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Chromatographic and Spectroscopic Elucidation of Phytoconstituents from *Momordica Charantia* and *Moringa Oleifera*

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KEYWORDS

Momordica charantia

Moringa oleifera

Flavonoids

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Spectroscopic characterization

ABSTRACT:

Introduction: Momordica charantia and Moringa oleifera are well-known medicinal plants valued for their therapeutic efficacy and nutritional properties. Despite growing interest in their bioactive compounds, comprehensive studies targeting flavonoid-rich fractions remain limited. This study aims to isolate, identify, and characterize the phytoconstituents from the leaves of both plants using advanced chromatographic and spectroscopic techniques.

Objectives: To perform systematic phytochemical screening, quantify total phenolic and flavonoid contents, isolate active flavonoid constituents using chromatographic methods, and characterize their structures through spectroscopic techniques.

Methods: Leaves of M. charantia and M. oleifera were extracted via successive maceration using petroleum ether, ethyl acetate, and methanol. Phytochemical tests identified major constituents including flavonoids, phenolics, alkaloids, terpenoids, and saponins. The total phenolic and flavonoid content were quantified using the Folin-Ciocalteu and aluminium chloride colorimetric techniques, respectively. Thin Layer Chromatography (TLC) guided the selection of mobile phases for Column Chromatography to isolate bioactive fractions. Isolated compounds were characterized using UV-Visible, FTIR, ^1H-NMR, and Mass Spectrometry.

Results: Methanol extracts exhibited the highest yields and richest phytochemical profiles. Total phenolic content varied from 70.96 mg GAE/g (M. charantia) to 109.18 mg GAE/g (M. oleifera), with total flavonoid content of 60.43 mg RE/g and 93.10 mg RE/g, respectively. TLC confirmed the presence of flavonoid-like compounds with Rf values matching standard catechins and myricetins. Column chromatography fractions (H from M. charantia and J from M. oleifera) revealed distinct UV absorption peaks at 290 nm and 289, 372 nm, respectively. FTIR confirmed the presence of hydroxyl, alkene, and alcohol groups. ^1H-NMR spectra exhibited characteristic polyhydroxylated aromatic proton patterns. The extracted compounds were identified by mass spectrometry as (2S,3R).M. charantia produces 2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol and 3,5,7-trihydroxyM. oleifera produces -2-(3,4,5-rihydroxyphenyl)chromen-4-one.

Conclusions: This study confirms that the leaves of M. charantia and M. oleifera are rich sources of bioactive flavonoids with potential therapeutic applications. The integrated use of chromatography and spectroscopy enabled the successful isolation and identification of key flavonoid compounds, supporting the traditional medicinal value of these plants. Further in vivo pharmacological investigations are recommended to explore their full therapeutic potential.

1. Introduction

The use of medicinal plants as therapeutic agents has been deeply rooted in traditional medicine systems across the world. Among various botanicals, *Momordica charantia* (commonly known as bitter melon) and *Moringa oleifera* (commonly referred to as drumstick tree) have gained considerable attention due to their diverse pharmacological activities and nutritional significance. Both plants are widely distributed in tropical and subtropical regions and are recognized for their multifaceted medicinal properties, including

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antioxidant, anti-diabetic, anti-inflammatory, and hepatoprotective effects.[1][2]

Modern phytochemical studies emphasize the importance of isolating and characterizing bioactive compounds from natural sources to validate their traditional uses and to explore their potential for novel drug development. In this context, secondary metabolites such as flavonoids, phenolics, alkaloids, terpenoids, and saponins play pivotal roles in exhibiting diverse biological activities.[3] *Momordica charantia*, a member of the Cucurbitaceae family, has been traditionally utilized for the management of diabetes and gastrointestinal disorders. Several bioactive compounds including flavonoids, cucurbitane-type triterpenoids, and polypeptide-p have been previously reported from different parts of this plant.[4] Similarly, *Moringa oleifera* from the Moringaceae family is revered as a "miracle tree" due to its highly nutritious profile and wide array of pharmacological properties. The leaves, in particular, are rich in phenolic acids, flavonoids like quercetin and kaempferol, as well as other phytochemicals known to impart potent antioxidant and anti-inflammatory effects.[5]

Despite the growing body of evidence supporting the therapeutic benefits of these plants, comprehensive studies focusing on the systematic extraction, isolation, and characterization of flavonoid-rich fractions from their leaves remain limited. Phytochemical investigation using advanced chromatographic and spectroscopic techniques facilitates the accurate identification of bioactive molecules and enhances our understanding of their structural characteristics. Techniques such as maceration extraction using solvents of varying polarities, Thin Layer Chromatography (TLC), Column Chromatography, UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) serve as indispensable tools in modern phytochemical research. [6]][7] Recent studies have highlighted the significant antioxidant capacity of methanolic extracts of both *M. charantia* and *M. oleifera*, which correlates with their high phenolic and flavonoid content.[8]

Such investigations are crucial in establishing scientific evidence supporting traditional claims and may contribute to the discovery of novel therapeutic agents. The current study aims to systematically explore the phytochemical constituents of *Momordica charantia* and *Moringa oleifera* leaves through successive extraction, followed by qualitative and quantitative analyses. Particular emphasis has been given to the isolation and structural elucidation of flavonoid compounds employing chromatographic separation and spectral characterization methods. The isolation of pure bioactive molecules from these plants could potentially open new avenues in pharmaceutical and nutraceutical applications. This research not only supports the ethno medicinal relevance of these plants but also highlights their promising potential as sustainable sources of natural antioxidants with therapeutic value.

2. Objectives

The primary objective of this study is to conduct a comprehensive phytochemical investigation of the selected plant material to identify and characterize its bioactive constituents, with a particular focus on flavonoid compounds.

The specific aims are as follows:

a. Systematic Phytochemical Screening

- Perform qualitative phytochemical tests to detect the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds.

- Utilize standard chemical assays and solvent extraction techniques to assess the plant's phytochemical profile, providing a foundation for further analysis.

b. Quantification of Total Phenolic and Flavonoid Contents

- Using the Folin-Ciocalteu method, determine the total phenolic content and present the findings in terms of gallic acid equivalents.

- Measure the total flavonoid content (TFC) via the aluminium chloride colorimetric assay, with quantification based on quercetin equivalents (QE).

- Correlate these values with potential antioxidant activity to assess the plant's therapeutic potential.

c. Isolation of Active Flavonoid Constituents Using Chromatographic Techniques

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- Employ column chromatography (CC), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) for the fractionation and purification of bioactive flavonoids.

- Optimize separation conditions to isolate individual flavonoid compounds in their purest form for structural characterization.

d. Structural Elucidation of Isolated Compounds via Spectroscopic Techniques

- Analyse pure flavonoids using modern spectroscopic techniques, such as nuclear magnetic resonance (NMR) spectroscopy (^{1}H-NMR, ^{13}C-NMR, 2D-NMR), mass spectrometry (MS), and infrared spectroscopy.

- Interpret spectral data to determine the precise chemical structures, including stereochemistry and functional group assignments.

By achieving these objectives, this research aims to contribute to the scientific understanding of the plant's bioactive components, potentially uncovering novel flavonoids with pharmacological significance. The findings may support future studies on drug discovery, nutraceutical development, and plant-based therapeutics.

3. Material and Methods

3.1 Plant collection

Leaves of Moringa oleifera Lam. and leaves of Momordica Charantia. L were received from B.N. College of Pharmacy in Udaipur, India. Plant materials was identified and authenticated by Department of Pharmacognosy and Phytochemistry, B.N College of Pharmacy Udaipur, India. Plant authenticated no. BNU/Pharm/Auth/23-24/02/(A,B)

3.2 Maceration extraction

The coarsely powdered of *Momordica charantia* and *Moringa oleifera* plant leaves were placed individually in a stoppered container with the solvents like petroleum ether, ethyl acetate, and methanol and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter had dissolved. The mixture then was strained, the marc (the damp solid material) was pressed, and the obtained extracts were filtrated. [9][10] The extraction yields of all extracts were calculated using the equation below:

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

3.3 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in petroleum ether, ethyl acetate and methanol extracts of *Momordica charantia* and *Moringa oleifera* using standard procedures.[11]

The extracts were subjected to following tests:-

1. Tests for carbohydrates:

- **Molisch test:** Add 2-3 drops of an alcoholic α-naphthol solution to 1ml of extract. Concentrated sulphuric acid was applied down the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.
- **Fehling's test:** A comparable amount of Fehling's solution A and B was added to 1 ml of extract and heated over a water bath for a few minutes. The formation of a brick red precipitate was seen.
- **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.
- **Barfoed's test:** 1 mL of extract and Barfoed's reagent were combined in a test tube and heated on a water bath for 2 minutes. The presence of monosaccharide is shown by the red hue produced by the production of cupric oxide.

2. Test for alkaloids:

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

- **Mayer's test**: To 2-3 ml of filtrate, put a few drops of Mayer's reagent along the tube's sides. Formation of white or creamy precipitate indicates the presence of alkaloids.
- **Hager's test**: In a test tube, a few drops of Hager's reagent were added to 1-2 milliliters of filtrate. Formation of yellow color precipitate indicates the presence of alkaloids.
- **Wagner's test**: To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. A reddish-brown precipitate is formed, which contains alkaloids.

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3. Test for flavonoids:

- **Lead acetate test**: A few drops of lead acetate solution were added to the extract. The presence of flavonoids may be indicated by the formation of a yellow precipitate.
- **Alkaline reagent test**: A few drops of sodium hydroxide were added to the extract separately in a test tube. When flavonoids are present, a brilliant yellow hue appears, which disappears once a few drops of diluted acid are applied.

4. Test for glycosides:

- **Borntrager's test**: Dilute sulfuric acid was added to 3 milliliters of extract, heated for 5 minutes, and then filtered. An equal volume of either chloroform or benzene was added to the cooled filtrate, and it was thoroughly shaken. Ammonia was added to the organic solvent layer after it had been separated. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.
- **Legal's test**: 1 ml of the extract was dissolved in pyridine. 1 ml of sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. The presence of cardiac glycosides is indicated by the color change from pink to blood red.
- **Keller-Killiani test**: In a test tube, combine two milliliters of extract, three milliliters of glacial acetic acid, and one drop of 5% ferric chloride. By the test tube's side, carefully add 0.5 cc of concentrated sulfuric acid. The presence of cardiac glycosides is indicated by the acetic acid layer turning blue.

5. Test for protein and amino acids:

- **Biuret's test:** In a test tube, 1 milliliter of a 10% sodium hydroxide solution was added to the extract, and it was then heated. To the combination above, a drop of a 0.7% copper sulphate solution was added. The presence of proteins is indicated by the production of a violet or pink color.
- **Ninhydrin test:** For ten minutes, three milliliters of the extract and three drops of a 5% Ninhydrin solution were heated in a water bath. When blue coloration forms, amino acids are present.

6. Test for saponins:

- **Froth test:** In a graduated cylinder, 1 milliliter of extract was mixed with 20 milliliters of distilled water and shaken for 15 minutes. A layer of continuous foam approximately 1 cm thick was observed to develop.

7. Test for triterpenoids and steroids:

- **Salkowski's test**: Chloroform was the solvent used to process the extract prior to filtration. A few drops of strong sulfuric acid were added to the filtrate, agitated, and left to stand. Sterol is present if the lower layers turn crimson. Triterpenes are present when a golden yellow coating forms at the bottom.
- **Libermann-Burchard's Test**: A portion of the extract was dissolved in distilled water. The mixture was mixed with two milliliters of ferric chloride solution (5% concentration). When a blue, green, or violet hue appears, phenolic compounds are present.

8. Test for tannin and phenolic compounds:

- **Ferric chloride test**: Distilled water was used to dissolve a portion of the extract. Two milliliters of a 5% ferric chloride solution were added to this mixture. Phenolic compounds are present when a blue, green, or violet tint develops.
- **Lead acetate test**: Distilled water was used to dissolve a portion of the extract. A few drops of lead acetate solution were added to this mixture. Phenolic chemicals are present when a white precipitate forms.
- **Gelatin Test**: The extract was dissolved in a certain amount of distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Phenolic compounds can be detected by the formation of a white ppt.

3.4 Quantitative Phytochemical estimation-

3.4.1 Spectrophotometric Quantification of Total Phenolic Content: -

The Folin-Ciocalteu Assay was used to determine the total phenolic content of plant extracts. Ethyl acetate and methanol extracts of Momordica charantia and Moringa oleifera were mixed with Folin-Ciocalteu's phenol reagent followed by a 7.5% Na2CO3 solution and deionized distilled water. The mixture was left in the dark for 90 minutes at 25°C, and the absorbance was measured at 760 nm. A calibration curve from a gallic acid solution was extrapolated to measure the Total Phenolic Compounds (TPC). The unit of measurement for the TPC was milligrams of gallic acid equivalents per gram of dry material.[12]

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3.4.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminium chloride method. [13] 0.5 ml of Momordica charantia and Moringa oleifera ethyl acetate and methanol extracts, 0.15 ml of NaNO2 (5%) and 0.15 ml of AlCl3.6H2O (10%) were mixed together in separate 10 ml test tubes. After 5 min, 2 ml of NaOH (4%) was added and volume up to 5ml with deionized distilled water. After thoroughly mixing the solution, absorbance at 510 nm was measured in comparison to a reagent blank. The standard deviation curve for total flavonoid was created using rutin standard solution (20 to 100µg/ml) using the previously described technique. The total flavonoid content was calculated as milligrams of rutin equivalents per gram dry fraction.[14]

3.5 Qualitative chromatographic analysis:-

(a) Preliminary Thin layer chromatography:-

Thin-layer chromatography is a "solid-liquid adsorption" chromatography. In this method stationary phase was TLC plates of silica gel 60 F254 pre coated with layer thickness of 0.2 mm using different solvent system. In this strategy, the mobile phase moves upward through the stationary phase.

Spots were applied manually using capillary tube, plates were air dried using and TLC chamber were developed at room temperature with respective solvent system.

Capillary action causes the solvent to go up the thin plate that has been saturated in it. During this approach, the mixture previously deposited on the bottom parts of the plate with a pipette is propelled upward at varied flow rates.

Thus the separation of analytes was achieved. The polarity of the substance, solid phase, and solvent determines the pace at which it moves upward.

$$\mathbf{R_f\ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Solvent system developed in preliminary TLC for methanol extract of *Momordica charantia* and *Moringa oleifera* extracts in which the maximum spots were visible in Toluene: Ethyl acetate: Acetic acid (6:6:1) and Toluene: Ethyl acetate: Acetic acid (3:3:0.6) mobile phase with std. flavonoids respectively. So that Toluene: Ethyl acetate: Acetic acid (6:6:1) for *Momordica charantia* and Toluene: Ethyl acetate: Acetic acid (3:3:0.6) for *Moringa oleifera* extracts, solvents were taken as mobile phase for column chromatography.

3.6 Isolation of column chromatography:-

Methanolic extracts was subjected separately to silica gel column chromatography for isolation of flavonoids from Momordica charantia and Moringa oleifera extracts.

Column chromatography uses a stationary solid phase to adsorb and separate substances that pass through it, which is assisted by a liquid mobile phase.

The Column was packed using wet packing technique using stationary phases, such as silica gel silica (60-120 mesh) which is placed in a vertical glass (usually) column (20 mm dia.). Slurry was prepared using toluene and was poured in to the column. Samples to be separated were mixed with silica and introduced at the top of the column and allowed to move with Toluene: Ethyl acetate: Acetic acid (6:6:1) solvent for Momordica charantia with toluene: Ethyl acetate: Acetic acid (3:3:0.6) solvent for Moringa oleifera extracts. The fractions/elutes collected were concentrated and TLC was carried out to find the presence of single compound.[15]

3.7 Spectroscopic characterization:-

3.7.1 UV-visible Spectroscopy

The isolated fractions (H & j) of MC and MO were scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded.[4]

3.7.2 FT-IR spectroscopy-

FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The isolate fractions (H & j) of MC and MO were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The samples pellets were placed into the sample holder and FT-IR spectra were recorded in the range 400-4000 cm-1 in FT-IR spectroscopy.[16]

3.7.3 NMR spectroscopy-

Jeol Resonance NMR was used to record 1H NMR spectra. TMS was used as an internal standard. NMR spectroscopy was performed for the isolated fractions (H & j) of MC and MO to identify the structure of the compound present in the isolated fraction. Chemical

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shifts ware shown in δ values (ppm) with TMS as an internal reference.

3.7.4 Mass spectroscopy-

Mass spectrometry converts molecules into ions and according to their mass and charge the ions or fragments can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fractions (H & j) of MC and MO were recorded on mass spectrometer instrument micrOTOF-MS.[17]

4. Results

4.1 Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Momordica charantia*	Leaves	1 Kg
2	*Moringa oleifera*	Leaves	1 Kg

4.2 Percentage yield

Table 2: Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Momordica charantia*	Petroleum ether	Dark Yellow to Brown	750.00	0.096	0.01
2.	*Momordica charantia*	Ethyl acetate	Dark Brown	749.80	4.060	0.54
3.	*Momordica charantia*	Methanol	Dark Brown	745.00	19.262	2.58
4.	*Moringa oleifera*	Petroleum ether	Dark Green to Brown	600.00	3.172	0.52
5.	*Moringa oleifera*	Ethyl acetate	Dark Green to Brown	596.70	8.021	1.34
6.	*Moringa oleifera*	Methanol	Dark Green to Brown	588.50	9.651	1.63

4.3 Qualitative Phytochemical Analysis of extracts of *Momordica charantia* and *Moringa oleifera*

Table 3: Phytochemical analysis of extracts of *Momordica charantia*

S. No.	Experiment	Result		
		Petroleum ether	Ethyl acetate	Methanol
Test for Carbohydrates				
1.	Molisch's Test	-	-	+
2.	Fehling's Test	-	-	+
3.	Benedict's Test	-	+	+
4.	Bareford's Test	-	+	+
Test for Alkaloids				
1.	Mayer's Test	-	+	+
2.	Hager's Test	+	+	+
3.	Wagner's Test	-	+	+
Test for Terpenoids				
1.	Salkowski Test	+	+	+
2.	Libermann-Burchard's Test	-	-	+
Test for Flavonoids				
1.	Lead Acetate Test	+	+	+
2.	Alkaline Reagent Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	-
2.	Lead Acetate Test	+	+	+
3.	Gelatine Test	-	+	+
Test for Saponins				
1.	Froth Test	-	-	+
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	-
2.	Biuret's Test	-	+	+
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	+	+	-
3.	Borntrager's Test	-	-	+

Table 4: Phytochemical analysis of extracts of *Moringa oleifera*

S. No.	Experiment	Result		
		Petroleum ether	Ethyl acetate	Methanol
Test for Carbohydrates				
1.	Molisch's Test	+	+	+
2.	Fehling's Test	+	+	+
3.	Benedict's Test	+	+	+
4.	Bareford's Test	-	-	+
Test for Alkaloids				
1.	Mayer's Test	+	+	+
2.	Hager's Test	-	+	+
3.	Wagner's Test	+	+	+
Test for Terpenoids				
1.	Salkowski Test	+	+	+
2.	Libermann-Burchard's Test	-	-	+
Test for Flavonoids				
1.	Lead Acetate Test	+	+	+
2.	Alkaline Reagent Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	-
2.	Lead Acetate Test	+	+	+
3.	Gelatine Test	-	+	+

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Test for Saponins				
1.	Froth Test	+	-	+
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	+
2.	Biuret's Test	+	+	+
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	+	+	-
3.	Borntrager's Test	-	-	+

4.4 Solubility determination of Methanolic extracts of *Momordica charantia* and *Moringa oleifera*-

Table 5: Solubility Determination of Methanolic extract of *Momordica charantia* and *Moringa oleifera*

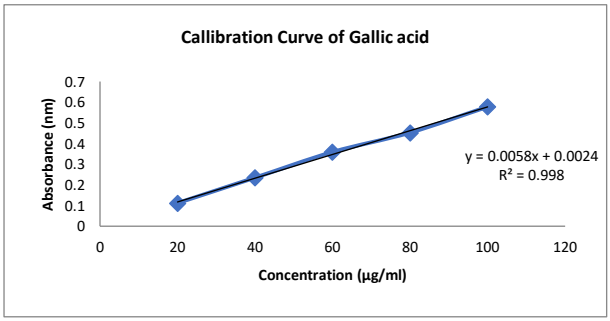
S. No.	Solvent	*Momordica charantia*	*Moringa oleifera*
1.	Water	Partially Soluble	Sparingly Soluble
2.	Ethanol	Soluble	Soluble
3.	Ethyl Acetate	Sparingly Soluble	Partially Soluble
4.	DMSO	Soluble	Soluble
5.	Petroleum Ether	Partially Soluble	Sparingly Soluble
6	Methanol	Soluble	Soluble
7	Chloroform	Soluble	Soluble
8	Acetone	Sparingly Soluble	Partially Soluble

4.5 Quantitative Phytochemical study of extracts of *Momordica charantia* and *Moringa oleifera*-

4.5.1 Total Phenolic Content (TPC) Estimation

Table 6: Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.112
2.	40	0.235
3.	60	0.359
4.	80	0.453
5.	100	0.578



Graph 1: Graph represent standard curve of Gallic acid

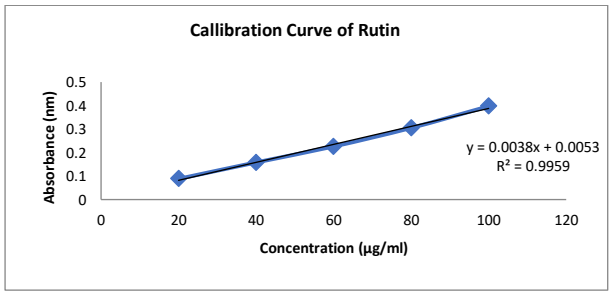
Table 7: Total Phenolic Content in *Momordica charantia* and *Moringa oleifera* extracts

Total Phenolic content (mg/gm equivalent to Gallic acid)				
Extracts	*Momordica charantia*		*Moringa oleifera*	
Solvents	Ethyl acetate	Methanol	Ethyl acetate	Methanol
Absorbance Mean±SD	0.2753±0.004	0.3568±0.003	0.4574±0.002	0.5479±0.003
TPC	54.66	70.96	91.08	109.18

4.5.2 Total Flavonoid Content (TFC) Estimation:

Table 8: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.088
2.	40	0.158
3.	60	0.226
4.	80	0.305
5.	100	0.397



Graph 2: Graph represent standard curve of Rutin

Table 9: Total Flavonoid Content in *Momordica charantia* and *Moringa oleifera* extracts

Total Flavonoid Content (mg/gm equivalent to Rutin)				
Extracts	*Momordica charantia*		*Moringa oleifera*	
Solvents	Ethyl acetate	Methanol	Ethyl acetate	Methanol
Absorbance Mean±SD	0.1465±0.003	0.1863±0.004	0.2511±0.003	0.2843±0.002
TPC	47.16	60.43	82.03	93.10

4.6 Preliminary TLC preparation for the estimation of active constitutes –

1. TLC of methanol extract of *Momordica charantia* and *Moringa oleifera*

For Flavonoid:- Mobile Phase- Toluene: Ethyl acetate: Acetic acid (6:6:1), **Mobile Phase-** Toluene: Ethyl acetate: Acetic acid (3:3:0.6)

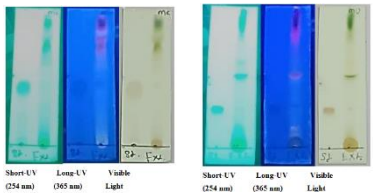


Figure1: **TLC estimation by UV lamp for MC and MO with Std. Flavonoid (Catechin), (Myricetin)**

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(Std.= Standard, MC = *Momordica charantia*) (Std.= Standard, MO = *Moringa oleifera*)

Table 10: TLC of methanol extract of *Momordica charantia extract*

S. No.	Solvent system	No. of spots	Colour of spots at Wavelength (365 nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Flavonoid)
`1.	**Toluene : Ethyl Acetate : Acetic acid (6:6:1)**	13	Dark Blue (Std.) Florescence (MC) Light Blue Florescence Florescence Pink Purple Pink Purple Florescence Purple Dark Blue Pink	Green (Std.) Light Green (MC) Light Green Green Green Light Green Green Green Light Green Light Green Green Dark Green Green	- 1.9/5=0.38 2.4/5=0.50 2.9/5=0.58 3.1/5=0.62 3.4/5=0.68 3.6/5=0.72 3.8/5=0.76 3.9/5=0.78 4.2/5=0.84 4.3/5=0.86 4.5/5=0.90 4.9/5=0.98	**2.4/5=0.50**

Table 11: TLC of methanol extract of *Moringa oleifera* extracts

S. No.	Solvent system	No. of spots	Colour of spots at Wavelength (365 nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Flavonoid)
`1.	**Toluene : Ethyl Acetate : Acetic acid (3:3:0.6)**	12	Blue (Std 365) Florescence (MO) Blue Florescence Light Pink Purple Florescence Florescence Florescence Florescence Pink Purple Dark Blue	Green (Std 254) Green (MO) Light Green Light Green Green Green Light Green Green Green Light Green Light Green Dark Green Dark Green	- 0.8/5=0.16 1.9/5=0.38 2.5/5=0.50 2.6/5=0.52 2.7/5=0.54 3.1/5=0.62 3.3/5=0.66 3.5/5=0.70 3.7/5=0.74 4.0/5=0.80 4.8/5=0.96	**1.9/5=0.38**

TLC of MC and MO extracts was performed on different solvent systems (solvent system was selected by literature survey). TLC performed in Toluene: Ethyl Acetate: Acetic acid (6:6:1) and Toluene: Ethyl Acetate: Acetic acid (3:3:0.6) that were clearly visible bands of MC and MO extracts with different Std. Flavonoids. The Rf values of MC and MO extracts were found to be 0.50 and 0.38 with different Std. Flavonoids were noted to be 0.50 and 0.38.

3.7 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of methanol extracts of *Momordica charantia* and *Moringa oleifera* were tested for the detection of various phyto compounds using TLC. The collected fractions/elutes were taken properly and do the UV spectrum.

3.7.1 Column Chromatography of MC and MO methanolic extract –

Table 12: Fraction collected from Column Chromatography of MC methanolic extract

Sr. No.	Eluent composition	Fraction collected	Remarks
1	**Toluene: Ethyl Acetate: Acetic acid (6:6:1)**	01 (A)	White coloured mixture of compound
2		02 (B)	Yellowish coloured mixture of compound
3		03-04 (C)	Creamy coloured mixture of compound
4		05 (D)	Dark greenish coloured mixture of compound
5		06 (E)	Greenish coloured mixture of compound
6		07 (F)	Light greenish coloured mixture of compound
7		08 (G)	Very light greenish coloured mixture of compound
8		09 (H)	Light yellowish coloured mixture of compound
9		10 (I)	Very light yellowish coloured mixture of compound
10		11 (J)	Creamy coloured mixture of compound
11		12 (K)	Yellowish coloured mixture of compound
12		13 (L)	Light yellowish coloured mixture of compound
13		14 (M)	Very light yellowish coloured mixture of compound
14		15 (N)	Creamy coloured mixture of compound
15		16 (O)	White coloured mixture of compound

Table 13: Fraction collected from Column Chromatography of MO methanolic extract

Sr. No.	Eluent composition	Fraction collected	Remarks
1	**Toluene: Ethyl Acetate: Acetic acid (3:3:0.6)**	01 (a)	White creamy coloured mixture of compound
2		02 (b)	Very light yellowish coloured mixture of compound
3		03 (c)	Dark yellowish coloured mixture of compound
4		04 (d)	Yellowish coloured mixture of compound
5		05 (e)	Light yellowish coloured mixture of compound
6		06-07 (f)	Creamy coloured mixture of compound

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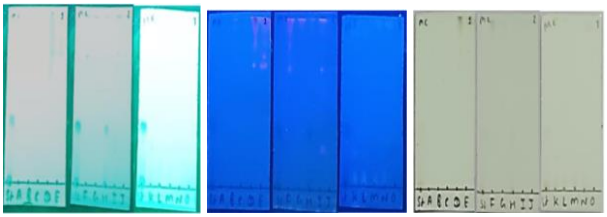
7		08 (g)	Dark greenish coloured mixture of compound
8		09 (h)	Greenish coloured mixture of compound
9		10 (i)	Light greenish coloured mixture of compound
10		11 (j)	Brownish coloured mixture of compound
11		12 (k)	Creamy coloured mixture of compound
12		13 (l)	Light greenish coloured mixture of compound
13		14-15 (m)	Greenish coloured mixture of compound
14		16 (n)	Very light greenish coloured mixture of compound
15		17 (o)	White creamy coloured mixture of compound

3.7.2 TLC of all collected fractions-

A) TLC of all collected fractions of MC methanolic extract –



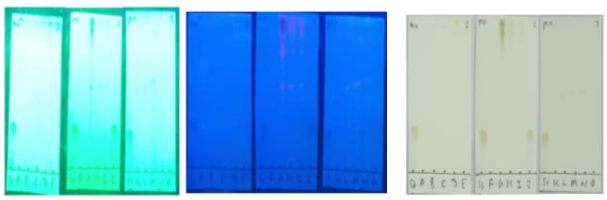
(a) Short-UV (254 nm), (b) Long-UV (365 nm), (c) Visible Light

Figure 2: TLC estimation by UV lamp for MC fractions after column chromatography with Std. Flavonoid.

Table 14: Rf values of all collected fractions of MC after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelength (365 nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Flavonoid)
1.	A	Toluene: Ethyl Acetate: Acetic acid (6:6:1)	-	-	-	-	2.5/5=0.50
2.	B		01	Pink	Light Green	4.9/5=0.98	
3.	C		-	-	-	-	
4.	D		06	Light Pink Fluorescence Fluorescence Purple Pink Dark Blue	Light Green Light Green Light Green Green Green Dark Green	3.4/5=0.68 3.5/5=0.70 3.9/5=0.78 4.2/5=0.84 4.3/5=0.86 4.6/5=0.92	
5.	E		06	Pink Purple Pink Purple Fluorescence Pink	Light Green Green Light Green Green No Spot	3.3/5=0.66 3.4/5=0.68 3.7/5=0.74	
					Light Green	3.8/5=0.76 4.3/5=0.78 4.8/5=0.96	
6.	F		05	Light Pink Pink Pink Purple Pink	Green No Spot No Spot Light Green Green	3.3/5=0.66 3.9/5=0.78 4.3/5=0.86 4.6/5=0.92 4.9/5=0.98	
7.	G		02	Purple Pink	Light Green Green	4.8/5=0.96 4.9/5=0.98	
8	H		02	Blue Fluorescence	Green Light Green	2.5/5=0.50 4.9/5=0.98	
9	I		03	Fluorescence Light Pink Pink	Light Green Light Green Green	1.9/5=0.38 3.3/5=0.66 4.9/5=0.98	
10	J		-	-	-	-	
11	K		02	Fluorescence Fluorescence	Light Green Green	0.8/5=0.16 1.8/5=0.36	
12	L		01	Fluorescence	Light Green	0.8/5=0.16	
13	M		01	Fluorescence	Light Green	0.8/5=0.16	
14	N		01	Fluorescence	Light Green	0.8/5=0.16	
15	O		-	-	-	-	

B) TLC of all collected fractions of MO methanolic extract –



(a) Short-UV (254 nm), (b) Long-UV (365 nm), (c) Visible Light

Figure 3: TLC estimation by UV lamp for MO fractions after column chromatography with Std. Sterol

Table 15:Rf values of all collected fractions of MO after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelength (365 nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Flavonoid)
1.	a		-	-	-	-	

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2.	b	Toluene: Ethyl Acetate: Acetic acid (3:3:0.6)	01	Light Blue	Light Green	4.8/5=0.96	1.9/5=0.38
3.	c		01	Light Blue	Light Green	4.8/5=0.96	
4.	d		02	Fluorescence Blue	Light Green Green	4.1/5=0.82 4.9/5=0.98	
5.	e		02	Fluorescence Blue	Light Green Green	4.1/5=0.82 4.9/5=0.98	
6.	f		-	-	-	-	
7.	g		06	Fluorescence Pink Fluorescence Pink Purple Dark Blue	Light Green Green Light Green Light Green Green Drak Green	2.5/5=0.50 2.6/5=0.52 3.5/5=0.70 3.7/5=0.74 4.0/5=0.80 4.6/5=0.92	
8	h		05	Pink Fluorescence Fluorescence Pink Purple	Green Light Green Light Green Green Green	2.6/5=0.52 3.7/5=0.74 4.0/5=0.80 4.6/5=0.92 4.9/5=0.98	
9	i		05	Fluorescence Fluorescence Fluorescence Pink Purple	Green Light Green Light Green Green Green	2.6/5=0.52 3.7/5=0.74 4.0/5=0.80 4.6/5=0.92 4.9/5=0.98	
10	j		01	Blue	Green	1.9/5=0.38	
11	k		-	-	-	-	
12	l		02	Fluorescence Fluorescence	Green Light Green	2.6/5=0.52 4.9/5=0.98	
13	m		03	Pink Fluorescence Fluorescence	Light Green Green Light Green	2.8/5=0.56 4.0/5=0.80 4.9/5=0.98	
14	n		02	Fluorescence Fluorescence	Light Green Light Green	2.9/5=0.58 4.9/5=0.98	
15	o		-	-	-	-	

Rf value Resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction (H) of MC and fractions (j) of MO methanolic extracts with mobile phase Toluene: Ethyl Acetate: Acetic acid (6:6:1) and Toluene: Ethyl Acetate: Acetic acid (3:3:0.6)by comparing with Std. Flavonoids (Catechin and Myricetin).

3.8 Spectroscopic characterization:-

3.8.1 Active constitutes estimation By UV-Spectroscopy-

UV spectra of the isolated fractions (H) and fractions (j) of MC and MO were recorded in solvent as Toluene: Ethyl acetate: Acetic acid (6:6:1) and Toluene: Ethyl acetate: Acetic acid (3:3:0.6) over a scanning range of 200-800 nm and λmax of isolated compound were determined. Toluene: Ethyl acetate: Acetic acid (6:6:1) and Toluene: Ethyl acetate: Acetic acid (3:3:0.6) solvents were used as blank. The wavelength of isolated fraction (H) of MC extract were noted to be one Peak at 290 nm and isolated fraction (j) of MO extract was noted to be two Peaks at 289 and 372 nm.

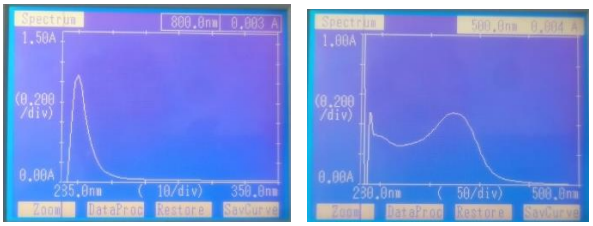

Figure 4: Active constitutes estimation By UV-Spectra of isolated fraction (H) of MC and isolated fraction (j) of MO extract after column chromatography

3.8.2 Active constitutes estimation By FTIR – Spectroscopy

(A) IR spectra of the isolated fraction (H) of *MC* and (j) of MO methanolic extract

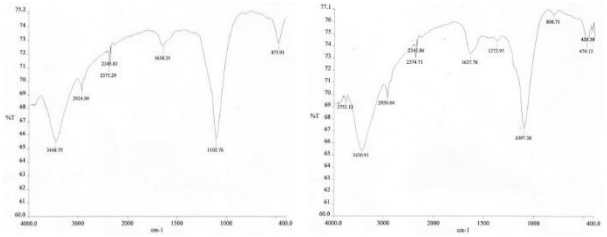

Figure 5: **IR spectra of the isolated fraction (H) of MC and (j) of MO methanolic extract**

Table 16: FTIR- Spectrum Frequency Range of the isolated fraction (H) of MC methanolic extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
9	H	3550-3200 (cm^{-1})	3448.75	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm^{-1})	2924.00	Medium	C-H stretching	Alkane
		2400-2000 (cm^{-1})	2375.29	Strong	C-H stretching	Alkane

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		1662-1626 (cm^{-1})	1638.35	Medium	C=C stretching	Alkene
		1124-1087 (cm^{-1})	1102.76	Strong	C-O stretching	Alcohol

In IR Spectra of isolated fraction (H) of MC methanolic extract showed that O-H stretching peak of Hydroxyl Group strong, broad appeared at 3448.75 cm-1, C-H stretching medium peaks of Alkane at 2924.00 & strong at 2375.29 cm-1, C=C stretching peak of Alkene at 1638.35 cm-1 and C-O stretching peak of Alcohol at 1102.76 cm-1.

Table 17: FTIR- Spectrum Frequency Range of the isolated fraction (j) of MO methanolic extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
10	j	3550-3200 (cm^{-1})	3430.91	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm^{-1})	2930.04	Medium	C-H stretching	Alkane
		2400-2000 (cm^{-1})	2374.71	Strong	C-H stretching	Alkane
		1662-1626 (cm^{-1})	1637.78	Medium	C=C stretching	Alkene
		1390-1310 (cm^{-1})	1373.95	Medium	O-H bending	Phenol
		1124-1087 (cm^{-1})	1097.38	Strong	C-O stretching	Alcohol
		840-790 (cm^{-1})	808.71	Medium	C=C bending	Alkene

In IR Spectra of isolated fraction (j) of MO methanolic extract showed that OH stretching of Hydroxyl group strong, broad peak appeared at 3430.91 cm-1, C-H stretching medium peaks of Alkane at 2930.04 & strong at 2374.71 cm-1, C=C stretching peak of Alkene at 1637.78 cm-1, OH bending peak of Phenol at 1373.95 cm-1, C-O stretching peak of Alcohol at 1097.38 cm-1and C=C bending peak of Alkene at 808.71 cm-1.

3.8.3 ^{1}H NMR - Spectroscopy-

^{1}H NMR spectra of isolated fraction (H) of MC and fraction (j) of MO methanolic extracts were recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

(A) 1H NMR spectra of the isolated Fraction (H) of MC –

In ^{1}H-NMR spectra isolated fraction (H) of MC methanolic extract showed H-2 protons appeared at 2.45-2.46 (dd) ppm and , H-1 proton appeared at 3.28 (d) ppm, H-1 proton appeared at 4.23 (ddd) ppm, H-1 proton appeared at 4.80 (d) ppm, H-1 proton appeared at 4.85 (d) ppm,H-2 protons appeared at 5.60-6.00 (5.62 (d) & 5.63 (d)) ppm,H-1 proton appeared at 5.83 (d) ppm, H-2 protons appeared at 6.60-6.77 (6.62 (dd) & 6.67 (dd)) ppm, ^{1}H-1 proton appeared at 7.00 (dd) ppm, ^{1}H-1 proton appeared at 7.92 (d) ppm and H-1 proton appeared at 7.99 (d) ppm.

(B) 1H NMR spectra of the isolated Fraction (j)of MO

In ^{1}H-NMR spectra isolated fraction (j) of MO methanolic extract showed that H-1 proton appeared at 2.52 (d) ppm, H-1 proton appeared at 2.60 (d) ppm, H-2 protons appeared at 3.25-3.30 ppm (3.26 (d) & 3.29 (d)) ppm, H-2 protons appeared at 5.60-5.65 ppm (5.62 (dd) & 5.63 (dd)) ppm, H-1 proton appeared at 6.10-6.13 (d) ppm, H-1 proton appeared at 6.29 (d) ppm, H-2 protons appeared at 7.15-7.20 ppm (7.16 (dd) & 7.19 (dd)) ppm.

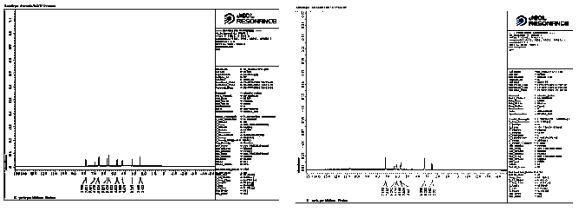

Figure 6: ^{1}H-NMR spectra of the isolated compound (Fraction H) of MC and Fraction (j) of MO methanolic extract

3.8.4 Mass – Spectroscopy-

A mass spectrum of isolated Fraction (H) and Fraction (j) of MC and MO extracts were recorded on Mass Spectroscopy.

(A)Mass spectra of the isolated Fraction (H) of MC Methanolic extract –

Mass spectra of isolated Fraction (H) of MC methanolic extract showed molecular ion [M^{+}] peaks at mlz 290.2000 which obtained (2S,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol compound in which presence of carbons (C_{15}), Hydrogens (H_{14}) and Oxygen (O_6). Finally the molecular formula of isolated Fraction (H) of MC methanolic

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extract was noted to be $C_{15}H_{14}O_6$ according to their fragments (109, 179, 244, 147, and 369 m/z).

(B)Mass spectra of the isolated Fraction (j) of MO methanolic extract –

Mass spectra of isolated Fraction (j) of MO methanolic extract showed molecular ion [M^+] peaks at mlz 318.2000 which obtained 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one compound in which presence of carbons (C_{15}), Hydrogens (H_{10}) and Oxygen (O_8). Finally the molecular formula of isolated Fraction (j) of MO methanolic extract was made to be $C_{15}H_{10}O_8$ according to their frangments(245, 178, 147, 290, 207 and 301 m/z).

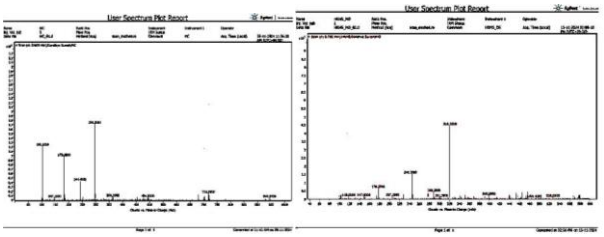

Figure 7: **Mass spectra of the isolated fraction (H) of MC and fraction (j) of MO Methanolic extract**

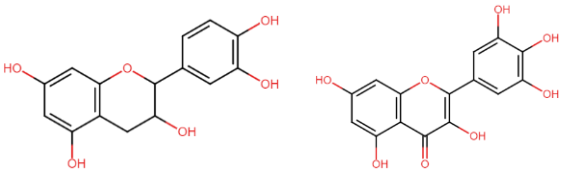

Figure 82: (2S, 3R)-2-(3, 4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) and 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one

5. Discussion

In the present study, methanolic, ethyl acetate, and petroleum ether extracts of Momordica charantia and Moringa oleifera leaves were prepared using maceration extraction. The percentage yield of methanolic extracts was found to be the highest for both plants, with 2.58% for Momordica charantia and 1.63% for Moringa oleifera. This aligns with previous findings indicating methanol's superior extraction efficiency due to its high polarity, facilitating the dissolution of a wide range of phytochemicals.[1] Qualitative phytochemical analysis revealed a diverse spectrum of secondary metabolites, including alkaloids, flavonoids, saponins, glycosides, tannins, and phenolic compounds. Methanolic extracts particularly showed strong positive responses in most tests, confirming the richness of bioactive constituents. This discovery is consistent with previous research that have highlighted the phytochemical richness of M. charantia and M. oleifera leaves, particularly in terms of flavonoids and phenolic compounds.[3][4] Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) further substantiated the qualitative findings. Methanolic extracts showed the highest TPC (70.96 mg GAE/g for M. charantia and 109.18 mg GAE/g for M. oleifera) and TFC (60.43 mg RE/g for M. charantia and 93.10 mg RE/g of M. oleifera). Moringa oleifera consistently exhibited higher TPC and TFC values than Momordica charantia, suggesting it as a more potent source of natural antioxidants. These results are comparable with recent reports by Alotaibi et al., (2023) who demonstrated Moringa leaves' superior polyphenolic content contributing to their medicinal properties. Preliminary TLC analysis with suitable solvent systems showed clear separations with Rf values consistent with standard flavonoids such as catechin and myricetin, supporting the presence of flavonoid-type compounds in both plant extracts. TLC-based fractionation confirmed that the isolated fractions, particularly fractions H (Momordica charantia) and J (Moringa oleifera), contained single prominent spots with fluorescence under UV light, correlating with flavonoid standards. Column chromatography followed by advanced spectroscopic characterization further validated the presence of flavonoid compounds. UV-Vis spectra demonstrated characteristic absorption peaks at 290 nm for M. charantia and 289, 372 nm for M. oleifera, corresponding to flavonoid structures. FT-IR analysis indicated the presence of functional groups typical of flavonoids such as O-H, C-H, C=C, and C-O stretching vibrations.[5] Further structural elucidation via ^{1}H NMR revealed distinctive proton signals associated with polyhydroxylated flavonoid skeletons. Mass spectrometry confirmed the molecular formulas C15H14O6 for the compound isolated from Momordica charantia and C15H10O8 for Moringa oleifera, identified as (2S,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol and 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one, respectively. These structures are consistent with catechin-type flavonoids, widely recognized for their antioxidant, anti-inflammatory, and therapeutic potential.[6][18] Overall, the

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results confirm that both M. charantia and M. oleifera leaves are rich sources of flavonoids and phenolics, with Moringa showing comparatively higher yields and compound diversity. The robust phytochemical profile and bioactive compounds identified in this study justify the traditional use of these plants in herbal medicine and validate their potential for development into natural therapeutic agents.

6. Conclusion

The present study successfully demonstrated the extraction, isolation, and characterization of phytochemicals from the leaves of Momordica charantia and Moringa oleifera using various solvents. The phytochemical analysis revealed the presence of significant bioactive compounds, including phenolics, flavonoids, alkaloids, and saponins, with methanol extract showing higher yields and richer phytochemical content. The isolated fractions were further confirmed through chromatographic and spectroscopic techniques such as TLC, UV-Visible, FTIR, NMR, and Mass Spectrometry, indicating the presence of potent flavonoid structures. These findings emphasize the potential of plant extracts as useful sources of natural medicinal chemicals, necessitating more pharmacological research.

Conflict of Interest

There is no conflict of interest.

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