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Original Article

In vitro Cytotoxicity Evaluation of *Hardwickia binata* Roxbs against Human Breast Cancer Cell Line MCF-7 and Human Cervical Cancer Cell Line HeLa

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ABSTRACT

Hardwickia binata belongs to the family Caesalpiniaceae is used traditionally folklore remedies for a variety of illnesses, such as fungus, gram-positive and gram-negative bacteria, cancer, diarrhoea, leprosy, worm infections, dyspepsia, leucorrhoea, chronic cystitis and gonorrhoea. In the present study the leaves of *Hardwickia binata* were treated to solvent extraction using ethyl acetate, chloroform, acetone, ethanol by soxhlet. The various solvent extract were investigated for the presence of phytochemicals such as phenolic substances, flavonoids, tannins, glycosides and proteins. The phytochemical were determined by conventional qualitative test and quantitative test like total phenolic, total flavonoid content. Antioxidant activity was done by different assay method using DPPH radical scavenging assay, nitric acid scavenging, reducing power assay method. Cytotoxicity efficacy was estimated using MTT assay method against human breast cancer cell line MCF-7 and human cervical cancer cell line HeLa. IC_{50} was calculated. This study showed that *Hardwickia binata* leaves may have potential to inhibit cancer cell growth in addition to focus on search of phytochemicals from leaves of *Hardwickia binata* roxbs due to their health promising effect including antioxidant and anticancer and can be used as leading drugs to treat cancer. The plant also has excellent antioxidant activity.

Keywords: Antioxidant, Cytotoxicity assay, Cancer cell line, *Hardwickia binata*, Phytochemical Analysis.

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INTRODUCTION

Cancer is a multifactorial ailments characterized by unusual and uncontrolled cellular proliferation, which can invade adjacent tissues and distant organs.[1] Alternative terms include malignant tumours and neoplasms. Cancer was responsible for 10 million mortality globally in 2020. Annually, almost 400,000 youngsters are diagnosed with cancer. The prevalence of cancers differs among nations. Cervical cancer is the major kind in 23 nations. Cancer is the second leading cause of death in the United States.[2,3] In 2024, approximately 2,001,140 new cancer cases will be diagnosed in the United State, resulting in 611,720 death. Chemotherapy is the predominant method employed for treating cancer. However, a significant risk linked to chemotherapy is the manifestation of serious side effects throughout the treatment process.[4] Natural products and secondary metabolites obtained from plant sources are considered as less harmful to normal cells.[5] The majority of current anticancer medicines utilized for cancer treatment are derived from plant sources. Secondary metabolites such as alkaloids, flavonoids, sesquiterpenes and polyphenols are extensively employed as anticancer drugs. Reports from the FDA indicate that 40% of approved anticancer molecules are derived from natural compounds, with a significant portion currently employed in anticancer therapies.[6] Polyphenols are essential phytochemicals found in medicinal plants, recognized for their significant role as anticancer agents. Polyphenols and flavonoids exhibits a diverse range of anticancer properties, including the prevention of oxidative stress from free radicals, the down regulation of pro-inflammatory signalling pathways, involvement in cell cycle arrest, induction of apoptosis and autophagy, as well as the suppuration of cancer cell proliferation and invasiveness. Polyphenols and flavonoids are significant subjects of research due to their potential as anticancer agents.[7,8] Medicinal plants represent a substantial reservoir of natural chemicals with diverse applications for human health. In contemporary society, people rely on plants for the majority of commercial products, including pharmaceuticals, healthcare items, food, beverages, textiles, cosmetics and fragrances.[9-11]

The beautiful drooping branchlets of the *Hardwickia binata*, sometimes called *"Anjan"*, make it an attractive medium-to

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large-sized deciduous ornamental tree. *Harongana madagascariensis* Chois and *Hardwickia trapeziformis* (R. Grah.) are synonyms for this monotypic genus of flowering plants. The Detarioideae subfamily of legumes is surviving. This plant is significant for both economic and medical purposes.[12,13] It has historically been employed in folkloric remedies for several ailments, including fungal infections, gram-positive and gram-negative bacterial infections, cancer, diarrhea, leprosy, helminthiasis, dyspepsia, leucorrhea, chronic cystitis and gonorrhea.[14-18] Researchers have reported that *Hardwickia binata* has antimicrobial activity.[19,20] The advancement of innovative antimicrobial chemotherapeutic agents has been recognized as a crucial focus within medical research.[21] The study's goal was to find out how well ethyl acetate, acetone, chloroform and ethanolic leaf extract of *Hardwickia binata* fight free radicals and cancerous cell. To orient future research towards the discovery of novel, powerful compounds, we present the results of such studies in this paper and secure anticancer and antioxidant compounds. Statistics indicate that more than 60% of the global population and approximately 80% in developing nations rely on traditional and medicinal plants for treatment purposes.[22] Numerous prior publications have revealed that medicinal herb have a significant role in cancer treatment, aiding in cancer control and inducing apoptosis in cancer cells.[23]

MATERIALS AND METHODS

Chemicals and reagents

All chemical and reagent utilized in the present study were of analytical grade.

Plant material

Leave of *Hardwickia binata* were gathered in month of September from Amaravati Road, Nagpur, Maharashtra, India. Plant specimen was authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

Preparation of Extract

Leaves of *Hardwickia binata* were dried and converted to coarse powder using mechanical grinder. The coarse powdered plant material underwent defatting with petroleum ether utilizing a Soxhlet apparatus. Defatted plant material underwent successive extraction using ethyl acetate, chloroform, acetone and ethanol in a Soxhlet apparatus. The extract was filtered and concentrated in a rotary evaporator under reduced pressure. The concentrated extract was stored in a vacuum desiccator for several days to eliminate residual solvent before to use.

Preliminary phytochemical screening of extract

A preliminary examination of the phytochemicals in the various solvent extracts was carried out to confirm the presence of distinct classes of phytoconstituents, such as tannin, flavonoid, alkaloids, saponins, glycosides and polyphenols.[24]

Quantification of phenolic and flavonoid content

The total flavonoid content of extracts was assessed using the specified method with $AlCl_3$ reagent, incorporating slight modifications. Quercetin is utilized as a standard as a flavonoid standard.[25] Flavonoids were quantified in mg quercetin equivalents/g extract. Estimated triplicates. Hagerman *et al*., (1998) utilized gallic acid as a phenolic standard. The TPC was assessed with Folin-Ciocalteu's reagent, as previously outlined with slight modifications. We measured the total phenolic content in triplicate using the gallic acid equivalent method.[26]

Antioxidant activity

DPPH Assay method

The antioxidant capacity was assessed through the IC_{50} value, which was derived from the ratio of radical scavenging activity using the following expression:

Free radicals DPPH inhibition (I %) was calculated using the equation below:

$$I\%=100\times(A\ blank-A\ sample)/A\ blank$$

The absorbance of the test compound is denoted as A_{sample}, while absorbance of control reaction is denoted as A_{blank}. Interpolating from a linear regression analysis yielded the IC_{50} value (g mL-1), which scavenged 50% of DPPH radical.[27]

Nitric oxide radical scavenging

Over a 5-hr period, sodium nitroprusside (5 μM) was incubated with various chloroform extract concentrations in 0.025 M phosphate buffer (pH 7.4) 25°C.[28] After 5 hr, 0.5 mL of incubation solution was withdrawn and diluted with 0.5 mL of Griess reagents (1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The chromophore produced has 546 nm absorbance. Triplicates were tested and scavenging activity was determined using 100-[100/blank absorbance x sample absorbance]. Same procedure, but with distilled water instead of extract. The activity were compared to standard antioxidant ascorbic acid.

Reducing power determination

Mixing different amounts of extract with 2.5 mL of 5% potassium ferriccyanate $[K_3Fe(CN)_6]$.[29] The mixture was incubated at 50°C for 20 min. 2.5 mL of 5% trichloroacetic acid was added and centrifuged for 10 min at 1000 rpm. 2.5 mL upper layer solution, 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloric were combined. At 700 nm, absorbance was measured. The blank was done similarly with distilled water instead of extracts. Rising reaction mixture absorbance suggested rising reducing power. The activity were compared to typical antioxidant ascorbic acid.

Cytotoxicity studies: MTT assay

The cytotoxicity of the supplied sample on the MCF-7 and HeLa cell lines (obtained from NCCS Pune) was assessed using the MTT assay. Ten thousand cells per well were cultured in a 96-well plated and incubated for 24 hr in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L-HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37ºC with 5% CO_2 in an air-jacketed CO2 incubator (Heal Force-HF90). On the subsequent day, cell were treated with varying amounts of the formulations, as specified in the Excel sheet. A stock solution of materials was produced in DMSO and subsequently diluted to obtain various quantities in incomplete cell culture medium (without FBS). Untreated cells were designated as control, while cell without MTT were classified as blank. Upon completion of the experiments, the culture supernatant was discarded and the cell layer matrix was solubilised in 100 µL of Dimethyl Sulfoxide (DMSO) and analysed using as ELISA plate reader (iMark, Biorad, USA) at 540 nm. The IC_{50} was determined. Results were expressed as Mean±SEM.

$$\% \text{ Viable cell} = (A_{test} / A_{control}) \times 100$$

($A_{test=}$Absorbance of test sample),

($A_{Control=}$Absorbance of Control).

RESULTS

Qualitative phytochemical analysis

Phytochemical study of different solvent extracts demonstrated the presence of flavonoid, phenolic compound, tannins, glycosides and proteins.

Quantification of phenolic and flavonoid content

The phenolic content of *Hardwickia binata* ethyl acetate extract was 14.51±1.85 mg/g GAE, chloroform extract yielded 17.42±0.308 mg/g GAE, acetone extract 38.54±0.247 mg/g and *Hardwickia binata* ethanolic extract 51.24±0.528 mg/g GAE.

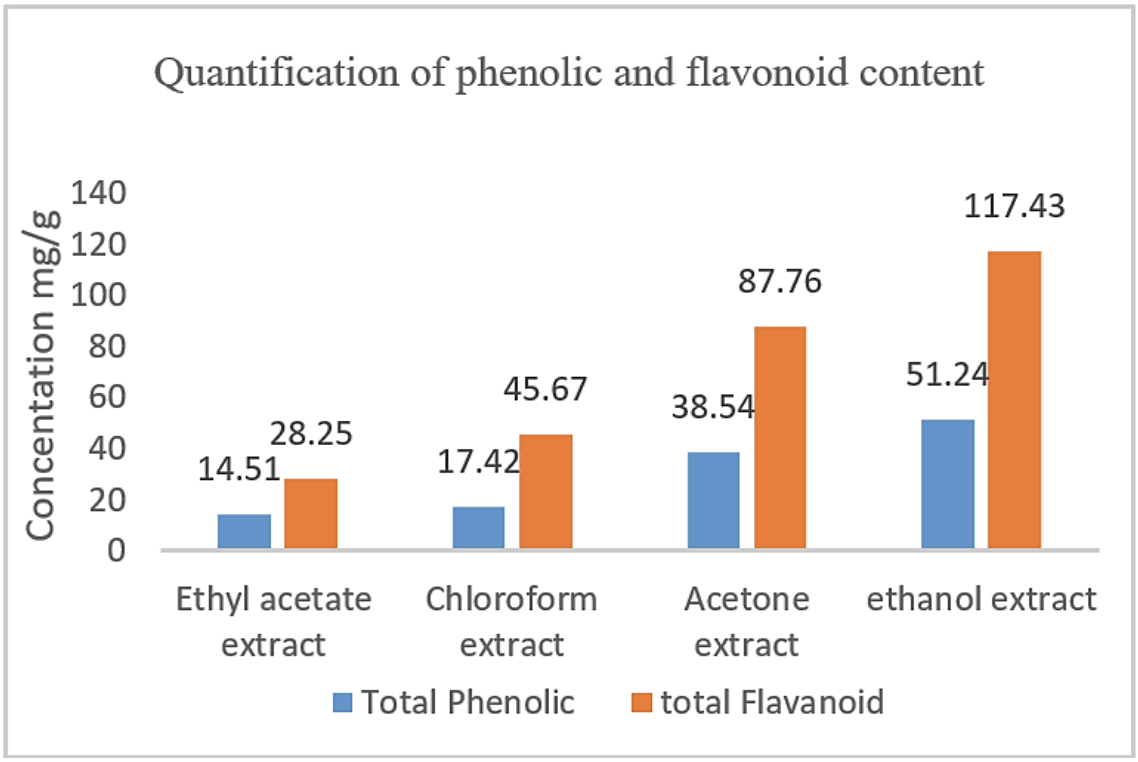

Figure 1: Quantification of phenolic and flavonoid content of various extract of leaves of *Hardwickia binata Roxbs.*

Table 1: IC_{50} value of different solvent extract of leaves of *Hardwickia binata* roxbs.

Sl. No.	Plant Extract	MCF-7	HeLa
		IC_{50} µg/mL	
1	Ethyl acetate extract	Not converged	85.8±0.53
2	Chloroform extract	83±0.074	Above Maximum Dose Limit
3	Acetone extract	67±0.05	55±0.11
4	Ethanol extract	52±0.053	31.3±0.023

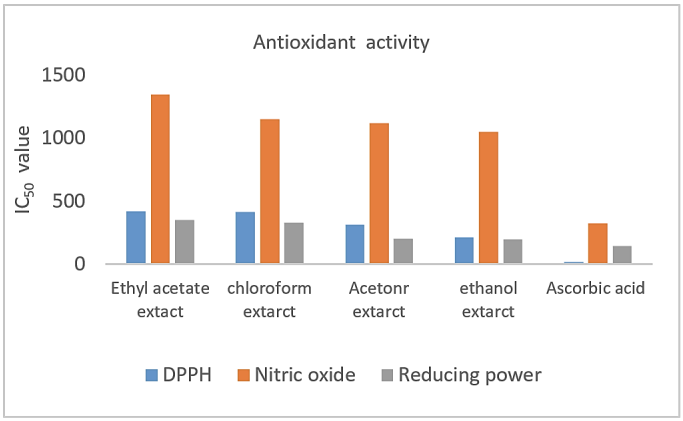

Figure 2: Antioxidant study of various solvent extract of *Hardwickia binata* Roxbs.

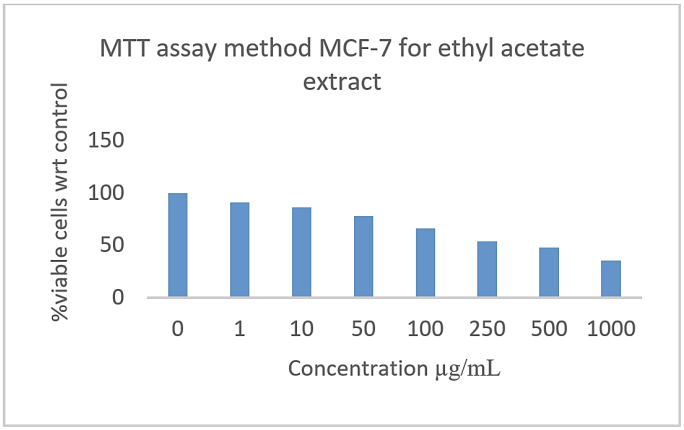

Figure 3: Inhibitory effect of ethyl acetate extract on the proliferation of human breast cancer cell line MCF-7.

According to the study, the ethanolic extract had the most flavonoid (117.43±0.236 mg GAE/g dry material). It was followed by the acetone, chloroform and ethyl acetate extracts, which all had polyphenolic chemicals. 87.76±0.285 mg/g RE, 45.67±0.276 mg/ g RE, 28.25±0.165 mg/ g RE. Figure 1 shown the amount of phytochemical present in various solvent extract of leaves of *Hardwickia binata.*

Antioxidant activity

Scavenging of DPPH radicals

Ascorbic acid, the positive control, had strong scavenging action with an IC_{50} of (9±0.01 µg/mL) Ethanol extract had the highest IC_{50} (211±0.41 µg/mL), followed by acetone (315±0.38 µg/mL), chloroform (416±0.43 µg/mL) and ethyl acetate (421±0.43 µg/mL). The results of antioxidant activity were disclosed in Figure 2.

Nitric oxide radical scavenging

The result shows the NO• scavenging activity, with an IC_{50} of (1049±0.09) µg/mL of ethanol extract, Ascorbic acid, acetone, chloroform and ethyl acetate extracts had IC_{50} values of (322±0.08), (1122±0.06), (1149±0.10) and (1350±0.48) µg/mL.

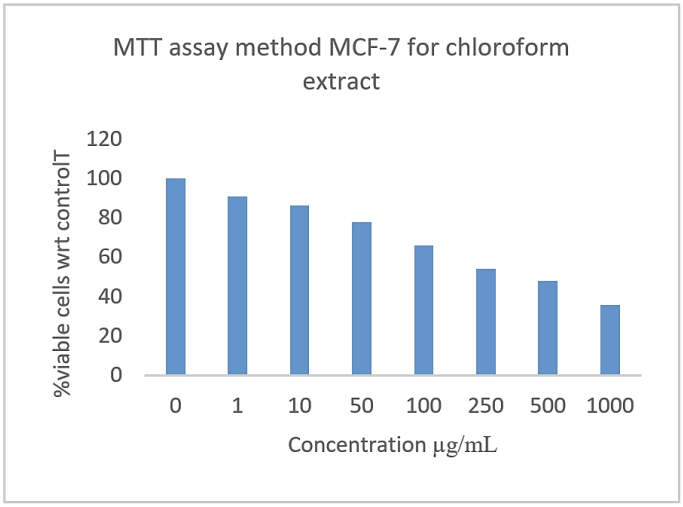

Figure 4: Inhibitory effect of chloroform extract on the proliferation of human breast cancer cell line MCF-7.

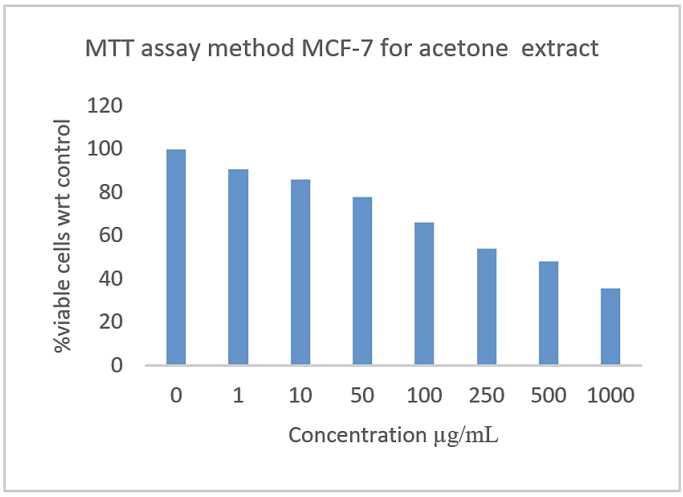

Figure 5: Inhibitory effect of acetone extract on the proliferation of human breast cancer cell line MCF-7.

Reducing power assay method

The reducing power sequence was ascorbic acid > ethanol > acetone > chloroform > ethyl acetate. All extracts prevented ferrous and ferrozine complex formation. Ascorbic acid had an IC_{50} value of 142.0±1.23% µg/mL, while the IC_{50} values for ethanol, acetone, chloroform and ethyl acetate extract were 198±1.01, 201±1.07, 330±1.82 and 350±1.52 µg/mL.

Cytotoxicity studies: MTT assay

The half-maximal inhibitory concentration (IC_{50}) of the ethyl acetate extract was determined to be not converged for MCF-7 and 85.8±0.53 µg/mL for HeLa, Chloroform extract 83±0.074 µg/mL for MCF-7 and above maximum dose limit for HeLa. Acetone extract 67±0.05 µg/mL for MCF-7 and 55±0.11 µg/mL for HeLa. Ethanol extract 52±0.053 µg/mL for MCF-7 and 31.3±0.023 µg/mL for HeLa. Ethanol extract was found to be more cytotoxic as compared to acetone extract. Ethyl acetate extract was found to

be cytotoxic against HeLa and doesn't show the activity against MCF-7. Chloroform extract was found to be cytotoxic against MCF-7 and doesn't show the activity against HeLa. The Results are shown in Table 1 and Figures 3-10.

DISCUSSION

It is common knowledge that cancer ranks as the second leading cause of mortality across the continent of the world. Cancer, which is a non-communicable disease, is a disease that is extremely lethal in people. Numerous external and internal risk factors contribute to the development of cancer; fundamentally, all cancers arise from a deficiency in the response of natural growth inhibitors. Herbs play a significant role in the medical field. Programmed cell death is an important step in maintaining cell homeostasis during body development. Cancer develops when damaged or abnormal cells are able to evade apoptosis and continue to proliferate unchecked. In terms of fatalities, breast cancer is second after lung cancer. One in ten women will be diagnosed with breast cancer at some point in her life. Numerous studies utilizing breast cancer cell lines have indicated that a range of natural plant products and antioxidants may reduce or inhibit carcinogenesis.[30] The advancement in this domain was essential to address a diverse array of ailments using natural herbs.[31] Since ancient times, plants and their derived phytoconstituents have been employed in the treatment of various diseases. Ayurveda, a time-honored and traditional medical system, primarily relies on the application of various herbs to address health issues.[32,33] The present study demonstrated that the various solvent extracts of *Hardwickia binata* contain a diverse array of secondary metabolites that exhibit antioxidant activity and anticancer properties on the MCF-7 and HeLa cancer cell line, based on

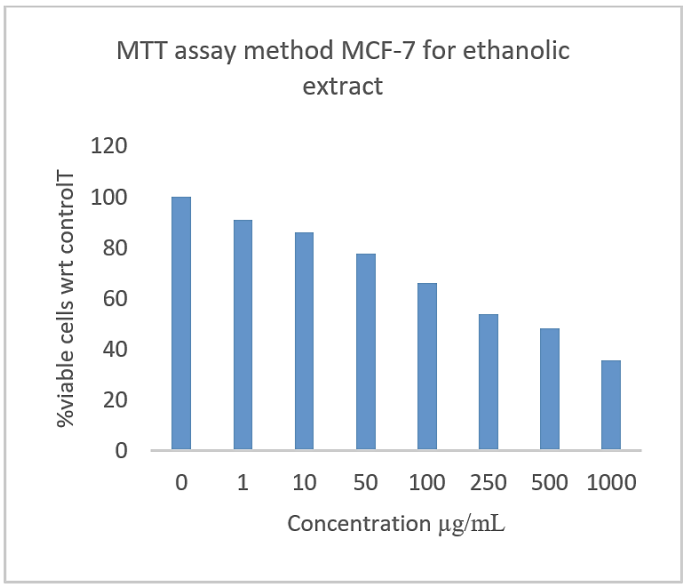

Figure 6: Inhibitory effect of ethanol extract on the proliferation of human breast cancer cell line MCF-7.

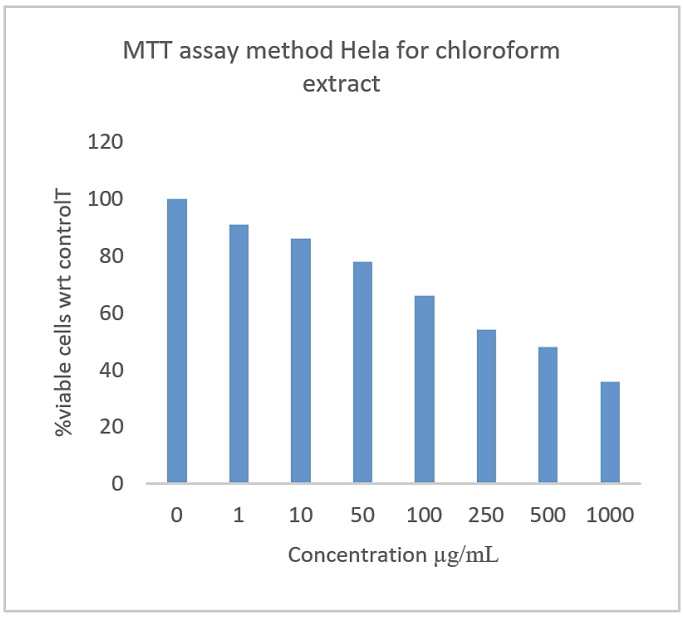

Figure 8: Inhibitory effect of chloroform extract on the proliferation of human cervical cancer cell line HeLa.

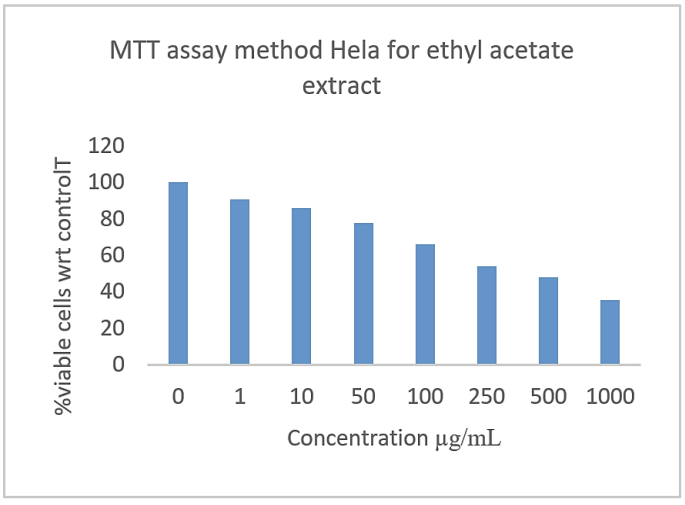

Figure 7: Inhibitory effect of ethyl acetate extract on the proliferation of human cervical cancer cell line HeLa.

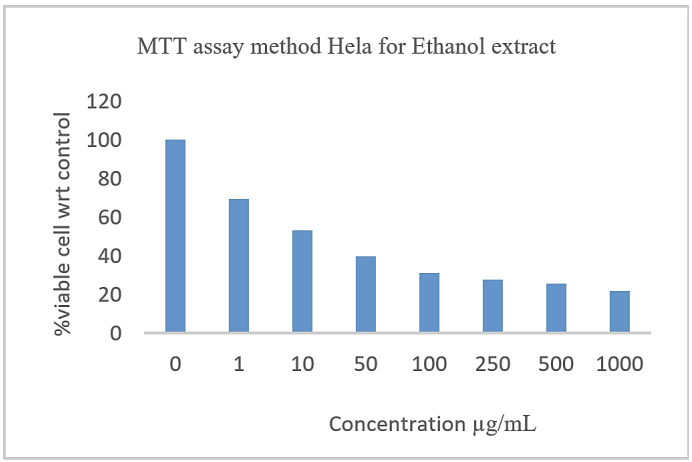

Figure 10: Inhibitory effect of ethanol extract on the proliferation of human cervical cancer cell line HeLa.

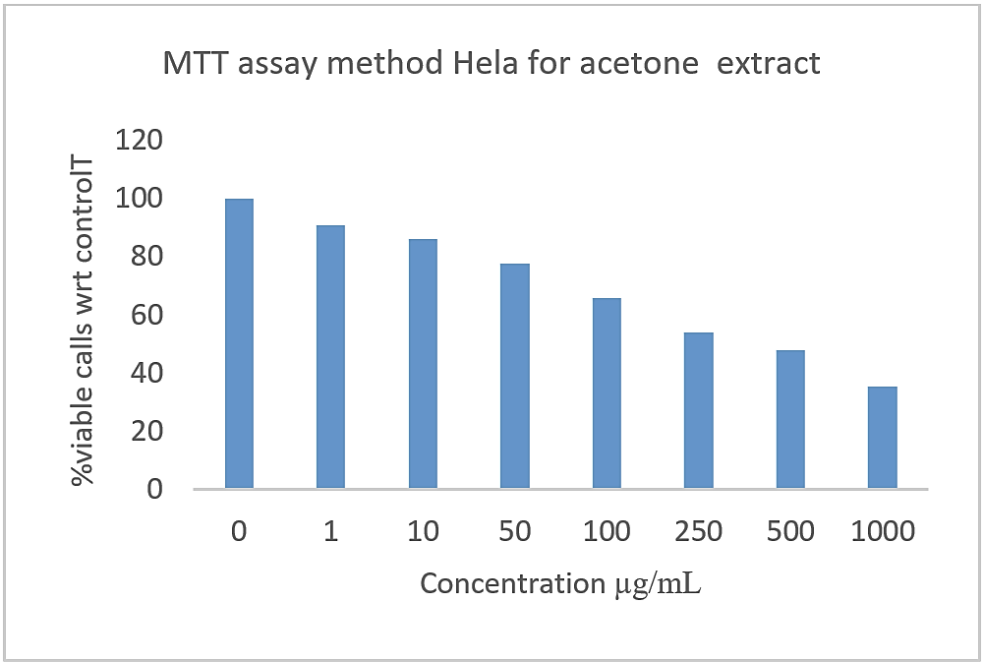

Figure 9: Inhibitory effect of acetone extract on the proliferation of human cervical cancer cell line HeLa.

the experiments conducted. *Hardwickia binata* is a recognized medicinal plant, exhibiting a range of medicinal properties such as antimicrobial, analgesic, antifungal, antibacterial, cardioprotective, anti-inflammatory, antitumor and DNA polymerase β inhibition activities.

CONCLUSION

In conclusion, the findings from our study indicate that *Hardwickia binata* possesses notable anticancer properties. The phytochemical analysis of the test plant extract, based on qualitative studies, indicated the presence of flavonoids, phenolic compounds, tannins, glycosides, proteins. The Study also suggest that acetone and ethanolic extract of *Hardwickia bianata* exhibit cytotoxicity as compared to ethyl acetate and chloroform extract. The plant's phytoconstituents might be useful in the search for new anticancer treatment agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

SPS: Executed the research work and prepared the manuscript, **RBK:** Guidance for conceptualization, designing and execution of research work.

ABBREVIATIONS

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **IC_{50}**: half-maximal inhibitory concentration; **DPPH-2:** 2-diphenyl-1-picrylhydrazyl; **GAE:** Gallic acid equivalent; **RE:** Rutin equivalent.

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