PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTI- NEOPLASTIC POTENTIAL OF *MIRABILIS JALAPA* LEAF EXTRACTS IN HEPATIC AND COLORECTAL CANCER CELLS

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ABSTRACT

This study investigates the phytochemical composition and *in vitro* anti-neoplastic activity of *Mirabilis jalapa* (*M. jalapa*) leaf extracts, focusing on HepG2 (hepatic) and HCT116 (colorectal) cancer cell lines. While extensive research exists on *M. jalapa*, its anti-neoplastic potential in these cancer models remains largely unexplored. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, triterpenoids, steroids, and phenolic compounds in both petroleum ether and 95% ethanol extracts. Based on extractive values and preliminary screening, ethanolic extract of *M. jalapa* (EEMJ) was selected for detailed phytochemical characterization and biological evaluation. TLC analysis using a toluene: ethyl acetate: methanol (5:4:1) mobile phase revealed six distinct spots. Spectral analysis of the isolated compound (MJ I) suggested structural similarity to kaempferol (3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one), a bioactive flavonoid. *In vitro* cytotoxicity studies using the MTT assay demonstrated that EEMJ exhibited stronger anti-neoplastic activity than the petroleum ether extract, with HepG2 cells showing greater sensitivity than HCT116 cells. These findings highlight the therapeutic potential of *M. jalapa* ethanol extract in cancer treatment. Further research, including molecular docking studies, could elucidate its mechanism of action, paving the way for novel nature-derived anticancer therapies.

Keywords: Anti-neoplastic activity, Phytochemical analysis, Molecular docking, HepG2 & HCT116 Cell Lines

INTRODUCTION

India has a rich history of traditional medicine, with systems such as Ayurveda, Siddha, Unani, and Homeopathy playing vital roles in healthcare. The country's Materia Medica extensively documents the therapeutic potential of natural substances. Despite advancements in modern medicine, traditional practices remain widely used, emphasizing the importance of natural remedies.Ayurveda, a holistic system rooted in Indian tradition, has significantly influenced medical practices. With over 800 plant species used in traditional healing, the exploration of herbal medicine continues to expand. However, the need for standardization and harmonization has emerged due to inconsistencies in preparation and quality control. [1]

The World Health Organization (WHO) defines herbal medicines as products derived from plant sources that offer therapeutic benefits. Herbal medicine classifications include raw plant materials, processed plant substances, and therapeutic formulations. Historical records from India, China, Egypt, Greece, and Rome highlight the longstanding use of medicinal plants. Texts such as the Charaka Samhita and Sushruta Samhita provide valuable insights into their applications.[2]

Herbal vs. Synthetic Medicines

While synthetic drugs have revolutionized healthcare, they often come with adverse effects. Herbal medicines, on the other hand, work in harmony with the body's natural functions, offering fewer side effects. Many modern pharmaceuticals are derived from plant sources, including morphine, aspirin, and atropine. Despite their efficacy, herbal medicines require scientific validation to ensure safety and effectiveness. Advances in technology have facilitated the extraction and identification of bioactive compounds, reinforcing the credibility of plant-based treatments. [3]

Herbal medicines against synthetic medicines

Although employing herbs for therapeutic purposes should be done carefully, they are frequently safer than medications. Both conventional drugs and natural therapies can be beneficial and harmful in the absence of professional guidance or knowledge because they create distinct physiological changes. Herbs support the body's natural processes, in contrast to the synthetic ingredients included in the majority of contemporary medications. Throughout history, plant-based diets and herbal medicines have proven to be effective. We can now recognize, separate, and extract essential plant components because of sophisticated technology. Pharmaceutical companies either make synthetic versions of medications using chemicals or extract them from plants, which can have unfavorable side effects when administered to patients.

For scientific research and medical applications, they believe it would be preferable to employ refined chemical compounds rather than the entire plant. After removing what they refer to as the "active" sections of the plants, they discard the other plants in this manner. They therefore referred to the other principles as "inactive" and firmly believed that employing the entire plant was a more sophisticated and scientific method than using these manufactured goods.

The two safest methods to use herbs are to buy certified organic items from a reliable supplier or produce your own. The quality, purity, strength, and labeling of pharmaceuticals are standardized in the US. Herbal remedies appear to be the most effective at treating persistent, recurring issues. The majority of herbal medicines can have significant effects with a lower risk of adverse side effects. Nevertheless, natural therapies, like synthetic drugs, have drawbacks. Herbal remedies can also be harmful if the incorrect herbs are suggested if low-quality herbs are utilized, or if insufficient amounts are given. [4]

Traditional Medicine and Cancer Treatment

Cancer remains a leading cause of mortality, necessitating novel therapeutic approaches. Traditional medicine offers a potential avenue for discovering anti-cancer compounds. Medicinal plants have contributed significantly to oncology, with drugs like

paclitaxel (from Taxus brevifolia) emerging from natural sources. Herbal therapies demonstrate cytotoxic, antioxidant, and immunomodulatory properties, making them promising candidates for cancer treatment. [5]

Detection of cancer

Early detection and prompt treatment are directly responsible for increased survival rates. Tools for cancer detection include-

- Self-exam
- Biopsy (the removal of living tissue for microscopic examination of cells)
- Ultrasound (the use of reflected high-frequency sound waves to differentiate various kinds of tissue)
- Computed tomography (CT) (the use of X-rays to produce a cross-sectional picture of body parts)
- Magnetic resonance imaging (MRI) (the use of magnetic fields and radio waves to show changes in soft tissue without the use of X-rays).[6-7]

Liver and Colorectal Cancer

Liver cancer, particularly hepatocellular carcinoma (HCC), poses a significant health burden. India contributes 18% of global liver cancer cases, with low survival rates despite advancements in treatment. The incidence of HCC continues to rise, emphasizing the need for effective interventions. Similarly, colorectal cancer originates from abnormal cell growth in the colon or rectum. Risk factors include genetic mutations, dietary habits, and lifestyle choices. Traditional medicine, with its emphasis on plant-derived compounds, offers potential solutions for cancer prevention and management.[8]

MATERIALS AND METHODS

Plant Collection and Authentication The leaves of *Mirabilis jalapa* were collected from Kumarapalayam, Namakkal District, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. Rajan, Officer in Charge (Rtd), Centre of Medicinal Plants Research in Homeopathy, Government of India. A specimen was deposited at the Pharmacognosy Lab, JKKN College of Pharmacy.[9]

Extraction Process The collected leaves were washed, shade-dried for one week, and pulverized to a fine powder. The powdered leaves (1 kg) were extracted using a Soxhlet apparatus with petroleum ether and 95% ethanol. The extracts were concentrated using a rotary vacuum evaporator, and their percentage yields were calculated.[10]

Phytochemical Screening Preliminary phytochemical analysis was conducted to identify active constituents such as alkaloids, flavonoids, tannins, saponins, and glycosides.

Tests including Dragendorff's, Mayer's, and Wagner's were used for alkaloids, while flavonoids and tannins were detected using Shinoda's and Ferric Chloride tests.[11]

Chromatographic Separation Thin-layer chromatography (TLC) was performed on silica gel plates using a solvent system of Toluene: Ethyl Acetate: Methanol (5:4:1) to isolate bioactive compounds. Column chromatography was used for further purification, followed by spectral characterization via IR, Mass Spectrometry, and NMR analysis.[12]

In *Vitro* Anticancer Activity The cytotoxic potential of extracts was assessed using the MTT assay on HepG2 (liver cancer) and HCT116 (colorectal cancer) cell lines. Cells were seeded in 96-well plates and treated with varying concentrations (62.5–500 µg/ml) of extracts. After 48 hours, MTT dye was added, and absorbance was measured at 570 nm. Cell viability was calculated using the formula:

% Cell Inhibition = 100 - (Abs (sample) / Abs (control) × 100).

Results were analyzed using Microsoft Excel for dose-response relationships. The extract showing the highest activity was further investigated for bioactive compound identification.[13]

Results:

The present research was designed to evaluate he phytochemical characterization and *vitro*anti-cancer activity of *Mirabilis Jalapa* Linn leaves.

PHASE I: PHYTOCHEMICAL STUDIES

1. Extraction of *M. Jalapa* Plant Leaves

Dried crushed *M. Jalapa* plant leaves were extracted with petroleum ether, and ethanol (95%v/v) continuously with as ox let apparatus and the results were tabulated Table 2.

Table1. Data Showing the Extractive Values of *M. Jalapa* Plant Leaves

S.No	Extract	Color/Physical nature	Percentageyield (% w/w)
1	Petroleum ether	DarkGreen/Semisolid	4.58
2	Ethanol(95%v/v)	Green/Semisolid	6.36

2. Preliminary Phytochemical Screening of M. *Jalapa* Plant Leaf Extracts

The extracts of *M. Jalapa* plant leaves were subjected to qualitative phytochemical screening to identify the active constituents which showed below- mentioned phytoconstituents in Table 3.

Table2. PreliminaryPhytochemicalScreeningof*M.jalapa* Plant Leaf Extracts [14]

1.	**Alkaloids**		
a.	Dragendorff'sTest	-	+
b.	Mayer'sTest	-	+
c.	Hager'sTest	-	+
d.	Wagner'sTest	-	+
e.	TannicAcidTest	-	+
2.	**Saponins**		
a.	FoamTest	-	+
b.	LeadAcetateTest	-	+
c.	HemolyticTest	-	+
3.	**Glycosides**		
a.	Legal'sTest	-	+
b.	BaljetTest	-	+
c.	Keller-KillianiTest	-	+
d.	Borntrager'sTest	-	+
4.	**Carbohydrates**		
a.	Molisch'sTest	+	-
b.	Fehling'sTest	+	-
c.	Benedict's Test	+	-
d.	Tollen'sTest	+	-
e.	Seliwanoff'sTest	+	-
f.	BromineWaterTest	+	-
5.	**Tannins**		
a.	GelatinTest	-	-
b.	FerricChlorideTest	-	-
c.	VanillinHclTest	-	-
d.	LeadAcetateTest	-	-
e.	PotassiumFerric Cyanide Test	-	-
f.	Potassium	-	-

	Dichromate Test		
6.	**Flavonoids**		
a.	Shinoda'sTest	-	+
b.	AlkalineReagentTest	-	+
c.	LeadAcetateTest	-	+
d.	Conc.SulphuricAcid Test	-	+
7.	**Steroids**		
a.	Libermann-Burchard's Test	+	-
b.	Salkowsky'sTest	+	-
8.	**Proteins**		
a.	BiuretTest	-	-
b.	NinhydrinTest	-	-
c.	XanthoproteicTest	-	-
d.	Millon's Test	-	-
9.	**Triterpenoids**		
a.	Knoller's Test	-	+
10.	**FixedOil &Fat**		
a.	Spot Test	+	-
b.	SaponificationTest	+	-

+Present -Absent

3. Thin Layer Chromatography (TLC)

EEMJ was subjected to thin layer chromatography on silica gel G which had shown good resolute ion of solutes system like Toluene: Ethylacetate:Methanol-5:4:1. The different spot developments in each system were identified using a corresponding detecting agent and RF values were calculated and presented in Table 4 and Figure 6. [15]

Table3. Thin layer chromatography of EEMJ [16]

Solvent system	**No. of spots**	**Visualizer**	**RfValues**
Ethyl acetate: Methanol(7:3)	2	TLC	0.61 0.65
Toluene: Ethylacetate(8:2)	2	TLC	0.59 0.66
Ethylacetate: Formic Acid (5:4:1)	3	TLC	0.62 0.64

			0.65
ylacetate: Methanol (5:4:1)	6	TLC	0.52 0.60 0.68 0.84 0.90

Figure 1. Thin layer chromatography of EEMJ

4. Column Chromatography

A cylinder-shaped glass column containing a stationary phase (silica gel) was encountered slowly from the top with a liquid solvent (mobile phase) that flowed down the column with the help of gravity or external pressure applied. This technique was used for the purification of compounds from a mixture. Once the column was ready, the sample EEMJ was loaded inside the top of the column. The mobile solvent was then allowed to flow down through the column. The compounds in the mixture have different interaction abilities with the stationary phase (silica gel), and mobile phase, thereby will flow along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture was achieved. The individual compounds were collected as fractions and analyzed further for structure elucidation.[17]

The isolation of the compound from fractions 33-36 obtained by column chromatography was selected and named MJ I. The compound was subjected to physical and spectral studies to confirm its purity and characterization.

Table 4. Column Chromatography of EEMJ [18]

action No.	Nature of Residue	Analysis by TLC	Colour of the spot	Rf Value
1-4	No residue	---	---	---
5-8	Yellowish Green	---	---	---
9-12	Yellowish Green	1spot with a tailing effect	---	0.61
13-16	No residue	----	---	---
17-20	Light Brown	2spots with a tailing effect	Green Brown	0.69 0.73
21-24	Yellowish Brown	2spots with a tailing effect	Brown Brown	0.75 0.81
25-28	Yellowish Brown	----	----	---
29-32	Yellow	----	----	---
33-36	llow Yellowish Green	1 spot	Brown	0.68
37-40	Light Yellowish Green	1spotwitha tailing effect	Light Yellow	0.64

Spectral Analysis of Isolated Compounds Compound MJ-I:

Compound **MJI** was also obtained as a yellow crystalline powder and its molecular formula was established as **$C_{15}H_{10}O_6$**from its Mass spectral data that showed $[M-H]^-$ion at m/z 284.91 which was further supported by its ^{13}C NMR spectral data. The IR spectra exhibited characteristic bands at 3420.16 cm^{-1} for aromatic –OH groups, 1653.23 cm^{-1} for the C=O group, 1168.20 cm^{-1} for the C-O group, and 1607.54 $cm^{-1}$for the C=C group.

The ^1H NMR spectrum of **MJ I** showed the presence of three meta-coupled aromatic doublets at δ 6.18, 6.43, and 6.87, a singlet at 6.62, one ortho-coupled aromatic proton and one ortho and meta-coupled aromatic proton appeared as a multiple at δ 7.37 are characteristic for a polyphenol. The ^{13}C NMR spectra showed the presence of fifteen aromaticcarbons(Table8).Resultsofspectraldatasuggestedthat**MJI**hadstructural similarities

with 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one which may have the presence of kaempferol.[19-20]

Table5.^{13}C NMR spectral data of compound MJ I

Carbon	**Signal(δ)**	**Carbon**	**Signal(δ)**
2	162.21	1’	121.57
3	105.62	2’	113.98
4	182.46	3’	146.48
5	164.60	4’	150.36
6	99.63	5’	116.70
7	164.82	6’	119.77

STRUCTURE OF KAEMPERFEROL[21]

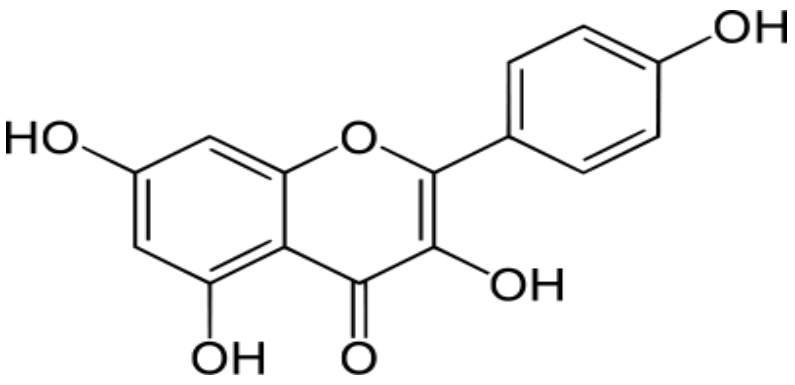


Figure 1. LC-MSofcompoundMJI

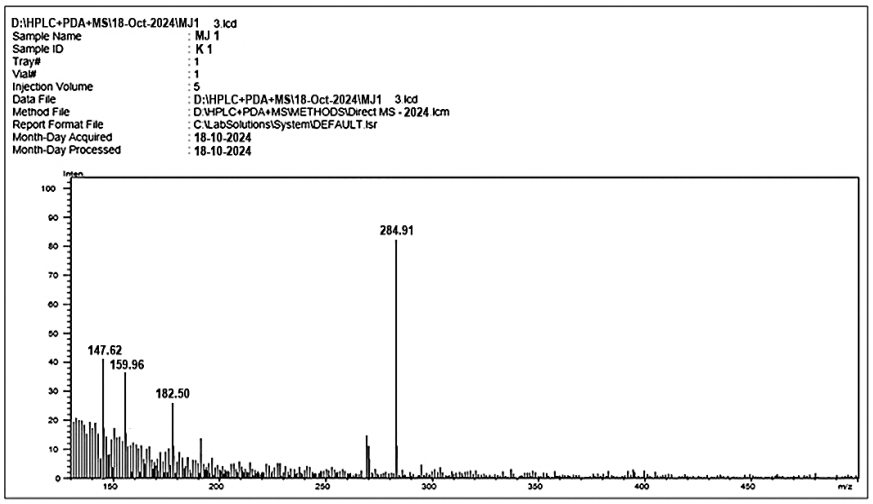
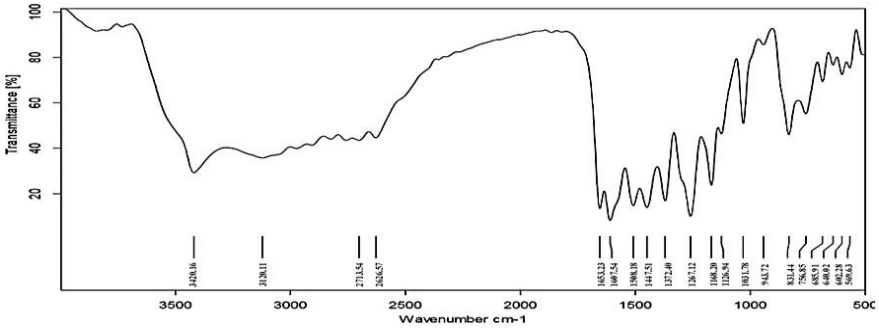


Figure 2. IR spectrum of compound MJI



Sample Name	Description	Saved or unsaved State	Spectrum quality check summary
MJ-1 (Kalai)	Sample 115 By Administrator Date Wednesday, October 09 2024	Saved	The Quality Checks give rise to multiple warnings for the sample.

Figure 3. 1HNMR spectrum of compound MJ

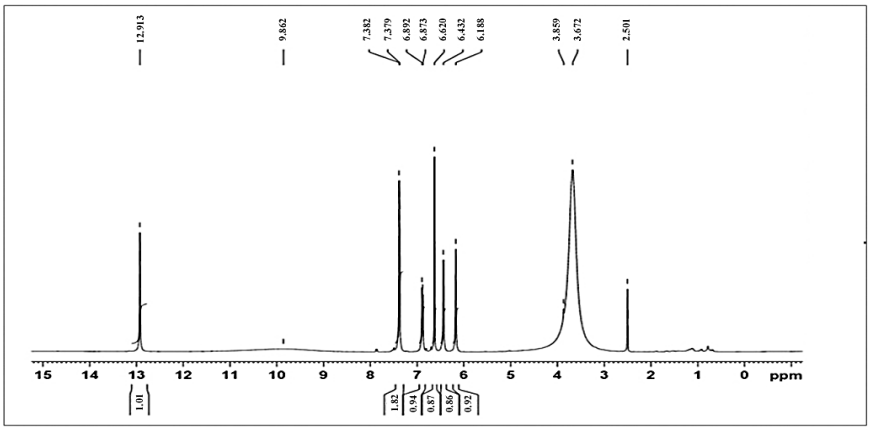
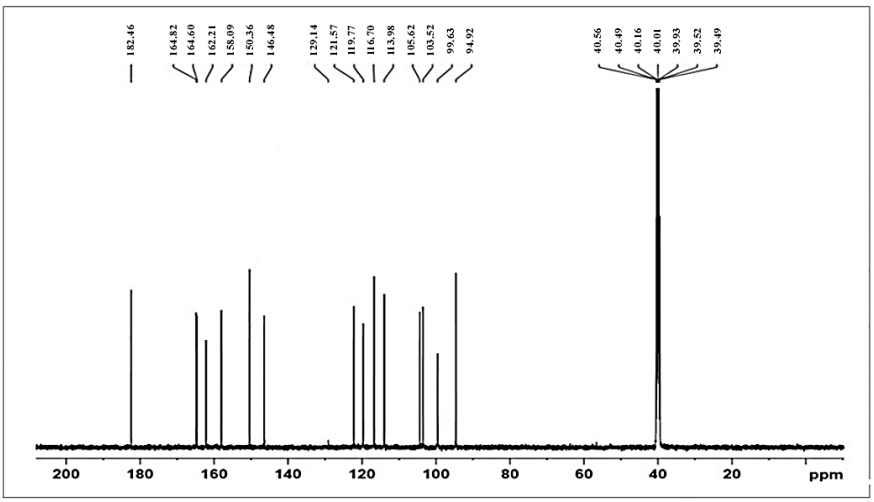


Figure 4. 13CNMR spectrum of compound MJI



PHASE II: PHARMACOLOGICAL STUDIES

In vitro Antineoplastic Activity

The anti-cancer activity of extracts of *M. Jalapa* was studied in a human live cancer cell line (HepG2) and the human colorectal adenocarcinoma cell line (HCT116) using the MTT assay method. The two plant extract swore subjected to HepG2 and HCT116 cell lines at the dose levels 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml, concentrations. The ethanol extract of *M. jalapa* (EEMJ) produced more significant anti-neoplastic activity than the petroleum ether extract in HepG2 and HCT116 cancer cell lines. EEMJ produced more significant anti-neoplastic activity inHepG2 cell lines when compared to HCT116 cancer cell lines.[22]

MTT Assay

MTT is a yellow water-soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple Formosan. Therefore, the amount of Formosan produced is directly proportional to the number of viable cells.

After 48 hr of incubation, 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well and incubated at 37^0 C for 4 hr. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using a microplate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample) / Abs (control) X 100.

A nonlinear regression graph was plotted between % Cell inhibition and was determined using Microsoft Excel software. [23-24]

TableNo.6. %CellInhibitionof*M.jalapa*PlantLeafExtractsin HepG2 Cell Line

	%CellInhibition	
ncentration (µg/ml)	Petroleum Ether Extract	Ethanol Extract
62.5	12.36	28.52
125	23.94	38.65
250	36.27	5.72
500	39.75	70.16
IC 50 Value	**620.42**	**253.24**

Figure 5. *Invitro*anti-neoplasticactivityof*M.jalapa*WholePlantExtractsin HepG2 Cell Line

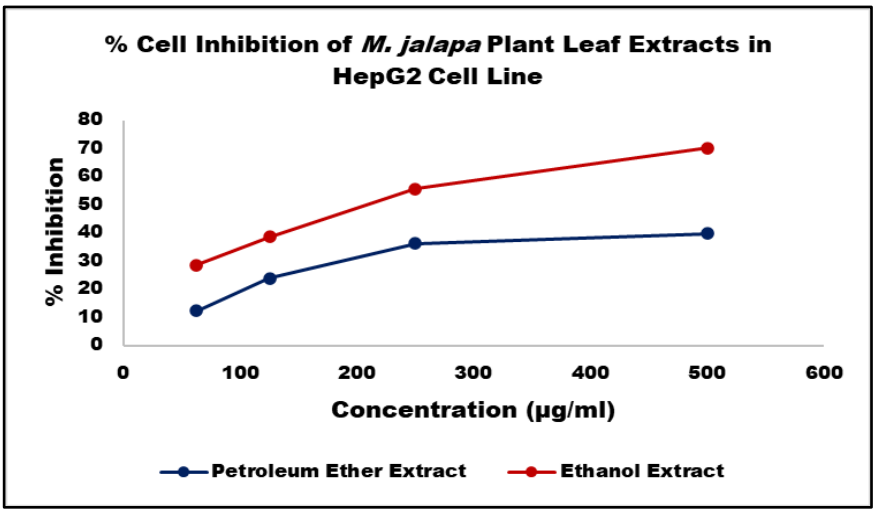
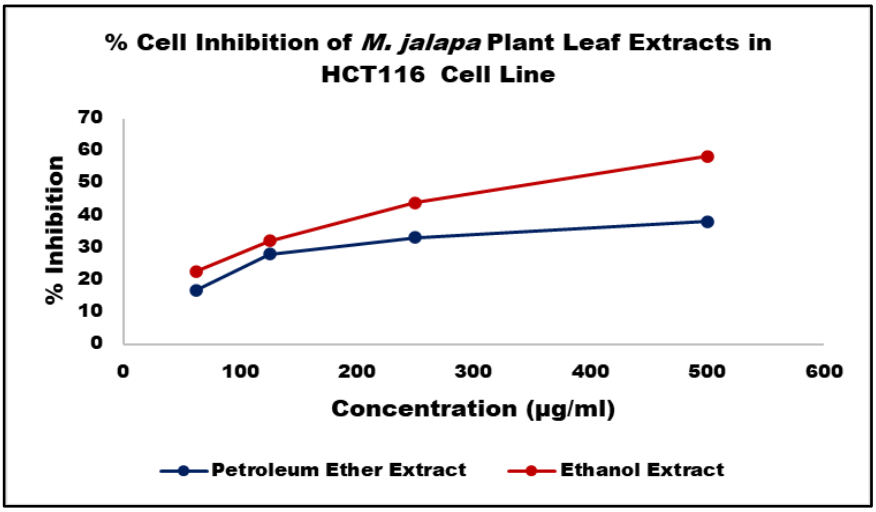


Figure6. *Invitro*anti-neo plastic activity of *M. Jalapa* Whole Plant Extracts in HCT116 Cell Line



CONCLUSION:

The results indicate that EEMJ exhibited significant cytotoxic effects on both HepG2 and HCT116 cell lines, with greater potency against liver cancer cells. The presence of kaempferol suggests that flavonoids contribute to the anticancer activity. The reduction in cell viability is attributed to apoptosis induction and oxidative stress mechanisms. Compared to standard chemotherapy, plant-derived compounds offer a natural alternative with potentially fewer side effects. Further research is needed to evaluate the in vivo effects and molecular pathways involved in *Mirabilis jalapa*'s anticancer activity.

CONFLICT OF INTEREST:

The authors declare no conflict of interest in this study.

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REFERENCES:

1. Patra K, Pareta S, Ranjit K, Harwansh K, Jayaramkumar. Traditional Approaches towards Standardization of Herbal Medicines: A Review. Journal of Pharmaceutical Science and Technology. 2010; 2(11); pp. 372-379.
2. Mukherjee KP. Quality Control of Herbal Drugs, Published by Business Horizons. New Delhi: Pharmaceutical Publishers; 2002;pp.22-24.

3. WHO Technical Report Series. Guidelines for the Assessment of Herbal Medicines.1996; pp.178-184,863.
4. Singh A, Saharan VA, Kharb V, Bhandari A. Current Status of Regulations for Herbal Medicines in Europe, United States and India. Journal of Nature Conscientia. 2011; pp. 406-422.
5. Sakarkar DM, Deshmukh VN. Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity. International Journal of Pharmaceutical Technology and Research. 2011; pp. 298-308.
6. Kleinsmith LJ, Person Benjamin Cummings. Principles of Cancer Biology. 2006; pp. 538-544.
7. Lewis, Sharon, Heitkemper, Margaret, Dirksen, Shannon. Medical-Surgical Nursing, Published by Mosby; 2000; pp. 270-274.
8. Rumzhum NN, Rahman MM, Islam MS. Cytotoxicity and Antioxidant Activity. 2008, Volume 1, Issues 1-2; pp. 85-88.
9. Sony Mone and S. Rajan. “Preliminary Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of Mirabilis jalapa L.". The Indian Pharmaceutical Congress Association proceedings, 2015;pp. C-1 to C-3.
10. S. Rajan, S. Thirunalasundari, and T. Jeeva. Extracts of Mirabilis jalapa., in the International Journal of Chemical Sciences, Volume 8, Issue 1;2010; pp. 561-567.
11. M. Kaur, A. Kaur, and P. Singh. Phytochemical Screening and Physical Constant Evaluation.; in the CABI Digital Library, Issue 1;2019; pp. 1-10
12. Jyotchna Gogoi, Khonamai Sewa Nakhuru, Rudragoud S Policegoudra, Pronodeshchattaopadhyay, Ashok Kumar Rai, Vijay veer. Isolation and characterization of bioactive compounds from *Mirabilis JalapaL.radix* ; 2015; 6(1); pp. 41-47.
13. Md. Mostafizur Rahman, Md. Shahid-Ud-Daula, Md. Zulfiker Mahmud. "Cytotoxicity and Antioxidant Activity of Extractives from Mirabilis *jalapa*" –Stamford Journal of Pharmaceutical Sciences, Volume 4, Issue 1;2011 ;pp. 94-100.
14. C.K. Kokate and A. P. Purohit. Pharmacognosy And Phytopharmaceuticals Textbook(49th Ed., 2014); pp. 284.
15. Hidlebert Wagner, Sabine Bladt, Plant drug Analysis, A thin layer chromatography Atlas, 2nd Edition, Springer Science & Business Media ;1996; pp. 355-367.
16. Peter E. Wall; Royal Society of Chemistry. Thin-Layer Chromatography: A Modern Practical Approach (1st Ed., 2005) ; pp. 56-68

17. Furnise BS, Mannaford AJ, Smith PWG, Tatchell AR, Vogel's Textbook of Practical Organic Chemistry, Pearson Education, 5th Edition, New Delhi; 2005; pp.197- 216.

18. Kalsi PS, Spectroscopy of Organic Compounds, New Age International (P) Ltd, 6th Edition; 2007; pp.65-163.

19. Dhurgham Khalid Abed Sarray, Liliia M.Horiacha, Iryna O. Zhuravel, Andrii I. Fedosov - HPLC Study of Phenolic compounds in *Mirabilusjalapa* raw material-National University of pharmacy,Kharkiv, Ukraine, 2020; pp.145-152.

20. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal of Immunological Methods, 1983; pp. 55-63.

21. Oyvind M. Andersen and Kenneth R. Markham- Flavonoids - Chemistry, Biochemistry and Applications" (1st Ed., 2006) – CRC Press; pp. 190-225.

22. Kokate. C.K, Purohit. A.P. & Gokhale. S.B. Pharmacognosy (54th Edition, Nirali Prakashan), (2019); pp. 556-585.

23. Edmond de Hoffmann and Vincent Stroobant, Mass spectrometry, principles and applications, England, 2nd Edition John Willey and son's Ltd; 2001;pp. 420.

24. Monks A, Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines, Journal of the National Cancer Institute, 1991; pp. 757-76