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Cytotoxic Effect of Ethanolic Extract Ofcipadessa Baccifera (Roth) Miq. Leaves on SH-SY5Y Neuroblastoma Cell Line Invitro

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ABSTRACT
Cipadessa baccifera (Roth) Miq. the plant possesses medicinal properties like antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anticancer and traditionally for diarrhea, toothache, cough, colds, indigestion, flu symptoms, dysmenorrhea, urinary tract infection,bleeding and swelling of gums, skin rashes, lowering blood sugar, and skin diseases.This work aim to study the Cytotoxic Effect of Ethanolic Extract of Cipadessabaccifera (Roth) Miq. leaves on SH-SY5Y neuroblastoma cell line invitro.Ethanol was used to extractCipadessabaccifera (Roth) Miq.leaves after they were gathered and shade-dried. For a full day, the SH-SY5Y neuroblastoma cells were cultivated and exposed to different doses of the ethanolic extract.The MTT assay was used to measure cell viability, cytotoxicity, and morphological changes were observed under an inverted microscope.On SH-SY5Y cells, the ethanolic extract ofCipadessa baccifera(Roth) Miq. leavesdemonstrated a dose-dependent cytotoxic impact. The morphological characteristics like condensed nuclei, vacuolization,cell shrinkage, rounding of cell were evident in treated cells.The study demonstrates thatCipadessa baccifera(Roth) Miq.ethanolic leaf extract exhibits significant cytotoxic activity against SH-SY5Y neuroblastoma cells, indicating that it may have promise as a natural anticancer drug.

Keywords: Cipadessa baccifera(Roth) Miq., SH-SY5Y neuroblastoma cell line, MTT assay, cytotoxicity , viability of cells.

I. INTRODUCTION

Cancer is defined by the unchecked proliferation and dissemination of abnormal cells.Metastasis is the process by which cancer cells expand into invasive tissues. According to the tissue or organ of the human body that is impacted, there are more than 100 distinct kinds of cancer. In addition to several internal variables like hormones, immunological disorders, hereditary mutations, and random mutations, it is brought on by a variety of external sources, including tobacco, chemicals, radiation, and infectious organisms[1].Globally, about 1 in 5 people will develop cancer in their lifetime.approximately 1 in 9 men and 1 in 12 women will pass away from it.In 2022, there were an expected 14,61,427 incident cases of cancer in India (crude rate: 100.4 per 100,000)and it estimates, the number of cancer cases will rise by 12.8% in 2025 compared to 2020.According to a 2025 study conducted at the Kerala Cancer Conclave, there are approximately 88,460 cases per year (45,350 women and 43,110 males)[2].One of the human cancers, neuroblastoma, exhibits spontaneous regression, changing from an undifferentiated stage to a fully cellular benign state. In children, this is the most common extracranial cancer and it comes from the sympathetic nervous system. Despite significant progress in diagnostic techniques and standard therapies over the last three decades, neuroblastoma continues to provide a terrifying challenge to scientific and clinical scientists[3].Herbal remedies are essential for both cancer prevention and treatment.Advanced molecular science knowledge and improvements in isolation and structure elucidation techniques have led to the identification of several anticancer herbs that work therapeutically by blocking hormones and enzymes that activate cancer, promoting DNA repair, encouraging the production of protective enzymes, inducing antioxidant action, and boosting the body's immunity[4].The plant Cipadessa baccifera (Roth) Miq.belonging to the family Meliaceae has antibacterial, antioxidant, anti-inflammatory, antidiabetic, and anticancer qualities and traditionally used for diarrhea, toothaches, coughs, colds, indigestion, flu symptoms, dysmenorrhea, urinary tract infections, gum bleeding and swelling, skin rashes, decreasing blood sugar, and skin illnesses[5].An established in vitro model for researching neuronal differentiation and identifying possible neurotoxic or

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neuroprotective drugs is the SH-SY5Y cell line, which is derived from human neuroblastoma. The present study aim to investigate the Cytotoxic Effect of Ethanolic Extract of Cipadessabaccifera (Roth) Miq. leaves on SH-SY5Y neuroblastoma cell line invitro.By assessing cell viability and morphological changes following treatment, this research seeks to elucidate the anticancer potential of Cipadessabaccifera (Roth) Miq. thereby contributing to the ongoing search for novel plant-based therapeutics in oncology.

II. MATERIALS AND METHODS

2.1Plant material

Cipadessa baccifera (Roth) Miq. Leaves were collected from kurupumthara, Kottayam, keralaand was authentified by Dr. Rojimonp. Thomas,HOD,Department of botany,CMS college Kottayam.

2.2 Preparation of plant extract

Weighed amount of dried and coarsely powdered Cipadessa baccifera(Roth) Miq.Leaves were extracted using Soxhlet extractor using ethanol (90%)as a solvent. Evaporation done with the help of rotary evaporator.

2.3Preparation of culture media for cell line culture

Dulbecco's modified Eagles medium, DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate(Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml) for culturing SHSY-5Y (Human Neuroblastoma cells) cell line[6].

2.4Preparation of cell for MTT assay

The SHSY-5Y cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay method carried out by Lee[7] was followed accordingly

- A 96-well tissue culture plate was seeded with 100µl of the cell suspension ($5x10^3$ cells/well) after a two-day-old confluent monolayer of cells had been trypsinized and suspended in 10% growth media. The cells were then kept at 37ºC in a humidified 5% CO_2 incubator.
- 1mg of Ethanolic Extract of Cipadessa baccifera (Roth) Miq. Leaveswas weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.
- The growth medium was removed after 24 hours,freshly prepared each compounds in DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25 µg, 12.5 µg, 6.25 µgin 500µl of DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO2 incubator. Non treated control cells were also maintained.
- Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.
- After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide,DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm.
- The percentage of growth inhibition was calculated using the formula: % of viability =(mean OD of SAMPLE ÷ mean OD of control∗100
- This assay measures the reduction in cell viability and any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

MTT mechanism

Tetrazolium salt reduction is generally accepted as a valid method for examining cell growth.In order to ascertain the impact of the test extract on cellular proliferation and viability, the 3-(4,5 Dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT) assay technique was used[7].Reduction of tetrazolium by dehydrogenase enzymes is character of

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metabolically active cell that produces NADH and NADPH. The formazan product (purple crystals) has low aqueous solubility. Formazan dissolved in dimethyl sulfoxide (DMSO) was quantified and the intensity of the product colour was measured at 570 nm. Which was directly proportional to the number of living cells in the culture(fig 1)[8].

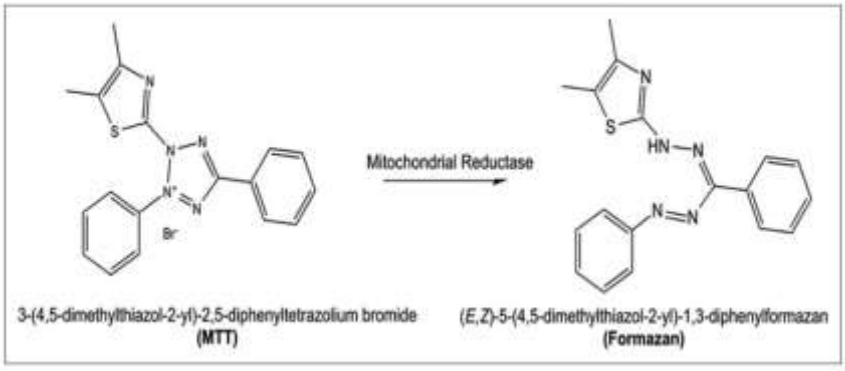
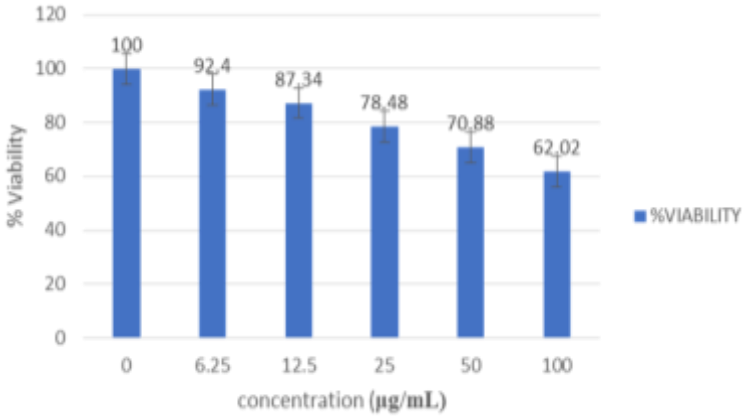


Figure 1: MTT mechanism

III. RESULTS AND DICUSSIONS



LC_{50} value- 196.21µg/ml

Figure 2: percentage cell viability of SH-SY5Y neuroblastoma cell line treated with leaf extract of Cipadessa baccifera (Roth) Miq.

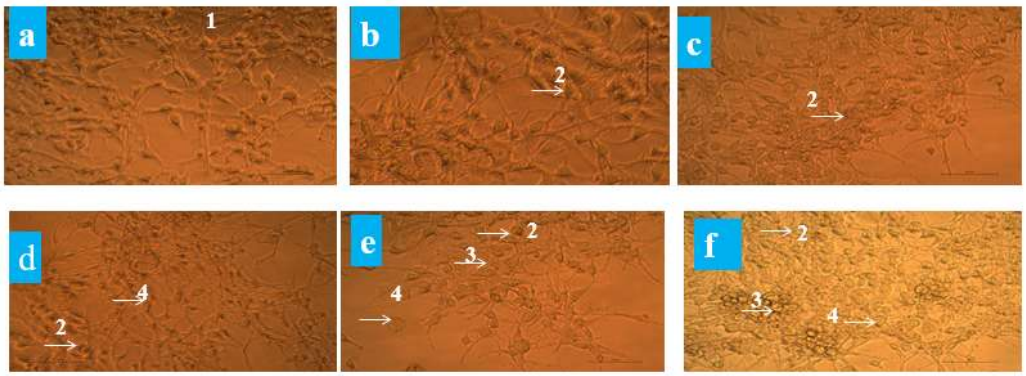


Fig 3: Phase contrast images of Neuroblastoma cell lines treated with control and different concentrations of Ethanolic leaf extract of Cipadessa baccifera (Roth) Miq.

a – Control, **b** – 6.25µg/ml, **c**–12.5µg/ml, **d** - 25µg/ml, **e** - 50µg/ml, **f** - 100µg/ml

Control cell (1),condensed nuclei(2), vacuolization (3),rounding of cells(4)

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Discussion

The ethanolic extract of Cipadessa baccifera(Roth) Miq. leaves have a dose-dependent cytotoxic effect on SH-SY5Y neuroblastoma cells. Both optical density (OD) and the percentage of cell viability gradually decreased as the extract concentration dose from 6.25 µg/ml to 100 µg/ml.At 6.25 µg/ml, cell viability was 92.40%, indicating minimal cytotoxic effect.At the highest tested concentration (100 µg/ml), the cell viability reduced to 62.02%.This decline in cell viability suggests that the extract possesses potential antiproliferative or cytotoxic activity against neuroblastoma cells. The optical density values are inversely proportional to the cytotoxicity: lower OD values at higher concentrations indicate reduced mitochondrial activity, reflective of cell death or reduced metabolic activity.The dose-dependent nature of the cytotoxicity suggests that components within the ethanolic extract interfere with the viability of SH-SY5Y cells, potentially through induction of apoptosis, cell cycle arrest, or oxidative stress.

IV. CONCLUSION

The Ethanolic leaf extract of Cipadessa baccifera (Roth) Miq. demonstrates a dose-dependent cytotoxic effect on SH-SY5Y neuroblastoma cells in vitro, by decreasing cell viability with increasing concentrations of the extract. These findings suggest that the plant contains bioactive compounds with potential anticancer activity. This study may help in opening new avenue for cancer research as well as plant – cancer treatment. Additionally, evaluating the extract's effects on normal (non-cancerous) cells is essential to determine its selectivity and safety for future therapeutic applications.

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