In- Vitro Anticancer Activity Of *Mimosa Hamata* Against A549, PA-1 And KB-3-1 Cell Lines And HPTLC Studies Of *Mimosa Hamata*

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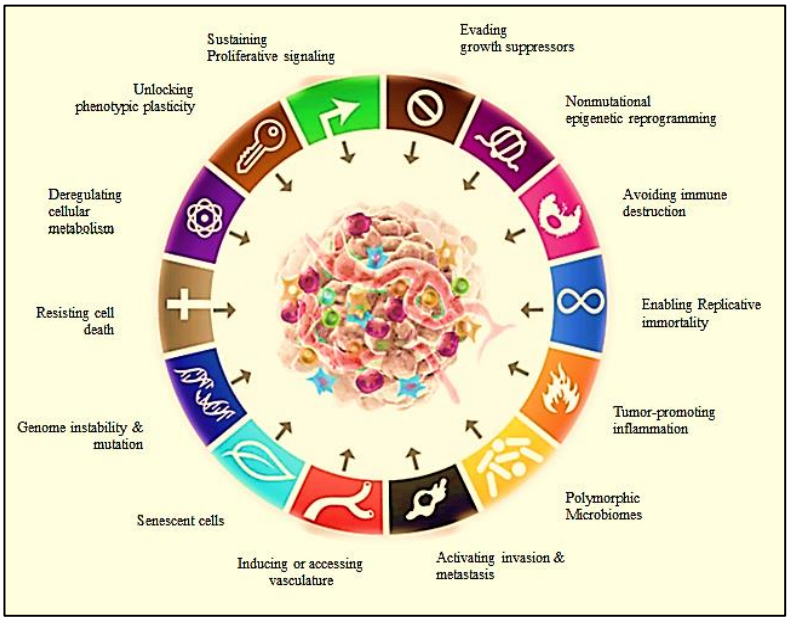
Abstract
The study explores the cytotoxic potential of the ethanol extract of plant *Mimosa hamata* against various cancer cell lines using the MTT assay. The extract demonstrated moderate cytotoxicity with IC50 values of 205.75 ± 3.38 µg/ml for A549 lung cancer.cells and 153.12 ± 2.33 µg/ml for PA-1 ovarian cancer cells. Notably, it exhibited the highest efficacy against KB-3-1 oral cancer cells, achieving an IC50 of 125.60 ± 2.04 µg/ml, indicating strong potential as an anti-cancer treatment, particularly for oral cancer. Additionally, high-performance thin-layer chromatography (HPTLC) fingerprinting revealed seven distinct bands in the extract, with a significant peak at Rf 0.43, likely attributable to quercetin. The presence of quercetin, along with other phytochemicals, suggests a multifaceted mechanism of action contributing to the extract's cytotoxic effects. These findings highlight the promise of the ethanol extract of *Mimosa hamata* as a viable candidate for cancer therapy and underscore the need for further investigations into its therapeutic mechanisms and clinical applications.

Introduction:
Cancer encompasses a wide range of diseases marked by the abnormal and uncontrolled proliferation of cells that arise from specific organs or tissues. These cancerous cells grow and divide uncontrollably, often leading to tumors or spreading to other parts of the body. The cancerous cells do not undergo programmed cell death like normal cell instead they continuously multiply, forming abnormal cells. As cancer cells proliferate, they compete with healthy cells for nutrients, resulting in the formation of tumor [1].
A tumor is a tissue mass resulting from abnormal, excessive, and uncoordinated cell proliferation, continuing even after the original growth stimulus has ceased. Cancer is marked by several distinct capabilities that enable tumor growth and progression. These capabilities often referred to as "hallmarks of cancer," were first outlined by Hanahan and Weinberg and have since been expanded to include emerging hallmarks. The hallmarks include sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, limitless replicative potential, induction of angiogenesis, activation of invasion and metastasis, genome instability and mutation, deregulation of cellular energetics, evasion of immune destruction, tumor-promoting inflammation, unlocking phenotypic plasticity, non-mutational epigenetic reprogramming, polymorphic microbiomes, accumulation of senescent cells [2].



Cancer is a major health challenge and is the leading cause of death and illness worldwide, with the number of cases steadily rising. It is estimated that by 2030, there will be approximately 21 million new cancer cases [3]. According to the Global Cancer Observatory (GLOBOCAN), the global estimate for new cancer cases in 2020 was approximately 19.3 million)[4].

Many anticancer drugs are developed to target cells that divide quickly. However, under normal conditions, various cells in our body also undergo rapid growth and division. Chemically synthesized drugs have been created for cancer treatment, but existing methods like chemotherapy have drawbacks because of their toxic impact on non-cancerous tissues, exacerbating human health issues[5]. Because of the numerous limitations associated with chemotherapy, there is a growing need for drugs that specifically target cancer cells while reducing harm to healthy tissues [6]. Consequently, the demand for alternative treatments, particularly those involving plant-based anticancer agents, is on the rise.

Mimosa hamata belonging to family mimosaceae is medium sized much branched armed shrub upto 2m tall. It has bipinnate leaves with small leaflets, long hooked thorns, compact clusters of pinkish-purple flowers, and cylindrical seed pods. These features make it a visually striking and distinctive plant species. Traditionally the plant is used as a tonic for general weakness and for the treating urinary complaints. Additionally, it is applied to burns and swollen glands[7].

Material and Methods

Collection and Authentication of Plant Material:

The plant *Mimosa hamata* Wild was collected from local area of Lonara, Koradi Nagpur and the plant was verified by Dr. Nitin. M. Dongarwar, Head, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The herbarium sheet of plant specimen was prepared in duplicate and deposited in Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for future reference. The herbarium voucher specimen number was 10568.

Extraction:

The aerial parts of *Mimosa hamata* were shade-dried and subsequently mechanically ground to obtain a coarse powder. Approximately 1 kg of the coarse powdered drug was extracted separately with petroleum ether at temperatures ranging from 50°C to 60°C using hot percolation in a Soxhlet apparatus for 72 hours. After the petroleum ether extraction, the residual marc was dried and then subjected to extraction with 95% ethanol at temperatures between 60°C and 70°C for up to 72 hours in the Soxhlet apparatus.

Phytochemical analysis

The ethanolic extract (EE) was subjected to different qualitative tests to indicate presence or absence of different phytochemical constituents like alkaloids, glycosides, phenolic compounds, flavonoids, saponins, sterols, tannins, fixed oils and fats, protein and amino acids, gum and mucilage and carbohydrates by adopting the standard procedure[8].

Cell viability assay by MTT for *Mimosa hamata*

Requirements

- Growth medium with 10 % FCS
- MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma),
- 5 mg/ml, filter sterilized, dissolved in Phosphate Buffer Solution (PBS)
- Dimethyl sulfoxide (DMSO)
- Dulbecco‟s modified eagle‟s medium (DMEM) (Thermofisher Scientific)
- Serum-free HEK 293 growth medium (Thermofisher Scientific)
- Fetal bovine serum (FBS) Cat No -10270106 (Gibco, Invitrogen)
- Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)
- 96-well plates

Cell culture:

The human lung adenocarcinoma epithelial cell line (A549), ovarian cancer cell line (PA-1) and oral cancer cell line (KB-3-1) were cultured in DMEM with low glucose and supplemented with 10 % FBS 1% penicillin-streptomycin antibiotic-antimycotic 100X solution and incubated in humidified atmosphere of 5 % CO_2 and 37 °C. The culture medium was changed every two days.

Preparation of working concentrations of plant extract:

Working concentrations of extracts of *Mimosa hamata* were prepared by incorporating desired quantity of the alcoholic extract into di-methyl sulfoxide prior to the experiment. The reactant mixtures were diluted with media and cells received treatment with different concentration ranges (6.25–100 µg/ml)

Principle: This colorimetric assay assesses the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. MTT penetrates the cells and enters the mitochondria, where it undergoes reduction yielding, formazan product which is insoluble and is dark purple coloured. Subsequently, the cells are treated with an organic solvent such as DMSO or isopropanol to solubilize the formazan product, which is then quantified spectrophotometrically. Since, MTT reduction exclusively occurs in the cells which are metabolically active, the extent of formazan production serves as an indicator of cellular viability and metabolic activity.

Procedure: MTT assay was used for evaluation of cytotoxicity.

Cells were initially seeded into a 96-well flat-bottom microplate and left to incubate for 12 hours at 37°C in an environment with 95% humidity and 5% CO2. Subsequently, different concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 µg/ml) of ethanolic extract from Mimosa hamata were introduced to individual cells, followed by another 48-hour incubation period. Afterward, the cells in three wells underwent two washes with phosphate buffer solution. Following the washes, 20 µl of MTT staining solution (5 mg/ml in phosphate buffer solution) was incorporated in every well, and the plate was incubated in darkness at 37°C for 4 hours, which results in the appearance of formazan crystals. Following this incubation, 100 µl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and the absorbance was measured at 570 nm using a microplate reader[9,10].

Formula:
Surviving cells (%) = Mean OD of test compound/Mean OD of Negative control x 100

HPTLC studies

Instrument Used

CAMAG TLC Scanner 3 "CAMAG TLC Scanner "Scanner 171005" S/N 171005 (2.01.02) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was winCATS.

HPTLC fingerprinting of *Mimosa hamata*

A number of developing solvent systems were tried. The solvent system, which gave best resolution, was considered valid and useful. The satisfactory resolution for *Mimosa hamata* was obtained in the ethyl acetate: toluene: formic acid (5:4.8:0.2) developing system, the standard used was quercetin. Both standard and the sample were prepared in methanol using concentration of 1mg/ml.

HPTLC Specification of standard and sample

Chamber type	Twin Trough Chamber 20x10cm
Mobile phase	Ethyl acetate: Toluene: Formic acid (5:4.8:0.2)
Saturation time	10min
Solvent front position	70.0 mm
Volume	10.0 ml
Drying device	Oven
Temperature	60 °C

HPTLC fingerprinting and chromatograms were developed and recorded. The peak values, table of peaks, graphical peak representation, and densitogram were observed (Rf vs. AU) [11].

Results and Discussion:

The ethanolic extract of *Mimosa hamata* revealed the existence of sugars, alkaloids, glycosides, tannins, flavonoids and saponins.

Cell viability assay by MTT for *Mimosa hamata*

The ethanol-based extract of Plant *Mimosa hamata* was subjected to the MTT assay to evaluate its cytotoxic effects against three different human cancer cell lines: A549 (lung carcinoma), PA-1 (ovarian teratocarcinoma), and KB-3-1 (oral carcinoma). The results are depicted in Table 1 and fig. 1 to 6

Table 1: IC50 values of ethanolic extract of *Mimosa hamata* for MTT assay comparing with standard drug Doxorubicin against A549, PA-1 and KB-3-1 cell lines.

IC- 50 values of *Mimosa hamata* against various cell lines (µg/ml)			
Treatment	**A549**	**PA-1**	**KB-3-1**
Ethanolic extract of *Mimosa hamata*	205.75 ± 3.38	153.12 ± 2.33	125.60 ± 2.04
Standard Drug (Doxorubicin)	7.70 ± 0.594	3.99 ± 2.21	3.08 ± 1.11
Negative control	100	100	100

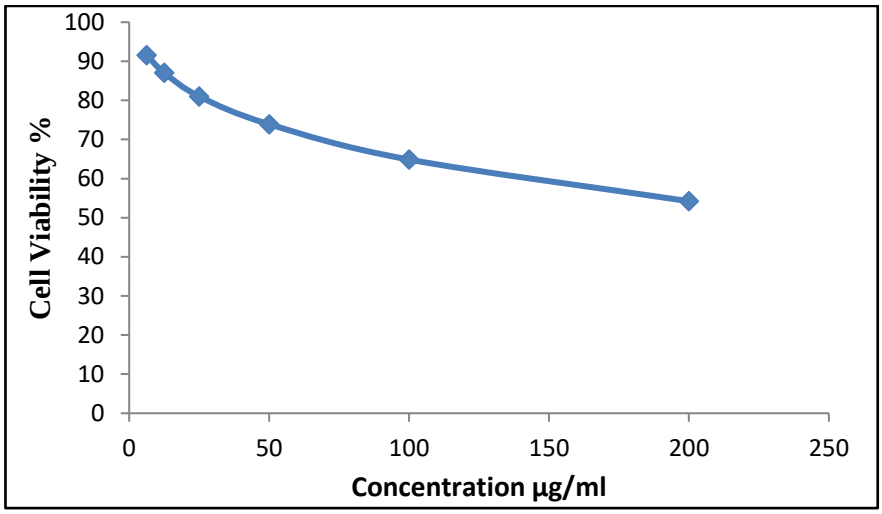


Figure 1: Percentage of cell growth inhibition at various concentration of *Mimosa hamata* against A549 cell line

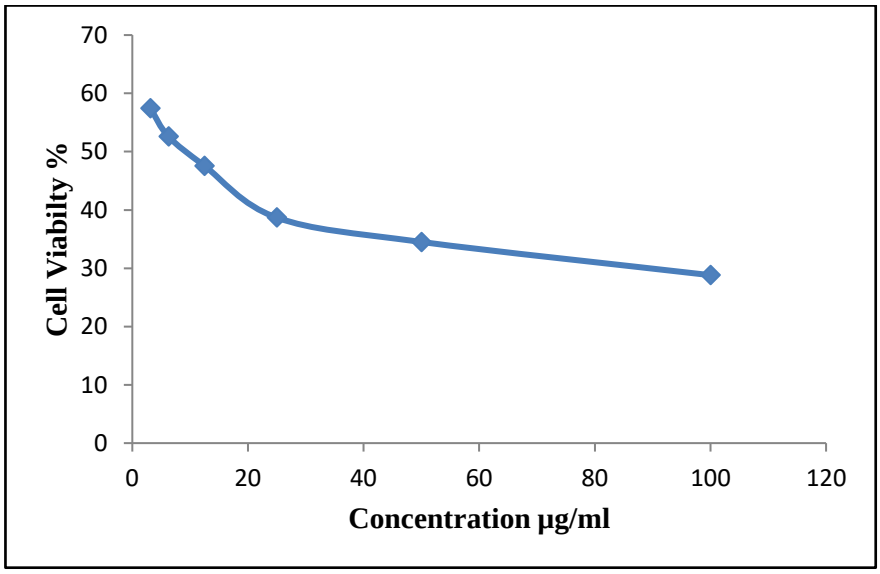


Figure 2: Percentage of cell growth inhibition at various concentration of Standard against A549 cell lines

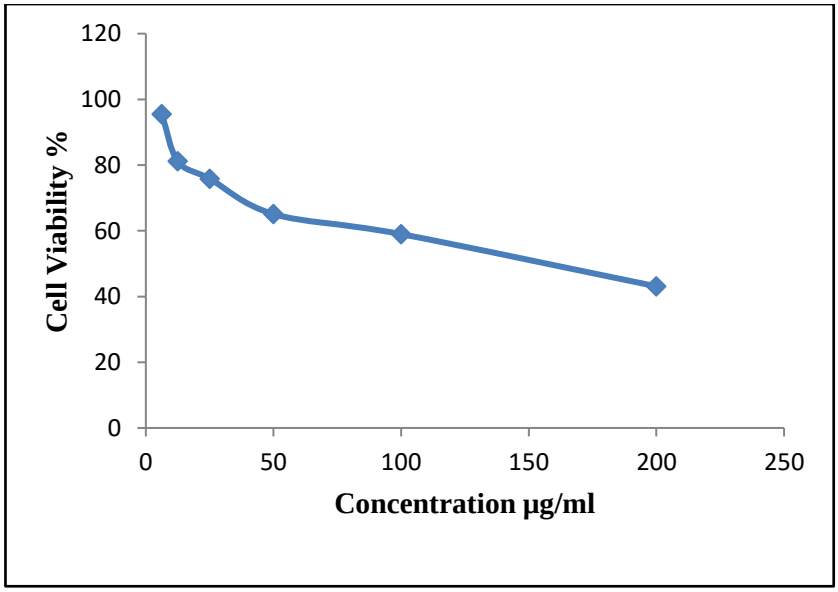


Figure 3: Percentage of cell growth inhibition at various concentration of *Mimosa hamata* against PA-1 cell lines

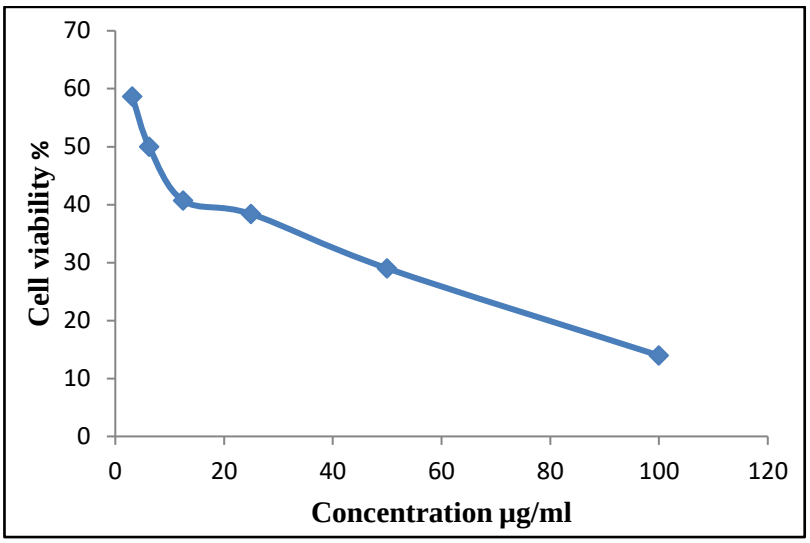


Figure 4: Percentage of cell growth inhibition at various concentration of standard against PA-1 cell lines

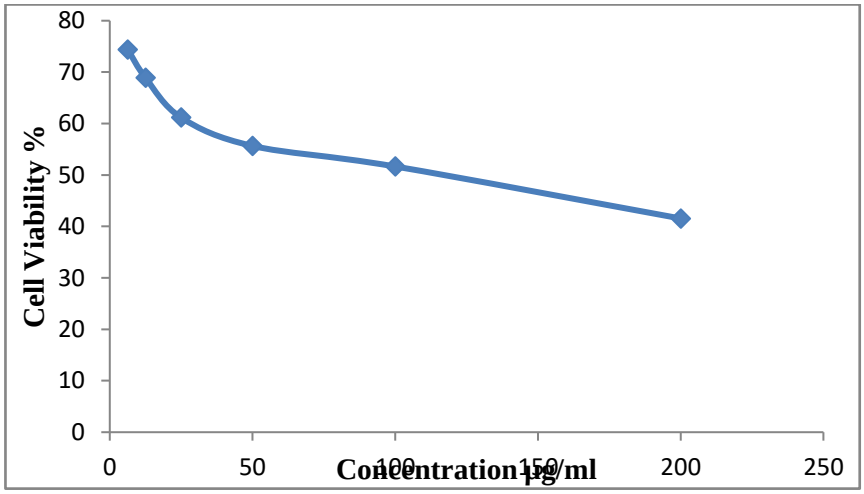


Figure 5: Percentage of cell growth inhibition at various concentration of *Mimosa hamata* against KB-3-1 cell lines

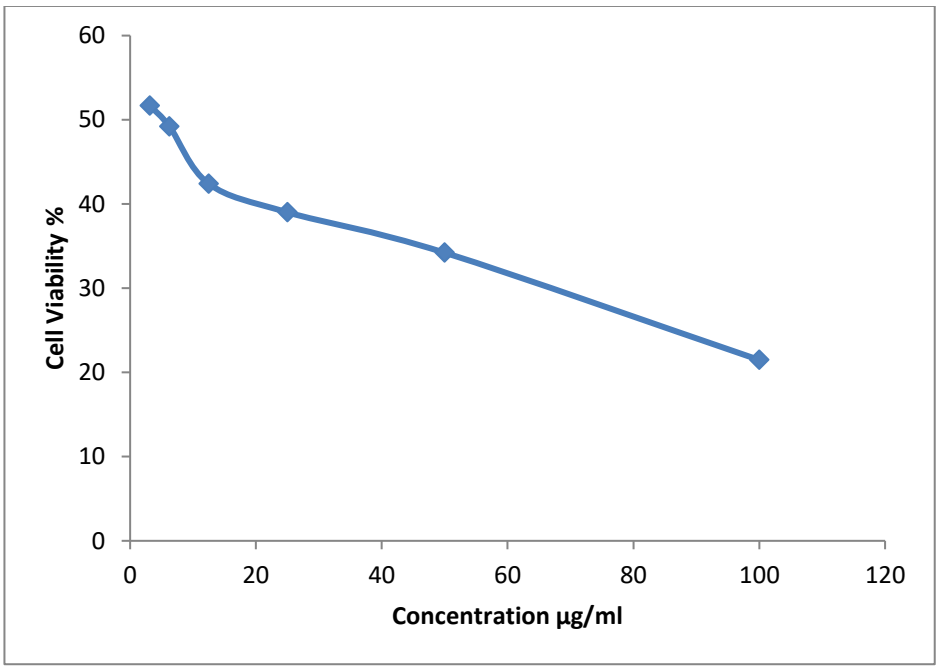


Figure 6: Percentage of cell growth inhibition at various concentration of standard against KB-3-1 cell lines

The IC50 value for the A549 lung cancer cell line was found to be 205.75 ± 3.38 µg/ml, indicating that the ethanol extract has moderate cytotoxic effects against these cells. For the PA-1 ovarian cancer cell line, the IC50 was lower at

153.12 ± 2.33 µg/ml, suggesting that the extract is more effective in reducing cell viability in ovarian teratocarcinoma cells. The KB-3-1 oral cancer cell line showed the highest sensitivity to the extract, with an IC50 of 125.60 ±2.04 µg/ml, highlighting its strong potential as a treatment for oral cancer. Overall, the extract shows promise as an anti-cancer agent across different types of cancer cells.

HPTLC study of ethanol extract of *Mimosa hamata*

HPTLC fingerprinting for ethanolic extract of *Mimosa hamata* was performed with standard quercetin using ethyl acetate: toluene: formic acid (5:4.8:0.2) as solvent system and visualized under 254nm and 366nm (figure7.13-7.20 and table 7.11-7.14). The densitogram of standard quercetin have Rf value 0.42. The densitogram of ethanol-based extract of *Mimosa hamata* indicated 7 bands with Rf values of 0.05, 0.26, 0.43, 0.49, 0.61, 0.74, 0.88 in which the 3rd peak exhibiting Rf value 0.43 is likely to be quercetin.(Table 2 - 5 and fig 7-14)

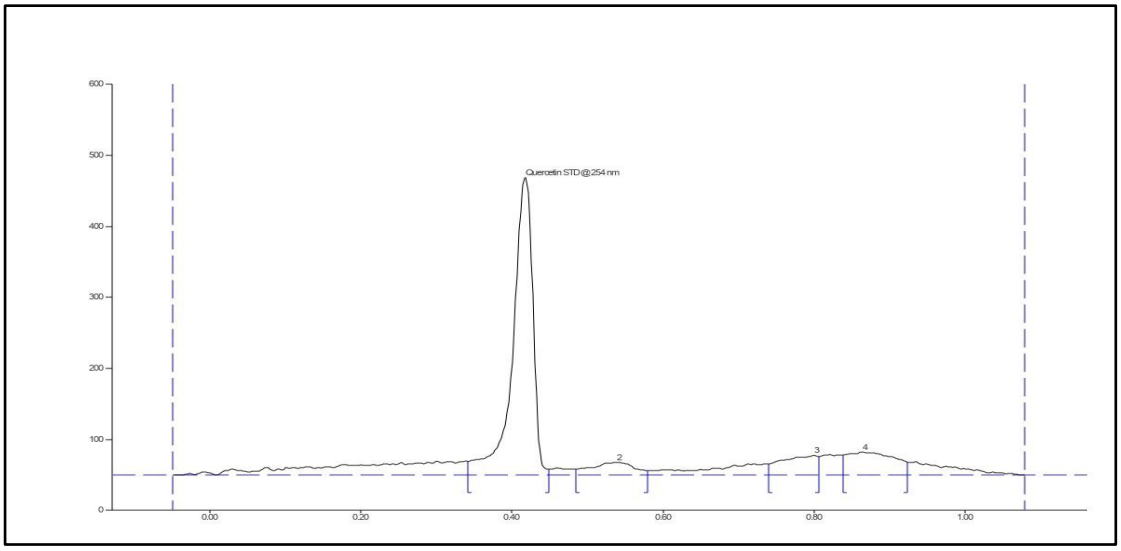


Figure .7: HPTLC Fingerprint of Standard Quercetin at 254nm

Table 2 Result of HPTLC analysis of Standard Quercetin at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.34	19.6	0.42	419.8	84.14	0.45	8.6	8056.9	71.22	Quercetin STD
2	0.49	8.4	0.54	18.3	3.67	0.58	6.6	764.4	6.76	unknown *
3	0.74	15.7	0.80	28.0	5.60	0.81	26.3	975.6	8.62	unknown *
4	0.84	28.4	0.87	32.9	6.59	0.93	18.3	1515.8	13.40	unknown *

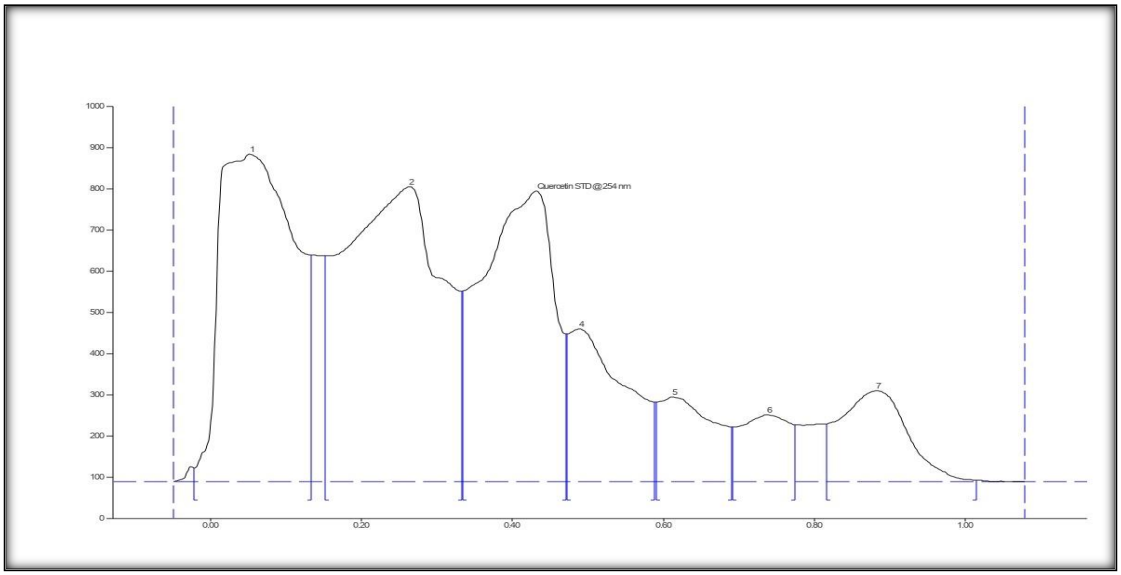


Figure 8: HPTLC Fingerprint of *Mimosa hamata* at 254nm

Table 3 Result of HPTLC analysis of ethanolic extract of *Mimosa hamata* at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	33.2	0.05	795.5	25.01	0.13	550.1	56973.8	25.24	unknown *
2	0.15	548.6	0.26	716.8	22.54	0.33	462.1	67019.4	29.69	unknown *
3	0.34	462.7	0.43	705.6	22.18	0.47	358.9	48599.8	21.53	Quercetin STD
4	0.47	359.1	0.49	371.1	11.67	0.59	193.9	20238.5	8.96	unknown *
5	0.59	193.9	0.61	206.3	6.49	0.69	133.6	10889.3	4.82	unknown *
6	0.69	133.7	0.74	163.7	5.15	0.78	139.2	7752.3	3.43	unknown *
7	0.82	140.7	0.88	221.8	6.97	1.02	4.0	14284.1	6.33	unknown *

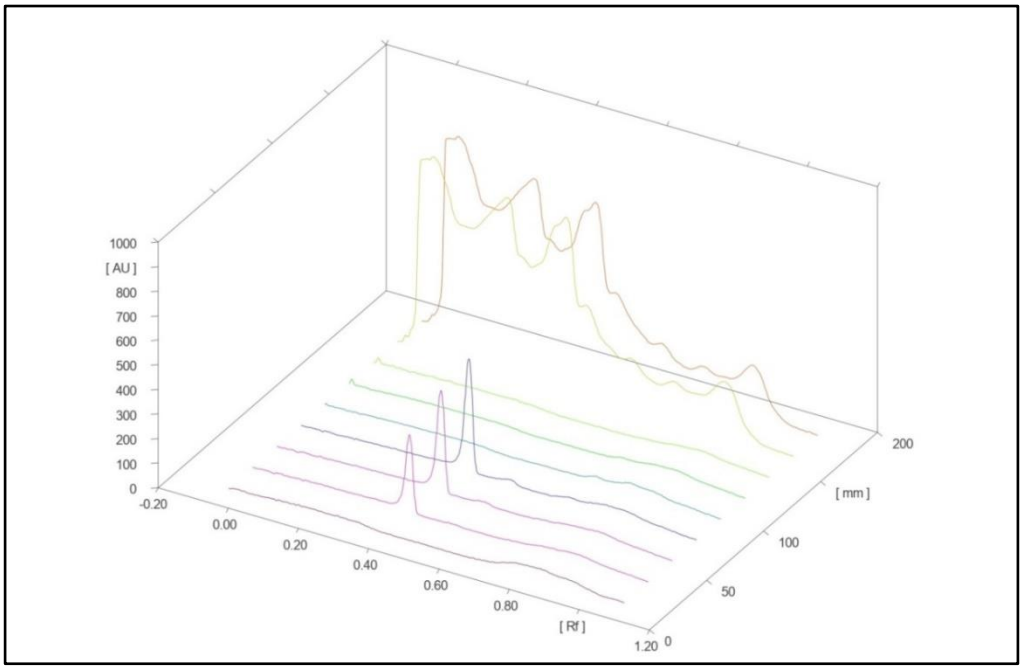


Figure 9: 3D chromatogram of ethanolic extract of *Mimosa hamata* at 254 nm

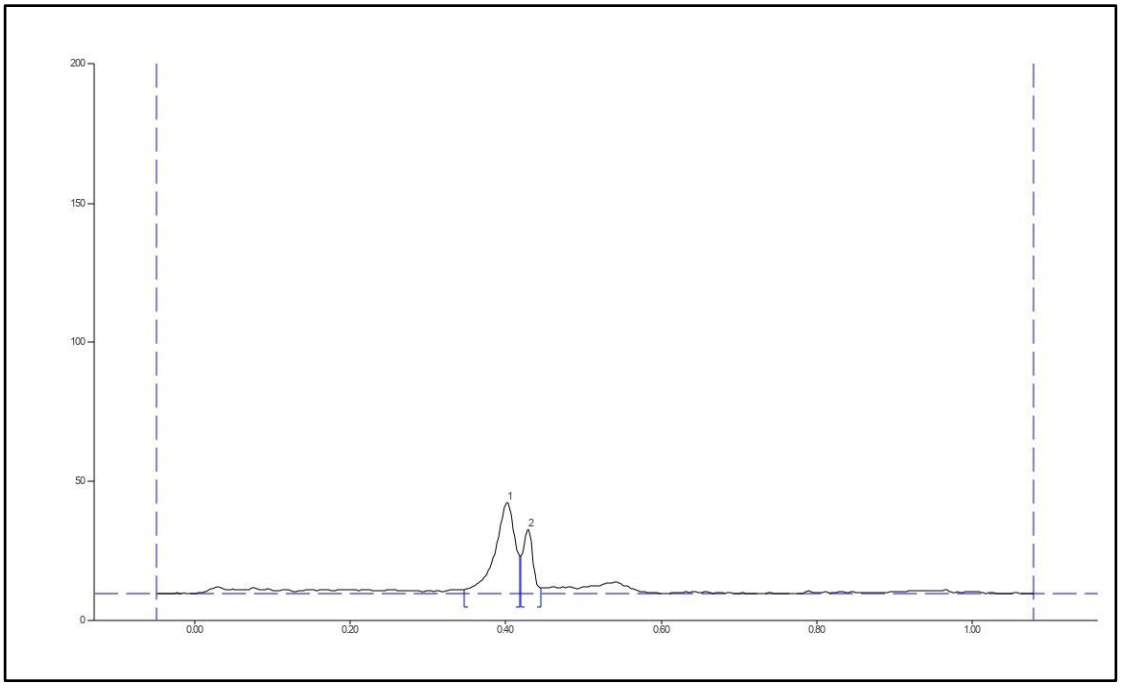


Figure 10 : HPTLC Fingerprint of Standard Quercetin at 366nm

Table 4 Result of HPTLC analysis of Standard Quercetin at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	1.7	0.40	32.9	58.39	0.42	13.6	616.8	73.73	unknown *
2	0.42	13.7	0.43	23.4	41.61	0.45	2.1	219.8	26.27	unknown *

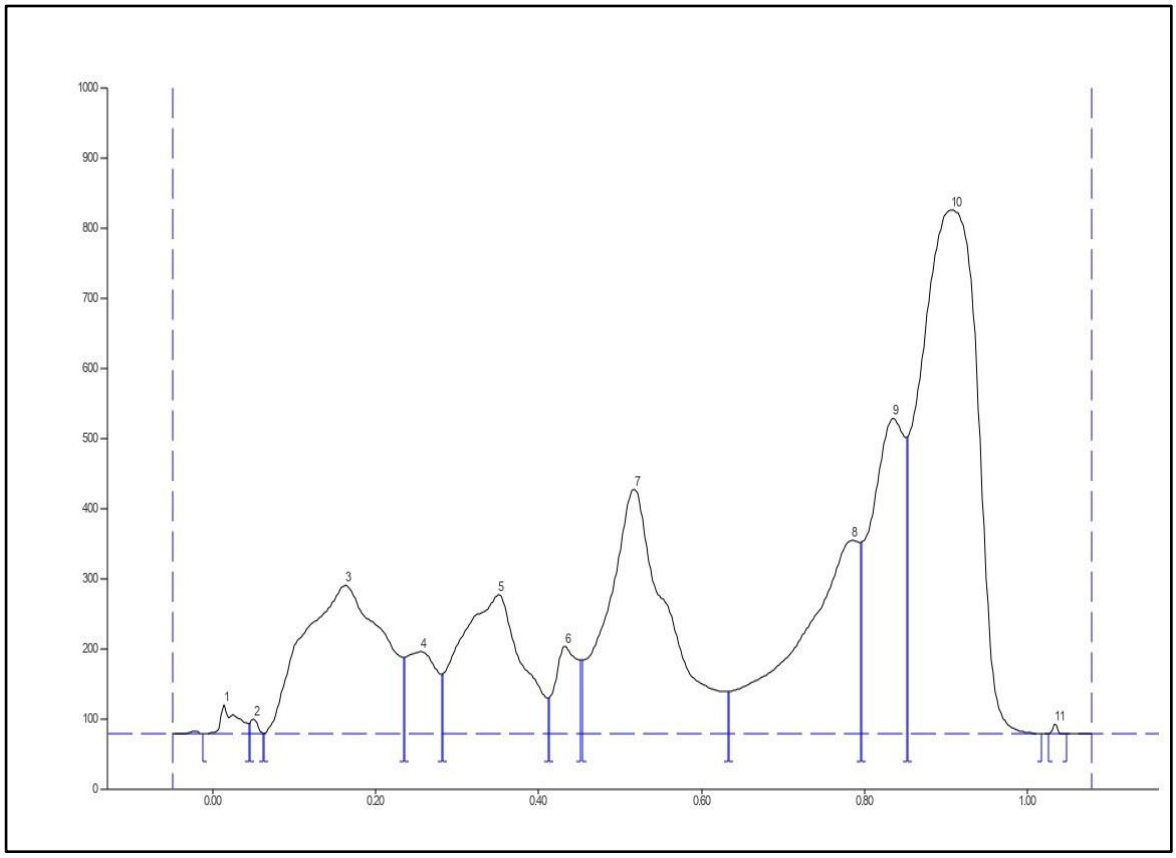


Figure 11: HPTLC Fingerprint of *Mimosa hamata* at 366nm

Table 5 Result of HPTLC analysis of *Mimosa hamata* at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.8	0.01	42.3	1.66	0.05	15.5	601.5	0.52	unknown *
2	0.05	16.6	0.05	22.3	0.87	0.06	1.4	143.0	0.12	unknown *
3	0.06	1.5	0.16	212.3	8.30	0.24	110.0	14793.7	12.71	unknown *
4	0.24	110.3	0.26	117.5	4.60	0.28	85.7	3119.0	2.68	unknown *
5	0.28	86.3	0.35	199.1	7.78	0.41	51.5	10651.1	9.15	unknown *
6	0.41	52.1	0.43	125.4	4.90	0.45	106.1	2503.0	2.15	unknown *
7	0.45	106.1	0.52	349.2	13.65	0.63	60.5	18676.1	16.05	unknown *
8	0.64	60.8	0.79	276.3	10.81	0.80	273.9	14724.5	12.65	unknown *
9	0.80	274.3	0.84	450.1	17.60	0.85	423.7	13356.0	11.48	unknown *
10	0.85	424.7	0.91	748.1	29.26	1.02	0.8	37760.5	32.44	unknown *
11	1.03	0.2	1.04	14.4	0.56	1.05	0.0	63.0	0.05	unknown *

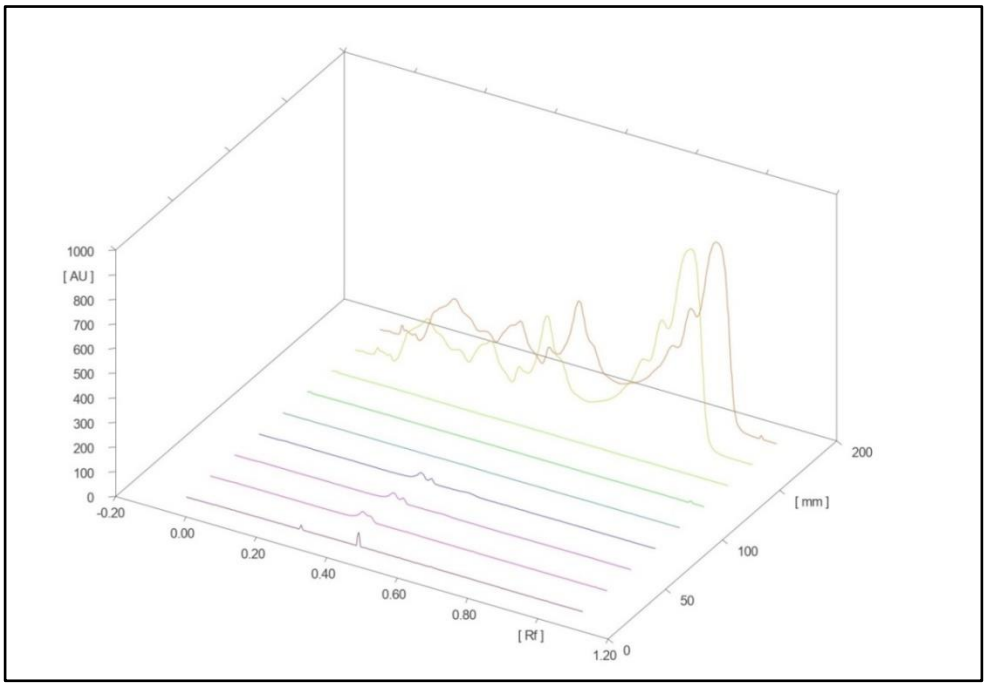


Figure 12: 3D chromatogram of *Mimosa hamata* at 366 nm

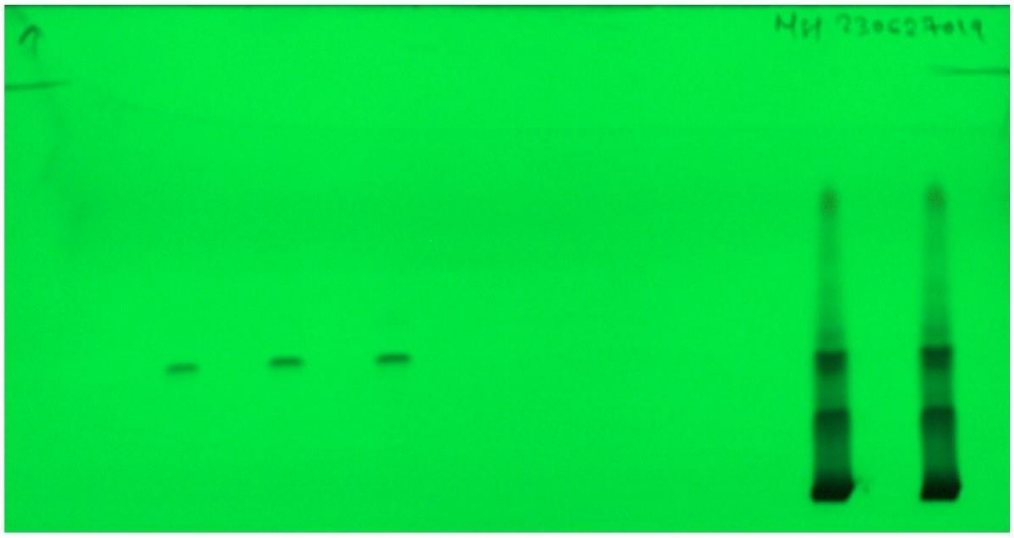


Figure 13: Chromatogram of ethanol extract of *Mimosa hamata* at 254nm

Figure 14: Chromatogram of ethanol extract of *Mimosa hamata* at 366nm

Conclusion

The study evaluated the cytotoxic effects of the ethanol extract of plant A on various cancer cell lines, revealing significant anti-cancer potential. The IC50 values indicated that the extract exhibited moderate cytotoxicity against A549 lung cancer cells (205.75 ± 3.38 µg/ml) and was more effective against PA-1 ovarian cancer cells (153.12 ± 2.33 µg/ml). Notably, the extract demonstrated the highest efficacy against KB-3-1 oral cancer cells, with an IC50 of 125.60 ± 2.04 µg/ml, underscoring its potential as a promising treatment for oral cancer.

Additionally, HPTLC fingerprinting analysis highlighted the presence of quercetin in the extract, identified by a prominent peak with an Rf value of 0.43, along with six other distinct compounds. This suggests that the extract contains a variety of phytochemicals that may contribute to its anti-cancer activity.

Overall, the findings support the potential of the ethanol extract of plant A as an effective anti-cancer agent across multiple cancer types, warranting further investigation into its mechanisms of action and clinical applications.

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