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In vitro anticancer activity of medicinal plants of Himalayan region

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Plant-derived products have great potential to develop novel cancer preventive and therapeutic agents. Himalayan region is known for its vast reserve of natural resources. The present study was planned with the objective to explore the anticancer potential of plants of Himalayan region. Total twenty lyophilized plant extracts were prepared after extraction with 70% ethanol. All plant extracts were evaluated for percent cytotoxicity against LC-540 Leydig Cell Testicular Tumor cell line (rats) at various concentration levels (20, 50, 100 and 200 µg/mL) and at different exposure time (24, 48, 72 h). The results revealed that the aqua-ethanolic extract of leaves of *Camellia sinensis* exhibited maximum anticancer activity among all twenty extracts screened, with 42.74±4.63% and 68±1.74% cytotoxicity at 100 and 200 µg/mL concentration respectively, followed by *Lantana camara, Tinospora cordifolia* and *Cedrus deodara*. Further exploration of *in vivo* antineoplastic effects and probable mechanism of action of its potent phyto-constituents could be highly useful in developing an effective herbal formulation in cancer therapeutics.

Keywords: Anticancer, *Camellia sinensis*, Cytotoxicity, Himalayan region, SRB assay

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The Himalayas are famous for their vast natural resources with high medicinal potential. Traditional system of medicine is based on the use of medicinal plants, which is catering the need of more than 80% of the human population[1]. Herbal plants are major component of various systems of medicine viz., Ayurveda, Unani and Siddha[2]. Cancer is one of the life threatening diseases despite of recent developments in the therapeutics, prevention and diagnostic measures[3] as cancer death rate constitute 2–3% of the annual deaths worldwide[4]. Among 18.1 million cases of human cancer incidence worldwide, 9.6 million cases of mortality have been reported[5]. The situation is more critical in economically challenged countries because of lack of medical and diagnostic facilities and high cost of treatment[6]. Current treatments include severe side effects such as toxicity, non-specificity, fast clearance and restriction in metastasis[7]. Nowadays, more stress is being given toward medicinal plant research as an alternative medicine in cancer therapeutics[8]. Natural products are cost effective, readily available and with fewer side effects[9] and approximately 60% of drugs currently used for cancer treatment have been isolated from plants[10]. Many plant constituents such as alkaloids, flavonoids, saponins, terpenes, taxanes, vitamins, minerals, glycosides, and other primary and secondary metabolites have been found to have significant anticancer action through various mechanisms viz., antiproliferative, antiangiogenic, apoptosis-inducing and metastasis-inhibiting properties[11,12]. Taking all these facts, the present investigation was planned with the objective to explore the anticancer potential of plants of Himalayan region by *in-vitro* cytotoxicity studies.

Materials and Methods

Collection and identification of plant samples

Twenty plant samples (leaves, stem, seeds) were collected from the various regions (Lahaul Spiti, Barot, Palampur) of Himachal Pradesh (H.P) from different plants viz., *Adhatoda vasica* Nees, *Aegle marmelos, Camellia sinensis, Carissa opaca, Cedrus deodara, Cinnamomum tamala, Cuscuta reflexa, Eucalyptus citriodora, Euphorbia helioscopia, Girardinia heterophylla, Hippophae rhamnoides, Lantana camara, Murraya koenigii, Pinus wallichiana, Rhododendron arboretum, Syzygium*

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cumini, Thuja occidentalis, Tinospora cordifolia and *Vitex negundo* (Table 1, Fig. 1).The selection of the plant material was done on the basis of the information provided by local villagers and farmers, and interactive sessions with the staff of Animal Husbandry Department posted at different Veterinary Hospitals of H.P. The plant samples were got identified from the Department of Biodiversity, CSIR-IHBT, Palampur, H.P.

Preparation of aqua-ethanolic extracts

The plant materials were shade dried at room temperature and grinded. The powdered material was weighed, macerated overnight with 70% ethanol and filtered using double layered muslin cloth. The filtrate was concentrated over rotary evaporator (BUCHI Rotavapor R-210, Switzerland) at 40°. The slurry was subjected to lyophilization (CHRIST Alpha 1-2 LD Plus, Germany) to obtain dried powdered plant extract. The percent (%) recovery of extracts was recorded on dry weight basis (w/w) and extracts were kept at 4°C till further use.

Evaluation of *in vitro* anticancer activity

Cancer cell line

The rat Leydig Cell Testicular Tumor cell line LC-540 (Passage number 15 (12-02-19) was procured from NCCS, Pune (NCCS/2260/18-19) and maintained in the Cell Culture Laboratory at CSIR-IHBT, Palampur, H.P.

Maintenance of cell line

RPMI-2640 medium (Sigma, USA) containing 10% Fetal Bovine Serum FBS (HiMedia, India), Sodium Bicarbonate (2 g/L) and supplemented with Penicillin (10,000 units/100 mL) and Streptomycin (10 mg/100 mL) (Sigma, USA) was used for routine maintenance of cells and were kept at 37ºC in an atmosphere of 5% $CO_2$and 90% relative humidity.

Sampling of cells

For sampling of cells, the stock solution of plant extracts was prepared at the concentration of 20 mg/mL. All the plant extracts were evaluated at four concentrations i.e., 20, 50, 100 and 200 µg/mL. For each concentration, volume of sample was calculated and final volume was made up to 1000 µL by adding remaining media.

Sulforhodamine B assay for measuring cytotoxicity

The Sulforhodamine B (SRB) assay was performed to assess the cell cytotoxicity[13]. The method was optimized for cytotoxicity screening of plant extracts to adherent cells in a 96-well format. After sufficient growth for experimentation, cells were trypsinized and counted by using hematocytometer. Seeding

Table 1 — List of plants samples collected from different regions of Himachal Pradesh

S.no.	Scientific name	Common/local name	Iucn red list category	Part of plant
1.	*Adhatoda vasica* nees	Vasala/basunti	Ne	Leaves
2.	*Aegle marmelos*	Bael/bilpatri	Nt	Leaves
3.	*Camellia sinensis*	Tea plant/chai patta	Dd	Leaves
4.	*Carisssa opaca*	Granda/garna	Lc	Leaves
5.	*Cedrus deodara*	Deodar/devdar	Lc	Leaves
6.	*Cinnamomum tamala*	Tejpatta	Lc	Leaves
7.	*Cuscuta reflexa*	Akashbel/amarbel	Ne	Stem
8.	*Eucalyptus citriodora*	Safeda	Lc	Leaves
9.	*Euphorbia helioscopia*	Dudhya/dudhali	Ne	Leaves
10.	*Girardinia heterophylla*	Bicchubutti	Ne	Leaves
11.	*Hippophae rhamnoides*	Sea buckthorn/charma	Lc	Leaves
12.	*Lantana camara*	Lal phulnu	Ne	Leaves
13.	*Murraya koenigii*	Curry patta	Ne	Leaves
14.	*Pinus wallichiana*	Himalayan pines/cheer	Lc	Leaves
15.	*Rhododendron arboreum*	Lal burans/brahs	Lc	Leaves
16.	*Syzygium cumini*	Jamun	Lc	Leaves
17.	*Thuja occidentalis*	Vidya/morpankhi	Lc	Leaves
18.	*Thuja occidentalis*	Vidya/morpankhi	Lc	Seeds
19.	*Tinospora cordifolia*	Giloy	Ne	Stem
20.	*Vitex negundo*	Negundo/bana	Lc	Leaves

International Union for Conservation of Nature (IUCN) Red List Category:NT-Near threatened, LC-Least concern, DD-Data deficient, NE-Not evaluated (https://www.iucnredlist.org)

Fig. 1 — Plants samples collected from various districts of Himachal Pradesh

100 µL (20,000 cells) per well in 96 well plate was done. Cells were incubated at 37°C in an atmosphere of 5% CO_2 and 95% humidity for overnight. Samples were prepared (working stocks) and sampling was done for 24, 48 and 72 h of incubation. DMSO and Vinblastin (10 µM) were used as vehicle and positive control, respectively. Cells were fixed by adding 50% TCA and supernatant was discarded after 1 h incubation at 4°C. TCA growth medium was removed after washing with distilled water and plates were dried at room temperature. Staining with SRB solution (0.4% in 1% glacial acetic acid) was done (100 µL/well) followed by the incubation in dark for 30 min at room temperature. The unbound dye was washed quickly with 1% glacial acetic acid, and the plates were dried at room temperature. The bound dye

was dissolved by addition of 10 mM Tris base (100 µL/well). The plates were gently stirred for 5 min on a mechanical shaker and the optical density (OD) was measured at 540 nm on a microplate reader (Bioteck Synergy, USA)[14].

Percent (%) cytotoxicity of the test samples was calculated as per the formula:

$$\% \text{ Cytotoxicity} = (OD_{control}-OD_{test})/(OD_{control}) \times 100$$

Statistical analysis

Statistical analysis was performed using IBM SPSS STATISTICS version 20 software by applying one-way ANOVA. The experimental results were obtained as the mean ± SEM. The plant extract concentrations versus percent cytotoxicity data at different time of incubation were collected in a spreadsheet (Microsoft Excel® 2016, Microsoft Corporation). The statistical significance was assayed at 5% ($p<0.05$) probability level.

Results and Discussion

The aim of the present study was to evaluate the antineoplastic activity of the various plants of Himalayan region through *in vitro* cytotoxicity method. The percent recovery of lyophilized aqua-ethanolic extracts varies from 6.15 to 20.07% for different extracts and is presented in Table 2. The percent cytotoxicity of plant extracts against LC-540 Leydig Cell Testicular Tumor cell line (rats) at various concentration levels (20, 50, 100 and 200 µg/mL) after exposure for different time intervals (24, 48, 72 h) is reported in Table 3. It is evident from the findings of SRB assay that the extracts exhibited significant increase in cytotoxicity in a concentration and time dependent manner. Microscopic images of LC-540 cell lines are shown in Figure 2. The percent cytotoxicity obtained at the highest concentration i.e., 200 µg/mL after 72 h exposure ranged from 12.22±4.65 to 68±1.74% for different extracts (Fig. 3). The results obtained in the present study revealed that the aqua-ethanolic extract of *Camellia sinensis* exhibited maximum anticancer activity among all twenty extracts with 42.74±4.63% and 68±1.74% cytotoxicity at 100 and 200 µg/mL concentrations respectively, followed by *Lantana camara*, *Tinospora cordifolia* and *Cedrus deodara*. This indicates that there must be some potent cytotoxic compounds present in these extracts which could be responsible for their potent antineoplastic activity. The results are in concurrence with the previous studies that suggested a possible use of theseplantsin cancer therapeutics[15-19].

Camellia sinensis is commonly called as "tea plant" or "tea shrub" in English and “chai patti’ in Hindi. It is distributed in Southeast China, gradually expanded to India, Sri Lanka and further into many tropical and sub-tropical countries[20]. Kangra valley of Himachal Prasheh is known for its commercial production of tea. The green tea used in the present study is “Kangra Local” variety of the CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, commercially available as Dhauladar Him Palam Tea. Tea polyphenols have been studied in cell culture and animal models, where they have been found to inhibit tumor onset and progression[21]. Green tea is rich in polyphenols such as epicatechin, epigallocatechin, epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) which are known to have health-promoting effects[22]. EGCG, main polyphenolic constituent of green tea,act by restoring expression of tumor suppression genes such as retinoid X receptor alpha, resulting in breast cancer inhibition by binding to many high affinity target proteins such as, 70 kDa zeta-associated protein (Zap-70)[23].

Many studies have reported that the anticancer potential of green tea poyphenols is achieved by the

Table 2 — Percent (%) recovery of lyophilized plant extracts

S. No.	Name of plant	Percent (%) recovery	S. No.	Name of plant	Percent (%) recovery
1.	*Adhatoda vasica* Nees	10.04	11.	*Hippophae rhamnoides*	10.71
2.	*Aegle marmelos*	11.04	12.	*Lantana camara*	13.24
3.	*Camellia sinensis*	17.69	13.	*Murraya koenigii*	12.92
4.	*Carissa opaca*	20.07	14.	*Pinus wallichiana*	6.37
5.	*Cedrus deodara*	18.73	15.	*Rhododendron arboreum*	15.54
6.	*Cinnamomum tamala*	14.84	16.	*Syzygium cumini*	15.29
7.	*Cuscuta reflexa*	17.18	17.	*Thuja occidentalis* (leaves)	16.05
8.	*Eucalyptus citriodora*	14.06	18.	*Thuja occidentalis* (seeds)	10.22
9.	*Euphorbia helioscopia*	15.69	19.	*Tinospora cordifolia*	10.01
10.	*Girardinia heterophylla*	6.15	20.	*Vitex negundo*	15.52

Table 3 — Percent (%) cytotoxicity of plant extracts in LC-540 leydig cell testicular tumor cell line (rats) at 20, 50, 100 and 200 µg/mL concentration after 24, 48 and 72 h exposure

S. No	Plant extract	Percent (%) cytotoxicity											
Concentration		20 µg/mL			50 µg/mL			100 µg/mL			200 µg/mL		
Incubation Time		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
1.	*A. marmelos*	-29.59 ± 0.56^{de}	-13.73 ±0.66^{b}	-10.76 ± 0.17^{a}	-27.41 ± 0.54^{de}	-13.65 ± 0.31^{bc}	-7.33 ± 0.60^{a}	-23.48 ± 0.02^{ef}	-6.19 ± 0.44^{bcd}	-5.01 ± 0.36^{g}	-24.39 ± 0.46^{fg}	16.82 ± 2.01^{h}	18.52 ± 2.21^{cde}
2.	*A. nees*	-25.12 ± 0.99^{cde}	-4.58 ±0.24^{abc}	1.19 ± 0.23^{a}	-27.30 ± 0.25^{cde}	-0.413 ± 0.62^{bcde}	7.37 ± 1.12^{ab}	-13.29 ± 0.42^{ef}	4.01 ± 0.8^{f}	29.86 ± 1.49^{ab}	-21.45 ± 0.59^{cde}	-3.06 ± 0.86^{abcd}	28.52 ± 3.82^{def}
3.	*C. opaca*	-4.415 ± 0.67^{a}	-3.18 ± 0.83^{a}	0.14 ± 0.73^{a}	0.10 ± 0.39^{a}	1.76 ± 0.52^{a}	3.61 ± 0.59^{a}	1.60 ± 0.10^{a}	4.94 ± 0.800^{a}	8.20 ± 0.53^{a}	8.04 ± 0.17^{a}	12.97 ± 2.52^{a}	20.41 ± 2.04^{a}
4.	*C. reflexa*	-10.28 ± 0.32^{a}	-7.76 ± 0.23^{a}	-4.76 ± 0.45^{abc}	-4.34 ± 0.66^{abc}	-3.21 ± 0.30^{abc}	-1.81 ± 0.11^{a}	-6.86 ± 0.06^{ab}	-1.87 ± 0.09^{abc}	-1.26 ± 0.11^{a}	12.85 ± 1.19^{bcd}	14.21 ± 2.23^{cd}	25.48 ± 1.60^{d}
5.	*C. tamala*	4.61 ± 0.96^{a}	4.27 ± 0.13^{a}	6.18 ± 0.94^{a}	11.86 ± 1.49^{a}	14.38 ± 1.77^{a}	19.91 ± 2.99^{a}	14.49 ± 1.25^{a}	19.82 ± 1.35^{a}	29.52 ± 2.14^{a}	12.97 ± 1.72^{a}	18.28 ± 1.52^{b}	32.68 ± 2.64^{a}
6.	*C. sinensis*	-16.76 ± 0.60^{a}	-9.83 ± 0.15^{ab}	18.23 ± 2.49^{cd}	5.40 ± 0.61^{cd}	13.65 ± 1.25^{ab}	22.29 ± 1.67^{cd}	4.45 ± 0.03^{bc}	22.76 ± 2.90^{cd}	42.74 ± 4.63^{de}	43.28 ± 3.26^{ef}	51.5 ± 4.04^{f}	65.22 ± 4.65^{f}
7.	*C. deodara*	-21.56 ± 0.47^{bc}	-6.00 ± 0.27^{b}	-5.62 ± 0.17^{a}	-6.77 ± 0.72^{ab}	0.59 ± 0.39^{bcd}	12.34 ± 0.20^{cd}	14.74 ± 1.30^{cde}	16.15 ± 1.76^{de}	18.68 ± 2.15^{de}	30.26 ± 1.79^{f}	36.88 ± 2.28^{ef}	40.53 ± 2.01^{f}
8.	*E. citriodora*	-20.15 ± 0.75^{cd}	-15.85 ± 0.93^{a}	-5.11 ± 0.25^{a}	-18.46 ± 0.74^{cd}	3.35 ± 1.14^{cd}	7.90 ± 1.82^{a}	-7.86 ± 0.58^{d}	3.08 ± 1.43^{bcd}	7.23 ± 1.13^{ab}	-31.36 ± 0.59^{bc}	17.17 ± 2.32^{bcd}	21.93 ± 2.11^{cd}
9.	*E. helioscopia*	-52.29 ± 0.30^{a}	-23.51 ± 1.28^{bc}	-19.97 ± 0.90^{bcd}	-27.90 ± 0.61^{b}	-14.70 ± 0.02^{d}	-2.26 ± 0.58^{cd}	-1.88 ± 0.01^{d}	-10.09 ± 0.58^{bcd}	4.41 ± 0.99^{d}	3.86 ± 0.41^{d}	23.27 ± 1.83^{e}	29.96 ± 2.56^{e}
10.	*G. heterophylla*	2.36 ± 0.34^{abc}	2.59 ± 0.23^{ab}	3.99 ± 0.34^{abcd}	3.81 ± 0.59^{abc}	3.06 ± 0.60^{a}	1.42 ± 0.17^{abcd}	10.34 ± 0.61^{cd}	13.85 ± 0.20^{cde}	22.33 ± 0.55^{cde}	16.18 ± 1.53^{de}	19.66 ± 0.78^{bcd}	26.76 ± 1.50^{e}
11.	*H. rhamnoides*	4.05 ± 1.23^{a}	13.31 ± 1.23^{abc}	15.27 ± 1.02^{abcde}	13.61 ± 0.31^{abcd}	17.10 ± 1.41^{bcde}	32.72 ± 1.22^{ef}	11.19 ± 0.82^{abc}	13.12 ± 0.59^{ab}	15.44 ± 0.42^{abcde}	25.65 ± 2.26^{de}	27.00 ± 1.61^{cde}	37.29 ± 1.65^{f}
12.	*L. camara*	-28.65 ± 0.37^{a}	-10.98 ± 0.97^{ab}	-7.74 ± 0.92^{abc}	-17.42 ± 0.92^{ab}	0.49 ± 0.17^{bc}	6.27 ± 1.25^{cd}	2.219 ± 0.47^{a}	19.20 ± 1.66^{de}	28.14 ± 2.43^{ef}	30.96 ± 2.61^{ef}	39.72 ± 2.21^{fg}	50.28 ± 4.63^{g}
13.	*M. koenigii*	-0.03 ± 0.43^{a}	0.24 ± 0.50^{f}	2.98 ± 0.69^{d}	2.37 ± 0.24^{ab}	7.335 ± 0.23^{b}	16.74 ± 1.84^{c}	7.14 ± 0.88^{ab}	19.06 ± 1.47^{fg}	32.16 ± 1.27^{g}	9.46 ± 1.26^{bc}	23.58 ± 2.73^{e}	30.46 ± 2.68^{g}
14.	*P. walliciana*	-8.31 ± 0.32^{b}	-5.71 ± 0.45^{ab}	-5.49 ± 0.23^{bc}	-15.04 ± 0.90^{bcd}	-5.09 ± 0.43^{a}	-2.70 ± 0.30^{bc}	-0.46 ± 0.89^{cd}	5.26 ± 0.67^{b}	8.73 ± 0.86^{d}	8.52 ± 1.76^{d}	27.88 ± 1.25^{e}	28.60 ± 2.24^{e}
15.	*R. arboreum*	-39.38 ± 0.52^{cd}	-24.09 ± 0.2^{b}	-12.92 ± 0.45^{a}	-26.39 ± 0.42^{de}	-22.37 ± 0.18^{b}	-3.301 ± 0.06^{b}	-23.59 ± 0.33^{e}	-19.23 ± 0.70^{b}	-2.22 ± 0.60^{b}	-46.66 ± 0.22^{c}	2.23 ± 0.46^{cde}	12.68 ± 1.74^{c}
16.	*S. cumini*	-16.96 ± 0.16^{ab}	-16.69 ± 0.71^{a}	-16.62 ± 0.06^{a}	-13.86 ± 0.20^{ab}	1.31 ± 0.22^{bc}	2.80 ± 0.58^{bc}	-6.73 ± 0.70^{abc}	14.68 ± 1.06^{cd}	15.27 ± 0.96^{cd}	18.72 ± 0.93^{de}	31.81 ± 2.17^{e}	33.22 ± 2.92^{e}
17.	*T. occidenalis* (leaves)	-32.40 ± 0.14^{a}	-22.91 ± 1.62^{b}	-9.10 ± 0.57^{bc}	-19.56 ± 0.55^{bc}	-6.74 ± 0.59^{bcd}	0.88 ± 0.05^{bc}	-13.98 ± 0.34^{bc}	4.28 ± 0.33^{cd}	10.57 ± 0.73^{bc}	5.39 ± 0.14^{de}	26.01 ± 0.91^{ef}	30.65 ± 2.69^{f}
18.	*T. occidentalis* (seeds)	-2.56 ± 0.80^{a}	6.51 ± 0.87^{a}	7.66 ± 0.70^{abcd}	-0.98 ± 0.39^{ab}	9.85 ± 0.61^{abcd}	24.80 ± 1.53^{cde}	18.51 ± 1.30^{e}	18.46 ± 0.62^{de}	33.36 ± 2.13^{cde}	5.75 ± 0.72^{e}	9.28 ± 0.12^{de}	18.65 ± 2.74^{cde}
19.	*T. cordiofolia*	1.31 ± 0.32^{a}	6.35 ± 0.47^{a}	9.50 ± 0.62^{a}	18.37 ± 1.29^{d}	25.31 ± 1.71^{d}	33.23 ± 2.28^{d}	17.31 ± 1.20^{d}	26.03 ± 2.16^{d}	42.03 ± 3.35^{bc}	31.56 ± 2.70^{b}	43.41 ± 2.72^{c}	48.17 ± 3.55^{a}
20.	*V. negundo*	7.46 ± 0.83^{bc}	11.03 ± 1.31^{bc}	11.64 ± 1.16^{b}	19.29 ± 2.04^{bc}	18.46 ± 1.01^{cd}	8.98 ± 1.03^{bc}	-2.95 ± 0.53^{a}	7.06 ± 0.92^{bc}	7.86 ± 0.67^{bc}	29.89 ± 0.08^{e}	33.54 ± 1.41^{de}	37.15 ± 1.37^{de}

Values (mean ± SEM, n = 3) with different superscript vary significantly with each other within a row ($p<0.05$)

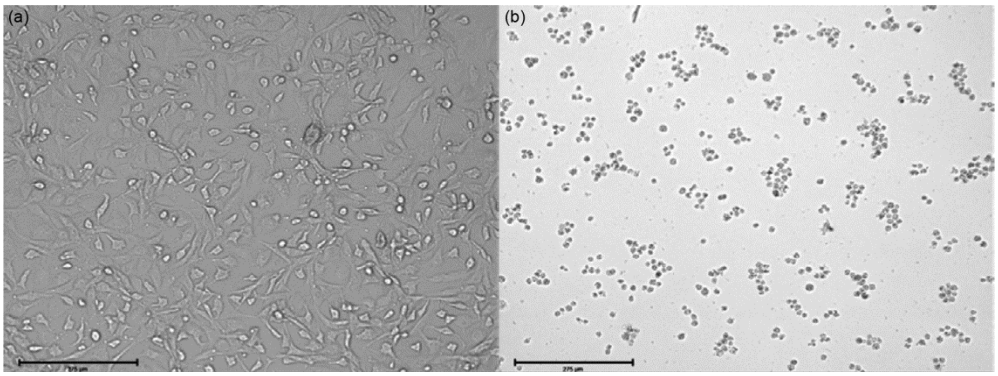

Fig. 2 — (a) Microscopic image of control LC540 cell lines (b) Microscopic image of LC540 cell lines exposed to *Camellia sinensis* extract for 72 h at 200 µg/mL concentration

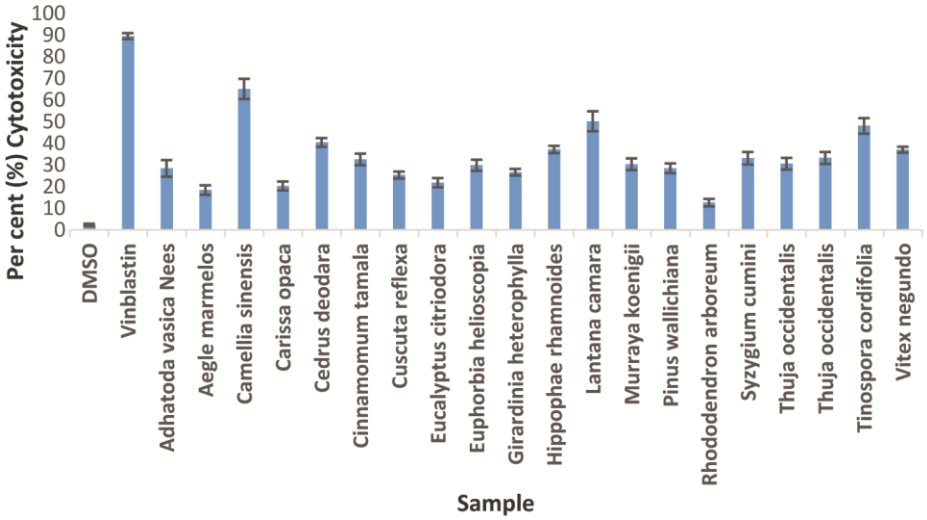

Fig. 3 — Percent (%) cytotoxicity of DMSO, Vinblastin (10 µM) and plant extracts (200 µg/mL) after 72 h exposure

regulation of different cancer-related processes and factors[24,25], viz., DNA methylation, histone modification, micro-RNA, apoptosis, invasion and angiogenesis in various types of malignancies[26,27]. Moreover, they have been found to modulate the functions of various cancer-related signaling molecules such as vascular endothelial growth factor (VEGF), cyclin D1 and caspase-3[28]. Studies have revealed that EGCGcan inhibit the proliferation and induce apoptosis by lung cancer stem cells[29,30] and suppress tumor spheroid formation by colorectal cancer stem cells[31]. These findings suggest that EGCG have strong anticancer effects both *in vivo* and *in vitro.* Thus, from the present study it could be concluded that *Camellia sinensis* extractpossess potent anticancer activity aginst LC-540 cancer cell lines. Further, exploration of *in vivo* antineoplastic effects and mechanism of action of its potent phyto-constituents could be useful in developing herbal formulations in cancer therapeutics.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

PB conceived and designed the study and drafted and revised the paper, ST performed the experiments and analyzed the data

References

1 Shanmugasundaram S, Complementary and alternative therapies in palliative care: A transition from modern medicine to traditional medicine in India, *J Cancer Pain Symptom Palliation*, 1 (2005) 25-29.

2 Das A M, Dhanabalan R & Palaniswamy M, Phytochemical screening and antibacterial activity of aqueous and methanolic leaf extracts of two medicinal plants against bovine mastitis bacterial pathogens, *Ethnobot Leaf*, 13 (2009) 131-139.

3 Qin W, Huang G, Chen Z & Zhang Y, Nanomaterials in targeting cancer stem cells for cancer therapy, *Front Pharmacol*, 8 (2017) 1.

4 American Cancer Society, A Biotechnology Company Dedicated to Cancer Treatment on Jan 26. Atlanta, Georgia: American Cancer Society; 2006. Available from: http://www.cancervax.com/info/index.htm.

5 Ferlay J, Colombet M, Soerjomataram I, Mathers C & Parkin DM, *et al*., Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, *Int J Cancer*, 144 (2019) 1941-1953.

6 Jemal A F, Bray M M, Center J, Ferlay E W & Forman D, Global cancer statistics, *CA Cancer J Clin*, 61 (2) (2011) 69-90.

7 Mukherjee S & Patra C R, Therapeutic application of anti-angiogenic nanomaterials in cancers, *Nanoscale*, 8 (2016) 12444-12470.

8 Denny B & Wansbrough H, The design and development of anti-cancer drugs, *Biotech J Cancer Drugs*, 12 (1995) 1-12.

9 Verma P & Kundu T, The ginger prophecy; a review of the underexplored genus, *Hedychium* against Cancer, *Indian J Pharm Sci*, 82 (1) (2019) 11-20.

10 Khan H, Medicinal plants in light of history: recognized therapeutic modality, *J Evid Based Complement Altern Med*, 19 (2014) 216-219.

11 Ijaz S, Akhtar N, Khan M S, Hameed A & Irfan M, *et al*., Plant derived anticancer agents: A green approach towards skin cancers, *Biomed Pharmacother*, 103 (2018) 1643-1651. doi: 10.1016/j.biopha.2018.04.113.

12 Kumar N, Singh R, Saminathan M, Singh K P & Dhama K, *et al*., Therapeutic effect of hydroethanolic extract of

Trianthema portulacastrum L. against N-Nitroso-N-Methylurea-induced mammary tumors in Wistar rats, *Indian J Tradit Know*, 19 (2) (2020) 406-415.

13 Skehan P, New colorimetric cytotoxicity assay for anticancer drug screening, *J Natl Cancer Inst*, 82 (1990) 1107-1112.

14 Vichai V & Kirtikara K, Sulforhodamine B colorimetric assay for cytotoxicity screening, *Nature Prot*, 1 (2006) 1112-1116.

15 Surh Y J, Cancer chemoprevention with dietary phytochemicals, *Nat Rev Cancer*, 3 (2003) 768–780.

16 Jagetia G C & Rao S K, Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascites carcinoma bearing mice, *Biol Pharm Bull*, 29 (3) (2006) 460-466. doi: 10.1248/bpb.29.460

17 Gaidhani S N, Singh A, Kumari S, Lavekar G S & Juvekar A S, *et al*., Evaluation of some plant extracts for standardization and anticancer activity, *Indian J Tradit Know*, 12 (4) (2013) 682-687.

18 Bhaskar D, Mashrea D S, Amresh N & Sathyamurthy B, *In vitro* studies on the effect of lantana camara linn. in liver cancer hepg 2 cell lines, *Eur J Pharm Med Res*, 4 (9) (2017) 539-545

19 Shi X, Du R, Zhang J, Lei Y & Guo H, Evaluation of the anti-cancer potential of *Cedrus deodara* total lignans by inducing apoptosis of A549 cells, *BMC Complement Altern Med*, 19 (1) (2019) 281. doi: 10.1186/s12906-019-2682-6.

20 Vishnoi H, Bodla B B & Kant R, Green tea (*Camellia sinensis*) and its antioxidant property: Review, *Int J Pharm Sci*, 9 (5) (2018) 1723-1736.

21 Bag A & Bag N, Tea polyphenols and prevention of epigenetic aberrations in cancer, *J Nat Sci Biol Med*, 9 (2018) 2-5.

22 Cooper R, Morré D J & Morré DM, Medicinal benefits of green tea: Part I. Review of noncancer health benefits, *J Altern Complement Med*, 11 (3) (2005) 521-528. doi: 10.1089/acm.2005.11.521.

23 Morris J, Moseley V R, Cabang A B, Coleman K & Wei W, *et al*., Reduction in promoter methylation utilizing EGCG (epigallocatechin-3-gallate) restores RXRα expression in human colon cancer cells, *Oncotarget*, 7 (23) (2016) 35313-35326. doi: 10.18632/oncotarget.9204.

24 Tsao A S, Liu D, Martin J, Tang X M & Lee J J, *et al*., Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions, *Cancer Prev Res* (Phila), 2 (11) (2009) 931-941. doi: 10.1158/1940-6207.CAPR-09-0121.

25 Singh B N, Shankar S & Srivastava R K, Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications, *Biochem Pharmacol*, 82 (12) (2011) 1807-1821. doi: 10.1016/j.bcp.2011.07.093.

26 Shi J, Liu F, Zhang W, Liu X & Lin B, *et al*., Epigallocatechin-3-gallate inhibits nicotine-induced migration and invasion by the suppression of angiogenesis and epithelial-mesenchymal transition in non-small cell lung cancer cells, *Oncol Rep*, 33 (6) (2015) 2972-2980. doi: 10.3892/or.2015.3889.

27 Ni J, Guo X, Wang H, Zhou T & Wang X, Differences in the effects of EGCG on chromosomal stability and cell growth between normal and colon cancer cells, *Molecules*, 23 (4) (2018) 788. doi: 10.3390/molecules23040788.

28 Liu L, Hou L, Gu S, Zuo X & Meng D, Molecular mechanism of epigallocatechin-3-gallate in human esophageal squamous cell carcinoma *in vitro* and *in vivo*, *Oncol Rep*, 33 (1) (2015) 297-303. doi: 10.3892/or.2014.3555.

29 Fujiki H, Watanabe T, Sueoka E, Rawangkan A & Suganuma M, Cancer prevention with green tea and its principal constituent, EGCG: from early investigations to current focus on human cancer stem cells, *Mol Cells*, 41 (2) (2018) 73-82. doi: 10.14348/molcells.2018.2227.

30 Zhu J, Jiang Y, Yang X, Wang S & Xie C, *et al*., Wnt/β-catenin pathway mediates (-)-Epigallocatechin-3-gallate (EGCG) inhibition of lung cancer stem cell, *Biochem Biophys Res Commun*, 482 (1) (2017) 15-21. doi: 10.1016/j.bbrc.2016.11.038.

31 Toden S, Tran H M, Tovar-Camargo O A, Okugawa Y & Goel A, Epigallocatechin-3-gallate targets cancer stem-like cells and enhances 5-fluorouracil chemosensitivity in colorectal cancer, *Oncotarget*, 7 (13) (2016) 16158-16171. doi: 10.18632/oncotarget.7567.