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Evaluation of anti-cancer activity of *Punica granatum* and *Murraya koenigii* by MTT assay

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Abstract
Punica granatum L is a deciduous shrub and many researchers studied the medicinal properties like anti-bacterial and anti-inflammatory. It has great nutritional values and numerous health benefits and it has been used in natural and holistic medicine to treat sore throats, coughs, urinary infections, digestive disorders, skin disorders, arthritis. *Murraya koenigii*, L.is a medicinally important herb of Indian origin, and very popularly used in Indian foods are the daily basis. Many Previous studies on the leaves, roots, and bark of this plant are reported rich sources of carbazole alkaloids, which produce potent biological activities and pharmacological activities like antioxidant, antidiabetic, anti-inflammatory and neuroprotective activities. In present work studied the anti-cancer activity of these two plant extracts with MCF7, HeLa, $CaCO_2$, $HepG_2$ and A549 cell lines by MTT assay. Punica granatum not much shows the anticancer activity with these five cell lines but murraya koenigii shows good anticancer activity in HeLa cell lines with IC_{50} of 62.29±0.317µg/ml followed by A549 (71.59±0.348), MCF7 (75.58±0.458), $CaCO_2$ (82.55±0.462) and $HepG_2$ (98.37±0.517) respectively.

Keywords: cancer, MTT, *Punica granatum* and *Murraya koenigii*, cell line

Introduction
In India, many years onwords has been using traditional medicine for the treatment of various infectious and inflammatory diseases [1]. As per the World Health Organisation (WHO), around 80% of the world's population still relies on plants as a source for primary health care [2]. The plants have been used extensively in folk medicine to treat ailments and diseases and are still used in the rural areas of developing countries [3]. More than 85,000 plant species that have been reported for medical use in all over the world and this indicates the plant derived natural products hold great promise for the discovery and development of new pharmaceuticals in a diverse human ailment [4]. Globally cancer is a disease which severely effects the human population and chemotherapy is using the most of treatments in cancers [5]. Due to resistance and side effects of chemotherapy treatment need a constant demand for new therapies to treat and prevent this life-threatening disease [6]. So, many researchers have investigating terrestrial plants extracts to develop the nanomaterial-based drugs for diseases including cancer [7].

The *Punica granatum* is a native shrub from western Asia and Mediterranean Europe that has a rich history of traditional use of medicine [8]. Punica granatum is ethnomedicinal important plant and depicted ameliorating medicinal value which used for the treatment of various diseases [9]. Extracts of granatum has been reported to have various medicinal values i.e. antioxidant, antibacterial, antidiabetic, cardioprotective and anticarcinogenic activity [10].

Murraya koenigii commonly called as curry leaves and belongs to Rutaceae family. Curry leaves are rich in carbohydrates, fibres, calcium, phosphorous, irons and vitamin A, vitamin B, vitamin E, iron, folic acid, carotenoids (Lutein and β-carotene) and Flavonoids (catechin and quercetin) [11]. It can improve digestion, lowers cholesterol and prevent greying of hair Curry leaves contain phytochemicals like alpha-pinene, beta caryophyllene, cinnamic acid, ferulic acid, girinimbine, myrcene, nerolidol, sabinene, and terpinen-4-ol [12]. A number of active constituents responsible for the medicinal nutritive properties have been isolated, characterised and reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic and cholesterol reducing activities [13].

The present study was focused to evaluate the cytotoxic effects and the possible use of these plants in the development of a new drug for the treatments in cancers.

Materials and Methods
Collection and authentication plant material
Punica granatum and Murraya *koenigii* plants were collected and authenticated by Dr. Madhava shetti, Taxonomist, Department of Botany, Tirupati. After collection of plant materials, washed thoroughly under running tap water until to the remove of adhering dust particles from the surface of the plants

Preparation of plant extracts
Plant materials were shade dried and grinded to powder. and 100 gm of dried powder of plant material socked in 1000 ml water in conical flask for extraction and kept it for 72 hrs. with occasional shaking. After 72 hrs, the extracts were filtered with No-42 whatman filter paper and collected water solvents concentrated on rotary vapor using round bottom flask. Concentrated extract was preserved in

sterilized air tight labelled bottle and preserved in refrigerator at 4˚c until required for further use.

Anticancer activity

Materials and methods

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from Eppendorf India.

Maintenance of cell line

The Human Cancer cell lines Breast cancer cell line (MCF-7), Cervical cancer cell line (HeLa), Colon cancer cell line ($CaCO_2$), Liver cancer cell line ($HepG_2$) and Lung cancer cell line (A549) were procured from NCCS, Pune and the cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0} C.

Preparation of plant extract

For MTT assay, each plant extract was weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/ ml and the cells were treated with series of concentrations from 5 to 100 µg/ ml.

Cell Proliferation assay (MTT Assay)

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the tryphan blue assay to know viable cells in cell suspension.

Cells were counted by hemocytometer and seeded at density of 5.0 X 10 ^{3} cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of plant extract in represented wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 ^{0} C for 3 hrs.

At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC_{50} value was determined by using linear regression equation i.e. y = mx + c. Here, y = 50, m and c values were derived from the viability graph.

Statistical analysis

Data were processed using Graph Pad Prism 7 software and the results were provided as a mean ± standard deviation (SD). The significance of a difference was considered in p-value < 0.05.

Results and Discussion

Results

The anti-cancer activity of *Punica granatum* and *Murraya koenigii* extracts with five cell lines studied by MTT assay. Cisplatin used as standard drug. For the MCF7, *Murraya koenigii* plant extract shows the IC_{50} of 75.58±0.458 followed by *Punica granatum* 127.55±0.572 µg/ml. Standard drug cisplatin shows the IC_{50} of 5.62±0.141 µM/ml respectively (Fig 2). For the HeLa, *Murraya koenigii* plant extract shows the IC_{50} of 62.29±0.317 followed by *Punica granatum* 110.36±0.416 µg/ml. Standard drug cisplatin shows the IC_{50} of 7.52±0.257 µM/ml respectively (Fig 3). For the $CaCO_2$, *Murraya koenigii* plant extract shows the IC_{50} of 82.55±0.462 followed by *Punica granatum* 124.16±0.317 µg/ml. Standard drug cisplatin shows the IC_{50} of 116.47±0.842 µM/ml respectively (Fig 4). For the $HepG_2$, *Murraya koenigii* plant extract shows the IC_{50} of 98.37±0.517followed by *Punica granatum* 103.78±0.475 µg/ml. Standard drug cisplatin shows the IC_{50} of 11.72±0.183µM/ml respectively (Fig 5). *Murraya koenigii* plant extract shows the IC_{50} of 71.59±0.348 followed by *Punica granatum* 116.90±0.609 µg/ml. Standard drug cisplatin shows the IC_{50} of 13.45±0.084 µM/ml respectively (Fig 6). The results are reported in Table 1.

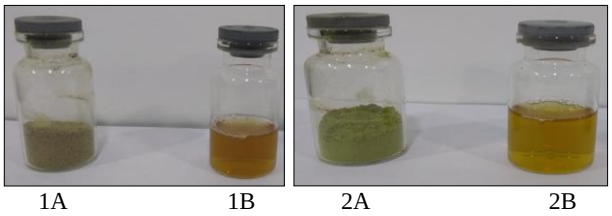

Fig 1: Plant Powder and Extracts. 1A) Punica granatum plant powder 1B) Punica granatum plant extract. 2A) Murraya koenigii plant powder 2B) Murraya koenigii plant extract.

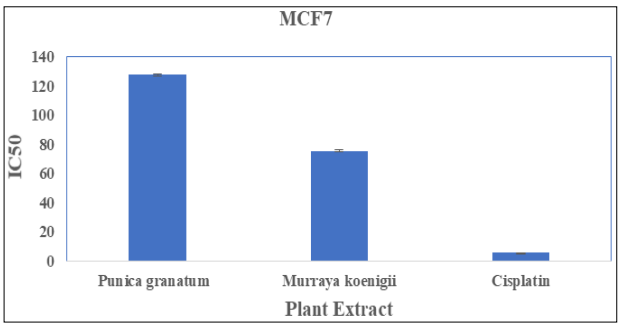

Fig 2: Cytotoxicity of MCF7 against *Punica granatum, Murraya koenigii* and standard Cisplatin.

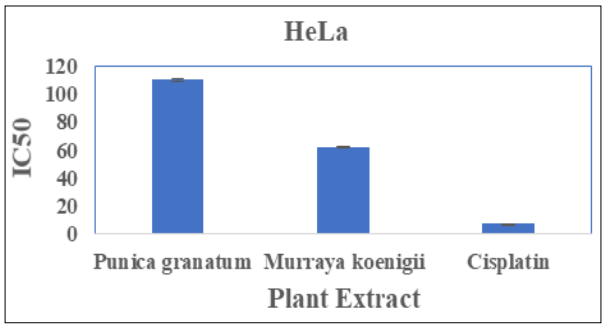

Fig 3: Cytotoxicity of HeLa against *Punica granatum, Murraya koenigii* and standard Cisplatin.

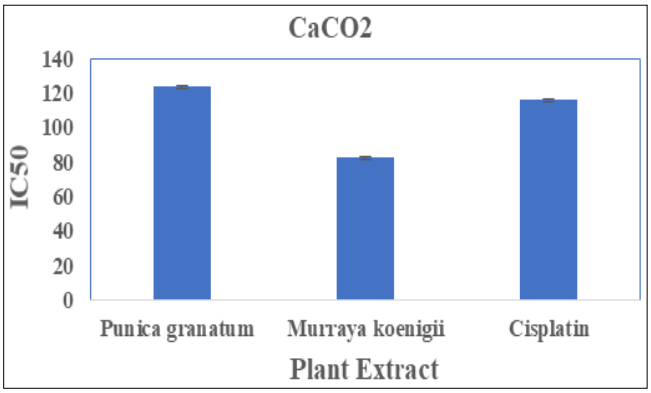

Fig 4: Cytotoxicity of $CaCO_2$ against Punica granatum, Murraya koenigii and standard Cisplatin.

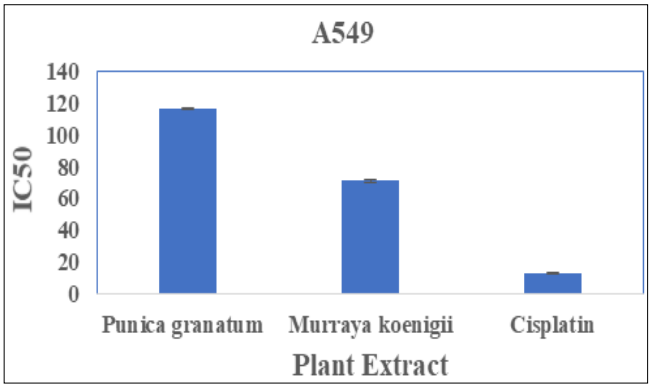

Fig 6: Cytotoxicity of A549 against *Punica granatum*, *Murraya koenigii* and standard Cisplatin.

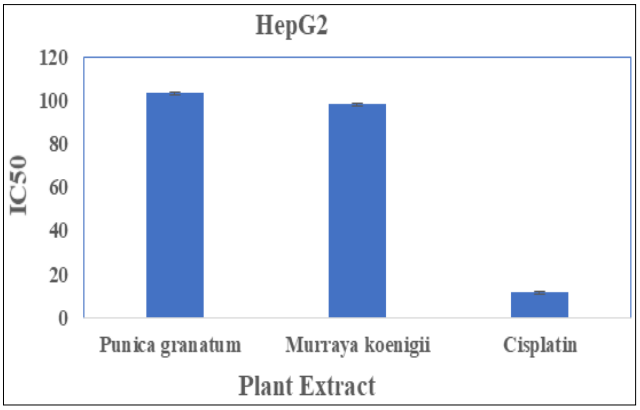

Fig 5: Cytotoxicity of $HepG_2$ against *Punica granatum*, *Murraya koenigii* and standard Cisplatin.

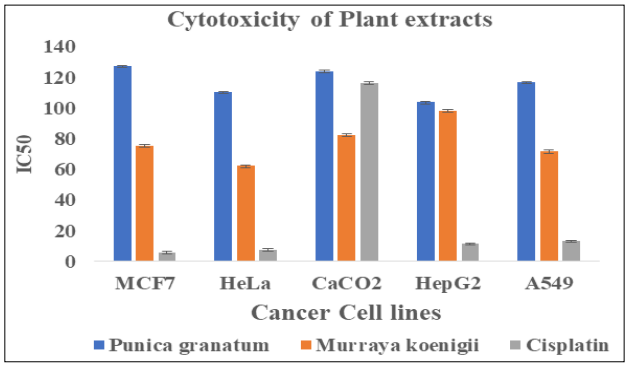

Fig 7: The cytotoxicity of the Plant Extracts is subjected to triplicates. The results are reported in mean SD. The IC_{50} are reported in µg/ml. Standard drug cisplatin IC_{50} are reported in µM/ml.

Table 1: The cytotoxicity of the Plant Extracts is subjected to triplicates. The results are reported in mean±SD. The IC50 are reported in µg/ml. Standard drug cisplatin IC50 are reported in µM/ml.

Plant extract	MCF7	HeLa	$CaCO_2$	HepG2	A549
Punica granatum	127.55±0.572	110.36±0.416	124.16±0.317	103.78±0.475	116.90±0.609
Murraya koenigii	75.58±0.458	62.29±0.317	82.55±0.462	98.37±0.517	71.59±0.348
Cisplatin	5.62 ± 0.141	7.52±0.257	116.47±0.842	11.72±0.183	13.45±0.084

Discussion

All over the world cancer has a major impact on society in developing and developed countries. Natural therapies, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects. Currently, a few plant products are being used to treat cancer [14]. AVIRAM *et al*., 2000 study reported that these extracts and pomegranate juice have been found to effectively reduce heart disease risk factors and atherosclerosis including LDL oxidation and macrophage oxidative status[15]. MENEZES *et al* 2006 study showed the antibacterial effects of extracts against dental plaque [16]. SEERAM *et al*., 2007 indicates that pomegranate juice may be effective against prostate cancer and osteoarthritis [17]. In our present study observed the *punica granatum* extract only showed the mild anti-cancer activity against liver cancer cell line HepG2 and not much anti-cancer activity observed in remaining cell lines.

Murraya koenigii have a lot of bioactive principles due to which plant has been proven as the medicinally important plant but not much research focused on this plant [18]. Presence of essential oils in the *Murraya koenigii* leaves shows the anti-bacterial against some of gram positive and gram-negative bacterial strains [19]. Ethanolic extract of *Murraya koenigii* leaves shows the anti-fungal activity against Rhizoctonia solani and Colletotrichum falcatum [20]. Murraya koenigii leaves were observed to have highest antioxidant potential when compared with four other leafy vegetables [21]. Extracted from Murraya koenigii bark extracted compounds Carbazole, girinimbine induces apoptosis in cells of HepG2 [22]. Major anti-cancerous bioactive molecule mahanine in *M.koenigii* shows anti-cancer activity against HL-60 cells [23]. Amna *et al*., also demonstrated the potential of leaves and proved it to be cytotoxic against HeLA cancer cell lines [24]. *M.koenigii* leaves extract studied in animal models using the intestine and colon cancer [25]. In our present study *murraya koenigii* leaves extract shows the anti-cancer activity in studied five human cancer cell lines. Cervical cancer cell line HeLa shows good anticancer activity with IC_{50} 62.29±0.317µg/ml and least anti-cancer activity in Liver cancer cell line $HepG_2$ with IC_{50} 98.37±0.517µg/ml was observed.

Conclusion

Anti-cancer activity of *Punica granatum* and *Murraya koenigii* studied with five types of human cancer cell lines and based on the present study concluding that *Muraaya koenigii* plant extra shows the good anti-cancer activity against five cell lines compared to the *Punica granatum*

plant extracts with five cancer cell lines and further studies need to requires to conclude the same.

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