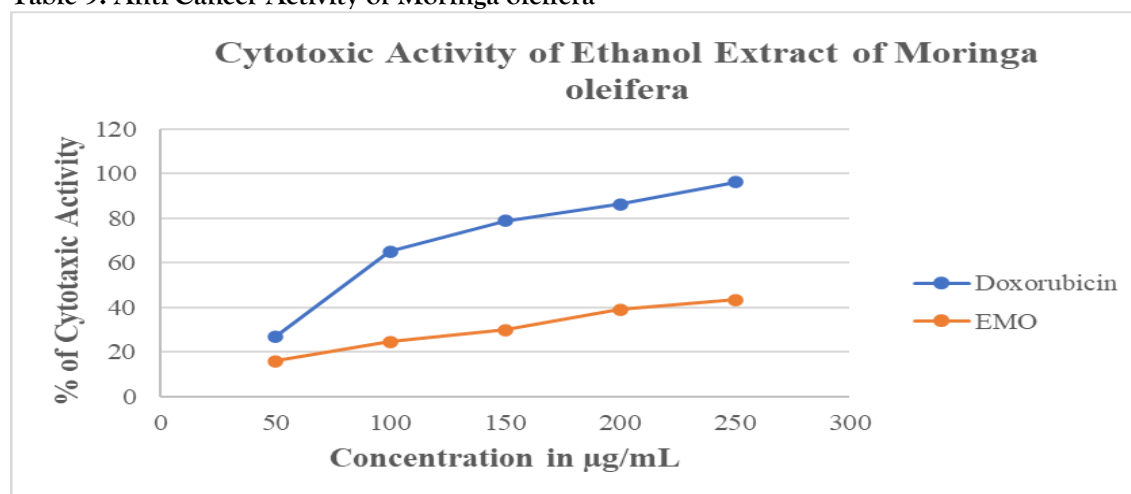


Fig 9: Anti-Cancer Activity of *Moringa oleifera*

RESULTS AND DISCUSSION:

Preliminary Phytochemical constituents of *Moringa oleifera* flower extracts:

The preliminary screening examinations for phytochemicals can prove to be beneficial in the identification of bioactive substances and in the advancement of novel pharmaceuticals. Upon the examination of plant extracts, a variety of phytochemicals were detected. The qualitative results obtained from the ethanol extracts of *Moringa oleifera* flowers unveiled a wide range of phytochemical compounds, such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins. Flavonoids, a type of phenolic compound commonly found in food, have positive impacts on human health by functioning as antioxidants and neutralizing free radicals. Tannins, a group of polymeric phenolic compounds, are accountable for localized tumors. Terpenoids and steroids were identified in *Moringa pterygosperma*, and they were noted to possess antibacterial characteristics. Saponins demonstrate anti-inflammatory effects through the precipitation and coagulation of red blood cells, while alkaloids are present in medications that alleviate fever and headaches (Chaudhari et al. 2024)

Total Phenolic and Flavonoid Content:

The examination demonstrated the measurement of important secondary metabolites. Contrasting the data showed that phenolic concentrations in *Moringa oleifera* plant matter exceed those of flavonoids. Flavonoids, one of the many polyphenolic secondary metabolites commonly present in plants, have been noted for their anti-inflammatory effects, capacity to eliminate free radicals, and ability to inhibit hydrolytic and oxidative enzymes. (Gad et al. 2024). Numerous researches have emphasized the crucial function of phenolics in supplying oxidative stability and antibacterial protection in food. Tannins can be found in almost all plant-based foods. Products containing tannins are employed as antioxidants, antiviral, antihelminthic, and antibacterial treatments. The selected plants contain significant levels of

various secondary metabolites, which could be responsible for the wound-healing mechanisms (Esparza et al. 2024).

DPPH Free radical Scavenging Activity:

1. The DPPH assay is a widely used method for assessing the antioxidant capacity of plant extracts. This method involves the conversion of the purple color of the methanolic DPPH solution to a light yellow shade due to the neutralization of free radicals by antioxidants through hydrogen donation (HAT-hydrogen atom transfer), resulting in a distinct absorbance at 517 nm. The plant extracts analyzed in this study exhibited strong scavenging activity against DPPH radicals. The presence of phenolic phytochemicals, particularly flavonoids, in the extract is believed to be responsible for its antioxidant properties. Plant phenolics are known for their role as primary antioxidants or free radical scavengers. Extensive research has explored the impact of antioxidant-rich plant extracts on promoting the process of wound healing, suggesting that their ability to neutralize free radicals contributes to the improvement of wound-healing mechanisms (Chaudhari et al. 2024; Das et al. 2024).

Determination of Ferric Reducing Ability power (FRAP) :

The reducing power assay provides a rapid and convenient way to evaluate antioxidant potential. As the concentration of the extract rose, so did its ability to reduce, as evidenced by the "Fe³⁺ to Fe²⁺ transformation" (measured by an increase in absorbance at 700 nm). At a concentration of 100 µg/mL, the extract displayed a maximum absorbance of 0.15, while the positive control, gallic acid, showed a maximum absorbance of 0.1 at the same concentration (Dhakad et al. 2024).

FTIR Analysis of flower extracts of *Moringa oleifera* :

The active components' functional groups were identified using FTIR analysis based on the peak values of the infrared spectrum. The spectrum revealed specific peaks, including Alkane at 2973.32 cm⁻¹, Alkane C-H stretching at 2920.28 cm⁻¹, Aldehyde C-H stretching at 2848.91 cm⁻¹, Ester C=O stretching at 1765.10 cm⁻¹, C-H bending at 1454.35 cm⁻¹, and Sulfate S=O stretching, Aliphatic ether C-O stretching, and Alkene C-C stretching at 1415.78 cm⁻¹, 1086.91 cm⁻¹, and 879.56 cm⁻¹ respectively. Furthermore, the FTIR absorption spectra indicated the reduction of Ag ions in a soluble extract, with absorbance bands ranging from 500 to 4000 cm⁻¹. These included peaks at 3287 cm⁻¹ for O-H carboxylic acid stretching, 2902 cm⁻¹ for C-H alkane stretching, 1776 cm⁻¹ for (RC(O))₂O anhydride stretch, 1732 cm⁻¹ for C=C alkenes stretch, 1701 cm⁻¹ for C=O anhydride and ester stretch, 1624 cm⁻¹ for CH₃-C(=O)-CH₃ ketone stretch, 1525 cm⁻¹ for NO₂ nitro stretch, 1361 cm⁻¹ for O-H stretching of alcohols and phenols, 1338 cm⁻¹ for R-O-R ether stretch, and 1209 cm⁻¹, 1189 cm⁻¹, and 497 cm⁻¹ for monosubstituted benzene stretching. The disappearance of specific bands indicated bioreduction as the primary cause of Ag ion reduction, leading to the emergence of a peak at 3287 cm⁻¹. (Dhakad et al. 2024).

GCMS Analysis of flower extracts of *Moringa oleifera* :

Research into the discovery of plant-based organic molecules and their functions is rapidly growing due to the abundance of plants as sources of advanced pharmaceuticals. Gas chromatography-mass spectrometry (GC-MS) has proven to be effective in accurately identifying bioactive components in plant-based research (Balamurugan et al.). Compounds such as butanoic acid, 1,5-heptadiene, 3,3-dimethyl(E), and 2-propanoic acid, 2-propenyl ester were found in the leaves, while squalene, 1-hexanol, 2-ethyl-2-propyl, 1,2-benzene dicarboxylic acid, hexanedioic acid, heptane, heptanoic acid, and iso-octanol were present in the flowers of *Moringa concanensis*, consistent with previous findings. These chemical constituents are believed to contribute to the plant's antibacterial, anticancer, analgesic, hepatoprotective, and anti-inflammatory properties, justifying its widespread use as a health supplement in traditional medicine. Furthermore, the results of this study indicated that organic solvents, such as methanol extracts of certain parts of *Moringa oleifera*, exhibited significant in vitro and in vivo antioxidant capabilities (Dhakad et al. 2024; Donli and Dauda 2003).

Anti-Cancer Activity of *Moringa oleifera*:

The MTT assay was used to investigate the cytotoxic effects of consecutively prepared extracts from *Moringa oleifera* flower on the HepG2 liver cancer cell line. The cells were exposed to different concentrations of the extracts, ranging from 12.5 to 100 µg/ml, for 72 hours. Both ethanol and aqueous extracts significantly reduced the viability of all tested cell lines in a concentration-dependent manner. Previous studies by Boonsri et al. (2008) explored the cytotoxic properties of *Moringa oleifera*, focusing on the extraction of wood and heartwood in dichloromethane, revealing potent cytotoxic effects on various cancer cell lines, including HepG2 cells. Furthermore, Al Baloushi et al. (2024) and Eilert, Wolters, and Nahrstedt (1981) isolated four quinones—mansonone-D, mansonone-H, thespone, and

thespesone—from *T. Populnea* flowers and evaluated their cytotoxicity against HepG2 liver cancer cells. Mansonone-D and Thespone, which generate superoxide anions believed to underlie the cell-killing effect, exhibited increased cytotoxic effects compared to the other two quinones. (Shahbaz et al. 2024). It is interesting to observe that *Moringa oleifera* contains cytotoxic compounds, with gossypol being the most extensively studied. Despite limited negative effects found in clinical trials, patient response rates were unsatisfactory. Hence, further investigation is needed to explore a new extract or a synergistic molecule. (Ebana et al. 1991)

CONCLUSION:

The results of this study highlight the strong antioxidant and cytotoxic properties of *Moringa oleifera* flower extract. Following an extensive analysis of phytochemicals, a wide range of bioactive compounds were discovered, including alkaloids, flavonoids, tannins, terpenoids, and polyphenols, all known for their potent antioxidant effects. Various methods such as DPPH and FRAP assays were used to evaluate the antioxidant abilities, demonstrating the extract's ability to combat free radicals and reduce oxidative stress, which are linked to various chronic diseases. Furthermore, the assessment of cytotoxicity showed significant activity against cancer cells, with higher apoptotic effects compared to the ethanolic extract. These findings support the traditional medicinal uses of *Moringa oleifera* in treating different conditions, including its potential in cancer therapy. The identification and characterization of these bioactive compounds suggest their potential as promising candidates for developing new anticancer drugs. Understanding the structural properties of these compounds could help uncover their mechanisms of action and lead to targeted therapeutic approaches against cancer.

Overall, the study underscores the pharmacological potential of *Moringa oleifera* flower extract, not only as an antioxidant but also as a valuable source of cytotoxic compounds with implications for cancer treatment. Further research into the precise mechanisms and clinical efficacy of these compounds is essential to fully harness their therapeutic advantages and integrate them into modern medical practices.

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