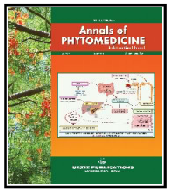
Annals of Phytomedicine 13(1): 522-528, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.54

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

In vitro anticancer activity of root bark of *Mallotus philippensis* (Lam.) Muell. Arg. on cervical cancer cell line

Malarkodi Velraj◆, Afroz Patan*, P. Shanmugasundaram*, S. Jayakumari, A. Vijayalakshmi, Shyam Sundar and M. Aruna

Department of Pharmacognosy, School of pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India

* *Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India*

Article Info

Article history
Received 7 December 2023
Revised 15 January 2024
Accepted 16 January 2024
Published Online 30 June 2024

Keywords
Mallotus philippensis (Lam.) Muell. Arg.
Anticancer activity
HeLa cell line
Phytochemical screening
TLC
GC-MS
Cytotoxicity studies

Abstract

One of the critically endangered but medicinally significant plants utilised in traditional medicine is *Mallotus philippensis* (Lam.) Muell. Arg., a member of the Euphorbiaceae family that has good potential for cultivation. TLC, GC-MS, anticancer activity, and preliminary phytochemical screening were carried out on the plant extract in order to investigate the chemical composition and anticancer activity of *M. philippenesis*. The plant was also examined for an anticancer investigation utilising the HeLa cancer cell line. Numerous root extracts underwent an initial phytochemical screening procedure that revealed the presence of multiple chemical constituents. TLC was used for the initial chemical investigation, and GC-MS characterisation reveals the bioactive chemicals. The MTT test was utilised to assess the *in vitro* cytotoxicity of plant extract and standard cisplatin on HeLa cells. Plant extract performed better than the control. The cytotoxicity effect on HeLa cell lines was noted in the tested sample concentrations after a 48 h treatment; further research also indicated that higher drug concentrations were associated with greater cytotoxicity against HeLa cell lines. The ethanolic root extract of *M. phillipens* is exhibited a significant IC_{50} concentration against HeLa cell lines, showed as 65.04 µg/ml and 4.43 µg/ml of plant extract and cisplatin, respectively. Consequently, the results of the ethanolic extract of *M. philippensis* root showed a considerable inhibition of cancer cells development.

1. Introduction

A cancer that emerges from the cervix is called a cervical growth. It is brought on by the erratic growth of cells that have the ability to invade or disseminate to different body parts (Ali *et al.,* 2016). HPVs (Human Papilloma Virus) infections (Das *et al.,* 2008) are related with an assortment of clinical conditions that ranges from less destructive injuries to hazardous cancer (http//globocan.iarc.fr/factsheet.asp, 2008). Human Papilloma virus composes 16 and 18 (Petrelli *et al.,* 2009) are the reason for 75% of cervical disease cases all inclusive, while 31 and 45 are the reasons for another 10%(Oldham and Dillman, 2009). India is unique in the world's ancient medical systems and possesses a great biodiversity of aromatic and medicinal plants (Mishra, 2011). Mallotus (family: Euphorbiaceae) is a broad genus of trees and shrubs that is mostly found in the old world's tropical and subtropical zones, with about 20 species found in India (Widén and Puri, 1980). The matured fruit consists of glandular hairs and are reddish brown in colour. The fruits are gathered by rubbing and shaking them with the hands. The material collected is grandular powder, fine, dull red and floats on water. This plant has long been used to treat bronchitis, abdominal illnesses, spleen enlargement, antimicrobial, antiparasitic, antifilarial (Singh *et al.,* 1997), immune-regulatory, antimicrobial, and anti-inflammatory properties. Additionally, it has been employed as an alexiteric, carminative, vulnerary, maturant, detergent, and purgative. *M. philippensis* is found naturally in a wide range of places, including the western Himalayas, southern China, India, Sri Lanka, Malesia, and Australia (Usmanghani *et al.,* 1997).

Corresponding author: Dr. Malarkodi Velraj
Professor, Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India
E-mail: malarkodisanna@gmail.com
Tel.: +91-9884242196

2. Materials and Methods

About 120 g of the root were roughly chopped into little pieces, shade-dried, coarsely powdered, and then extracted using the Soxhlet process using an organic solvent called ethanol. After that, the extract was gathered, distilled out at atmospheric pressure over a water bath, and the remaining of the solvents were eliminated. This extract was then utilised for cytotoxic research, TLC, GC-MS, and preliminary phytochemical screening.

2.1 Phytochemical screening

Numerous techniques were used to analyse the phytochemicals of the various plant extracts (Kokate, 1994; Houghton and Raman, 1998; Overton, 1963). The existence of different varieties of secondary metabolites such as terpenoid phenolic groups, alkaloids and flavonoids were explored.

2.2 Thin layer chromatography

Mallotus philippensis (Lam.) Muell.Arg. ethanolic extract was assessed using TLC plates, which provide evidence for the initial phytochemical screening (Figure 1 and Table 3).

2.2.1 Principle

The principle of separation is adsorption. A solvent serves as the mobile phase, and a thin layer of adsorbent deposited on a chromatographic plate serves as the stationary phase. Based on their affinity for the stationary phase, components that are mixed together and spotted on a TLC plate are separated. Greater affinity components move more slowly, while lower affinity components move more quickly.

2.2.2 Procedure

2.2.2.1 Preparation of TLC plate

After weighing 50 g of silica gel G, 100 ml of distilled water was shaken for 2 min to produce a uniform slurry. The slurry was poured onto the TLC plate (Schulz, 1983; Hardborne, 1973) spreader which was adjusted to 0.5 mm thickness. 20 TLC plates of same size (20 × 10) are stocked on a base plate and were coated in a single passage of the spreader over them, 10 min were waited to the plated to be settle down. The plates were dried for 30 min at 1100^{O}C in a hot air oven. After drying, the plates were put in a desiccator to be used later.

2.2.2.2 Application of test sample

The test sample is placed in a capillary tube, dissolved in the solvent, and spotted on a TLC plate 2 cm above the bottom. The applied spots ranged in equal diameter from 2-3 mts and were as evenly spaced as possible.

2.2.3 Development of chromatographic chamber

The spotted plates are stored in a glass chamber. To prevent an unfavourable edge effect and inadequate chamber saturation, a thin sheet of filter paper was positioned in a U-shaped chromatographic chamber and let to soak in the developing solvent. The paper was soaked and then pressed up against the room wall to make it stick to the wall. At room temperature, the experiment was conducted.

2.2.4 Solvent system

Many evolving solvent systems were tested, but only the solvent systems produced a suitable resolution. Ethyl acetate: methanol: formic acid for the development, plates were air dried and sprayed with iodine using water 5:0.2:0.3:0.6 for flavonoids. The number of spots was counted. The formula was used to determine the R_f values:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

2.3 Analysis of gas chromatography-mass spectrometry (GC-MS)

2.3.1 Gas chromatograph

A 15 m Alltech, Inc. EC-5 columns (250 µ I.D., 0.25 µ film thickness) and an even, deactivated 2 millimetre direct injector liner were installed in a Shimdzu GC-2010 Plus gaseous chromatograph. For introducing the sample, a split ratios (10:1) was injected. The oven was set to begin at 35°C and maintain that temperature for 2 min. After that, it was to rise by 20°C each minute to 450°C and stay there for a duration of 5 min. The flow rate of helium carrier gas was kept at 2 ml/min (constant flow mode), as stated by (Harborne, 1973; Khan *et al.,* 2013; Elhan Khan and Iffat Zareen Ahmad, 2021).

2.3.2 Mass spectrum

The electron ionisation (EI) mode of a capillary column's metallic quadrupole mass filter pre-rod mass spectrometer were directly linked to a GC-MS techniques solution ver. 2.6 computer, which was utilised for all investigations. At the resolution strength of 1,000 (20% height definition) and an inter-scan delay of 0.2 sec, low-resolution images mass spectra were obtained, with each scan taking 0.3 sec to cover the range of m/z 25 to m/z 1000. With a resolution strength of 5000 (20% height definition), high-resolution mass spectrum images were obtained, spanning with magnet's range from m/z 65 to m/z 1000 in 1 sec each scan.

2.3.3 Mass spectrometry library search

By comparing the recorded spectra of the chemical components with the mass spectra stored in the NIST libraries V 11 data bank, the components were identified. A similarity search based on retention index was carried out with the GC-MS technology metabolomics database.

2.4 Cytotoxic studies of ethanolic extract of *M. philippensis* against Hela cell line on cervical cancer

2.4.1 Cell suspension preparation

After discarding the culture medium, a subculture of HeLa cells cultured in dulbecco's modified eagle's medium (DMEM) was incubated with trypsin independently (Vijayalakshmi *et al.,* 2022). The flask holding the broken-up cells was filled with 25 millilitres of of DMEM that contained 10% FCS. The cells were gently passed through a pipette to homogenise them and suspend them in the liquid. Each well of a 24 well culture plate received one millilitre of the homogenised cell suspension as a process of seeding of cells, along with varying concentrations of the standard medication cisplatin or the test extract sample (0 to 200 µg/ml). After that, the plates were kept in a humidified CO_2 incubator with 5% CO_2 at 37°C. After incubating for 48 h, the cells were inspected under an inverted tissue culture microscope. The cytotoxicity test was carried out once the cell confluence reached 80% (Abondanza *et al.,* 2008; Shobhit *et al.,* 2022).

2.4.2 Cytotoxicity assay

In the analysis, (3,(4,5-dimethylthiazol 2yl) 2,5-diphenyl tetrazolium bromide) (MTT) was utilised. Viable cells' mitochondrial succinate dehydrogenase and reductase cleave MTT to produce a quantifiable purple product called formazan. The number of viable cells and the degree of cytotoxicity are directly and inversely correlated with the purple formazan production. The wells were supplemented with MTT after 48 h of incubation, and they were then left at room temperature for 3 h. Using a pipette, the contents of each well were cleared. After that, 100 µl of SDS in DMSO was applied to dissolve the formazan crystals. Using a read well touch microplate reader, the absorbances were measured at 570 nm (Stahl Tim Mosmann, 1983).

3. Results

The present work enclose a detailed studies on the extraction, preliminary phytochemical screening of the ethanolic extract of roots, GC-MS analysis, TLC and cytotoxic studies on cervical cancer cell line.

Soxhlet extraction was carried out for the roots. The percentage yield was calculated as per standard procedure and results were tabulated in Table 1.

Table 1: Extraction of root of *M. philippensis*

S.No.	Method of extraction	Colour of the extract	Amount of extract obtained in grams	Percentage of extract obtained w/w
1.	Soxhlet	Dark brown colour	5.38	3.58

To identify the chemical components in *M. philippensis* root extracts, a preliminary phytochemical test was conducted. The presence of alkaloids, flavanoids, phenolic compounds and terpenoids were observed in the root extract of *M. philippensis*. The results were expressed in Table 2.

Table 2: Phytochemical analysis of *M. philippensis*

Chemical test	Powder	Ethanolic extract	Aqueous extract
Alkaloids	+	+	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Proteins	-	-	-
Amino acids	-	-	-
Saponins	-	-	-
Flavanoids	+	+	+
Phenolic compounds	+	+	+
Tannins	-	-	-
Terpenoids	+	+	+
Oils and fats	-	-	-
Steroids	-	-	-

Different solvent systems were used in TLC to demonstrate the chemical test. Ethyl acetate was the system of solvents in which high resolution was attained. Ethyl acetate:formic acid:water:methanol in the ratio of 5:0.2:0.3:0.6. Three spots were identified in the ethanolic extract. In Table 3, the value of R_f was shown to be 0.9, 0.6, 0.5.

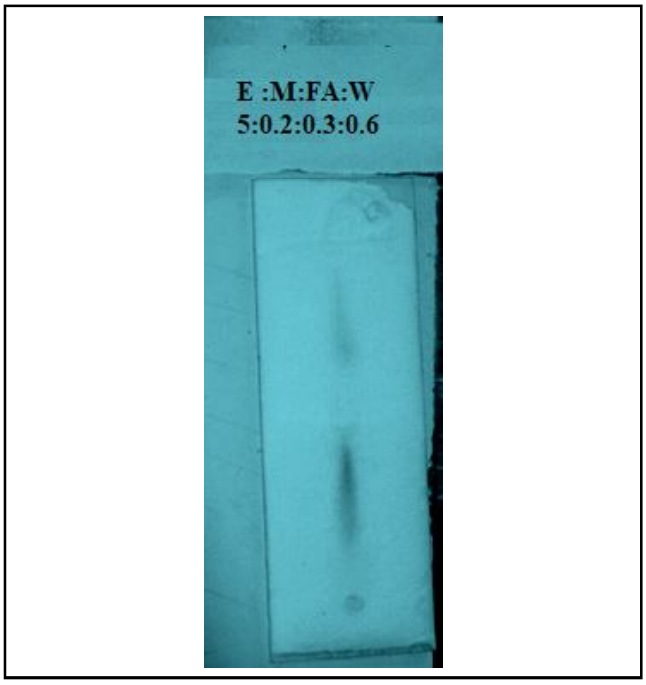


Figure 1: Thin Layer Chromatography.

The chemical contents were ascertained by GC-MS analysis via comparison of the mass spectra with those of the analogues provided by NIST (National Institute Standard Technology), which consist of over 62,000 patterns.

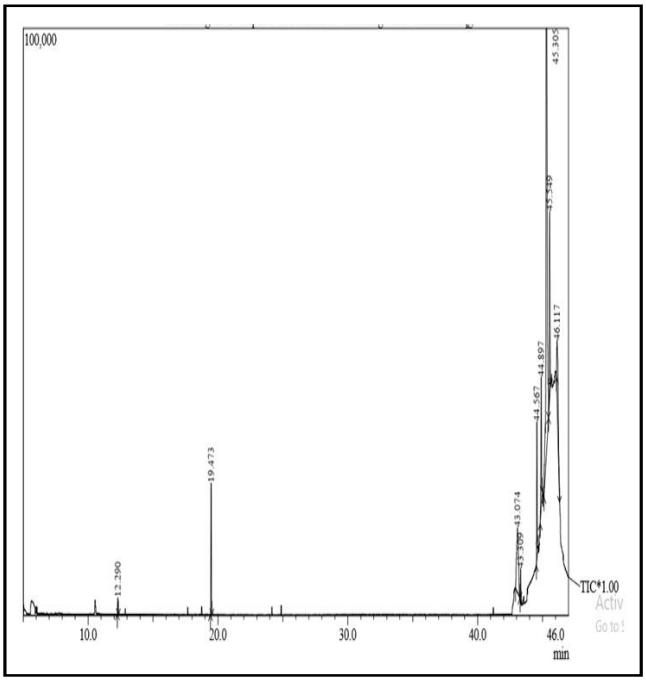


Figure 2: Chromatogram of GC-MS characterized compounds of *M. phillipensis*.

Table 3: Thin layer chromatography of ethanolic root extracts of *M. philippensis*

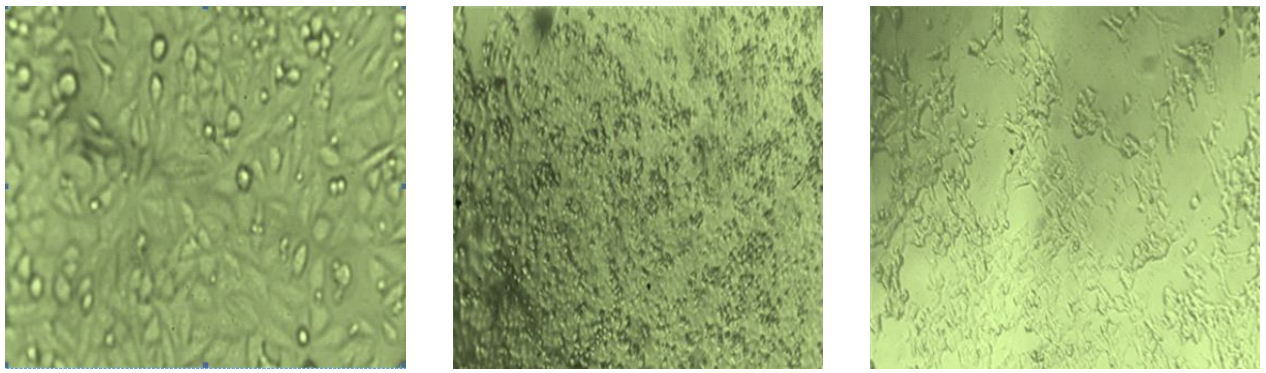
Test extract	Solvent system	Detecting agent	Number of spots	R_f valu e(cm)
Ethanol	Ethyl actetate: Methanol:Formicacid: Water (5:0.2:0.3:0.6)	Iodine vapour	3	0.90.60.5

Table 4: GC-MS spectral analysis of ethanolic extract of *M. Philippensis*

S.No.	Retention time	Name of the compounds	Area %	Height %	Molecular formula	Molecular weight
1	12.290	Benzenepropanoyl bromide	0.56	0.96	C_9H_9BrO	213.074 g/mol
2	19.473	Heptanoic acid,	4.80	7.59	$CH_3(CH_2)_5COOH$	130.187 g/mol
3	43.074	3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile	8.05	3.74	$C_{11}H_{18}FNS_2$	247
4	43.309	Propanoic acid, 2-methyl-, 2-propenyl ester	0.96	1.86	$C_7H_{12}O_2$	128
5	44.567	Borane, diethyl(decyloxy)	4.40	7.94	$C_{14}H_{31}BO$	226
6	44.897	1,2-Pentanediol, 5-(6-bromodecahydro -2-hydroxy-2,5,5a,8a-tetramethyl-1 -naphthalenyl)-3-methylene-	6.07	7.46	$C_{20}H_{35}BRO_3$	402
7	45.305	Urs-12-en-3-ol, acetate, (3.beta.)-	60.63	55.98	$C_{32}H_{52}O_2$	468
8	45.549	Octacosanoic acid, 2,4,6,8-tetramethyl-, methyl ester	7.51	10.85	$C_{33}H_{66}O_2$	494
9	46.117	Urs-12-ene	7.02	3.62	$C_{30}H_{50}$	410

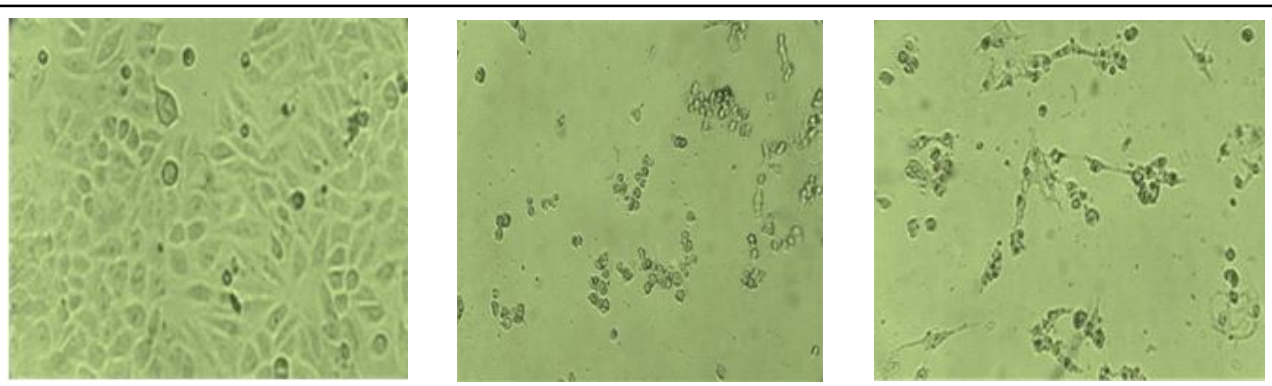
Table 5: *In vitro* cytotoxicity effect of ethanolic extract against HeLa cell lines

Sample concentration (µg/ml)	Ethanolic extract	Cisplatin
0	100.00	100.00
1.5625	94.41 ± 0.02	68.85 ± 0.02
3.125	77.35 ± 0.02	47.65 ± 0.03
6.25	68.37 ± 0.02	17.56 ± 0.03
12.5	52.94 ± 0.03	7.24 ± 0.03
25	46.15 ± 0.03	7.16 ± 0.02
50	35.95 ± 0.02	7.02 ± 0.02
100	26.55 ± 0.03	7.02 ± 0.02
200	15.25 ± 0.03	6.95 ± 0.03
IC_{50}	35.93	7



Cell viability on control | Cell viability on conc. 100 mg/ml | Cell viability on conc. 200 mg/ml

Figure 3: Ethanolic extract's anticancer properties against HeLa cell lines.



Cell viability on control | Cell viability on conc. 100 mg/ml | Cell viability on conc. 200 mg/ml

Figure 4: Cisplatin's anticancer properties against HeLa cell lines.

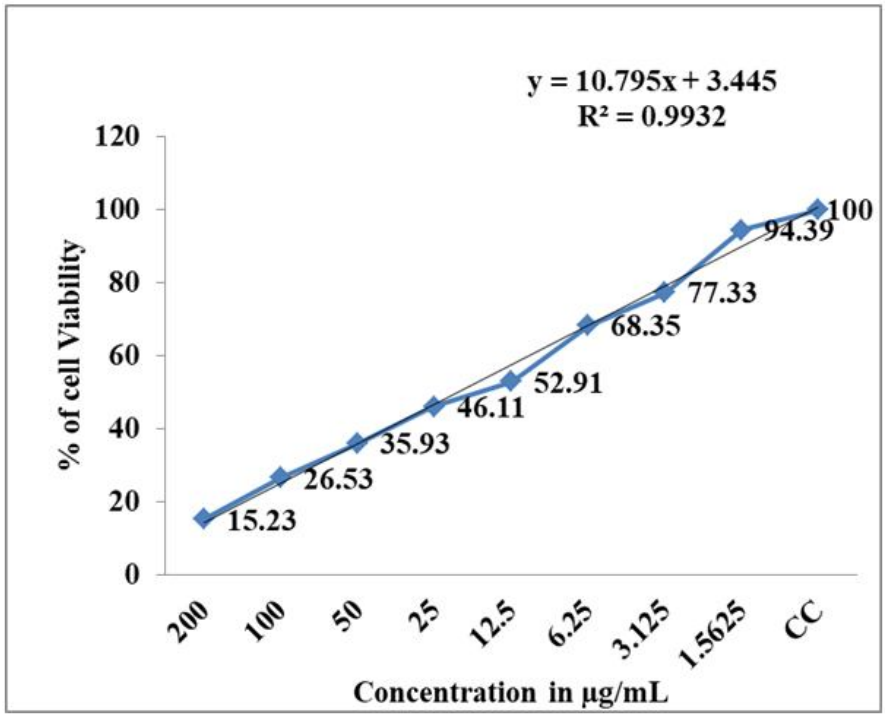


Figure 5: Ethanolic extract's anticancer properties against HeLa cell lines.

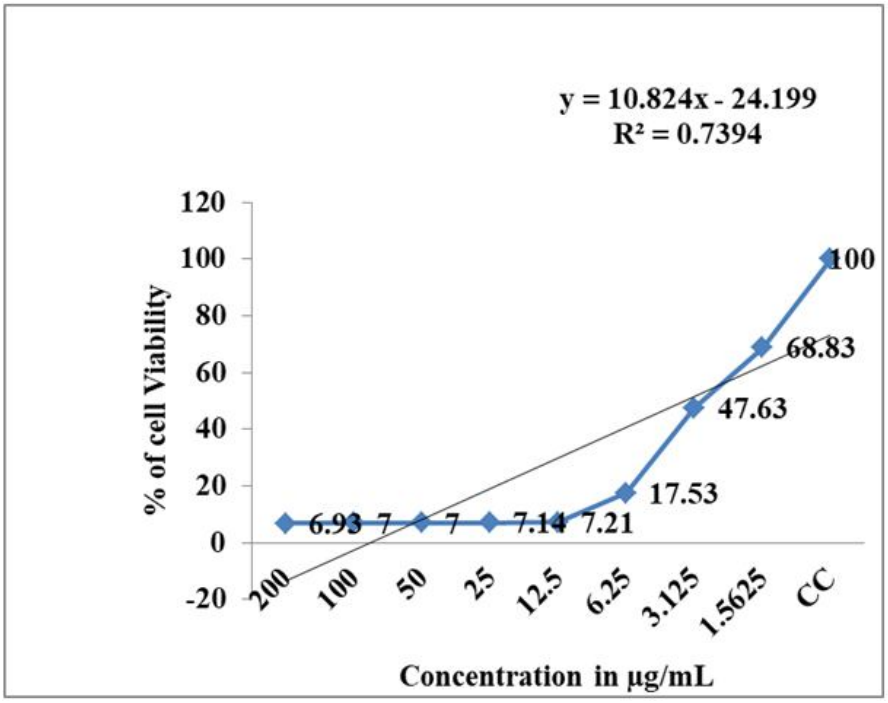


Figure 6: Cisplatin's anticancer properties against HeLa cell lines.

4. Discussion

Extraction of the plant root of *M. philippensis* by the Soxhlet method and amount of extraction obtained as 5.38 g and percentage yield of extraction is 3.58% w/w. The presence of alkaloids, flavanoids, phenolic compounds and terpenoids were observed in the root extract of *M. philippensis*. And for the detection of phytochemical constutients, TLC was performed and the R_f value was found to be 0.9, 0.6,0.5.

The chemical contents were ascertained by GC-MS analysis *via* comparison of the mass spectra. By using GC-MS analysis, the ethanolic extract was shown to include benzenepropanoyl bromide, heptanoic acid, 3,3-bis-tert-butylsulfanyl-2-fluoro-acrylonitrile, propanoic acid, 2-methyl-, 2-propenyl ester, borane, diethyl (decyloxy), and other substances. 1,2-pentanediol octacosanoic acid, 2,4,6,8-tetramethyl-, methyl ester, Urs-12-ene, acetate, 3-ol, and 6,5-bromodecahydro-2-hydroxy-2,5,5a, 8a-tetramethyl-1-naphthalenyl)-3-methylene (Table 4). The components of the ethanolic root extract were concealed in terms of their molecular weight, names, chemical structures, and molecular formulas. According to GC-MS analysis, the extract of ethanolic roots contained tabulations of values for the peak area, retention duration, molecule formula, molecular mass, and % of several phytochemicals (Preethi Dubey *et al.,* 2020). Skin cancer treatment uses heptanoic acid; antibacterial uses include 6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl -3-methylene; diethyl (decyloxy) as a wound disinfectant; and 1,2-Pentanediol. are some of the discovered phytochemical components; for example, urs-12-ene is utilised as an inflammatory medicine.

According to the *in vitro* cytotoxicity activity investigations HeLa cell lines were found to be cytotoxic when tested with test extract and standard substance cisplatin shown in Table 5. The cytotoxicity effect on HeLa cell lines was noted in the tested sample concentrations after a 48 h treatment; further research also demonstrated that higher drug concentrations were associated with greater cytotoxicity against the HeLa cell lines (Figures 3-6). The ethanolic root extract of *M. phillepensis* shown a noteworthy IC_{50} value of 35.93 (µg/ml) for plant extract and 7.00 (µg/ml) for cisplatin against HeLa cell lines, respectively (Bakhodir Mamarasulov *et al.,* 2020; Yuva Bellik *et al.,* 2020).

5. Conclusion

Medicinal plant forms the back bone of traditional medicine and potential source of new and leading components of therapeutic value in drug development. But isolating certain phytochemical components and putting them through defined biological action will undoubtedly produce useful findings for subsequent research.

Natural extracts have potential as a therapeutic adjuvant to stop the proliferation of tumour cells.

When the *in vitro* MTT assay method was used to examine the ethanolic root extract's cytotoxic efficacy against various cancer cell lines, it showed notable cytotoxic activity when compared to the conventional medication cisplatin. Secondary metabolites such as alkaloids, flavanoids, terpenoids, or phenolic chemicals may be the cause of the cytotoxic action.

Important cytotoxic studies are also required to see whether the natural extract could be utilised in addition to the chemotherapy that is currently being used to treat various tumours. Furthermore, additional research studies need to be planned to look at the molecular mechanism underlying anticancer action with broad area of research.

Acknowledgments

I extremely thankful to Dr.Ishari K.Ganesh, Chairman, Vels Institute of Science Technology and Advanced Studies (VISTAS) for providing technical support and facilities to carry out my research article.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abondanza, T.S.; Oliveria, C.R. and Barbosa, C.M.V. (2008). Bcl-2 expression and apoptosis induction in human HL-60 leukaemic cells treated with a novel organotellurium compound RT-04. Food Toxicol., **46**:2540-2545.

Ali, C.I.; Makata, N.E. and Ezenduka, P.O. (2016). Cervical cancer: A health limiting condition. Gynecol Obstet., **6**(378):2161-0932.

Bakhodir Mamarasulov; Kakhramon Davranov and Dilfuza Jabborova (2020). Phytochemical, pharmacological and biological properties of *Ajuga turkestanica* (Rgl.) Brig (Lamiaceae). Ann. Phytomed., **9**(1):44-57.

Das, B.C.; Hussain, S.; Nasare, V. and Bharadwaj, M (2008). Prospects and prejudices of human papillomavirus vaccines in India. Vaccine. **26**(22):2669-2679.

Globocan. Cancer incidence, mortality and prevalence worldwide. **(2008).** Available at: http//globocan.iarc.fr/factsheet.asp.

Elhan Khan and Iffat Zareen Ahmad (2021). An insight into the prophylactic and therapeutic activities of golden apple (*Cydonia oblonga* Miller), cultivated in Kashmir valley of India, for the future cancer care and prevention: A review. Ann. Phytomed., **10**(2):22-35.

Harborne J.B. (1973). Phytochemical methods. Chapman and Hall. London, pp:117.

Houghton, P.J. and Raman, A. (1998). Laboratory Handbook for the Fractionation of natural extracts. London, Chapman and Hall, pp:154-162.

Khan, M.; Qureshi, R.A.; Hussain, M; Mehmood K. and Khan RA. (2013). Hexane soluble extract of *M. philippensis* (Lam.) Muell. Arg. root possesses anti-leukaemic activity. Chemistry Central Journal, **7**(1):157.

Kokate, C.K. (1994). "Practical Pharmacognosy", 4th edition, Vallabh Prakashan, Delhi, India, pp:107-113.

Kumar, V. P.; Chauhan, N. S.; Padh, H. and Rajani, M. (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology, **107**(2):182-188.

Mishra, M. (2011). Conversation of biodiversity in the natural forests of central India: A case of critically endangered medicinal species safed musli in Bhopal forest (MP) India. Bioscience Discovery, **2**(3):299-308.

Oldham, R.K. and Dillman, R.O. (2009). Editors. Principles of Cancer Biotherapy. Springer Science and Business Media, pp:229.

Overton, K.H. (1963). Isolation, purification and preliminary observation in elucidation of structure by physical and chemical methods. Interscience, pp:509.

Petrelli, N.J. (2009). Clinical Cancer Advances: Major research advances in cancer treatment, prevention, and screening:A report from the American Society of Clinical Oncology. J. Clin. Oncol., **27**(35):6052-6069.

Preety Dubey; Shiv Kumar Jayant and Nalini Srivastava (2020). Preliminary phytochemical screening, FTIR and GC-MS analyses of aqueous, ethanolic and methanolic extracts of stem of *Tinospora cordifolia* (Guduchi) for search of antidiabetic compounds. Ann. Phytomed., **9**(2):183-197.

Singh, R.; Singhal, K. C. and Khan, N. U. (1997). Antifilarial activity of *Mallotus philippensis* Lam. on Setaria cervie (Nematoda: Filarioidea) *in vitro*, Indian Journal of Physiology and Pharmacology, **41**(40):397-403.

Shobhit Srivastava; Kuldeep Singh; Sujeet Kumar Gupta; Shomprakash Kushwaha; Arun Kumar; Piyush Kumar and Parveen Kumar (2022). Recent advances in isatin-thiazole hybrids as potential anticancer agents. Ann. Phytomed., **11**(2):33-41.

Stahl Tim Mosmann. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, **65**:55-63.

Usmanghani, A.; Saeed, K. and Alam, M. T. (1997). Indusynic medicine. Karachi Research Institute of Indusyunic Medicine, pp:285-287.

Vijayalakshmi, A.; Prabha, T.; Lalitha, V.; Hemalatha, S.; Jagadeeswaran, M.; Chaitanya, M.V.N.L.; Selvamani, P. and Latha, S. (2022). Dietary carotenoid fucoxanthin as a promising biomarker to target the cancer cells: A focused review. Ann. Phytomed., **11**(1):164-174.

Widén, C.J. and Puri, H.S. (1980). Natural occurrence and chemical variability of phloroglucinols in Kamala. Planta Medica, **40**(11):284-287.

Yuva Bellik; Mostapha Bachir-Bey; Wided Fatmi; Mokhtaria Kouidri; Yasmina Souagui and Sidi Mohammed Ammar Selles (2020). Micronutrients and phytochemicals against COVID-19: Mechanism and molecular targets. Ann. Phytomed., **9**(2):15-29.

Citation **Malarkodi Velraj, Afroz Patan, P. Shanmugasundaram, S. Jayakumari, A. Vijayalakshmi, Shyam Sundar and M. Aruna (2024). *In vitro* anticancer activity of root bark of *Mallotus philippensis* (Lam.) Muell. Arg. on cervical cancer cell line. Ann. Phytomed., 13(1):522-528. http://dx.doi.org/10.54085/ap.2024.13.1.54.**