ijlpr 2023; doi 10.22376/ijlpr.2023.13.3.L38-L50

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Moringa oleifera against Colorectal Cancer

In-silico Analysis of Phytocompounds Identified by GC-MS from *Moringa oleifera* Leaves of Kolli Hills at Namakkal Against Colorectal Cancer

Sowmiya S[1], R. Jasmine[1*], Sherlin Rosita A[1], Bharathi V[2] and Keerthiga M[3]

[1]*Department of Biotechnology & Bioinformatics, Bishop Heber College (Autonomous), Affiliated to Bharathidasan University, Trichy, Tamil Nadu – 620 017*

[2]*Department of Microbiology, Vivekanandha Arts and Science College for Women, Veerachipalayam, Sankari West, Sankari, Salem, Tamil Nadu - 637 303*

[3]*Department of Biotechnology, Cauvery College for Women, Trichy, Tamil Nadu – 620 018*

Abstract: Plants have been used for medicinal purposes long before the prehistoric period. The use of plants and natural substances to cure a wide range of human ailments has become increasingly popular due to the population explosion, insufficient drug supply, high cost of treatments, adverse effects of various synthetic medications, and the emergence of drug resistance. Because of its potent healing properties, India views *Moringa oleifera* as a "mystical plant." These phytochemicals made by plants have the potential to take the place of antibiotics, antihelminthicides, and antivirals in the fight against a range of pathogenic diseases. The aim of this current study is to evaluate the *M. oleifera* leaf aqueous extract compounds against Colorectal cancer through *In-silico* analysis. The objective of this research is to analysis the phytocompounds present in the extract of *Moringa oleifera* from the Kolli Hills region, identify the biologically active compounds through GC-MS, and determine whether *In-silico* analysis reveals anticancer activity. The GC-MS analysis, which indicates 10 peaks of biomolecules such as Hexatriacontane, 2- Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R- [R*, R*-(E)], Neophytadiene, Docosane, Benzenepropanoic Acid, Alpha. -[(Trimethylsilyl)Oxy]-, Trimethylsilyl, Tetracosane, Succinic acid, epicotyl 3-methylbut-3-enyl ester , 2,3,4,5,6-Pentafluorobenzyl Crotonate , Acetic acid, 2-(9-phenylimino-10-acridanyl)- , ethyl ester , Butane, 1,1-diethoxy-3-methyl- . Docking studies with β-catenin revealed that Acetic acid, 2-(9-phenylimino-10-acridanyl)-ethyl ester, has minimum docking scores and high binding affinity against β-catenin of Human Colorectal cancer, indicating that *M. oleifera* from Kolli Hills region has a promising natural bioactive source and might be a valuable source for future anti-cancer therapy especially Colorectal cancer. The novelty of this study is that no report has yet been cited on the effectiveness of *M. oleifera* extracts obtained from the Kolli hills region as an anti-cancer agent against Colorectal cancer.

Keywords: *Moringa oleifera,* GCMS, β-catenin, Acetic acid, Colorectal cancer, Kolli hills, Namakkal

***Corresponding Author**

Dr.R.Jasmine , Department of Biotechnology & Bioinformatics, Bishop Heber College (Autonomous), Affiliated to Bharathidasan University, Trichy, Tamil Nadu – 620 017

Received On 15 December, 2022
Revised On 8 February, 2023
Accepted On 18 February, 2023
Published On 1 May, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Sowmiya S, Dr. R. Jasmine, Sherlin Rosita A, Bharathi V and Keerthiga M , In-silico Analysis of Phytocompounds Identified by GC-MS from Moringa oleifera Leaves of Kolli Hills at Namakkal Against Colorectal Cancer.(2023).Int. J. Life Sci. Pharma Res.13(3), L38-L50 http://dx.doi.org/10.22376/ijlpr.2023.13.3.L38-L50

This article is under the CC BY- NC-ND Licence (https://creativecommons.org/licenses/by-nc-nd/4.0)

Copyright @ International Journal of Life Science and Pharma Research, available at www.ijlpr.com

1. INTRODUCTION

Since prehistoric days, people have employed plants and extracts of their various parts for their therapeutic benefits and to treat specific illnesses, as well as general tonics, foods, and other techniques to boost the body's immunity and vitality [1-3]. *M. oleifera* is well renowned for having tremendous therapeutic properties[4]. As a food and medicinal plant, *M. oleifera* is used throughout Asia for its great nutritional value and plenty of vegetable oil, proteins, vitamins, beta carotene, calcium, riboflavin, iron, and polyphenolic compounds. The plant has a wide range of therapeutic applications and is employed in traditional medicine to treat skin ailments, respiratory problems, ear and dental infectious diseases, high blood pressure, metabolic disorders, nutritional deficiencies, and cancer[5]. Furthermore, the pharmacological significance of the leaf extract containing bioactive compounds includes its use as an antioxidant[6], anti-carcinogenic[7], antispasmodic, diuretic, anti-inflammatory, antiulcer, antibacterial, antifungal, and antinociceptive[8]. Many studies have demonstrated the utilization of *M. oleifera*'s various sections to have strong *in-vitro* or *in-vivo* antioxidant and anticancer potential. *M. oleifera* is a potent anti-cancer agent because its use on a small scale is natural, safe, and highly reliable[9]. *M. oleifera* is going to prove to be an excellent source of information with low production costs for preventing malnutrition, nutritional deficiencies, and a variety of pathologies such as child blindness caused by dietary vitamin and element deficiencies[10]. *M. oleifera* extract might be an effective treatment for Colorectal cancer[11]. Colorectal cancer is the third most widespread cancer type globally, with nearly 2 million cases diagnosed in 2021. It is the second leading cause of cancer death, accounting for nearly 1 million deaths per year. According to the International Agency for Research on Cancer (IARC), the worldwide incidence of Colorectal cancer will start rising by 56% between 2020 and 2040, reaching more than 3 million new cases per year. The estimated increase in disease-related deaths is 69%, to approximately 1.6 million deaths worldwide in 2040[12]. Wnt/ β-catenin signaling pathway is essential for intestinal homeostasis and is aberrantly activated in most Colorectal cancers (CRC) through mutation of the tumor suppressor Adenomatous Polyposis Coli (APC). APC is an essential component of a cytoplasmic protein complex that targets β-catenin for destruction[13]. The current study seeks to determine the presence of phytochemical compounds from the *M. oleifera*, Kolli Hills region, Namakkal which would be confirmed using *in-vitro* GC-MS and *in-silico* molecular docking analysis to determine its inhibition against the target β-catenin in Colorectal cancer.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

M. oleifera leaves were collected from the Kolli Hills region, Namakkal, Tamil Nadu, India. Dr. S. Soosairaj, Assistant Professor, confirmed the plant's authenticity in John Britto's Flora of Central and Northern TamilNadu (2019). The herbarium specimen can be accessed and deposited as 2979 at the Dept. of Botany, St. Joseph's College, Trichy-02. Leaves were then dried in the shade at room temperature for several days before being crushed into a powder[14].

2.2. Preparation of leaf extracts

Plant material *M. oleifera* leaves have been air–dried in the shade before being powdered for solvent extraction. Powdered *M. oleifera* leaves (100g each 1:10 w/v) were extracted by maceration (24h) and infusion (30 min) in room temperature and boiling water (100°C) respectively. The extract was turned into a dark-brown residue known as a crude extract by being evaporated under lower pressure at a temperature below 40°C. The percentage yields of aqueous extracts were calculated after freeze drying[15].

2.3. Preliminary qualitative phytochemical analysis

In order to determine the qualitative phytochemical analysis for carbohydrates, steroids, saponins glycosides, flavonoids, alkaloids, proteins, and resins many tests were performed using the standard procedure for aqueous extracts from plant powdered samples**.** The GC-MS analysis was then performed on the aqueous extract because of their higher presence of phytoconstituents[16].

2.4. GC-MS Analysis

The aqueous extract of *M. oleifera* was submitted to GC-MS analysis at the Heber Analytical Instrumentation Facility at Bishop Heber College, Trichy, in order to determine its components. The GC-MS Agilent Technologies 7820A GC system was used to analyze the extracted phytochemicals from *M. oleifera* leaves. Agilent Technologies 5977 MSD Gas Chromatogram coupled with an Agilent Technologies GCMS capillary column HP-5MS (30 m, 0.25 mm ID, 0.25) composed of 5% diphenyl and 95% dimethyl polysiloxane. The electron ionization system used had an ionizing energy of 70 eV. The carrier gas was helium gas (99.99%) at a constant flow rate of 1 mL/min and an injection volume of 1µl at a split ratio of 50:1, the injector temperature was 60°C, and the ion source temperature was 250°C. A voltage of 70 eV was used to record mass spectra. By comparing its average peak, the relative percentage amount of each component was calculated[17].

2.5. Identification of Phytocompounds

Based on their retention duration, percentage of peak area, a pattern of mass spectra, and comparison with the data of the NIST11 library, the phytocompounds were identified (National Institute of Standards and Technology's LIB-NIST).

2.6. Anticancer activity – In- silico analysis

2.6.1. Protein Preparation

The three-dimensional crystal structure of Beta-catenin and Htcf-4(PDB ID: 1JDH) at a resolution of (1.90 Å)[18] was retrieved from the Protein Data Bank database (https://www.rcsb.org/) (Figure. 1). The alpha helices are in red and the loops are in green. The protein was in a complex with a peptide inhibitor (Htcf-4) and an A chain. The protein molecule was then optimized for molecular docking by removing the water molecules, inhibitors, and other heteroatoms and used for docking analysis.

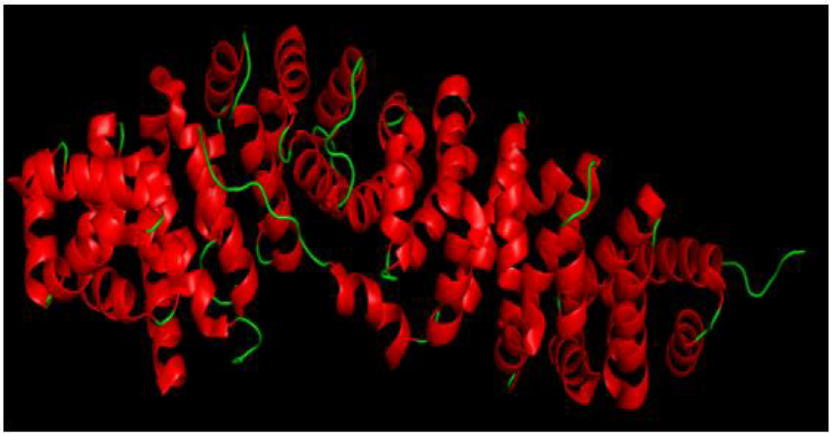


Fig 1: Three-dimensional structure of Beta-catenin and Htcf-4(PDB ID: 1JDH)

2.6.2. Ligand Preparation

The compounds derived from the leaf extracts of *M. oleifera* obtained from GC-MS analysis were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Canonical Smiles of ligands, IUPAC names, and the molecular formula and its 2D structure were retrieved (Tables 3 and 4). Further activities of the derived ligands were also mined from the literature sources, which consisted mainly of recently published articles.

2.6.3. Active site prediction

An important step is determining the active sites of the target protein. The active sites of Beta-catenin and Htcf-4(PDB ID: 1JDH) were predicted using the Biovia Drug Discovery Studio Visualizer 2021[19].

2.6.4. Molecular Docking

The molecular docking was performed using PyRx Auto Dock VINA. Docking scores were obtained from the generated log files. The compounds derived from the leaf extracts of *M. oleifera* were imported into the PyRx 0.8 tool and subjected to energy minimization[20]. The ligands were standardized and converted into PDBQT format using the PyRx Virtual Screening Tool. The conjugate gradient algorithm was used to minimize energy with the universal force field (UFF), and a grid box was set to the active site of the receptor Beta-catenin and Htcf-4 (PDB ID: 1JDH) with the following dimensions in: center (X, Y, Z) = (-2.63, 10.89, 48.42), dimensions (X, Y, Z) = (28.39, 21.74, 40.38). The docking scores were calculated as binding affinity in kcal/mol. The ligand-protein interactions were generated with Discovery Studio version v21.1.0.20298 (BIOVIA, San Diego, CA, USA) (Dassault Systèmes BIOVIA, 2017).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

M. oleifera, a member of the Moringaceae family, is an effective treatment for malnutrition. Preliminary phytochemical analysis of plant aqueous extracts revealed the presence of resins, carboxylic acid tannin, flavonoids, carbohydrates, glycosides, protein, phenol, alkaloids, saponin and the absence of steroids, and gum (Table 1). The phytoconstituents discovered from the aqueous extract of *M. oleifera* leaves can be used as foremost cancer treatments and they also possess the ability to inhibit cancer cell growth [21]. Hence based on the literature studies, the initial phytochemical screening was done and the aqueous extract was subjected to GC-MS for further investigation. Numerous studies revealed that the content of secondary metabolites varied between plants of the same genus and even within the same plant[22]. This is caused by a variety of factors, including environmental heterogeneity, whose effect is highly scale-dependent. It might promote great niche variety, enabling species to coexist on a vast scale. Additionally, the tremendous complexity and heterogeneity of soil, such as (soil quality, textures, thickness, water-retention qualities, and oxygenation, generate a great diversity in the chemical components even within the same country[23]. According to this hills region has plenty of phytochemical compounds in their nature. Cancer, diabetes, hypertension, malaria, the common cold, and cough are all treated with alkaloids. In addition to these other applications, phenols are used in the production of pharmaceuticals, because its having antimicrobial, antifungal, and antioxidant properties[24]. Phenolic, alkaloids, and saponins are one of the most important natural protections that inhibit the growth of harmful microorganisms. These compounds can also act as antimicrobials agent against *Bacillus sp., Streptococcus sp., Pseudomonas sp., E.coli.,*[25].

Table 1: Phytochemical Screening of *Moringa oleifera* extracts

	Test	Procedure	Observations (Indicating Positive Test)	Mole
		Test for Resin		
1)	Acetic anhydride test	1mL plant extract + Acetic anhydride solution + 1mL con H_2SO_4	Colour change from Orange to yellow	+
		Test for Alkaloids		
1)	Dragendroff's/ Kraut's test	Few mL filtrate + 1-2 mL *Dragendorff's reagents*	Formation of a reddish-brown precipitate	+
2)	Hager's test	Few mL filtrate+ 1-2 mL *Hager's reagents*	Formation of a creamy white precipitate	+

3)	Mayer's/ Bertrand's/ Valser's test	Few mL filtrate + 1-2 drops of *Mayer's reagent* (Along the sides of test tube)	Formation of a creamy white/yellow precipitate	+
		Test for Carbohydrates		
1)	Molish's test	2mL filtrate + 2 drops of alcoholic α –naphthol + 1mL conc.H_2SO_4 (along the sides of test tube)	Formation of a violet ring	+
2)	Seliwanoff's Test	1mL extract solution + 3mL *seliwanoff's reagent* + heated on water bath for 1 min.	Formation of rose red colour {ketoses}	+
3)	Test for starch	Aqueous extract + 5mL 5% KOH solution	Formation of a cinary colouration	+
		Test for Glycosides		
1)	10% NaOH test	1mL dil. H_2SO_4 + 0.2mL extract + boiled for 15min. + allowed cooling + neutralize with 10% NaOH + 0.2mL *Fehling's solution A & B*	Formation of a brick red precipitate	+
2)	Aqueous NaOH test	Alcoholic extract + dissolved in 1mL of water + few drops of aqueous NaOH solution	Formation of yellow colour	+
		Test for Proteins and Amino acids		
1)	Biuret test	2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets	Formation of pink-coloured sol. (in ethanoic layer)	+
2)	Millon's test	2mL filtrate + few drops of *Millon's reagent*	Formation of white precipitate	+
3)	Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	Formation of purple colored sol. {Amino acids}	+
		Test for Flavonoids		
1)	Alkaline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colorless on the addition of diluted acid	+
2)	Lead acetate test	1mL plant extract +a few drops of 10% lead acetate solution	Formation of yellow precipitate	+
3)	Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	Formation of a green precipitate	+
		Test for Phenolic compounds		
1)	Iodine test	1mL extract + few drops of dil. Iodine sol.	Formation of transient red colour	+
2)	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol.	Dark green/bluish black colour	+
3)	Hot water test	Warm water in beaker + mature plant part is dipped + warmed for a min.	Black or brown colour ring at the junction of dipping	+
		Test for Tannins		
1)	Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	Formation of white precipitate	+
2)	Braymer's test	1mL filtrate[d] + 3mL distilled water + 3 drops 10% Ferric chloride solution	Formation of Blue-green colour	+
		Detection of Carboxylic acid		
1)	Effervescence test	1mL plant extract + 1mL sodium bicarbonate solution	Appearance of Effervescence	+
		Detection of Gums		
1)	Alcohol test	Dissolve 100mg extract in 10mL distilled water+ 25mL absolute alcohol (Constant stirring)	Formation of White or cloudy precipitate	_
		Test for Steroids		
1)	Acetic anhydride test	0.5mL plant extract + 2mL of acetic anhydride + 2mL conc. H_2SO_4	Colour change from violet to blue/green	-
2)	Hesse's response	5mL aq. extract + 2mL chloroform + 2mL conc. H_2SO_4	Formation of Pink ring /Red colour (in lower chloroform layer)	
3)	Sulphur test	Extract solution + pinch of Sulphur powder	Formation of Red –rose colour	
		Test for Saponins		
1)	Foam test	0.2gm plant extract + 5mL distilled water; shaken well; heated to boiling	Appearance of creamy miss of small bubbles	+
2)	Olive oil test	Aq. extract + 5mL distilled water; shaken vigorously + few drops of olive oil + shaken vigorously	Appearance of foam	+
3)	Hemolysis test	Drop of fresh blood on glass slide + plant extract	Zone of hemolysis	+

(+, - indicates the presence and absence of phytoconstituents respectively)

3.2. *Identification of Components by GC-MS*

The National Institute of Standard and Technology's (NIST's) database, which contains more than 62,000 patterns, was used to interpret the mass spectrum of the GC-MS. The mass spectra of the known and unknown components that were housed in the NIST library were compared. Some of the test materials components names, molecular weights, and mass fragmentation were determined. The 10 components from the aqueous extract of the *M. oleifera* plant were derived from the GC-MS analysis (Supplementary File 1) based on the presence of peaks in the GC-MS chromatogram, suggesting the presence of phytoconstituents named as Hexatriacontane (21.3%), 2- Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- (20.03%),Neophytadiene (13.67%) (Figure 2a), Docosane (10.48%), Benzenepropanoic Acid, Alpha.-[(Trimethylsilyl)Oxy]-, Trimethylsilyl (9.11%), Tetracosane (6.16%), Succinic acid, eicosyl 3-methylbut-3-enyl ester (6.41%) (Figure 2b), 2,3,4,5,6-Pentafluorobenzyl Crotonate (5.5%),Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester (3.82%) (Figure 2c), Butane, 1,1-diethoxy-3-methyl- (3.51%). It appears that the compound 2,3,4,5,6-Pentafluorobenzyl Crotonate structure not reported yet. The 2D structures of the phytoconstituents were downloaded from the Pubchem database, and for conversion of chemical structure into 3D, the structures were drawn using ChemDraw and presented in Figure 3. This is used for further *In- silico* analysis.

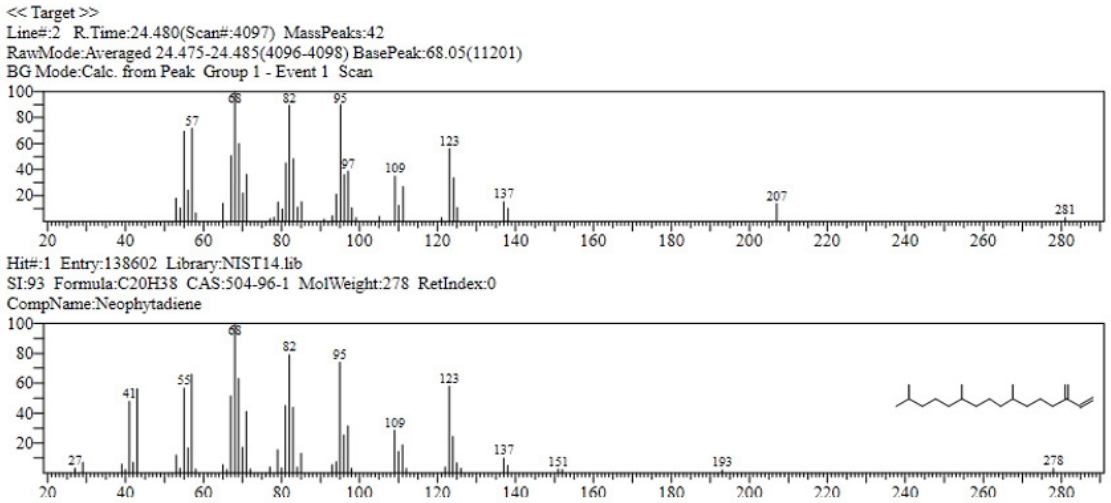


Fig 2a: GC-MS Chromatogram of Aqueous extract of *Moringa oleifera* for Neophytadfiene

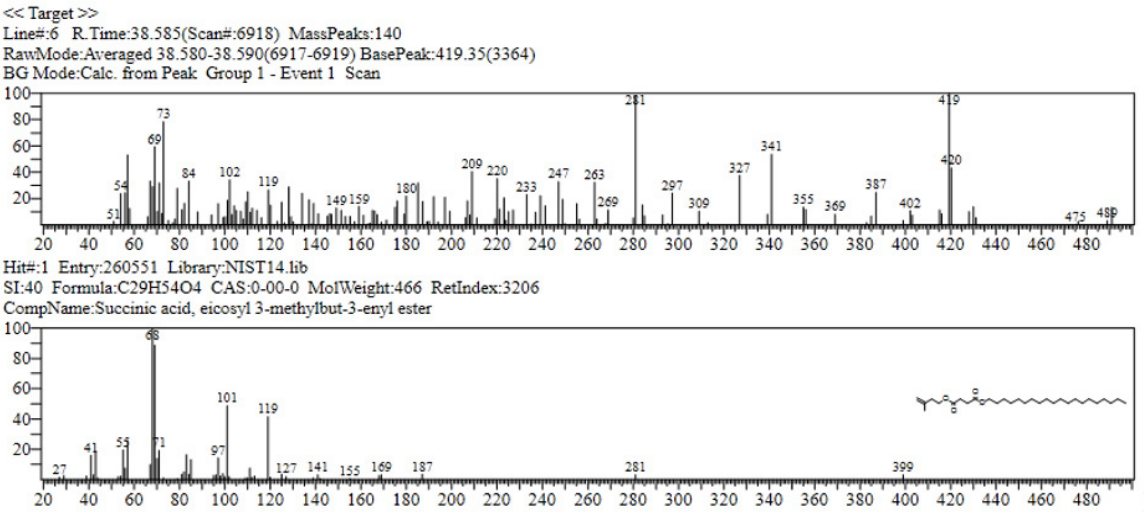


Fig 2 b: GC-MS Chromatogram of Aqueous extract of *Moringa oleifera* for Succinic acid, eicosyl 3-methylbut-3-enyl ester

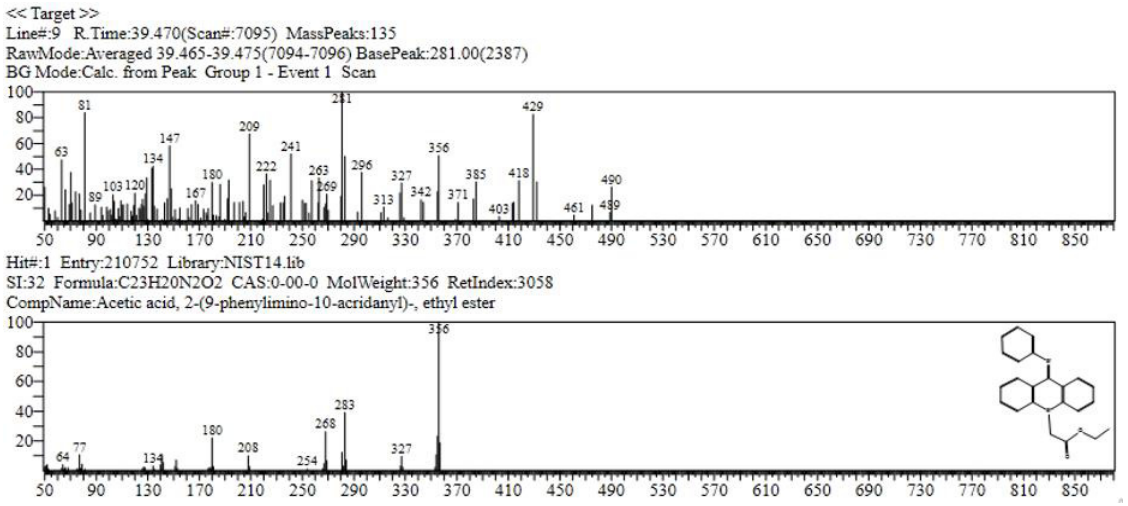


Fig 2 c: GC-MS Chromatogram of Aqueous extract of *Moringa oleifera* for Acetic acid, 2-9-Phenlyimino-10 acridanyl)- ethyl ester

The predominant compounds identified from *M. oleifera* were Butane, 1,1-diethoxy-3-methyl- of area 21.3% followed by Neophytadiene (area- 20.03%) which is reported to have antimicrobial and anti-inflammatory properties. Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r- [r*, r*-(e)]]- of area- 13.67% is the third predominant compound along with molecular weight, peak area and molecular formula were reported in Table 2. The computed details of the Phytochemical constituents derived from *M. oleifera* which includes its IUPAC name, smiles notation along with its reported activity in literatures were presented in Table 3. Based on the literature review mentioned (Table 4), it has been found that the three compounds (Neophytadiene, Succinic acid, eicosyl 3-methylbut-3-enyl ester, Acetic acid, 2-(9-phenylimino-10-acridanyl)- ethyl ester had anticancer activity, therefore those compounds have been chosen for further *in -silico* analysis.

Table 2: Phytochemical constituents identified in the Aqueous extract of *Moringa oleifera* using gas chromatography-mass spectrometry.

S.No	Name of the Compound	Area %	Height%	Molecular Weight (g/mol)	Molecular formula
1	Butane, 1,1-diethoxy-3-methyl-	21.3	6.32	160.25	C9H20O2
2	Neophytadiene	20	15.9	278.5	C20H38
3	Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	13.7	17.1	296.5	C20H40O
4	Tetracosane	10.5	6.67	338.7	C24H50
5	Docosane	9.11	10.9	310.6	C22H46
6	Succinic acid, eicosyl 3-methylbut-3-enyl ester	6.41	5.86	466.7	C29H54O4
7	Hexatriacontane	6.16	18.9	507	C36H74
8	Benzenepropanoic acid, .alpha.-[(trimethylsilyl)oxy]-, trimethylsilyl ester	5.5	7.01	310.53	C15H26O3Si2
9	Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester	3.82	6.22	356.4	C23H20N2O2
10	2,3,4,5,6-Pentafluorobenzyl Crotonate	3.51	5.29	266	C11H7F5O2

Table 3: Details of computed descriptors of Phytochemical constituents of *Moringa oleifera*

S.No	Name of the Compound	IUPAC Name	SMILES	Reported activity/ Applications
1.	Butane, 1,1-diethoxy-3-methyl-	1,1-diethoxy-3-methylbutane	CCOC(CC(C)C)OCC	Flavoring agent in distilled beverages [26] Food additives [27]
2.	Neophytadiene	7,11,15-trimethyl-3-methylidenehexadec-1-ene	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C	Carminative, Anti-ulcerative, Gastrin inhibitor, Anti-protozoal (Leishmania), Histamine release inhibitor , Anti-parasitic, anti-

				inflammatory and antimicrobial agents[28] Antipyretic, Analgesic, Antioxidant activity[29]
3.	Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	[(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl] acetate	CC(C)CCCC(C)CCCC(C)CCCC(=CCOC(=O)C)C	Antimicrobial & Anti-inflammatory [30]
4.	Tetracosane	Tetracosane	CCCCCCCCCCCCCCCCCCCCCCCC	Anti-inflammatory[31], Antioxidant and Antimicrobial [32]
5.	Docosane	Docosane	CCCCCCCCCCCCCCCCCCCCCC	Anti-tuberculostatic activity[33,] Anti-bacterial & anti-oxidant[34]
6.	Succinic acid, eicosyl 3-methylbut-3-enyl ester	1-O-icosyl 4-O-(3-methylbut-3-enyl) butanedioate	CCCCCCCCCCCCCCCCCCCCOC(=O)CCC(=O)OCCC(=C)C	Anticancer activity [35]
7.	Hexatriacontane	Hexatriacontane	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Anti-inflammatory & Antimicrobial[36]
8.	Benzenepropanoic acid,.alpha.-[(trimethylsilyl)oxy]-, trimethylsilyl ester	trimethylsilyl (2Z)-3-phenyl-2-trimethylsilyloxyiminopropanoate	C[Si](C)(C)OC(=O)C(=NO[Si](C)(C)C)CC1=CC=CC=C1	Anti- microbial , Antifungal and Anti-inflammatory [37]
9.	Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester	ethyl 2-(9-phenyliminoacridin-10-yl)acetate	CCOC(=O)CN1C2=CC=CC=C2C(=NC3=CC=CC=C3)C4=CC=CC=C41	Anticancer and anti-inflammatory[38]
10.	2,3,4,5,6-Pentafluorobenzyl Crotonate	**-**	**-**	No reported activity Structure drawn using Chemsketch

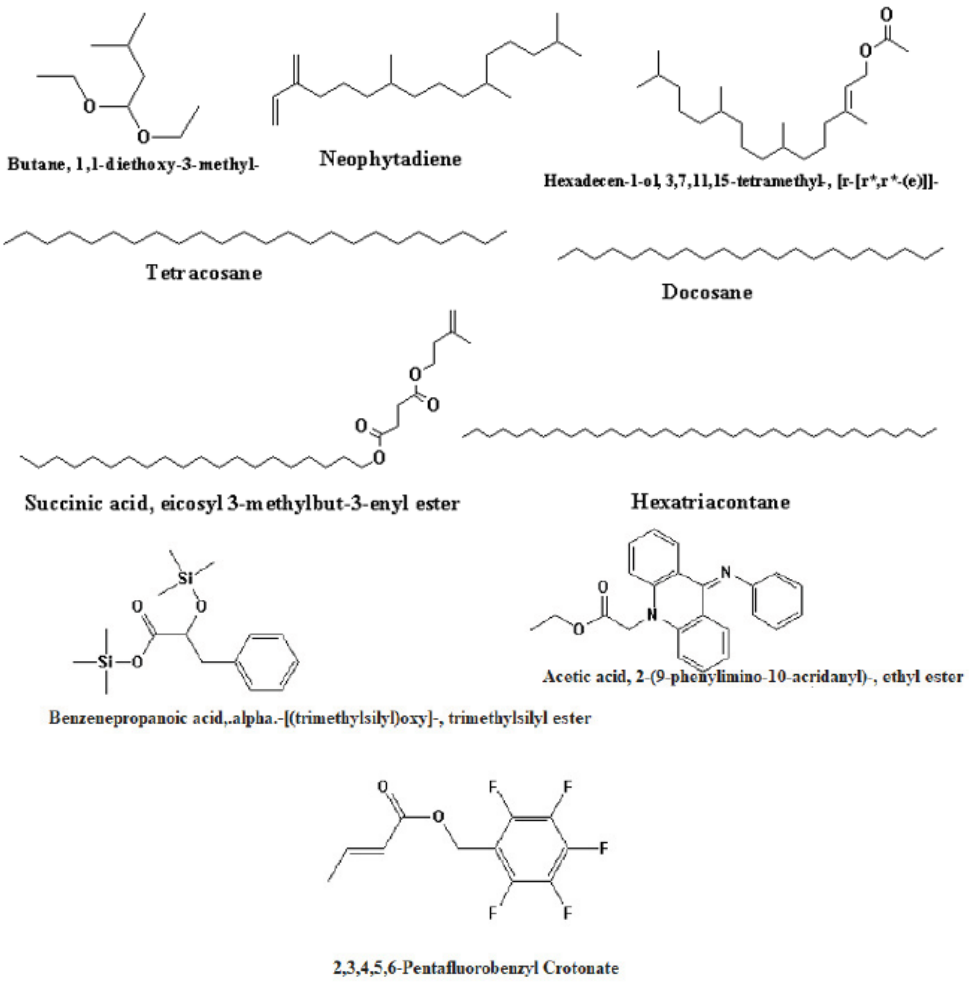
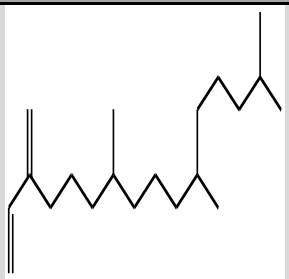
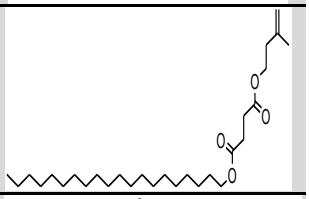
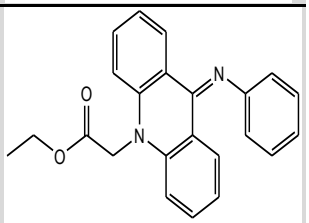


Fig 3 : 2D structure of bioactive compounds taken for the present study

Table 4 : Selected compounds for docking study

S. No	Compound Name	Pubchem ID	SMILES	IUPAC Name	2D Structure
1	Neophytadiene	CID 10446	CC(C)CCCC(C)CCCC(C)CCCC(=Cxdd)C=C	7,11,15-trimethyl-3-methylidenehexadec-1-ene	
2	Succinic acid, eicosyl 3-methylbut-3-enyl ester	Open Babel 07212212052D	O=C(OCCCCCCCCCCCCCCCCCC)CCC(OCCC(C)=C)=O	4-O-(3-methylbut-3-enyl) 1-O-octadecyl butanedioate	
3	Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester	CID 632055	CCOC(=O)CN1C2=CC=CC=C2C(=NC3=CC=CC=C3)C4=CC=CC=C41	ethyl 2-(9-phenylimino acridin-10-yl)acetate	

3.3. Anticancer activity – In silico Analysis

The inhibitory impact of selected compounds identified in *M. oleifera* leaf extract was evaluated using molecular docking. The binding affinity and interactions of selected compounds in the active region of β - catenin, which is responsible for the early stages of Colorectal cancer, were determined using molecular docking[39]. The higher the negative docking scores, the better

the binding affinity. The chosen three compounds derived from the aqueous extracts showed good binding affinity against beta-catenin (PDB ID: 1jdh), as summarized in Table 5. The interaction between the three docking complexes has been contrasted based on their binding energies, which in turn involve hydrogen bonding and other bonding between proteins and ligands[40].The interaction between the compounds Acetic acid 2-(9-phenylimino-10-acridanyl)-, ethyl ester provided the most potent binding affinity against beta-catenin (-6.4 kcal/mol) than the other two compounds, and the complex formed three hydrogen bonds, namely ASN A:415, ARG A:386; the other interactions observed in the complex are Pi-Pi (TRP A:383), Pi-Anion (GLU B:28), Pi-Alkyl (CYS A: 419) (Figure 4a & 4b). In contrast, the docking scores for succinic acid and eicosyl 3-methylbut-3-enyl ester were -5.7 kcal/mol with three hydrogen bonds, i.e., TRP A:338; ASN A:387; and ARG A:386 (Figure 5a & 5b). The docking scores of Neophytadiene (-5.0 kcal/mol) indicate a lower binding affinity with no bonding interactions (Figure 6a and 6b). Consequently, these docking scores exposed that the compounds found within *M. oleifera* have minimum docking scores and high binding affinity with the target β - catenin. According to Mumtaz, phenolic compounds such as gallic acid, p-coumaric acid, and 4-hydroxy-3-methoxycinnamic acid exhibited − 5.8, − 5.6, and − 5.7 Kcal/mol, respectively. Docking results justified those compounds exhibited good docking energy values Kcal/mol[41]. In our research shows less than -5 indicates hills region having more potential than normal region.

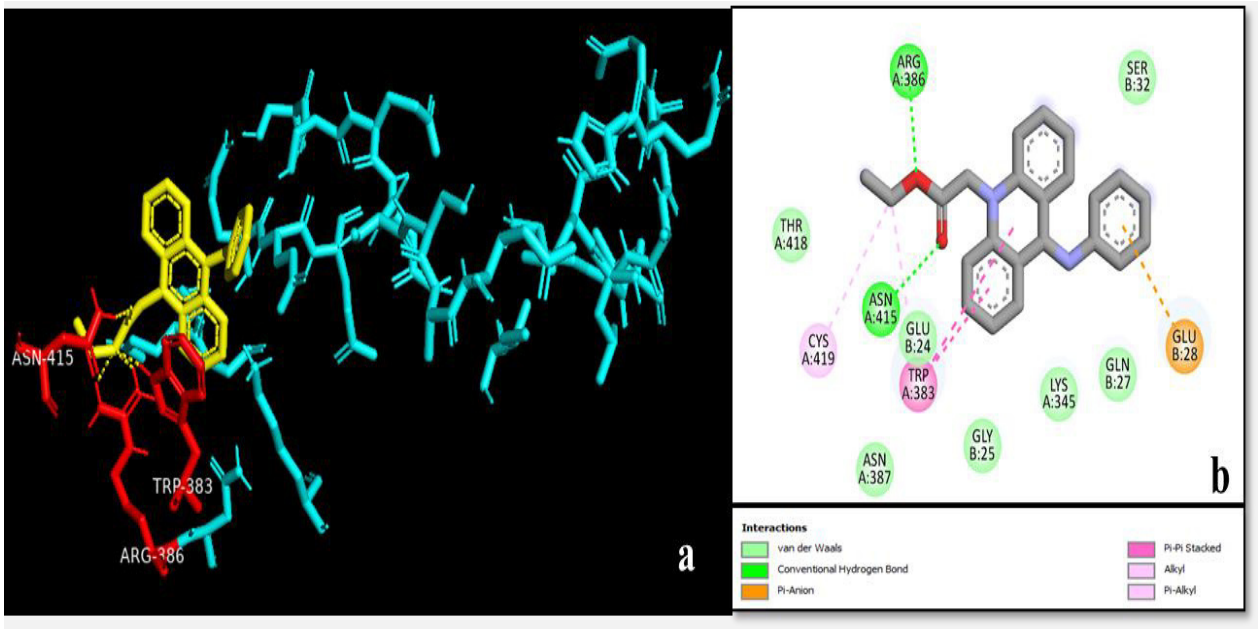


Fig 4 a): 3D view of the best selected conformation of Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester(yellow), hydrogen bonding interactions(red), against the target β -catenin(cyan) 4 (b) 2D view of the binding site interactions.

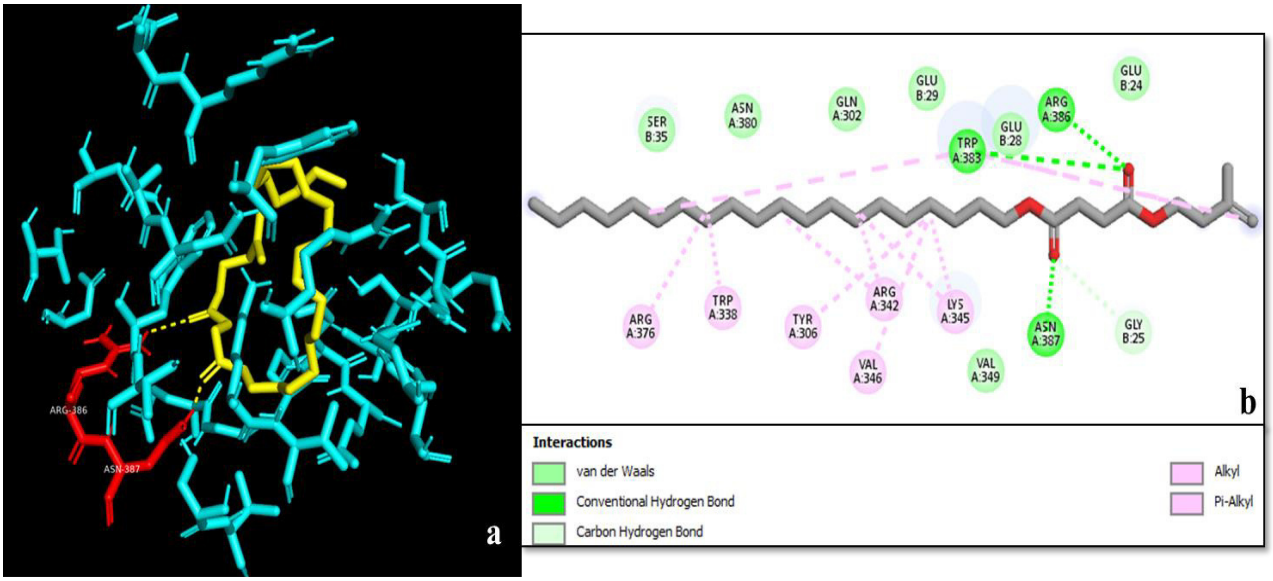


***Fig 5 (a)* 3D view of the best selected conformation of Succinic acid, eicosyl 3-methylbut-3-enyl ester (yellow), hydrogen bonding interactions(red), against the target β -catenin(cyan) 5 (b) 2D view of the binding site interact**

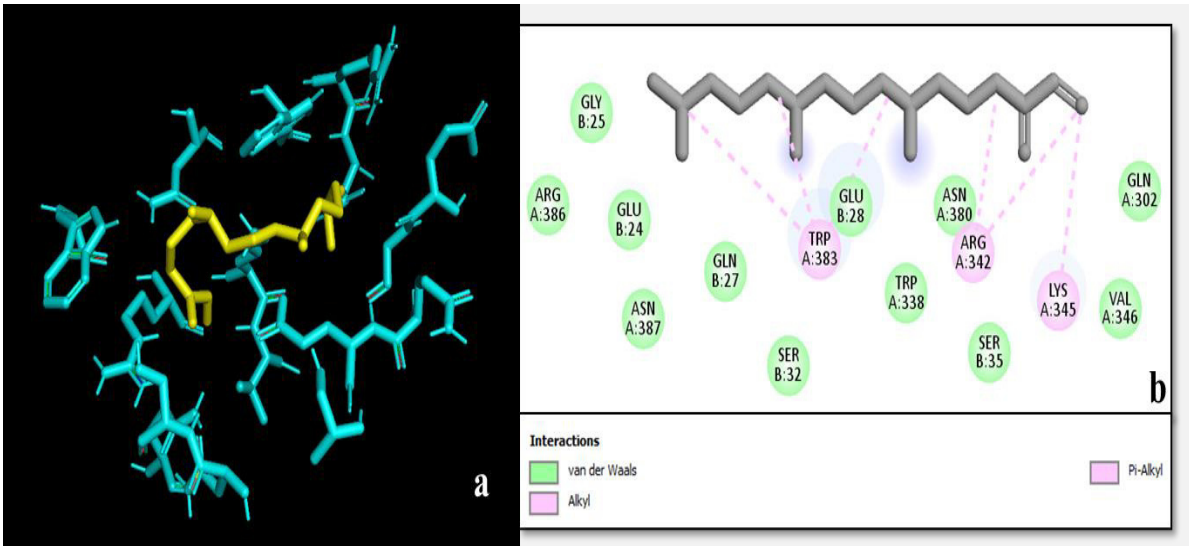


Fig 6 (a) 3D view of the best selected conformation of Neophytadiene (yellow), against the target β - catenin(cyan) 6 (b) 2D view of the binding site interactions

Some investigation revealed that the structural details of flavonoids, the molecular basis of β-catenin/TCF4 inhibition. The Wnt/-catenin signaling pathway is a known cause of colorectal cancer and is among the most prominent signaling pathways[42]. The modification and degradation of β -catenin, a functional effector molecule of Wnt signaling, are critical events in the Wnt signaling pathway and the development and progression of Colorectal cancer[43]. As a result, the Wnt signaling pathway is crucial in disease pathogenesis, particularly in the pathogenesis of Colorectal cancer (CRC). Inhibition of β - catenin significantly reduces the viability of β - catenin driven colorectal cancer cells[44]. Therefore, based on the molecular docking analysis the compound Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester inhibits β--catenin with minimal binding score and has more affinity towards the target for inhibition (Figure. 7) . Hence the compound (Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester) deserves further consideration in the design and development of new inhibitors for the treatment of Colorectal cancer.

Table 5: The docking scores and the interacting residues of the compounds found within *Moringa oleifera L.* extracts against the β - catenin protein

S.No	Protein ID	Compound Name	Binding energy (kcal/mol)	H-bonding interactions	Other interactions
1.	1jdh	Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester	**-6.4**	(ASN A:415, ARG A:386)	Pi – Pi (TRP A:383), Pi-Anion(GLU B:28), Pi-Alkyl(CYS A: 419)
2.	1jdh	Succinic acid, eicosyl 3-methylbut-3-enyl ester	**-5.7**	(TRP A:338, ASN A:387, ARG A: 386)	Pi – Pi (TRP A: 383, TYR A:306, VAL A:346, ARG A:342, LYS A:34VAL A:349) VW (GLU B:28, ASN A:385)
3.	1jdh	Neophytadiene	**-5.0**	----------	Pi – Pi (TRP A: 383—GLU :28, ARG A:382, LYS A:345)

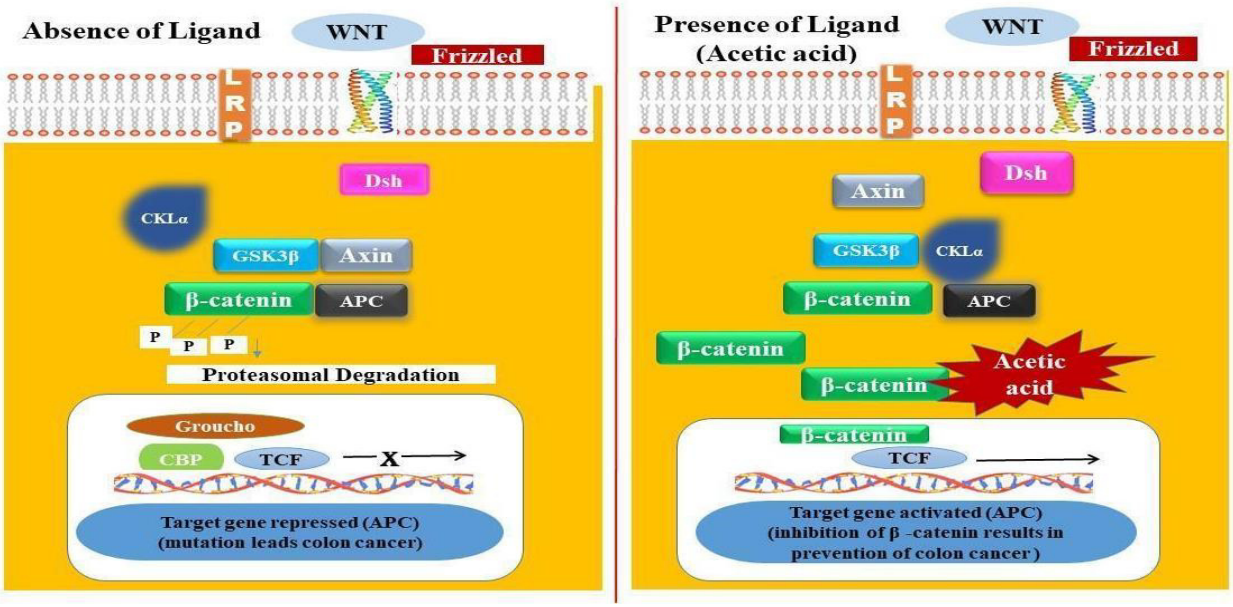


Fig 7: Wnt/β -catenin signaling proposed pathway with *M. oleifera* bioactive compound Acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester

4. CONCLUSION

The search for newer treatments has always been influenced by the cultural understanding of medicinal plants. The precise mechanism of this irony has yet to be discovered. Environmental factors and geographical locations affect the nutrient levels of the leaves and other parts of any plant. Our research revealed that *M. oleifera* leaves from the Kolli Hills region have rich phytochemical components exhibiting antimicrobial and anticancer properties. This is confirmed by the therapeutic potential of secondary metabolites in *Moringa oleifera* leaves through phytochemical characterization and GC-MS analysis. We have reported the binding capabilities of phytocompounds such as acetic acid, 2-(9-phenylimino-10-acridanyl)-, ethyl ester, succinic acid, and Neophytadiene with β -catenin in the context of Colorectal cancer for future study and assessment towards possible therapy.

5. AUTHORS CONTRIBUTION STATEMENT

The study was conceptualised and designed by Dr. Jasmine and S. Sowmiya, and the phytochemical analysis was performed by S. Sowmiya and V. Bharathi. M. Keerthiga and S. Sowmiya interpreted the phytochemical compounds using GC-MS analysis, and Dr. Sherlin Rosita evaluated the *in-silico* analysis. All authors read and approved the final version of the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

7. REFERENCES

1. Ullah R, Alqahtani AS, Noman OMA, Alqahtani AM, Ibenmoussa S, Bourhia M. A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. Saudi J Biol Sci. 2020 Oct 1;27(10):2706-18. doi: 10.1016/j.sjbs.2020.06.020, PMID 32994730.
2. Gamal EEG, Khalifa SAK, Gameel AS, Emad MA. Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia. J Med Plants Res. 2010 Dec 18;4(24):2680-3. doi: 10.5897/JMPR09.556.
3. Purena R, Seth R, Bhatt R. Protective role of *Emblica officinalis* hydro-ethanolic leaf extract in cisplatin induced nephrotoxicity in Rats. Toxicol Rep. 2018 Jan 1;5:270-7. doi: 10.1016/j.toxrep.2018.01.008, PMID 29487802.
4. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: a review on nutritive importance and its medicinal application. Food Sci Hum Wellness. 2016 Jun 1;5(2):49-56. doi: 10.1016/j.fshw.2016.04.001.
5. Tanga TT. *Moringa oleifera* as a Gift of Nature to Human Beings. Int J Pharm Bio Med Sci. 2022. Apr 19;2(4):50-6.
6. Verma AR, Vijayakumar M, Mathela CS, Rao CV. S, & Rao C. V. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem Toxicol. 2009;47(9):2196-201. doi: 10.1016/j.fct.2009.06.005, PMID 19520138.
7. Bharali R, Tabassum J, Azad MR. Chemomodulatory effect of *Moringa oleifera*, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pac J Cancer Prev. 2003;4(2):131-9. PMID 12875626.
8. Adeyemi S, Larayetan R, Onoja AD, Ajayi A, Yahaya A, Ogunmola OO et al. Anti-hemorrhagic activity of ethanol extract of *Moringa oleifera* leaf on envenomed albino rats. Sci Afr. 2021 Jul 1;12:e00742. doi: 10.1016/j.sciaf.2021.e00742.
9. Rode SB, Dadmal A, Salankar HV. Nature's gold (*Moringa oleifera*): miracle properties. Cureus. 2022 Jul 7;14(7):e26640. doi: 10.7759/cureus.26640, PMID 35949760.
10. Mushtaq BS, Hussain MB, Omer R, Toor HA, Waheed M, Shariati MA et al. *Moringa oleifera* in Malnutrition: A Comprehensive Review. Curr Drug Discov Technol.

2021;18(2):235-43. doi: 10.2174/1570163816666191105162722, PMID 31692437.
11. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. *Moringa oleifera* as an anti-cancer agent against breast and colorectal cancer cell lines. PLOS ONE. 2015 Aug 19;10(8):e0135814. doi: 10.1371/journal.pone.0135814, PMID 26288313.
12. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M. The global burden of Colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN *Gut*, Published online 8; 2022 [cited September 2022]. Available from: http://dx.doi.org/10.1136/gutjnl-2022-327736.
13. Parker TW, Neufeld KL. APC controls Wnt-induced β-catenin destruction complex recruitment in human colonocytes. Sci Rep. 2020 Feb 19;10(1):2957. doi: 10.1038/s41598-020-59899-z, PMID 32076059.
14. Abdallah EM. Antibacterial properties of leaf extracts of *Moringa oleifera Lam.* growing in Sudan. J Adv Med Pharm Sci. 2016;5(1):1-5. doi: 10.9734/JAMPS/2016/21386.
15. M. Fahal E, Babitha Rani AM, Aklakur MD, Chanu TI, Saharan N. Qualitative and Quantitative phytochemical analysis of *Moringa oleifera* (Lam.) Pods. IntJCurrMicrobiolAppSci. 2018;7(5):657-65. doi: 10.20546/ijcmas.2018.705.080.
16. Olivier MT, Muganza FM, Shai LJ, Gololo SS, Nemutavhanani LD. Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of *Asparagus suaveolens* aerial parts. S Afr J Bot. 2017 Jan 1;108:41-6. doi: 10.1016/j.sajb.2016.09.014.
17. Bhalla N, Ingle N, Patri SV, Haranath D. Phytochemical analysis of *Moringa oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications. Saudi J Biol Sci. 2021 Dec;28(12):6915-28. doi: 10.1016/j.sjbs.2021.07.075. PMID 34866991, PMCID PMC8626243.
18. Graham TA, Ferkey DM, Mao F, Kimelman D, Xu W. Tcf4 can specifically recognize β-catenin using alternative conformations. Nat Struct Biol. 2001 Dec;8(12):1048-52. doi: 10.1038/nsb718, PMID 11713475.
19. Prasanth DSNBK, Murahari M, Chandramohan V, Guntupalli C, Atmakuri LR. Computational study for identifying promising therapeutic agents of hydroxychloroquine analogues against SARS-CoV-2. J Biomol Struct Dyn. 2022;40(22):11822-36. doi: 10.1080/07391102.2021.1965027. PMID 34396938.
20. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comp Chem. 2010 Jan 30;31(2):455-61. doi: 10.1002/jcc.21334, PMID 19499576.
21. Bhadresha K, Thakore V, Brahmbhatt J, Upadhyay V, Jain N, Rawal R. Anticancer effect of *Moringa oleifera* leaves extract against lung cancer cell line via induction of apoptosis. Advances in Cancer Biology - Metastasis. 2022 Dec 1;6:100072. doi: 10.1016/j.adcanc.2022.100072.
22. Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: overview. Curr Trends Biotechnol Pharm. 2015;9(3):293-304.
23. Abd Rani NZ, Husain K, Kumolosasi E. *Moringa* Genus: a review of Phytochemistry and Pharmacology. Front Pharmacol. 2018 Feb 16;9:108. doi: 10.3389/fphar.2018.00108, PMID 29503616, PMCID PMC5820334.
24. Bhattacharya A, Tiwari P, Sahu PK, Kumar S. A Review of the Phytochemical and Pharmacological Characteristics of *Moringa oleifera*. J Pharm Bioallied Sci. 2018 Oct-Dec;10(4):181-91. doi: 10.4103/JPBS.JPBS_126_18, PMID 30568375, PMCID PMC6266645.
25. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014 Dec 1;46:412-29. doi: 10.1016/j.foodcont.2014.05.047.
26. Bharat CR, Krishna GD. GC-MS Analysis of Young Leaves of Allophylus cobbe (L.) Raeusch. and Allophylus serratus (Roxb.) Kurz. Indian J Pharm Educ Res. 2017 Jul 1;51(3):472-9. doi: 10.5530/ijper.51.3.75.
27. Chandra Mo S, Anand T. Comparative Study of Identification of Bioactive Compounds from Barringtonia acutangula Leaves and Bark Extracts and its Biological Activity. J Appl Sci. 2019 May;19(6):528-36. doi: 10.3923/jas.2019.528.536.
28. Adnan M, Nazim Uddin Chy M, Mostafa Kamal ATM, Azad MOK, Paul A, Uddin SB et al. Investigation of the Biological Activities and Characterization of Bioactive Constituents of Ophiorrhiza rugosa var. prostrata (D.Don) & Mondal Leaves through In Vivo, In Vitro, and In Silico Approaches. Molecules. 2019 Apr 8;24(7):1367. doi: 10.3390/molecules24071367, PMID 30965575.
29. Al-Rajhi AMH, Qanash H, Almuhayawi MS, Al Jaouni SK, Bakri MM, Ganash M et al. Molecular interaction studies and phytochemical characterization of *Mentha pulegium L.* constituents with multiple biological utilities as antioxidant, antimicrobial, anticancer and anti-hemolytic agents. Molecules. 2022 Jul 28;27(15):4824. doi: 10.3390/molecules27154824, PMID 35956775.
30. El-Shahaby O, El-Zayat M, El-Fattah A, El-Hefny M. Evaluation of the biological activity of *Capparis spinosa var. aegyptiaca* essential oils and fatty constituents as Anticipated Antioxidant and antimicrobial Agents. Prog Chem Biochem Res. 2019;2(4):211-21. doi: 10.33945/SAMI/PCBR.2019.4.6.
31. Suganandam K, Jeevalatha A, Kandeepan C, Kavitha N, Senthilkumar N, Sutha S et al. Profile of phytochemicals and GCMS analysis of bioactive compounds in natural dried-seed removed ripened pods methanolic extracts of *Moringa oleifera*. J Drug Delivery Ther. 2022 Oct 15;12(5-S):133-41. doi: 10.22270/jddt.v12i5-S.5657.
32. Paudel MR, Chand MB, Pant B, Pant B. Assessment of antioxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. Biomolecules. 2019 Sep 12;9(9):478. doi: 10.3390/biom9090478, PMID 31547263.
33. Fandaklı S, Yaylı N, Kahriman N, Uzunalioğlu E, Ulaş Çolak NU, Yıldırım S et al. The chemical composition of volatile oil and biological activity of *Rhododendron caucasicum*. Rec.Nat.Prod.. 2019 Jul 1;13(4):316-23. doi: 10.25135/rnp.107.18.09.952.
34. Mollica A, Stefanucci A, Macedonio G, Locatelli M, Luisi G, Novellino E et al. Chemical composition and biological activity of *Capparis spinosa L.* from Lipari Island. S Afr J Bot. 2019 Jan 1;120:135-40. doi: 10.1016/j.sajb.2018.02.397.
35. Kasarci G, Ertugrul B, Iplik ES, Cakmakoglu B. The apoptotic efficacy of succinic acid on renal cancer cell

lines. Med Oncol. 2021 Oct 23;38(12):144. doi: 10.1007/s12032-021-01577-9, PMID 34687367.
36. Taha H, Awang-jamil Z, Aminuddin MF, Basri AM, Zaidi BQ, Ahmad N. Phytochemicals and antimicrobial analysis of selected medicinal plants from *Brunei Darussalam*. Biodiversitas. 2021 Jan 13;22(2). doi: 10.13057/biodiv/d220211.
37. El Moussaoui A, Bourhia M, Jawhari FZ, Salamatullah AM, Ullah R, Bari A et al. Chemical profiling, antioxidant, and antimicrobial activity against drug-resistant microbes of essential oil from *Withania frutescens L.* Appl Sci. 2021 Jan;11(11):5168. doi: 10.3390/app11115168.
38. Mofeed J, Deyab M, Sabry AE, Ward F. In vitro anticancer activity of five marine seaweeds extract from Egypt against human breast and colon cancer cell lines.
39. Rath S, Jagadeb M, Bhuyan R. Molecular docking of bioactive compounds derived from *Moringa oleifera* with p53 protein in the apoptosis pathway of oral squamous cell carcinoma. Genomics Inform. 2021 Dec;19(4):e46. doi: 10.5808/gi.21062. PMID 35012289, PMCID PMC8752987.
40. Shady NH, Mostafa NM, Fayez S, Abdel-Rahman IM, Maher SA, Zayed A et al. Mechanistic wound healing and antioxidant potential of *Moringa oleifera* Seeds extract supported by metabolic profiling, in silico network design, molecular docking, and in vivo studies. Antioxidants (Basel). 2022 Sep 1;11(9):1743. doi: 10.3390/antiox11091743, PMID 36139817.
41. Mumtaz MZ, Kausar F, Hassan M, Javaid S, Malik A. Anticancer activities of phenolic compounds from *Moringa oleifera leaves*: *in vitro* and *in silico* mechanistic study. Beni Suef Univ J Basic Appl Sci. 2021 Dec;10(1):1-.
42. Oliveira LFS, Predes D, Borges HL, Abreu JG. Therapeutic potential of naturally occurring small molecules to target the Wnt/β-catenin signaling pathway in colorectal cancer. Cancers. 2022 Jan 14;14(2):403. doi: 10.3390/cancers14020403, PMID 35053565.
43. Moreno-Jiménez MR, Rocha-Guzmán NE, Larrosa M, Bressa C, Segura-Sánchez CA, Macías-Salas A et al. Constituents of *Quercus eduardii* leaf infusion: their interaction with gut microbiota communities and therapeutic role in Colorectal cancer. J Funct Foods. 2022 Oct 1;97:105258. doi: 10.1016/j.jff.2022.105258.
44. Schatoff EM, Leach BI, Dow LE. Wnt signaling and colorectal cancer. Curr Colorectal Cancer Rep. 2017 Apr;13(2):101-10. doi: 10.1007/s11888-017-0354-9. PMID 28413363, PMCID PMC5391049.