EXPLORING THE ANTIPROLIFERATIVE ACTIVITIES OF METHANOL EXTRACT OF LEAVES OF *GIVOTIA ROTTLARIFORMIS LINN[NILAGIRI]*

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Abstract: The present study was performed to explore the anti proliferative property of selected rare herb of Nilagiri District through tribal healer source of information.Collected herb were further screened for taxonomy to assertain the authenticity of the collection. The selected herb of *Givotia rottlariformis* leaves were shade dried and the extract obtained using methanolic solvent whch is screened for further studies. Since the earlier studies on antioxidant potential by dot-plot assay, DPPH radical scavenging assay and hydroxyl radical scavenging assay indicates its basic anti proliferative activity. Hence the present study is focused to assertain the anti proloferative activity of the selected plant using cell line studies. The methanol extract of the leaves of *G. rottlariformis* showed higher toxicity at lower concentration in liver cancer cell line with less influence on the normal cell line indicates its safety in administration .Since the results are quite promising it can be further extended to screeen the potent bioactive compound followed by pre clinical and clinical trials to bring out as a potent anti proliferative drug.

Keywords: Antioxidant, HepG2, Nilgiri plant, TLC.

Introduction: The Nilgiri district popularly known as "The Blue Mountains" is a vital place for medical, ethno-botanical as well as anthropological studies. It is located in the Western Ghats and the tribes of this district are Kotas, Kurumbas, Irulas, Paniyas, and Kattunayaks [4].There are many tribal healers have their own method of diagnosis and therapeutics for different diseases challenging the modern medicine. Most of their drugs are plant based safe drugs with out side effects. Regarding the potent tribal drugs, they remain unexplored due to lack of scientific authentication. Present medical practice indicates and accepts that few of the herbs have anti cncerous property hence there is an increasing demand for phyto drugs and some medicinal herbs have proven potential to cure dreadful diseases like cancer, paralysis, bronchial asthma etc. Medicinal herbs and their extracts are widely used in the treatment of liver disorders like hepatitis, cirrhosis and loss of appetite [1]. These plants contain the therapeutic [2]bioactive compounds called as secondary metabolites which are responsible for curing various diseases, since the synthetic drugs show lot of side effectes. As we are aware that liver is the main organ known to filter toxins and drug particle from blood. Hence it has become a great challenge for the synthetic drug manufacturers to find out an alternative and safe treatment for liver diseases, which resulted in exploring the herbal sources [3]. Cancer is a challenging disease in the last 100 years and many works have been attributed to eradicate the disease .Hence WHO and medical councils of different countries prepared to spent billions of dollars to find out a safe therapeutic for cancer.Although there are many drugs are available in the modern medicine which controls the disease to small extent only at very early stages but there is no proper medicine is available to complete the disease. Hence in the present study it has been proposed find out a potent drug through tribal source of information. *G. rottlariformis* has been reported to be effective in curing indigestion, sunburn, skin diseases and external tumors. Hence the present study is aimed to explore the efficacy of *G. rottlariformis* as anti-proliferative compounds. [5]

Materials and Methods:

Materials: The plant leaves of *G. rottlariformis* was collected from Kotagiri zone of Nilgiri district and identified by S. Aroumougame CAS in Botany University of Madras. Leaves of *G. rottlariformis* were washed with tap water, rinsed with distilled water and shade dried. The dried leaves were ground to obtain coarse powder and subjected to solvent extraction using methanol. [6]

Methods:

(i) Cytotoxic Activity On HepG2 & Pbmc Cell Line [18-19] The cytotoxic activity of MELH was carried out by the MTT assay method. HepG2 cell line and PBMC cell line were used and effective doses were calculated from the dose-response curve.

(ii) Cell Culture on Liver Cancer Cell: HepG2 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/ml), gentamycin (100µg/ml) and amphotericin B (1mg/ml) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were allowed to grow to confluence over 24 h before use.

(iii) Isolation of Pbmc and Determination of Cytotoxicity: Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood from healthy donors (aged 18–45) as described previously. Cells were obtained by Ficoll-Hypaque density centrifugation. PBMCs were suspended in DMEM medium and the cells were counted. 0.2x106 cells/0.2 ml/well seeded in 96-well cell culture plates.

(iv) Chemicals and Reagents: MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

(v) Cytotoxic Activity by MTT Assay Method: Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, HepG2 and PBMC cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.11–100µg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/ml) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments. The cell viability was calculated by the following formula.

Cell Viability (%) = 1-(OD of Treated Cells – OD of Control Cell) X 100

Results: Table 1 indicates that the methanol extract of leaves of G. rottlariformis on HepG2cell line shows that 50% of the cells were viable at the concentration of 300 µg/ml from which the safe dose of adminstration were fixed through IC 50 calculations as 300.84 [Table 3]

"Table 1- Cell viability of methanol extract of leaves of G. rottlariformis on HepG2cell line"

S.No	Concentration (µg/ml)	Cell viability (%)	Cytotoxicity (%)
1	100	61.90	38.10
2	200	56.22	43.78
3	300	50.14	49.86
4	400	43.31	56.69
5	500	30.63	69.37

"Fig 2- Cytotoxic activity of methanol extract of leaves of G. rottlariformis on HepG2 cell line"

"Table 3- IC_{50} *of methanol extract of leaves of G. rottlariformis on cytotoxic activity in cell line"*

S. No	Cell line	IC_{50} (µg/ml)
1	HepG2	300.84

Fig 2 evidence the cytotoxic effect of the selected plants to acces its safe therapeutic property indicates that even at high doses it produces very insignificant toxic signs.

Discussion: The present study is aimed to screen the pharmacological potential of the selected drug in controlling cancer. From the results it is clear that methanol extract of the leaves of *G. rottlariformis* showed higher toxicity at lower concentration in liver cancer cell line, which indicates its safety in administration as drug..The methanol extract of leaves of *G. rottlariformis* in *invitro* liver cancer cell line shows high pharmacological property due to the presence of potent bioactive compound against cancer. The present investigation creates platform for further researches to find out the therapeutic bioactive compound to develop a potent drug for cancer through preclinical study followed by clinical study...

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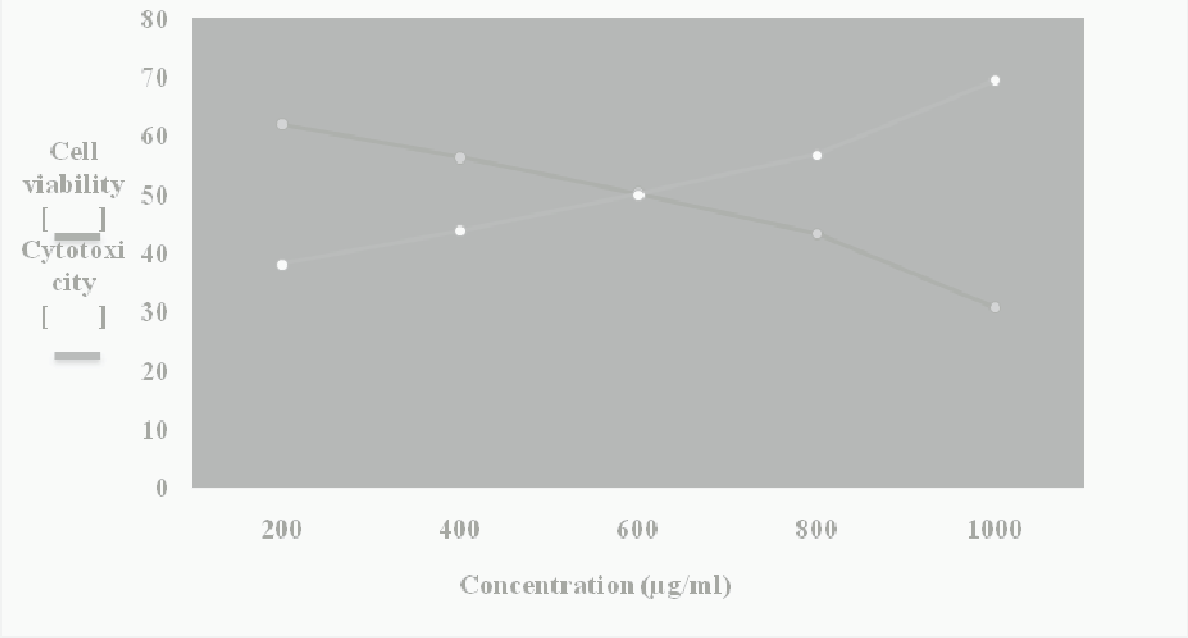

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