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Anticancer activity of leaves extract of *Hypericum mysorense* L.

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Abstract
Medicinal plants have been used for the treatment of several diseases since ancient times, but their anti-cancer properties have not been well studied. Herbal medicine has been used as a major treatment for cancer in various countries in South East Asia and the Middle East and Europe a long time ago. People looked for drugs in nature to retaliate diseases based on experience and there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure. Although many advanced countries have considered traditional herbal medicine as an official treatment for cancer, only 5-15% of these herbs have been investigated to detect their bioactive anticancer compounds. Several plant-derived compounds have shown promising anticancer therapeutics and attempts have been made to characterize the effectiveness of active constituents to be isolated from natural sources as chemo-preventive agents for rapid relief. *H. mysorense* (Hypericaceae) found at high elevation in the Western Ghats of India especially in Nilgiris. *H. mysorense* are mentioned in traditional system of folklore for its skin-related disorders, wound healing, spasmolytic, antipsychotic antifungal, and antiviral and nerve-calming properties. The present study was conducted to determine the anticancer activity of methanol leaf extract of *H. mysorense* on HT29 and A549 cells by MTT assay method. The maximum dead cells of HT29 were 59.67±0.73% and dead cells of A549 were 63.27±1.80 at 100 µg/mL concentration by leaf extract of *H. mysorense*.

Keywords: Cytotoxicity, HT29 cells, A549 cells, MTT assay

Introduction
Cancer is one of the leading causes of morbidity and the second leading cause of death globally [1]. Although great advancements have been made in the treatment and control of cancer progression, significant deficiencies, and room for improvement remain due to undesired side effects of chemotherapy. The use of plant-derived products as natural therapies, in cancer treatment, may reduce the adverse side effects. The *in vitro* study results showed few plant products are being used to treat cancer indigenously that have shown very promising anticancer activity [2]. In recent years, various plant-derived compounds have shown promise as anticancer agents and will outline their potential mechanism of action. While the ancient peoples used medicinal plants primarily as simple pharmaceutical forms as decoctions, infusions, and macerations and in Middle Ages, particularly between the 16th and 18th centuries, the demand for compound drugs was increasing which comprised of medicinal plants along with drugs from the animal. The oldest written evidence of medicinal plants and used for the preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised of 12 recipes for drug preparation and referring over 250 medicinal plants.

In the human body, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated during physiological reactions and are quite reactive as well as harmful to the cells. If the generated radicals are not scavenged or are not utilized during the combustion process, in which foods are converted into energy, they can damage important molecules, such as proteins, DNA, and lipids. If the protein gets oxidized by radicals (ROS and RNS) it leads to the development of a variety of diseases, including aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes, and neurodegeneration [3]. There is an increasing interest in natural antioxidants, such as phenols and flavonoids, present in the medicinal plants and herbs, as they might help to prevent oxidative damage in cellular constituents. Flavonoids are a large group of plant polyphenols possessing a wide range of biological activities like anti-inflammatory, anti-hepatotoxic, and anti-carcinogenic action [4]. Phenolic compounds such as phenolic acids and flavonoids are good free radicals scavenger, through electron transfer reaction [5]. During biological reactions, the most dangerous.

OH radicals are formed through the Fenton reaction in the presence of metal ions act as a catalyst and the flavonoids prevent the formation of highly reactive •OH radicals by chelating metal ions, like iron and copper [6]. If the reactive oxygen species (ROS) have been detected in cancer cells at elevated rates, they promote many aspects of tumor development and progression. A challenge for novel therapeutic drugs will be the fine-tuning of intracellular ROS signaling to effectively deprive cells of ROS-induced tumor-promoting events. Therefore, a combination of ROS inhibitors of antioxidants with pharmacological agents within tumor cells may be needed to selectively kill cancer cells and overcome drug resistance [7].

Hypericum is a well-known genus in herbal medicine that belongs to the family Hypericaceae, and one of its species *Hypericum mysorense* is a well-known folklore medicine for its important therapeutic potential such as spasmolytic, hypotensive, and antifungal activities [8]. It is a shrub and small tree native to the Nilgiri hills in India and found primarily at high elevation in the Western Ghats. *H. mysorense* is reported in Ayurveda for having antiviral, wound healing, antiviral, and nerve-calming properties [9]. It exhibited significant antiviral activity against herpes simplex virus type-1 as well as antitumor and antimicrobial activities [10]. It is an erected, glabrous shrub of 1-3 meter height. Leaves are simple alternate stipulate petiolate. Leaves with petiole 1-4 mm; blade narrowly lanceolate to oblong-lanceolate or broadly ovate, (1.7-) 2.5-7.8 × (0.7-) 1-3.2 cm, abaxially paler or ± glaucous; laminar glands short streaks to dots; abaxial glands dense to sparse or absent; main lateral veins (2 or) 3- or 4-paired, without visible tertiary reticulation; base narrowly cordate to subcordate, apex acute to rounded. Inflorescence 1-5-flowered, from an apical node, nearly round-topped; bracts deciduous, lanceolate, or narrowly oblong to obovate-spatulate. Pedicels 3-16 mm. The bark is externally brownish and internally light reddish-brown. It occurs in the curved or sometimes flat pieces with a size of 7-8 × 18-20 cm and a thickness of about 1.5-3 cm. It has a mucilaginous taste which is followed by a bitter sensation [11]. The odor is characteristic, unpleasant, and has a slight astringent effect on the throat. The bark shows a fibrous fracture. The bark shows the number of masses of moss and fungal growth. The outer surface of the bark has got numerous lenticels. The number of ringles and undulations are also seen on the outer surface, while the inner surface shows the presence of numerous striations. The bark is smooth and has a glistering appearance due to the numerous shinning calcium oxalate crystals in sunlight. Overall the bark is compact, hard, and lighter in weight. Fresh bark detached from the trunk of the tree is yellowish a turns to brown and then reddish-brown on storage [12].

Fig 1: *Hypericum mysorense*

Materials and Methods

Preparation of Extract

The leaves of *Hypericum mysorense* were collected from Ooty, Tamilnadu, India. The leaves were washed, shade dried for 10 days, and powdered by the mechanical blender. About 20 g of leaves powder was weighed and soaked in 200 mL of methanol and kept at room temperature for 72 h. The supernatant was filtered and condensed by a rotary evaporator at 50 °C, which yields orange-red gummy extract.

Anticancer activity

Chemicals and reagents

MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange was obtained from Sigma, USA. All other fine chemicals were obtained from Sigma, Aldrich.

Cell culture

HT29 (colon cancer) and A549 (lung cancer) cells were obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose-well Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) 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.

MTT assay

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, HT29 and A549 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.001-100 µg/mL) of methanol leaves extract of *H. mysorense* was added and incubated for 48 h. After treatment, the cells were incubated with MTT (10 µL, 5 mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data are represented as the mean values for three independent experiments [13].

$$\text{Cell viability (\%)} = \left[\frac{\text{Mean OD}}{\text{Control OD}}\right] \times 100$$

Results and Discussion

Cancer is a global burden and there is a constant demand for new therapies to treat and prevent this life-threatening disease. Around 70% of deaths in low- and middle-income countries are due to cancer. Since ancient times, natural products have been a good source for cancer treatment due to the presence of diverse active compounds [14]. For many centuries, indigenous traditional herbal medicines have been used to treat many diseases including cancer around the world. In India, only a small number of plants have been reported to have anti-cancer properties, and more research is required to validate their medicinal activity for developing novel drugs to fight such killer diseases. The plant kingdom produces naturally occurring secondary metabolites which are being tested for the anticancer activity for the development of new clinical drugs. For many years herbal plants have been

used as medicine for their natural antiseptic properties and the primary source of medical treatment in developing countries [15].

Table 1: Anticancer activity of methanol leaves extract of *H. mysorense* on HT29 (colon cancer) and A549 (lung cancer) cells.

S. No	Concentration (µg/mL)	Cell death (%)	
		HT29	A549
1	0.001	18.52±0.61	15.80±0.73
2	0.01	27.04±1.05	21.30±1.79
3	0.1	33.97±0.08	30.65±0.78
4	1	42.23±1.19	39.06±1.47
5	10	47.36±0.90	48.54±1.71
6	100	59.67±0.73	63.27±1.80

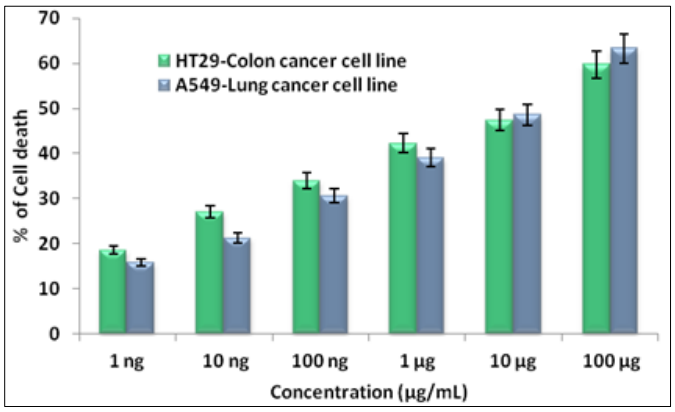

Fig 2: Anticancer activity of leaves extract of *H. mysorense* on HT29 (colon cancer) cells and A549 (lung cancer) cells.

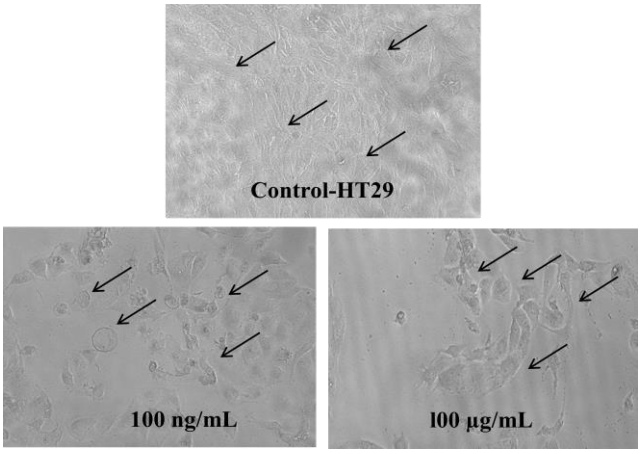

Fig 3: Morphological changes of HT29 cells after treatment by the leaves extract of *H. mysorense*

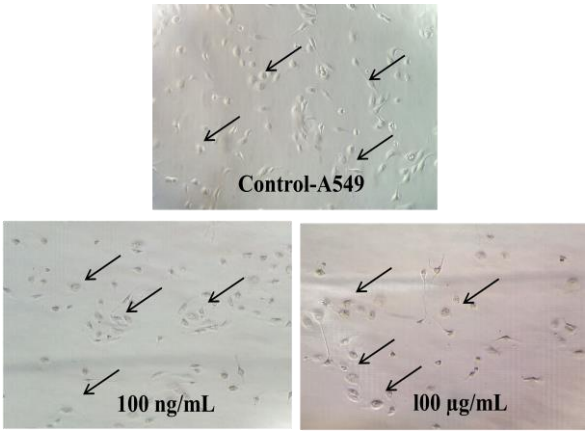

Fig 4: Morphological changes of A549 cells after treatment by the leaves extract of *H. mysorense*

The anticancer activity of leaves extract of *H. mysorense* results showed a variable in cellularity against HT29 and A549 cancer cells. The maximum cell death of HT29 (colon cancer) cells by the leaves extract was 59.67±0.73% at 100 µg/mL concentration and the IC_{50} was 10.56 µg/mL concentration. The maximum cell death of A549 (lung cancer) cells by the leaves extract was 63.27±1.80% at 100 µg/mL concentration and the IC_{50} was 10.30 µg/mL concentration (Table 1; Fig. 2). The majority of cells have round or ovoid shapes, but sometimes they can be polygonal or elongated after treating the leaves extract. The microscopic image revealed that the leaves extract of *H. mysorense* has a significant effect on treated HT29 and A549 cells compared to untreated cells (Fig 3; Fig 4). At 100 µg/mL concentration, enlargement of the nucleus of cells was observed with variable sizes and shapes and the dead cells increase with increasing concentration of the leaves extract.

Conclusion

Herbal products are considered to be one of the best choices of medicine for preventing and treating the incidence of cancer. This is mainly due to the plants contained different types of active substances, which work against many types of cancers in a synergetic way. These compounds can be extracted and can be used alone or in combination with other anticancer drugs. In comparison with synthetic drugs, these compounds are found to be naturally available, cheaper, and easy to administered orally and have low or minimal side effects, and they are found to be rich in various biologically potential chemotherapy compounds. The demand for naturally derived compounds from medicinal plants and their properties for cancer treatment leads to potential targets for clinical trials. The results showed that the anticancer activity of leaves extract of *H. mysorense* induces apoptosis at the lowest IC_{50} values, which indicates safe for normal cells in toxicity concern. Besides, the prevalence of cancer-screening tests and the identification of high-risk patients, including those with inherited cancer syndromes, are important for improving survival.

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