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Research Article

Anticancer Activity of Ethanolic Extract of Coleus Amboinicus

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ABSTRACT

The present study was designed to evaluate the anticancer activity of ethanolic extract of coleus amboinicus against hepatic cell line (HepG2 cells). In this study we investigate the antioxidant property of the plant. The anticancer activity of the plant extract was evaluated by MTT assay and the cell cytotoxicity was determined. The doses of test used to determine the cell cytotoxicity are: 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml. The test is compared with the standard solution of doxorubicin (~15.98µg/ml). In this study, HepG2 cells were treated with recommended concentrations of the test compound (HB) along with negative and positive controls for an incubation of 24hrs. The experiment results suggested that the test compound (HB) showed dose dependent cytotoxicity, against the HepG2 cell line, with IC50 value of ~ 447.09 µg/ml, after 24hrs of incubation. Coleus amboinicus contain phytochemicals like alkaloids, flavonoids, glycosides phenolic and poly phenolic compounds with potential anti-oxidant properties.

INTRODUCTION

Cancer stands out as a major cause of death and illness, especially among older individuals.[1]cancers are caused by mutations that may be inherited, induced by environmental factors, or result from DNA replication errors.[2]in fact, a risk of developing cancer that is ten times higher compared to those under 65.the development of cancer arise from both internal and external factors, culminating in a series of genetic changes-a process referred to as multistep oncogenesis .[1]its widely acknowledged that current cancer treatments are often fall short of being both highly ineffective and completely safe. Consequently, the search for new and improved anticancer drugs remain crucial to overcome the irritations of existing chemotherapy. remarkably, around half of the 200 or so many chemical compounds approved in recent decades for cancer treatment either directly derived from natural

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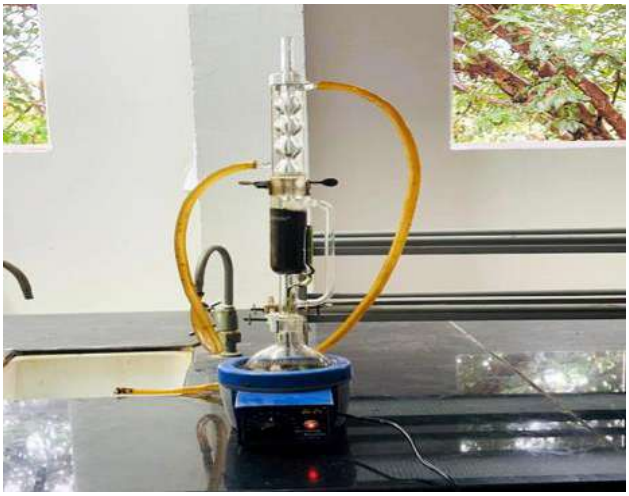
sources or are modified versions of compounds found in nature, enhanced for safety and efficacy.[2] Across the globe, numerous plant species are utilised in various aspects of human health.These plants frequently contain active components like alkaloids, phenols, tanins, cryogenics, glycosides, and terpenoids.[3] These substances have a history of safe use including applications as sweeteners, anti infectives and antibacterials.For millennia medicinal plants have played a role in treating human illenesses.The importance of these plants and traditional health practises in addressing global health changes is gaining increased recognition.Plant derived anticancer agents are now employed in cancer therapy.Examples of these agents include vincristine, taxol, vinblastine, derrivatives of these, irinotecan and topetecan, as well as etoposide, which is derived from epipodophyllotoxin;these are used in clinical trials worldwide.[4,5] Coleus amboinicus(lour), also known as plectranthus amoboinicus or coleus aromaticus is a freshly and strongly aromatic plant belonging to the Lamiaceae family. It is commonly reffered to as Mexican mint,Spanish thyme, Cuban oregano or indian borage. In traditional folk medicine, this plant is used to treat a range of conditions, including asthma, headaches, skin problems, coughs, constipation, cold and fevers.[6] To date several phytochemicals have been identified in coleus amboinicus including various compounds such as phenolics, terpenoids, phenolic acids, flavonoids, falvones, and tanins.[7] research , both invitro and invivo, has documented numerous pharmacological properties of crude extracts from coleus amboinicus including antitumour,antibacterial, antifungal, antiprotozoal, anti-inflammatory, anti-oxidant, antidiabetic, wound healing, analgesic, antirheumatic and other therapeutic properties.[8,9]

MATERIAL AND METHODS

Collection Of Sample: Fresh leaves of Coleus amboinicus (Plectranthus amboinicus) were plucked from local area of Thaliparamba,Kannur.

Drying Of Sample: The leaves of Coleus amboinicus were plucked, washes with clean water and dried under shade dry for 1 week and dried leaves of Coleus amboinicus were subjected to powdering. BLENDING: Dried leaves of Coleus amboinicus were put in a mechanical blender and made a fine powder.These powder of Coleus amboinicus were further subjected to extraction process.

Extraction: The fine powder of Coleus amboinicus(60g) into the thimble of 250g Soxhlet apparatus and wetted with ethanol and the solvent is heated in the round bottom flask of the Soxhlet apparatus.Once the extraction process is completed it is subjected to evaporation until the product is obtained.



METHODOLOGY

MTT ASSAY: MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm (Alley, M. C et al., 1986, Mosmann et al., 1983). [10]

MATERIALS:

1. Cell line: HepG2 (Human Hepatocellular Carcinoma Cell Line (NCCS, Pune))
2. Cell culture medium: McCoy's 5A Media (#AL057A, Himedia)
3. Fetal Bovine Serum (#RM10432, Himedia)
4. Antibiotic Antimycotic Solution-Penicillin & Streptomycin (#A001A, Himedia) 5. Trypsin-EDTA solution (#TCL155, Himedia) 6. D-PBS (#TL1006, Himedia) 7. DMSO (#PHR1309, Sigma) 8. MTT Reagent (# 4060, Himedia) 9. Standard drug: Doxorubicin (#D1515, sigma) 10. T25 flask (#12556009, Biolite - Thermo) 11. 96-well plate for culturing the cells (Corning, USA)
5. Trypsin-EDTA solution (#TCL155, Himedia)
6. D-PBS (#TL1006, Himedia)
7. DMSO (#PHR1309, Sigma)
8. MTT Reagent (# 4060, Himedia)
9. Standard drug: Doxorubicin (#D1515, sigma)
10. T25 flask (#12556009, Biolite - Thermo)
11. 96-well plate for culturing the cells (Corning, USA)
12. 1.5 ml centrifuge tubes (TARSON)
13. 50 ml centrifuge tubes (# 546043 TARSON)
14. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor (#Eppendorf).
15. 10 to 1000 µl tips (TARSON)

Equipment's:

1. Centrifuge (Remi: R-8oC).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted microscope (Biolinkz, India)
4. 37°C incubator with humidified atmosphere of 5% CO2 (Memmert, Germany)
5. 96well microplate reader (ELX-800, BioTek, USA)

Assay Controls:

- Medium control (medium without cells)

- Negative control (medium with cells but without the experimental drug/compound)
- Positive controls (medium with cells and ~15.98 µg/ml of Doxorubicin)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alphatocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

Steps Followed: 1. Seed 200µl cell suspension for adherent in a 96-well plate at required cell density, without the test agent. Allow the cells to grow for about overnight. 2. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet). 3. Incubate the plate for 24hrs at 37°C in a 5% CO2 atmosphere. 4. After the incubation period, takeout the plates from incubator, and remove spent media only for adherent cell line and add MTT reagent to a final concentration of 0.5mg/mL of total volume. 5. Wrap the plate with aluminium foil to avoid exposure to light. 6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time s should be kept constant while making comparisons.) 7. Add 100 microlitres of DMSO. gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. 8. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm. 9. The IC50 value was determined by using Logarithmic equation i.e., Y=Mln(x)+C Here, Y = 50, M and C values were derived from the viability graph.[11]

Table 1: Details Of Test Compound Concentrations

SL. No	Test Compound	Cell Line	Concentration treated to cells
1	Untreated	HepG2	No treatment
2	Standard	HepG2	Doxorubicin ~ 15.98 µg/ml
3	Blank	HepG2	Only Media without cells
4	HB	HepG2	5 (100,200,300,400 and 500 µg/ml)

RESULT AND DISCUSSION

The result is represented as statistical analysis by computerised method. In this study, given test compound is evaluated to analyse the cytotoxicity effect on HepG2 cell line. The concentrations of the test compound used to treat the cells are as follows:

Table 2: Measure Of Cell Viability

Concentration	Cell Viability %
Untreated	100 ± 0.0070
Standard (Doxorubicin)	46.31 ± 0.0021
100µg/ml	82.08 ± 0.0028
200 µg/ml	68.23 ± 0.0014
300 µg/ml	58.57 ± 0.0084
400 µg/ml	52.66 ± 0.0035
500 µg/ml	47.14 ± 0.0084

IC50 VALUE = ~ 447.09 µg/ml

Table No.5: Measure Of Cell Viability

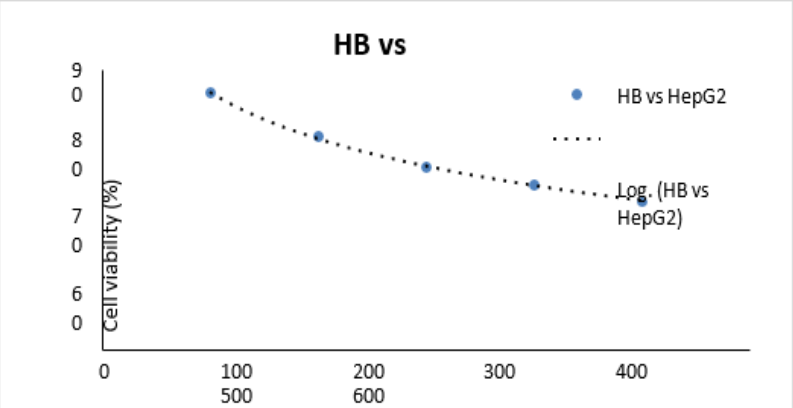


Fig1: Graphical Representation of Concentration Of HB(µg/Ml) Vs Cell Viability (%)

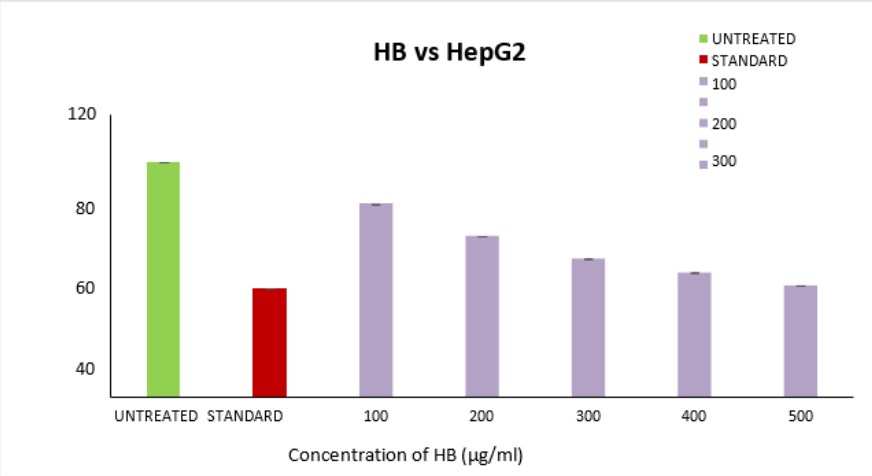
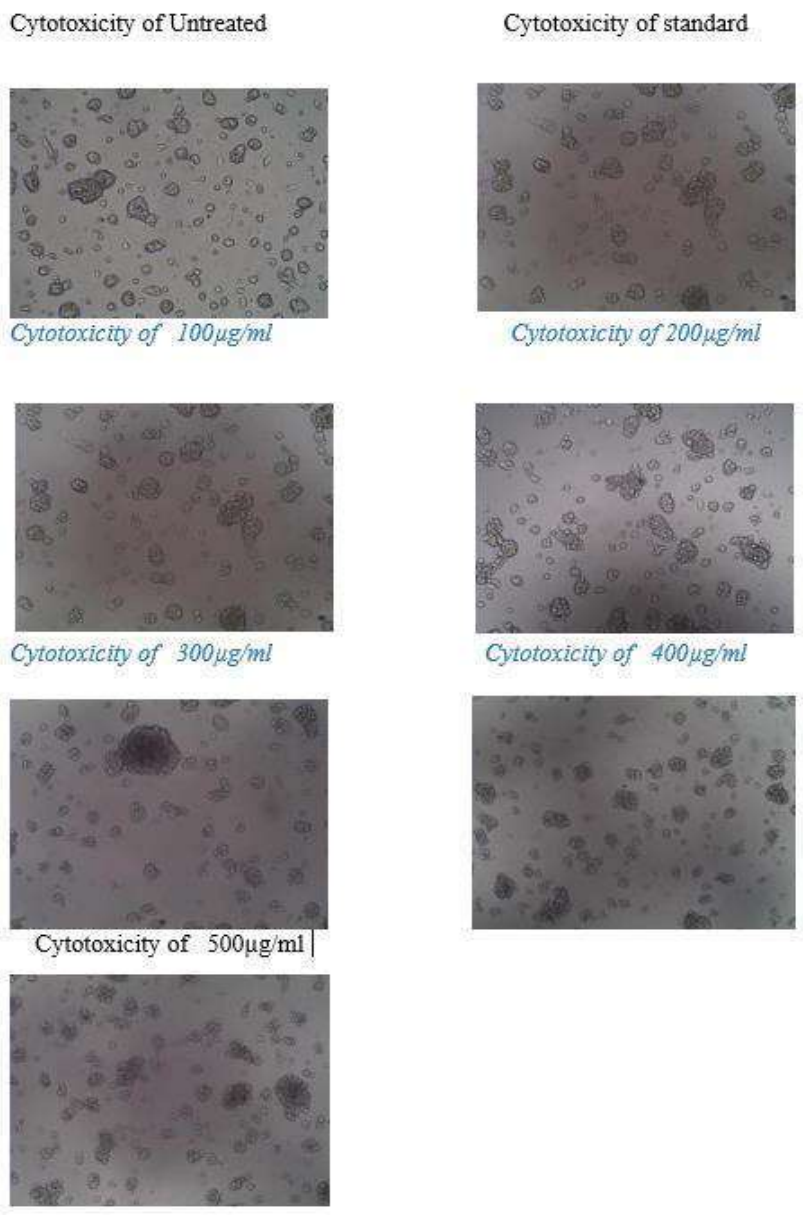


Fig 2: Mean % cell viability of HepG2 cell line after exposing to test compound for 24hrs.



CONCLUSION

In this study, HepG2 cells were treated with recommended concentrations of the test compound (HB) along with negative and positive controls for an incubation of 24hrs. The cytotoxicity of the test sample was evaluated by MTT assay. The experiment results suggested that the test compound (HB) showed dose dependent cytotoxicity, against the HepG2 cell line, with IC50 value of ~ 447.09 µg/ml, after 24hrs of incubation. *Coleus amboinicus* contain phytochemicals like alkaloids, flavonoids, glycosides phenolic and poly phenolic compounds with potential anti-oxidant properties. However further research is necessary to fully understand their mechanism of action and to determine their effectiveness in cancer treatment. Therefore, further investigation need to be carried out to isolate and identify the anti-oxidant constituents present in the plant extract.

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