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Research Article

Invitro Cytotoxic Activity of Ethanolic Extract OF *Reissantia indica* on the Human Colon Cancer Cell Line (HT-29)

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ABSTRACT

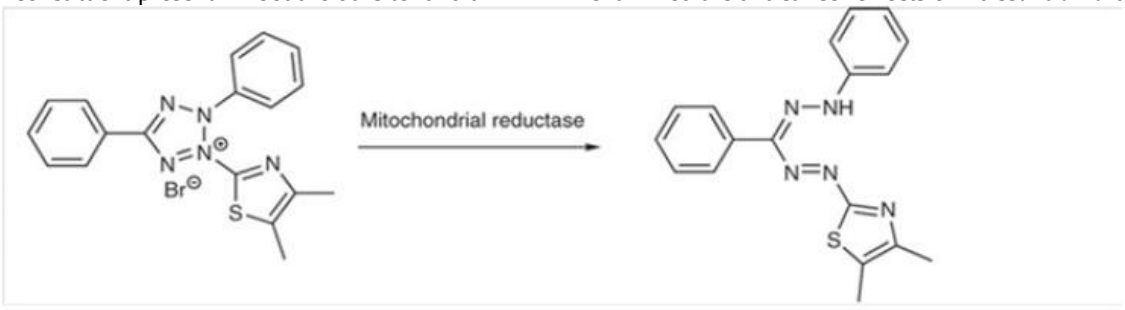
Reissantia indica belongs to the family Celastraceae shrub, in dryish forest or wood land or on river banks, often in stony ground of Senegal to Cameroun and occurring south in the transvaalin tropical Asia from India and Ceylon to the Philippines at altitude range of 210–1170 m. Root bark is used for the treatment of respiratory troubles. Stems are considered as febrifuge. Leaves are scorched and given to women during confinement. Powdered leaves and roots are applied to sores and wounds. Effect of anticancer activity was studied on HT- 29 cell lines by MTT assay using the ethanolic extract of *Reissantia indica* . Different doses of plant extract and standard were taken and introduced into cancer cells were recorded at 24 hrs respectively it clearly showed us the dose dependent response for the inhibition of cells. The most potent anticancer activity has been shown at the concentration 1000 µg/mL of *Reissantia indica*. extract on HT-29 colon cancer cell line.

Keywords: *MTT Assay, HT-29 colon cancer cell line, Reissantia indica*.

INTRODUCTION

Many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Thus, research has developed into investigating the potential properties and uses of plant extracts for diseases like cancer[1]. *Reissantia indica* medicinal plant which belongs to the family Celastraceae (Spike-thorn family) Synonym *Hippocrateaindica*. In India it was identified in Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Karnataka, Kerala, Maharashtra, Orissa, Tamil Nadu, West Bengal. *Reissantiaindica* root bark was used for the treatment of respiratory troubles. Stems are considered as febrifuge. Powdered leaves and roots are used to treat sores and wounds. Leaves part are scorched and given to women during confinement. The chemical constituent present in root are dulcitol and an antibiotic principle Pristimerin which is considered to be activity against Gram positive bacteria. It is believed that pristimerin is also works against the strains of mycobacterium which causes tuberculosis. It is most useful as an antibiotic therapy in respiratory inflammations.

Cancer is an uncontrollable cellular growth that remains an aggressive killer disease in worldwide. Treatment for cancer is always been costly and express several side effects with respect to treatment like radiation or chemotherapy[2]. Alternative methods of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenesis and associated inflammatory processes. In the current study we initially examined the anticancer effects of *Reissantiaindica*



(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))

MTT

(2E,4Z)-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan

(Formazan)

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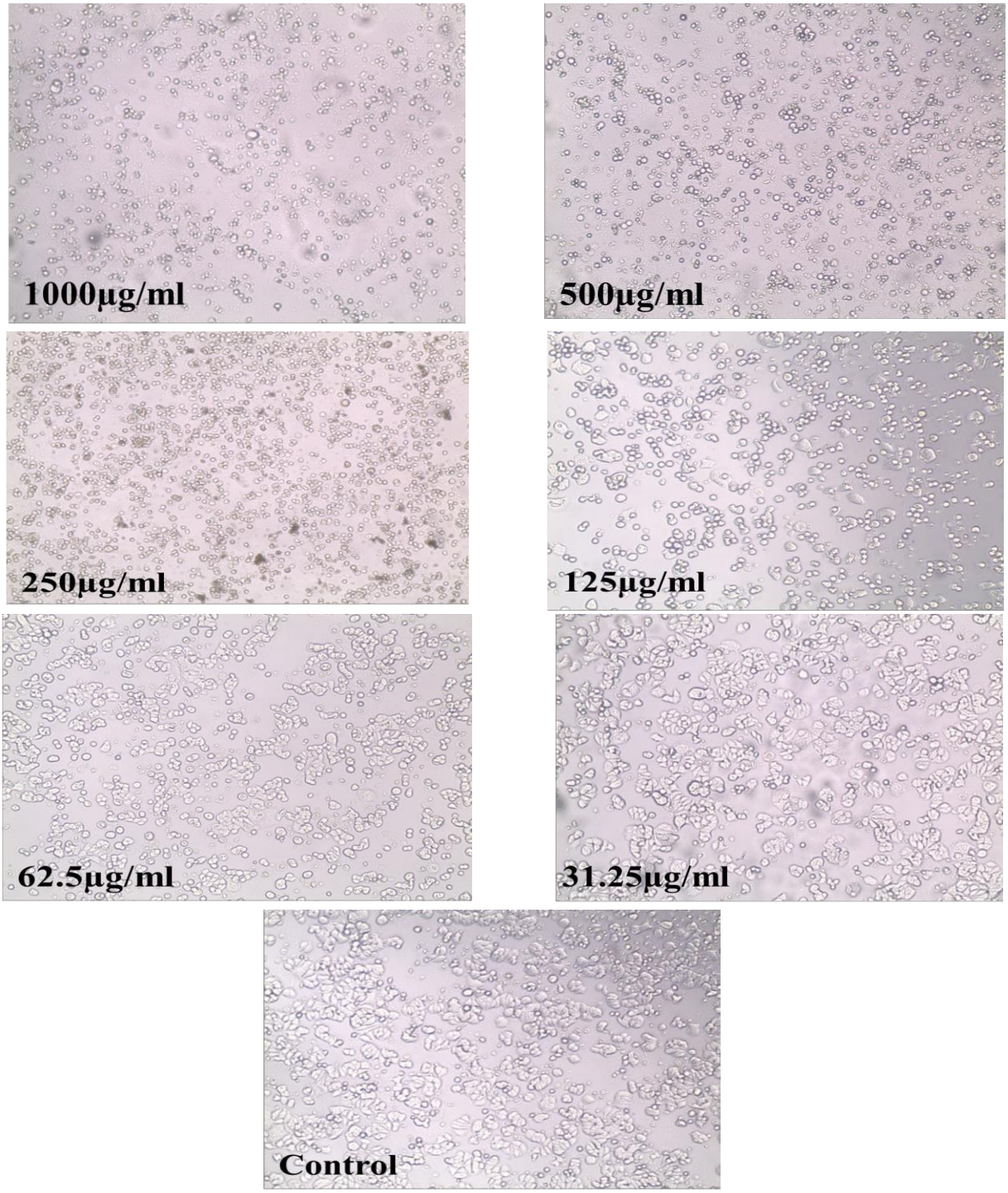
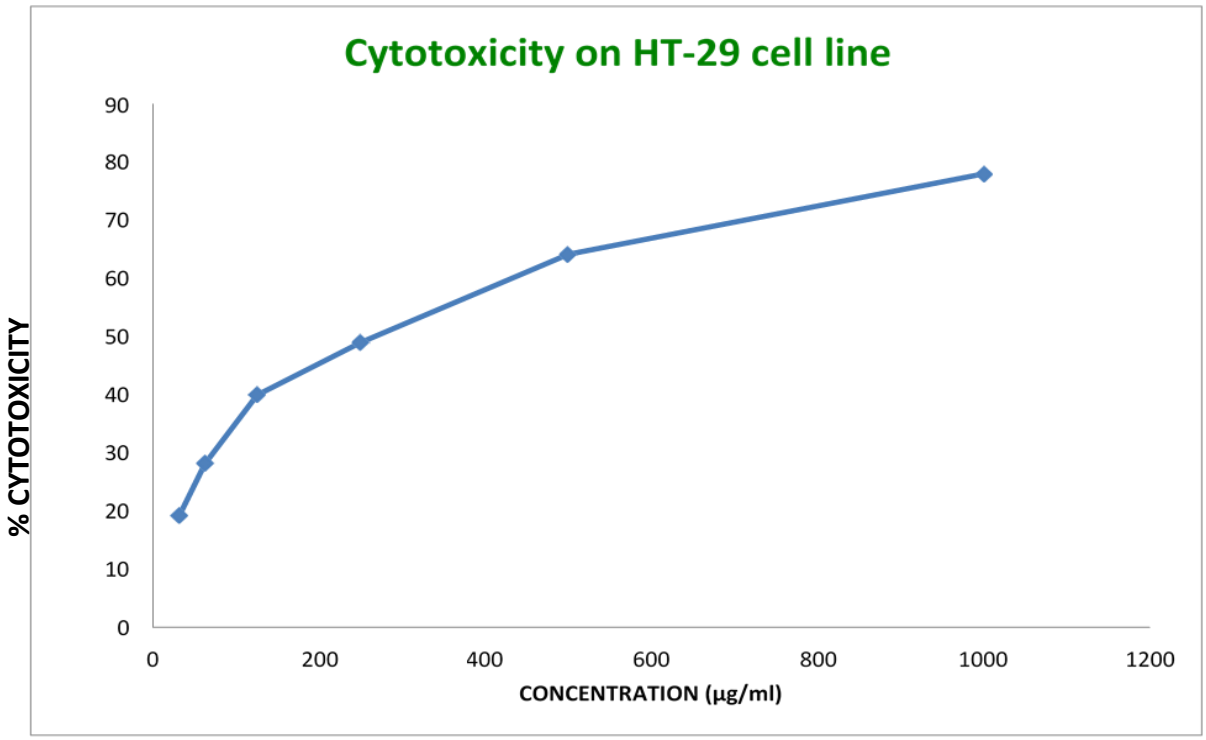


Figure 1: Cells after the administration of various concentrations of plant extract

extracts (ethanol extraction) on human tumor cell lines as well as human primary cancer cultures. The ethanolic plant extracts were then selected for additional research focusing also on the nature of cell death caused by these plant extracts.

The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide), it was a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT was converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol)[3,4]. The resulting purple solution was spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.



Graph 1: % cytotoxicity Vs Concentration (µg/ml).

METHODS AND MATERIALS

Collection and processing of the plant

The aerial parts of the *Reissantiaindica*was collected from sengotai, Tirunelveli, Tamilnadu, India in the month of November, 2016. Plant material was identified and authenticated by Mr. V.Chelladurai, Retired research officer botany, C.C.R.A.S. Govt of India, Tirunelveli. The collected plant was free from diseases and also free from contamination of other plants. The collected plant was air dried for few days.

Preparation of extract:

The aerial parts of the *Reissantia indica* powder was cold-macerated with ethanol for 72hrs ,48hrand 24hrs respectively. The ethanolic extract was then concentrated to a syrupy mass under reduced pressure until entire solvent get evaporated then air-dried and preserved in a Desiccators. The phytochemical analysis of the extract was also performed.

Cytotoxic assay

Materials and Reagent

MTT reagent (the solution is filtered through a 0.2 µ m filter and stored at 2–8 °C for frequent use or frozen for extended periods)

Pen strip,

CO2 incubator

Tecan Plate reader 5.DMEM,

DMSO 7.FBS,

Trypsin procured from Invitrogen.

Method

Preparation of test solutions

For cytotoxicity studies, serial two fold dilutions (0-1000µg/ml) were prepared from this for carrying out cytotoxic studies.

Table 1: OD and Cytotoxicity

Concentration (µg/ml)	OD (570 - 650 nm)	% Cytotoxicity
1000	0.522	78.07950728
500	0.834	64.97760358
250	1.115	53.1774916
125	1.431	39.90761478
62.5	1.734	27.18365062
31.25	1.906	19.96080627
Control	0.470	49.09247

Cell lines and culture medium

HT-29 (Human colon cancer cell line) was procured from ATCC, stock cells was cultured in medium supplemented with10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml)in an humidified atmosphere of 5% CO2 at 37^{O}C until confluent. The cell was dissociatedwith TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS) and centrifuged. Further, 50,000 cells / well of Jurkat was seeded in a 96well plate and incubated for 24 hrs at 37^{O}C, 5 % CO2 incubator.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 hr, when partial monolayer was formed, the supernatant was flicked off, washed the monolayer oncewith medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37^{O}C for 24hrs in 5%CO2 atmosphere. After incubation the test solutions in the wells were

discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 hrat 37º C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbancewas measured using
a microplate reader at a wavelength of 590 nm. The percentage growthinhibition was calculated using the following formula and concentration of test drug neededto inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves foreach cell line[3-6].

Calculating Inhibition

% Inhibition = 100 – (OD of sample/OD of Control) x 100

RESULTS AND DISCUSSION

The phytochemical analysis of the ethanolic extract of aerial parts *Reissantia indica* reveals that the plant has components such as alkaloids, terpenoids, steroids, flavonoids, phenolic compounds, and tannins. Effect of Cytotoxicity activity of *Reissantia indica* plant extracts were carried out against HT-29 cell line at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay. MTT assay of *Reissantia indica* shows highest cytotoxicity of this extract against HT-29 cell line was found at1000**µ**g/ml concentration with 78.079 percent of cell growth inhibition.

The Fig 1 clearly shows the inhibition of growth of HT-29 cells which decreases with increasing of dose administered. At the final dose, there was only minimal amount of cells survived. In the graph[5], within the time span of 24 hours we are able to the growth cessation for cancer cells. The growth inhibition of the extract was found to be 1000µg/ml at 24 hours. Obtaining results nearing 78.07% as inhibition value.

Graph 1.It was found that the percentage of growth inhibition to be increasing with increasing concentration of test compounds, and IC50 value of this assay was 218.04 µg/ml

CONCLUSION

The phytochemical, in vitro anticancer activities of aerial part of *Reissantia indica* have been studied. Since the IC_{50} of the extract is found to be 218.04 µg/ml. which shows that the extract has good cytotoxic effect. The image of the strain indicates that the extract induced apoptosis in HT-29 cell line in dose dependent manner. It shows highest cytotoxicity effect against HT-29 cell line at 1000**µ**g/ml concentration .Thus our data suggested that, it should be considered as a promising anticancer agent. It maybe a potential chemotherapeutic agent based on its ability to induce apoptosis in cancer cells. Therefore it can be used for the preparation of herbal formulation for cancer.

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