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Anticancer activities of ethanol tuberous root extracts of *Decalepis hamiltonii* Wight & Arn and *Hemidesmus indicus* (L.) R.Br.

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Abstract
Herbal medicines containing different alkaloids of medicinal plants are used for prevention and treatment of dreadful diseases such as cancer, HIV and tuberculosis. They include traditional medicines of ancient times and the present day standardized plant drugs are all herbal drugs. In the age of clinical medicines, the main challenge is drug resistance. So, multidrug resistance somehow hampers random use of clinical medicines anymore, resulting in the use of herbal medicines. Use of herbal medicines is cheaper due to its easy availability. Modern day medicines have already accepted herbalism as a form of alternative medicine. Clinical medicines however use many plant-derived metabolites in pharmaceutical drugs, for example- opium, aspirin, digitalis, quinine etc; but scope of using herbal medicines is further extended as it consists of many more unexplored herbals, minerals, fungal and algal products. The aim of the present study was to evaluate the anticancer activity of ethanol tuberous root extract of *D. hamiltonii* and roots of *H. indicus* on Hep G2 cells and cytotoxicity on Vero cells by MTT assay method. The maximum cell death of Vero cells by tuberous root extract of *D. hamiltonii* was 23.66±0.003% and that of *H. indicus* was 20.69±0.003% at 160 µg/mL concentration. The maximum cell death of HepG2 cells by tuberous root extract of *D. hamiltonii* was 76.58±0.005% and in the case of *H. indicus* it was 72.11±0.004% at 160 µg/mL concentration.

Keywords: Anticancer, cytotoxicity, vero cells, HepG2 cells, MTT assay

Introduction
Cancer has been a constant battle globally despite a lot of development in cures and preventative therapies. The disease is characterized by cells in the human body continually multiplying with the systemic inability to be controlled or stopped. Consequently, tumours of malignant cells have potential to be metastatic [1] and current treatments include chemotherapy and radiotherapy which are expensive. Treatments of chemotherapy subject the patients into a lot of strain and further damage to their health. Therefore, there are a using alternative treatments and therapies against cancer as recent medicines [2]. For many years herbal medicines have been used and are still being used in developing countries as the primary source of medical treatment. Medicinal plants have been in use as drugs for their inherent therapeutic properties. Thus, research continues on the medicinal properties and uses of plant extracts in the preparation of potential drugs for diseases including cancer [3]. Many plant species are already being used in herbal medicine to treat or prevent cancer in the developing countries. Compounds which are characteristic to the plant kingdom and are necessary for plant survival and "housekeeping" of the organisms are being investigated for their ability to inhibit growth and initiate apoptosis of cancerous cells. *Decalepis hamiltonii* Wight & Arn., commonly known as "Maakali kizhangu", belongs to the family Asclepediaceae. It grows largely in southern parts of India in the hilly and forest areas of the Western Ghats. It is a twining shrub with branchlets jointed. Leaves measure 6 x 4.5 cm, obovate-elliptic or orbicular, apex obtuse, base cuneate, membranous; petioles 1.5 cm. Cymes trichotomously branched; calyx deeply 5 lobed, 2 mm oblong; corolla tube 1 mm, long, lobes 3 x 2 mm, oblong, recurved, white pubescent inside; corona of 10 scales, alternately long hooked and short; filaments 1 mm, anthers attached to style apex; ovaries 1 mm [4]. It is utilized in tribal and traditional Indian and Chinese medicines for the treatment of a wide range of ailments including those of the digestive system, lungs and circulatory system. The roots of *D. hamiltonii* are also used in folk medicine and in Ayurvedic preparations [5]. Ancient tribes in the Western Ghats of India used its roots particularly for inflammation [6].
Hemidesmus indicus (L.) R. Br., called 'anantamul' in vernacular name, is a plant species of Apocynaceae family commonly found in India. It is a climbing plant that grows indifferent areas of western and southern parts of India. It is a slender, laticiferous, semi-erect, endangered

shrubby plant producing creeping or twining shoots from a woody root stock and specifically known for its immense medicinal values. It is used for venereal diseases, herpes, skin diseases, arthritis, rheumatism, gout, epilepsy, insanity, chronic nervous diseases, abdominal distention, intestinal gas, debility, impotence and turbid urine. The root is valuable and used as demulcent, diaphoretic, diuretic and tonic [7]. It is used in the treatment of appetite loss, dyspepsia, fever, skin diseases, syphilis, leucorrhoea, genitourinary diseases and chronic coughs [8]. In 1831, the 'anantamul' was introduced into European medicine [9]. Today, the 'anantamul' is receiving renewed attention as an Ayurvedic medicine and herbal product that may have health benefits for blood purification, kidney and urinary disorders, and skin infections.

a. *Decalepis hamiltonii* ***b.*** *Hemidesmus indicus*

Fig 1: Tuberous roots of *D. hamiltonii* and roots of *H. indicus*

2. Materials and Methods

2.1 Collection of root tubers and preparation of extracts

The fresh tuberous roots of *D. hamiltonii* were collected from Gandhi market, Tiruchirappalli, Tamil Nadu, India. The tuberous roots were cut into small pieces and soaked in ethanol for 72 h. Then the supernatant was filtered by using filter paper and condensed by rotary evaporator at 50℃, which yields reddish brown viscous mass. The roots of *H. indicus* were collected from Thirukovillur, Villupuram district Tamil Nadu, India. The above procedure was followed, which yields yellowish green viscous mass.

2.2 Cytotoxicity and anticancer activity

2.2.1 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.

2.2.2 Cell culture

Vero and HepG2 cells 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.

2.2.3 Cell growth inhibition studies by MTT assay

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, Vero and HepG2 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (5–160µg/mL) ethanol extract of tuberous roots of *D. hamiltonii* and ethanol extract of roots of *H. indicus* were added and incubated for 48 h. After treatment, the cells were incubated with MTT (10µL, 5mg/mL) at 37 ℃ 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 [10].

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

3. Results and Discussion

Cancer is one of the major causes of death in the world, and it is the second leading cause of mortality after cardiovascular diseases [11]. Common treatments such as radiotherapy and chemotherapy cause some complications. The cancer cell reproduces in an abnormal way by asexual reproduction, that is, it ignores signals related to regulation of cell's growth around it and obtains invasion characteristics and causes changes in surrounded tissues. Herbal extracts have antioxidant compounds that can induce apoptosis and inhibit cell proliferation.

Table 1: Cytotoxicity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on Vero cells.

S. No	Concentration (µg/mL)	Cell death (%)	
		Decalepis hamiltonii	***Hemidesmus indicus***
1	5	06.51±0.003	01.01±0.003
2	10	09.97±0.003	03.40±0.003
3	20	15.12±0.004	07.40±0.003
4	40	18.94±0.002	13.65±0.003
5	80	21.76±0.003	16.62±0.003
6	160	23.66±0.003	20.69±0.003

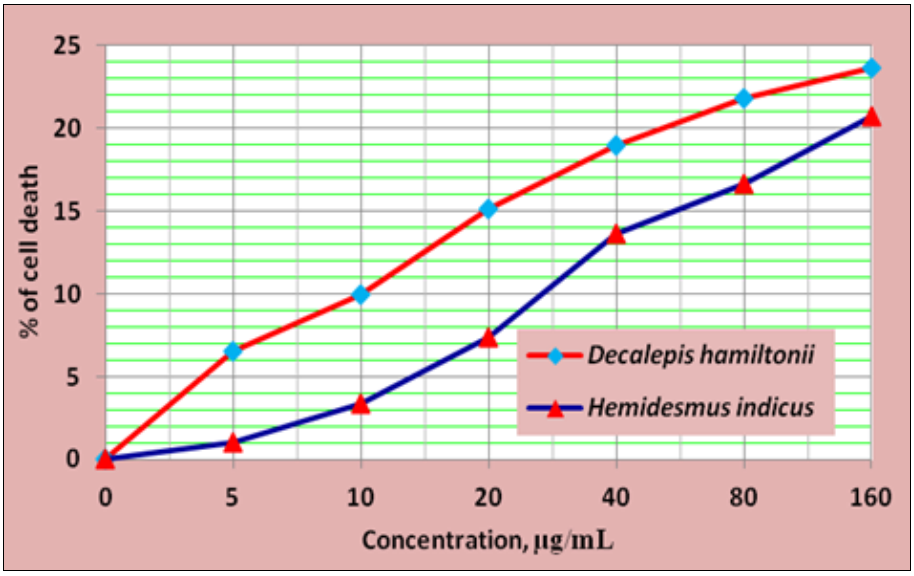

Fig 2: Cytotoxicity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on Vero cells.

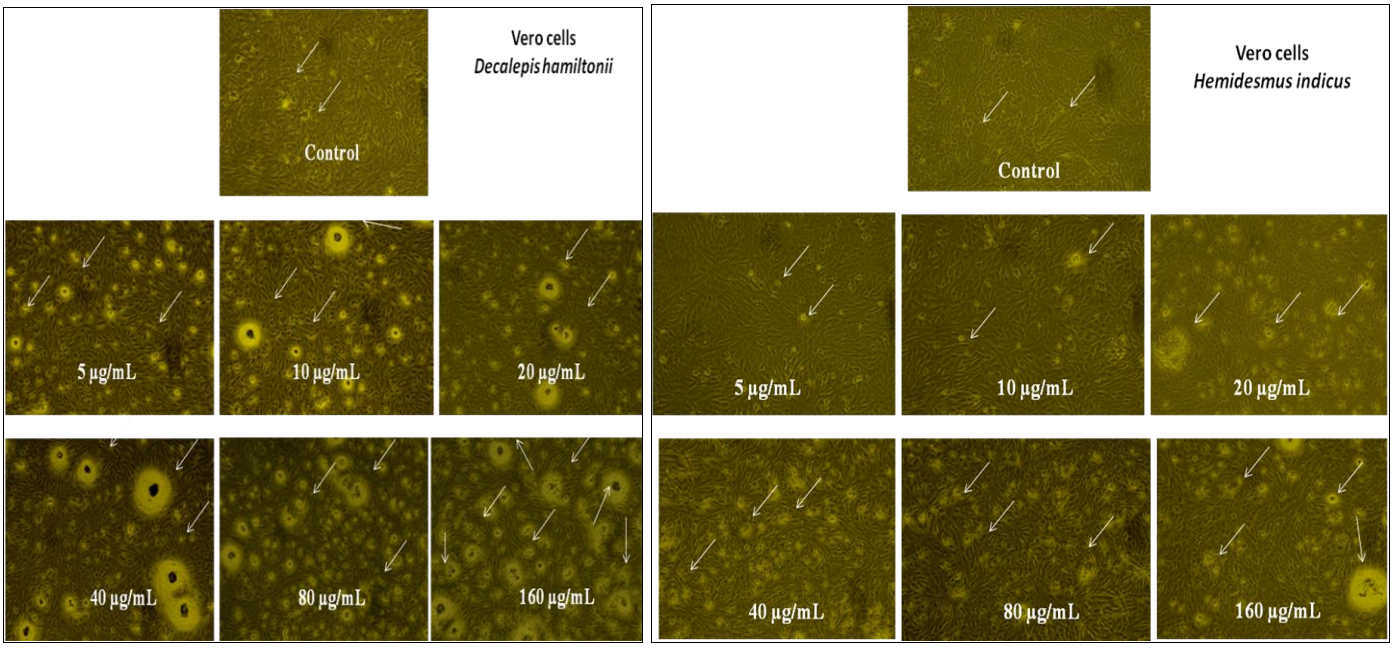

Fig 3: Cytotoxicity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on Vero cells.

Table 2: Anticancer activity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on HepG2 cells.

S. No	Concentration (µg/mL)	Cell death (%)	
		Decalepis hamiltonii	***Hemidesmus indicus***
1	5	03.77±0.004	01.13±0.003
2	10	10.35±0.003	04.96±0.003
3	20	30.29±0.004	20.55±0.004
4	40	46.89±0.004	31.66±0.004
5	80	65.64±0.004	60.99±0.003
6	160	76.58±0.005	72.11±0.004

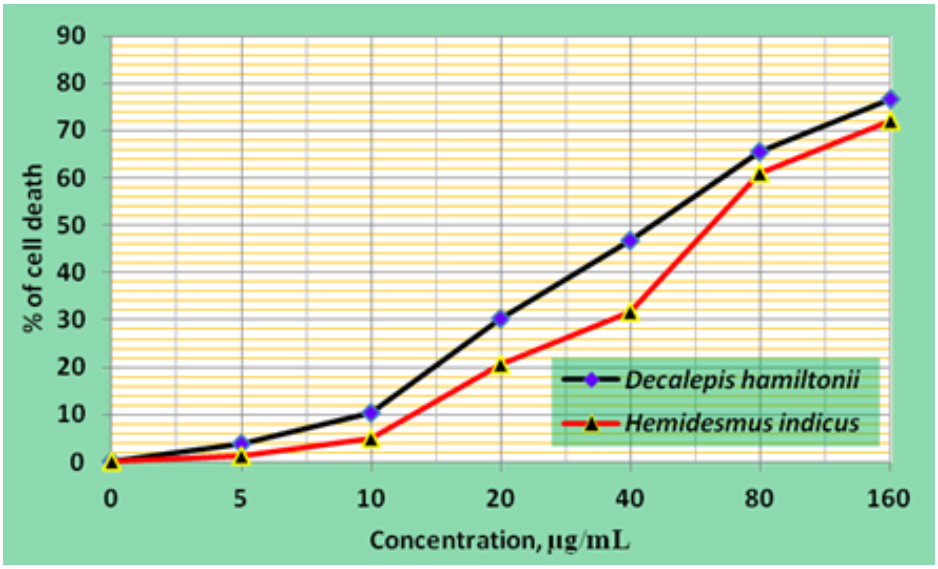

Fig 4: Anticancer activity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on HepG2 cells.

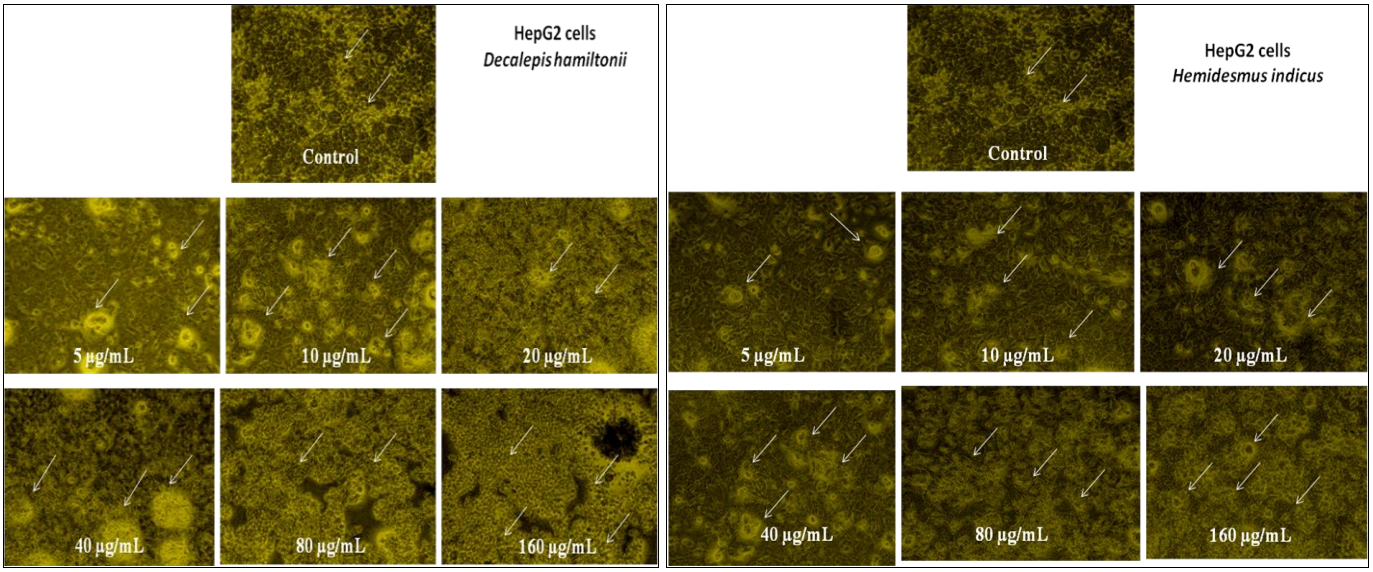

Fig 5: Anticancer activity of tuberous root extracts of *D. hamiltonii* and *H. indicus* on HepG2 cells.

Modern chemotherapy utilizes many substances of plant and aquatic origin. These compounds have cytotoxic properties with many different mechanisms of action, such as inhibition of tumour cell growth, induction of apoptosis, DNA damage, inhibition of topoisomerases I and II, etc. Studies have shown that plant-derived compounds in combination with anticancer drugs have great potential to destroy tumour cells without affecting normal cells such as lymphocytes and fibroblasts. The anticancer allopathic drugs used previously exhibited relatively high toxicity not only to the tumour cells, but also to the normal cells of the body part in which the cancer had developed. Currently, the search for novel anticancer drugs is being conducted among terrestrial plants [12], and those of marine environments [13]. Plants have been used for centuries to treat diseases. In various parts of the world, several plants are consumed for their health benefits as a part of traditional folk medicine. The increase in the incidence of various types of cancer creates a need for new anticancer drugs. Numerous anticancer drugs isolated from plant materials are tested on various cancer cell lines. This study reveals the anticancer potentials of tuberous root extracts of *D. hamiltonii* and *H. indicus* on HepG2 cell line, which was compared with the cytotoxicity on Vero cell lines. The maximum cell death of Vero cells by tuberous root extract of *D. hamiltonii* was 23.66±0.003% at 160 µg/mL concentration and the IC_{50} was 338.12 µg/mL concentrations. The maximum cell death of Vero cells by root extract of *H. indicus* was 20.69±0.003% at 160 µg/mL concentrations and the IC_{50} was 386.66 µg/mL concentrations (Table 1). The maximum cell death of HepG2 cells by tuberous root extract of *D. hamiltonii* was 76.58±0.005% and by root extract of *H. indicus* was 72.11±0.004% at 160 µg/mL concentration. The IC_{50} of tuberous root extract of *D. hamiltonii* on HepG2 cells was 42.65 µg/mL concentrations while that of *H. indicus* was 65.58 µg/mL concentrations (Table 2). Morphological alteration of HepG2 and Vero cells upon exposure using *D. hamiltonii* and *H. indicus* extracts were observed under phase contrast microscope. The cells indicated the most prominent effects after exposure to the *D. hamiltonii* and *H. indicus* extracts. The microscopic observations revealed the *D. hamiltonii* and *H. indicus* extracts were to be having outstanding effect on treated HepG2 cells compared to treated Vero cells and untreated cells (Fig 2; Fig 3; Fig 4; Fig 5). The number of dead cells increased correspondingly with increasing of the extracts. At high extract concentration, enlargement of the cells was conspicuously observed.

4. Conclusion

Cancer is a painful fatal disease and fighting against such a disease is very essential for public health. Regarding the fast progress in the phytochemical study of herbal products, plants are transformed into popular anticancer medicines. In cancer, initial tumours will be treated by chemical supplement therapies or surgery. The current investigation showed that tuberous root extracts of *D. hamiltonii* and root extract of *H. indicus* have a promising anticancer activity on HepG2 cells with very low cytotoxicity on Vero cells. Hence both the extracts could be used in folk medicine as well as in Ayurvedic medicine preparation for cancer treatment.

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