ANTIPROLIFERATIVE ACTIVITY OF *WRIGHTIA TINCTORIA* AND *NERIUM INDICUM* PLANT EXTRACTS AGAINST HUMAN CANCER CELL LINES

Gokulakannan Shanmugam*, Sudhakar Pachiappan, Sabarinath Chandrasekar, Gayathiri Muthusamy, Poorana Pushkalai Saravanan

Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal-637 205, Affiliated to The Tamil Nadu Dr. MGR. Medical University, Chennai-600 032, India

***Corresponding Author**

Gokulakannan shanmugam,

Assistant Professor,
Department of Pharmacology,
Swamy Vivekanandha College of Pharmacy,
Elayampalayam, Namakkal-637 205,
Affiliated to The Tamil Nadu Dr. MGR. Medical University,
Chennai-600 032, India

Abstract

Cancer remains one of the leading causes of mortality worldwide, driving the search for new, effective treatments. Medicinal plants are a rich source of bioactive compounds with potential anticancer properties. This study evaluates the antiproliferative activity of *Wrightia tinctoria* and *Nerium indicum* extracts against human cancer cell lines. Plant materials were collected, dried, and extracted using chloroform, ethanol, and water. The cytotoxic effects were assessed using the brine shrimp lethality assay, while the MTT assay was used to evaluate antiproliferative activity against HeLa (cervical cancer) and MCF7 (breast cancer) cells. The ethanol extracts exhibited the highest cytotoxicity with *Nerium indicum* being more potent than *Wrightia tinctoria*. In the MTT assay, both ethanol extracts showed significant, dose dependent inhibition of HeLa and MCF7 cell growth. The highest inhibition was observed at the concentrations of 50-60 mg/mL for *Wrightia tinctoria* and 40–55 mg/mL for *Nerium indicum*. These findings suggest that the bioactive compounds in *Wrightia tinctoria* and *Nerium indicum* might be have potent anticancer properties. The ethanol extracts, particularly from *Nerium indicum*, demonstrated stronger antiproliferative effects, making them promising candidates for further research in cancer treatment.

Keywords*:* *Wrightia tinctoria, Nerium indicum,* Cytotoxicity, MTT assay, Cancer, Medicinal plants.

1. Introduction

Cancer is a major global health concern, responsible for millions of deaths each year. Despite advancements in chemotherapy, radiotherapy, and immunotherapy, cancer treatment remains challenging due to drug resistance, severe side effects, and high treatment costs[1]. Therefore, there is a growing interest in exploring natural compounds derived from medicinal plants as alternative or complementary therapies for cancer Medicinal plants have been used for centuries in traditional medicine for treating various diseases, including cancer[2]. Many plant-

derived compounds, such as paclitaxel (from *Taxus brevifolia*) and vincristine (from *Catharanthus roseus*), have been successfully developed into anticancer drugs[3]. This has led to increased research into identifying new plant-based bioactive compounds with potential anticancer effects. *Wrightia tinctoria* and *Nerium indicum* are widely used in traditional medicine for their antimicrobial, anti-inflammatory, and wound-healing properties. However, their potential anticancer effects remain underexplored[4, 5]. Previous studies have reported the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic compounds, which may contribute to their cytotoxic properties[6-8]. This study aims to provide an overview of the anticancer potential of *Wrightia tinctoria* and *Nerium indicum*, with a focus on their phytochemical composition, cytotoxic effects, and mechanisms of action. By summarizing recent findings, this study highlights the significance of plant-based compounds in cancer research and their potential role in the development of novel anticancer therapies.

2. Materials and Methods

2.1. Collection and Authentication of Plant Materials.

The plant materials of *Wrightia tinctoria* and *Nerium indicum*, were collected from the in and around area of Komarapalayam and Sankagiri regions of Namakkal District, Tamil Nadu, India. The collected plant materials were certified by Dr. Jayaraman, Botanist, Chennai, Tamil Nadu, under the authentication number CARC/ 2014/3069. After collection, the leaves of both plants were precisely washed with distilled water to remove dust and impurities. Then the leaves were shade- dried at room temperature for 10 – 15 days to retain their bioactive mixtures. The dried leaves were also pulverized into a fine powder using a mechanical grinder and stored in an airtight holder until added use.

2.2 Preparation of Plant Extracts

To gain bioactive composites from the plant materials, consecutive solvent extraction was performed using Sechelt apparatus[9, 10]. The powdered plant materials (1 kg) were subordinated to successive solvent extraction using three different detergents of adding polarity Chloroform (non-polar), Ethanol (semi-polar), Water (polar). Each extraction process was carried out for 10 - 12 hours with 400 mL of each detergent. After extraction, the crude passages were filtered using Whatman No. 1 filter paper to remove plant debris. The filtrates were concentrated under reduced pressure at 60 °C using a rotary vacuum evaporator to get dry excerpts. These excerpts were also stored at 4° C in watertight holders for further analysis.

2.3. Phytochemical Screening

The primary phytochemical analysis was carried out to identify the major chemical ingredients present in the chloroform, ethanol, and water extracts. Standard qualitative tests were performed for Alkaloids (Mayer's Test) [11], Flavonoids (Shinoda Test) [12], Glycosides (Borntrager's Test) [13], Saponins (Foam Test) [14], Phenolic composites and Tannins (Ferric Chloride Test) [14], Phytosterols (Liebermann – Burchard Test) [16], Proteins and Amino Acids (Biuret Test) [17], Carbohydrates (Molisch's Test) [18]. The results were recorded as either positive or negative on the presence or absence of the constituents.

2.4. Brine Shrimp Lethality Bioassay (Cytotoxicity Test)

The brine shrimp lethality bioassay was conducted to assess the cytotoxicity of the plant extracts[19]. The assay was carried out using brine shrimp (Artemia salina) nauplii, which were incubated in artificial seawater.

Preparation of Test Solutions

Each extract (chloroform, ethanol, and water) was dissolved in dimethyl sulfoxide (DMSO) to prepare stock results (4 mg/ mL). Periodical dilutions were made to gain attention of 200

µg/ Ml, 100 µg/ mL, 50 µg/ mL, 25 µg/ mL, 10 µg/ mL, 5 µg/ mL, 3 µg/ mL, 1 µg/ mL. A positive control was prepared using vincristine sulfate, a well- known cytotoxic agent. DMSO (0.1) served as the negative control. Brine shrimp hatching and exposure brine shrimp eggs (Artemia salina) were incubated in artificial seawater under nonstop aeration at 27°C for 48 hours[20]. Ten nauplii were placed in each test tube containing 5 mL of artificial seawater. Different concentration of extract and controls were added to the tubes. After 24 hours, the number of survival was counted under a microscope, and the mortality chance was nauplii calculated[21].

LC_{50} Determination

The median lethal concentration (LC_{50}) was calculated using Probit analysis, conniving the logarithm of attention vs chance mortality. A lower LC_{50} value indicates advanced cytotoxicity[22].

2.5. *In Vitro* Anticancer Activity (MTT Assay)

The MTT assay was performed to estimate the anti-proliferative exertion of the factory excerpts against mortal cancer cell lines[23].

Cell Lines and Culture Conditions

The following mortal cancer cell lines HeLa (Live Cervical Cancer Cell Line), MCF7 (mortal bone Cancer Cell Line) were obtained from NCCS, Pune, India. The cells were dressed in Minimum Essential Medium (MEM) supplemented with10 Fetal Bovine Serum (FBS), 40 µg/ mL Gentamicin, 29 µg/ mL L- Glutamine. Cells were maintained at 37°C in a 5 CO_2 humidified incubator[24, 25].

2.6. MTT Assay Procedure:

Cells were planted into 96- well plates at a viscosity of 17,000 cells/ well and incubated for 24 hours to form a monolayer. The monolayers were treated with different concentration of extracts (prepared in DMSO). Untreated cells served as a negative control, and vincristine

sulfate was used as a positive control. Incubation After 48 hours, 100 µL of MTT result (0.5 mg/mL) was added to each well. MTT Reduction -The plates were incubated for 4 hours, allowing feasible cells to reduce MTT to formazan crystals. Solubilization–Formazan chargers were dissolved using 100 µL of DMSO, and absorbance was measured at 550 nm using a microplate anthology[26, 27].

2.7. Statistical analysis

All trials were performed in triplet, and data were expressed as mean ± standard deviation (SD). Statistical comparisons were made using one- way ANOVA followed by Dunnett's post hoc test in Graph Pad Prism software[28]. Data Representation: Brine shrimp lethality bioassay results were colluded as log attention vs. mortality percentage. MTT assay data were anatomized as absorbance vs. medicine attention to determine cell viability and IC50 values.

2.8. Ethical Considerations:

The study followed standard ethical guidelines for in vitro experiments. Human cancer cell lines were attained from authenticated cell culture repositories. Brine shrimp bioassay was performed in agreement with established toxin webbing protocols.

3. RESULTS

3.1. Yield of Plant Extracts:

The extraction process was conducted using three solvents **(**chloroform, ethanol, and water**)** to obtain different fractions of plant bioactive compounds. The percentage yield of each extract was calculated (Table 1).

Table 1: Percentage Yield of Selected Plant extracts

Extract	***Wrightia tinctoria***	***Nerium indicum***
Chloroform	2.83%	1.48%
Ethanol	8.68%	9.86%
Aqueous	14.5%	13.5%

Aqueous and ethanol extracts showed the respectively higher yield in both the plants, indicating the presence of a greater number of bioactive compounds in aqueous and ethanol soluble fractions**.** Chloroform extracts had the lowest yield, suggesting fewer non-polar compounds in these plants**.**

3.2. Phytochemical Screening

The preliminary phytochemical analysis of the extracts revealed the presence of various secondary metabolites, which are known to exhibit anticancer activity**.** Both the plant extracts showed the presence of flavonoids, saponins, phenolic compounds, and tannins. *Wrightia tinctoria* contained alkaloids and glycosides, which were absent in *Nerium indicum*. *Nerium indicum* extracts were rich in phytosterols and steroids, which might contribute to their stronger anticancer activity.

3.3. Cytotoxicity Assessment (Brine Shrimp Lethality Bioassay)

The brine shrimp lethality assay was used as a preliminary cytotoxicity screening to determine the toxic potential of plant extracts. The LC_{50} values indicated that ethanol extract of *Wrightia tinctoria* had the highest cytotoxicity (LC_{50} = 3.00 µg/mL), making it a promising candidate for further anticancer studies. Chloroform extract of *Nerium indicum* was also highly toxic (LC_{50} = 10.10 µg/mL), suggesting strong bioactive potential. Aqueous extracts had the weakest cytotoxic effects which suggest that water soluble compounds may not contribute significantly to anticancer activity (Figure 1). The results align with previous research where ethanol extracts of *Wrightia tinctoria* demonstrated potent cytotoxicity in various cell lines. Studies on *Nerium indicum* have reported its apoptosis-inducing properties in cancer cells, supporting our findings (Table 1) (Figure 2).These cytotoxic effects further validate the traditional medicinal use of these plants for treating various diseases, including cancer.

Table 2. LC_{50} Values of Plant Extract

Extract	LC_{50} (µg/mL)	
	Nerium indicum	***Wrightia tinctoria***
Chloroform	10.10 ± 0.57	25.02 ± 1.44
Ethanol	25.22 ± 3.22	3.00 ± 0.07
Aqueous	80.20± 1.70	90.00 ± 3.58

N=3, Values are expressed as mean ± SD

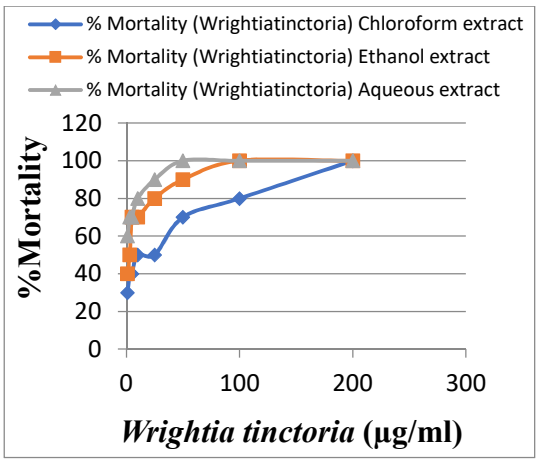

Figure 1: Brine Shrimp Lethality Bioassay of *Wrightia tinctoria*

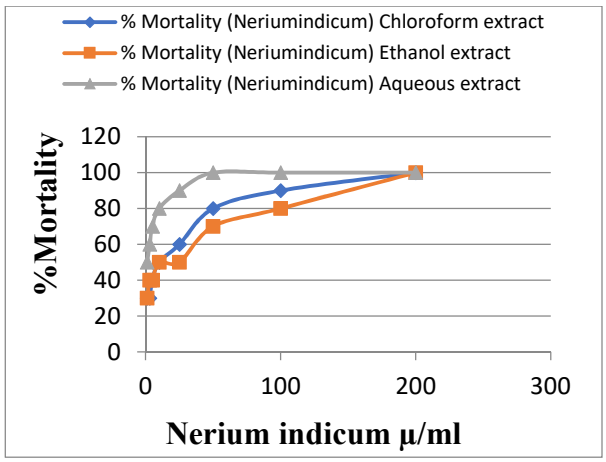

Figure 2 : Brine Shrimp Lethality Bioassay of *Nerium indicum*

3.4. *In Vitro* Anticancer Activity on cancer cell lines (MTT assay)

The MTT assay evaluated the ability of plant extracts to inhibit cancer cell growth. The results in Table 3 revealed that ethanol extract of *Wrightia tinctoria* showed the highest inhibition of HeLa cells (IC_{50} = 30 mg/ml), indicating a strong cytotoxic effect on cervical cancer cells (Figure 3&4). Ethanol extract of *Nerium indicum* was more effective against MCF7 cells (IC_{50} = 40 mg/ml), suggesting selective cytotoxicity towards breast cancer cells (Figure 5 & 6). Chloroform extracts also exhibited significant inhibition, but less than ethanol extracts, indicating that ethanol extracts contain more potent bioactive compounds.

Table 3. IC_{50} Values for Anticancer Activity

Extract	IC_{50} (mg/mL)	
	HeLa Cells	MCF7 Cells
Chloroform (WT)	50 ± 0.23	60 ± 4.11
Ethanol (WT)	30 ± 2.03	45 ± 1.20
Chloroform (NI)	40 ± 1.22	55 ± 0.43
Ethanol (NI)	35 ± 5.20	40 ± 1.00

N=3, Values are expressed as mean ± SD. WT- *Wrightia tinctoria,* NI- *Nerium indicum*

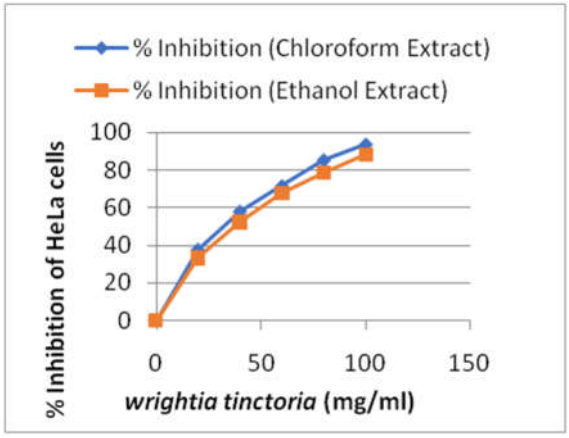

Figure 3.Antiproliferative activity of Wrightia tinctoria in HeLa cells.

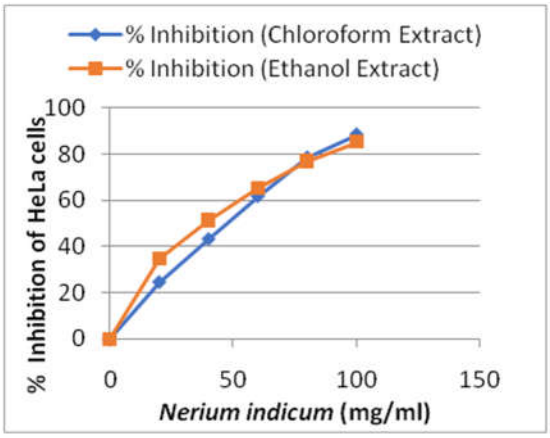

Figure 4.Antiproliferative activity of *Nerium indicum* in HeLa cells.

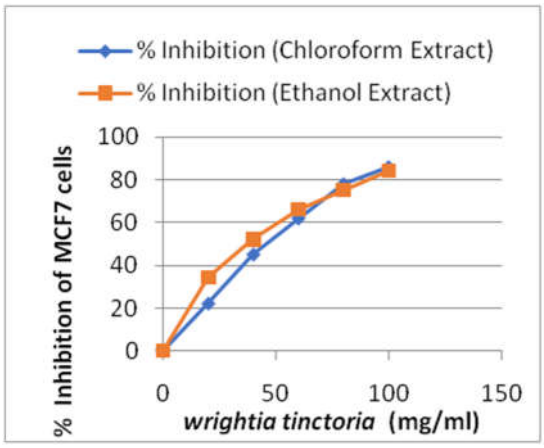

Figure 5. Antiproliferative activity of of *Wrightia tinctoria* in MFC7 cells.

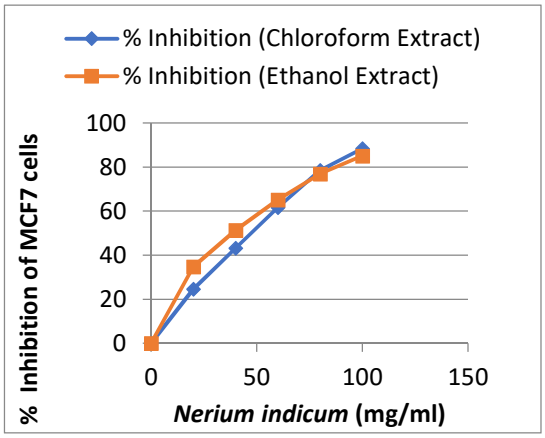

Figure 6. Antiproliferative activity *Nerium indicum* in HeLa cells.

4. DISCUSSION

Plants have long been recognized as an abundant and diverse source of bioactive compounds, largely due to their ability to synthesize a wide range of secondary metabolites[29]. These compounds often exhibit significant biological activity, including potent toxicity against a variety of microorganisms such as bacteria and fungi. The ecological role of these metabolites is primarily protective, allowing plants to defend themselves against pathogens, herbivores, and environmental stressors. However, their diverse chemical structures and bioactivities also make them valuable leads in the search for novel therapeutic agents. Despite the rapid advances in synthetic chemistry, molecular biology, and combinatorial drug design, natural products derived from plants continue to hold a prominent place in pharmaceutical research and development. Many clinically approved drugs, including anticancer and antimicrobial agents, have been inspired by or directly extracted from plant-derived compounds[30]. A commonly employed method for preliminary toxicity screening of plant extracts is the brine shrimp lethality assay. This bioassay utilizes the larvae of *Artemia salina* (brine shrimp) as a model organism to assess cytotoxic activity[31]. It is widely appreciated for its simplicity, low cost, and minimal requirement for specialized equipment or reagents. The test is particularly useful in the early stages of drug discovery for identifying potential cytotoxic and antitumor agents in crude plant extracts. The brine shrimp lethality assay provides a rapid and effective means of evaluating the general toxicity of extracts, and has been correlated with more complex biological systems[32]. Several studies have reported a strong correlation between toxicity in brine shrimp and lethality in human cancer cell lines, thereby supporting its predictive value in cytotoxicity screening[31, 33]. In the current study, the crude extracts of *Wrightia tinctoria* and *Nerium indicum* were subjected to the brine shrimp lethality assay to evaluate their potential cytotoxic properties. Both plants have a long-

standing history of use in traditional medicine systems, particularly in the treatment of cancer and skin-related ailments. The results of the assay indicated significant toxicity for both plant extracts, which lends scientific credibility to their ethnopharmacological use. More specifically, the leaf extracts of both species exhibited greater toxicity than other aerial parts, aligning with previous reports that highlight the therapeutic superiority of leaves due to higher concentrations of bioactive compounds. To further investigate the nature of the active constituents, the crude extracts were subjected to solvent fractionation using water, ethanol, and chloroform. The fractionation process aimed to separate compounds based on polarity and solubility, providing insight into the type of phytochemicals responsible for the observed bioactivity. Among the various fractions, chloroform and ethanol extracts exhibited the highest toxicity in the brine shrimp assay, suggesting that non-polar and semi-polar constituents contribute significantly to the cytotoxic potential of these plants. This observation is consistent with previous findings in studies involving plants such as *Achillea santolina*, *Typhonium flagelliforme*, *Schisandra sphenanthera*, and *Scutellaria barbata*, where non-polar phytoconstituents were identified as key contributors to cytotoxic and antitumor activities. Subsequent *in vitro* assays were conducted to assess the cytotoxic effects of the aqueous extracts of *Wrightia tinctoria* and *Nerium indicum* on human cancer cell lines. Interestingly, these polar fractions demonstrated minimal toxicity, reinforcing the notion that the biologically active compounds are predominantly non-polar. The aqueous extracts showed weak anti-proliferative activity, indicating that water-soluble compounds in these plants are less likely to contribute to anticancer effects. This further emphasizes the importance of extraction solvent choice in isolating pharmacologically relevant compounds from plant materials. Further evaluation of the ethanol extracts, particularly from *Nerium indicum*, revealed dose- and time-dependent cytotoxic effects across several human cancer cell lines, including HeLa (cervical cancer) and MCF-7 (breast cancer) cells. The cytotoxic

response was not uniform across all cell types, indicating possible cell-line specific mechanisms of action and suggesting a need for more detailed mechanistic studies. Among the tested extracts, the ethanol fraction of *Nerium indicum* exhibited the most potent cytotoxic effects, outperforming *Wrightia tinctoria* in both HeLa and MCF-7 cell lines. These results are in accordance with prior research indicating the pro-apoptotic and anti-proliferative effects of *Nerium indicum* on a wide range of cancerous cell types. Given these promising results, further investigation into the chemical composition of *Nerium indicum* is warranted. Detailed phytochemical profiling, bioassay-guided fractionation, and structure-activity relationship (SAR) studies are needed to isolate and characterize the active constituents responsible for the observed effects. The identification of such compounds could potentially lead to the development of novel chemo preventive or chemotherapeutic agents derived from natural sources.

5. CONCLUSION

In conclusion, the findings of this study not only validate the traditional uses of *Wrightia tinctoria* and *Nerium indicum* in cancer treatment but also emphasize the value of brine shrimp lethality assay as a preliminary screening tool in natural product research. The greater efficacy of ethanol and chloroform fractions in both bioassays highlights the importance of extraction strategy in isolating pharmacologically active compounds. Moving forward, detailed pharmacological and phytochemical investigations are essential to harness the full therapeutic potential of these plants, particularly *Nerium indicum*, which has shown significant promise in this study. The ongoing search for safe and effective plant-based anticancer agents remains a vital endeavor in the development of modern medicine.

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