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Assessment Of Anticarcinogenic Activities Of Endangered Plant Species Of Western Ghats In Cytotoxicity Studies For Mcf-7 Cell Line By Mtt Assay Method

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ABSTRACT

Cancer is a major public health problem in most developed countries, and is one of the leading causes of death around the world. The traditional approaches to treat cancer such as surgery, hormone therapy, radiotherapy and chemotherapy methods can cause adverse side effects. With this context, in the current we aimed to evaluate anticarcinogenic activities of endangered plant species of western ghats in cytotoxicity studies for MCF-7 cell line by MTT assay method. Results depicted that extracts of all the four endangered plant species *viz. Curcuma zedoaria*, *Syzygium travancorium*, *Plectranthus vettiveroides*, and *Utleria salcifolia* have showed increased inhibition activity with increased concentration of the extracts. The leaf extract *U. salcifolia* and *P. vettiveroides* exhibited IC_{50} value of 34.24 µg/ml and 199 µg/ml respectively. While, stem extracts of *C. zedoaria* and *S. travancorium* exhibited IC_{50} value of 289 µg/ml and 73 µg/ml respectively. In conclusion, stem extract of *U. salicifolia* exhibited low IC50 value, and therefore it showed highly efficient cytotoxic effect than other tested endangered plant species of western ghats *viz. C. zedoaria, S. travancorium*, and *P. vettiveroides*. Hence, stem extract of *U. salicifolia* could be considered for the development of natural anticarcinogenic drugs.

Keywords: Western ghats, Endangered plants, *U. salicifolia*, Cytotoxicity, MCF-7 cell line

INTRODUCTION

Cancer is one of the leading causes of death around the world, coming in at a close second to cardiovascular diseases, and it is responsible for increasing mortality rate.[1] There were 19.3 million cancer cases and 10 million deaths recorded in 2020.[2] As per world health organization (WHO), numbers of deaths caused by cancer are one among the highest after heart disease. Cancer is a major public health problem in most developed countries; however, there have been notable improvements in the survival rate of patients over the past three decades owing to early detection and progress in medical treatment.[3] It has been estimated that cancer cases rose to around 18 million and the death toll crossed 9 and a half million deaths in the year 2018. Studies say that by 2030, the death toll of cancer will raise to 17 million.[4]

Initially, at the start of cancer, normal cells after developing mutations (being called neoplastic cells) grow and formation of tumor occurs. Later on, it spreads to other normal body cells through metastasis,[1] and their spread to other parts of the body.[5] Breast cancer, colorectal cancer, lung cancer, stomach cancer and liver cancer are major types of cancer. Treatment of cancer depends upon the facts that what factors are involved as causative agents and at what stage cancer is.[1]

The traditional approaches to treat cancer are surgery, hormone therapy, radiotherapy and chemotherapy. However, these prevailing methods each cause adverse side effects.[6] Some of the important reasons that drive scientists and researchers to explore new and faster anticancer drugs are the increasing death rate and the ill effects caused by these drugs.[7] As a result of these disadvantages, there has been continual research to find plant-based compounds that could have anti-cancer properties.[8]

Medicinal plants continue to be an important source of lifesaving drugs for humankind, especially in the developing nations. The World Health Organization has estimated that more than 80% of the world population in developing countries depends primarily on herbal medicine for basic health care. The increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics has renewed the interest in the use of plants and plant-based drugs.[9]

The plant kingdom represents an extraordinary reservoir of molecules with a variety of astonishingly diverse structural features derived from complex biosynthetic steps. Medicinal plants are a rich bio-resource of drugs for traditional systems of medicine and modern medicines, nutraceuticals and food supplements. Threatened medicinal plants have an important role in traditional herbal medicinal practices and are being widely exploited, leading to near extinction.[10] Humans have been exploring different plant species for thousands of years in an effort to treat illness and improve overall health. Therefore, they have found many bioactive compounds in plants that have great medicinal promise. Flavonoids, carotenes, alkaloids, and phenolics are four of the most studied plant chemicals having medicinal effects, including anticancer potential.[5]

Western Ghats is one of the major repositories of medicinal plants. It houses around 700 species of medicinal plants of which 450 species are threatened and currently the number of species added to the red list category in that region is increasing. Therefore, the valuable genetic resources are being lost at a rapid rate.[9] With this scenario, in the current study we aimed for assessment of anticarcinogenic activities of endangered plant species of western ghats.

MATERIALS AND METHODS

Collection of Plant Species

The endangered plant species of western ghats *viz. Curcuma zedoaria*, *Syzygium travancorium*, *Plectranthus vettiveroides*, and *Utleria salcifolia* (Figure 1) have been selected in the present study were collected from the Foundation for Revitalisation of Local Health Traditions (FRLHT), Yelahanka, Bengaluru. The plants were classified and authenticated for taxonomic identity. The plants and their parts were collected in plastic bags and transported to the laboratory. Roots, stem and leaves are shade dried, powdered and stored in refrigerator until the time of analysis.

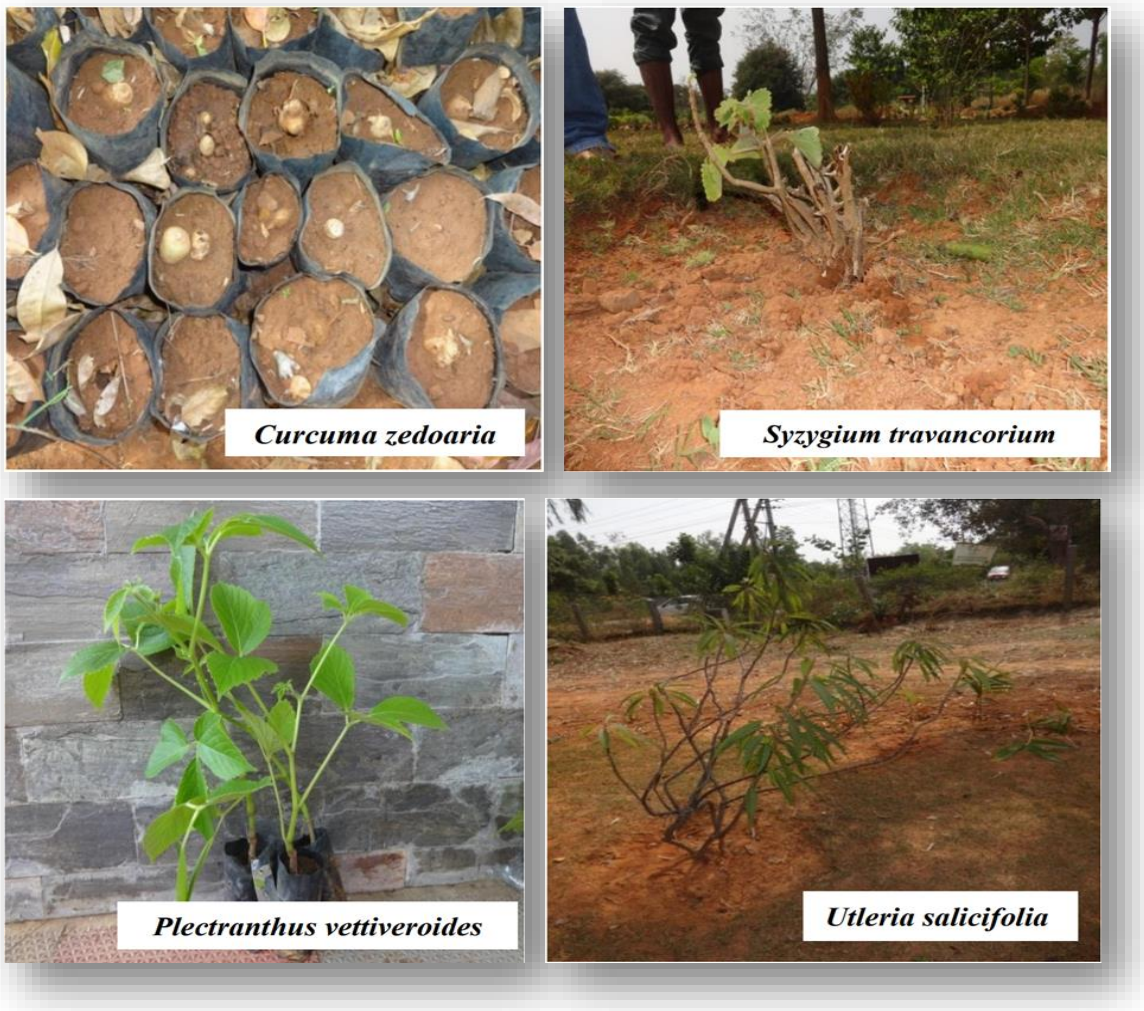


Figure 1. Showing endangered plant species of western ghats collected from FRLHT, Yelahanka, Bengaluru

Extraction

The collected samples were separated into leaves, stem and roots. All the parts were cleaned and shade dried and powdered in a blender. 15g of powdered sample without moisture content was elicited in the Soxhlet apparatus with methyl alcohol. The extraction was run for a minimum of 20 cycles or till the solvent in the sample container turned colorless (Figure 2).

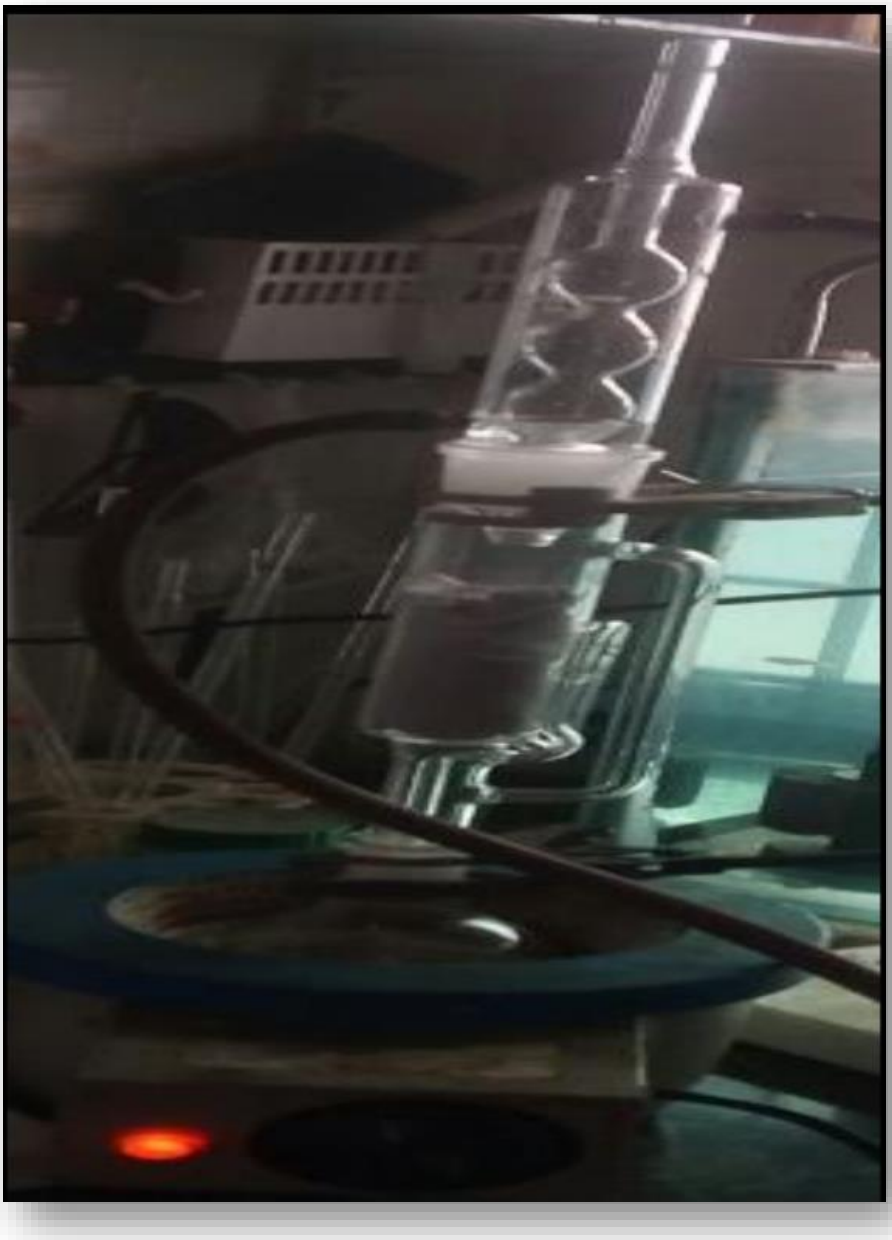
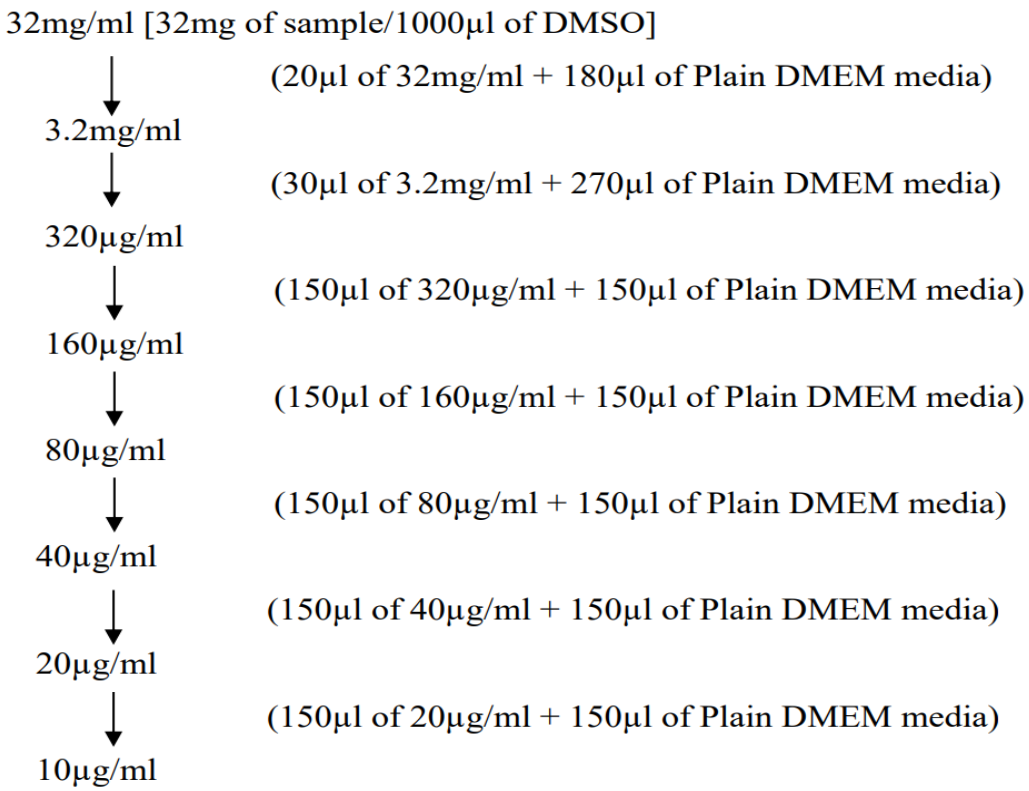


Figure 2. Showing Soxhlet extraction of phytoactives from endangered plant species of western ghats

Determination of Anti-cancer Activities of Plant Extracts

The cytotoxicity studies for MCF-7 cell line by MTT assay was determined by the method as described by Hayon et al.[11] 32mg/ml stocks were made with the use of DMSO. Serial two-fold dilutions were changed from 320µg/ml to 10µg/ml using DMEM plain media for treatment as shown below.



Cell lines and Culture Medium

ATCC was used to produce all cell lines, the stock cells were cultured in streptomycin (100µg/ml), DMEM supplemented with penicillin (100 IU/ml) and 10% inactivated FBS in an atmosphere of 5% CO_2 and increased humidity at 37°C until they were confluent. Cell dissociating solution (0.2% trypsin, 0.05% glucose in PBS, 0.02% EDTA) was used to dissociate the cell. The cells were examined for viability and centrifugation was conducted. Then, 50,000 cells were seeded in a 96 well plate and placed in a 5% CO_2 incubator, maintained at 37°C for one day.

Procedure: Trypsinization of the monolayer cell culture was carried out and respective media containing 10% FBS to adjust the cell count to 1.0 x 10^5 cells/ml. 100µl of the diluted cell suspension (50,000cells/well) was added to each well in the 96 well microtiter plate. After a complete day a partial monolayer was observed and the supernatant was flipped off. It was then washed once with the medium and 100µl of various concentrations of test drugs were added to the partial monolayer in microtiter plates. Incubation of the plates was carried out at 37°C for 24 hours in a 5% carbon dioxide atmosphere. Then, the test solutions were disposed and 100µl of 5mg/10ml of MTT in PBS was dropped onto each well. Incubation of the plates was carried out for 4 hours at 37°C, in a 5% carbon dioxide atmosphere. After removal of the supernatant, 100µl of DMSO was mixed and the plates were shaken in a gentle manner to make the formazan formed more soluble. A microplate reader at 590 nm was used to measure the absorbance. The inhibition percentage was evaluated using the below equation and IC_{50} values of test drug was calculated for every cell line from the dose response curves.

% Inhibition = 100-(OD of sample/OD of Control) x 100
Where,
OD, Optical density

RESULTS

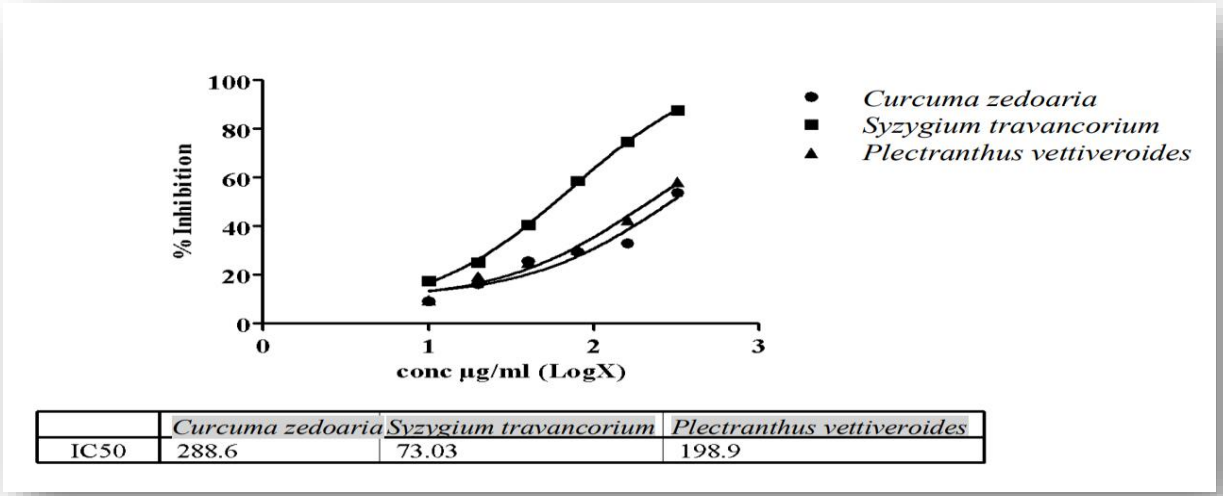
Effect of extracts of endangered plant species of western ghats *viz. C. zedoaria, S. travancorium, P. vettiveroides, and U. salcifolia* on cytotoxicity studies for MCF-7 cell line by MTT assay was represented in Table 1 and depicted in Figure 3, Figure 4, and Figure 5. Results depicted that all the four plant extracts showed increased inhibition activity with increased concentration of the plant extracts. Stem extract of *C. zedoaria* stem has shown anticarcinogenic activity of 53% inhibition on cancer cells at 320 µg/ml, Stem extract of *S. travancorium* stem has shown anticarcinogenic activity of 87% inhibition at 320 µg/ml. Leaf extract *P. vettiveroides* leaves showed anticarcinogenic activity of 58%

inhibition at 320 µg/ml and leaf extract of *U. salcifolia* showed anticarcinogenic activity of 58% inhibition at 320 µg/ml. The leaf extract *U. salcifolia* and *P. vettiveroides* exhibited IC_{50} value of 34 µg/ml and 199 µg/ml respectively. While, stem extracts of *C. zedoaria* and *S. travancorium* exhibited IC_{50} value of 289 µg/ml and 73 µg/ml.

Table 1. Effect of extracts of endangered plant species of western ghats on cytotoxicity studies for MCF-7 cell line by MTT assay

Variables	Conc. (µg/mL)	Inhibition %	IC_{50} (µg/mL)
Control	0.00	0.00	
Stem extract of *C. zedoaria*	10	9.29 ± 0.294	288.6
	20	16.22 ± 0.675	
	40	25.52 ± 0.351	
	80	29.50 ± 0.379	
	160	33.04 ± 0.055	
	320	53.67 ± 0.330	
Stem extract of *S. travancorium*	10	17.62 ± 0.423	73.03
	20	25.04 ± 0.117	
	40	40.55 ± 0.296	
	80	58.69 ± 0.325	
	160	74.89 ± 0.295	
	320	87.68 ± 0.329	
Leaf extract of *P. vettiveroides*	10	9.718 ± 0.072	198.9
	20	19.46 ± 0.417	
	40	25.10 ± 0.352	
	80	30.54 ± 0.157	
	160	42.56 ± 0.105	
	320	58.27 ± 0.238	
Leaf extract of *U. salcifolia*	10	5.27 ± 0.00	34.24
	20	14.57 ± 0.072	
	40	29.60 ± 0.417	
	80	41.03 ± 0.157	
	160	50.45 ± 0.105	
	320	56.61 ± 0.235	

Values were expressed as mean ± standard error of mean (SEM)



	Curcuma zedoaria	*Syzygium travancorium*	*Plectranthus vettiveroides*
IC50	288.6	73.03	198.9

Figure 3. Showing determination of IC_{50} values of endangered plant species of western ghats in cytotoxicity studies for MCF-7 cell line by MTT assay

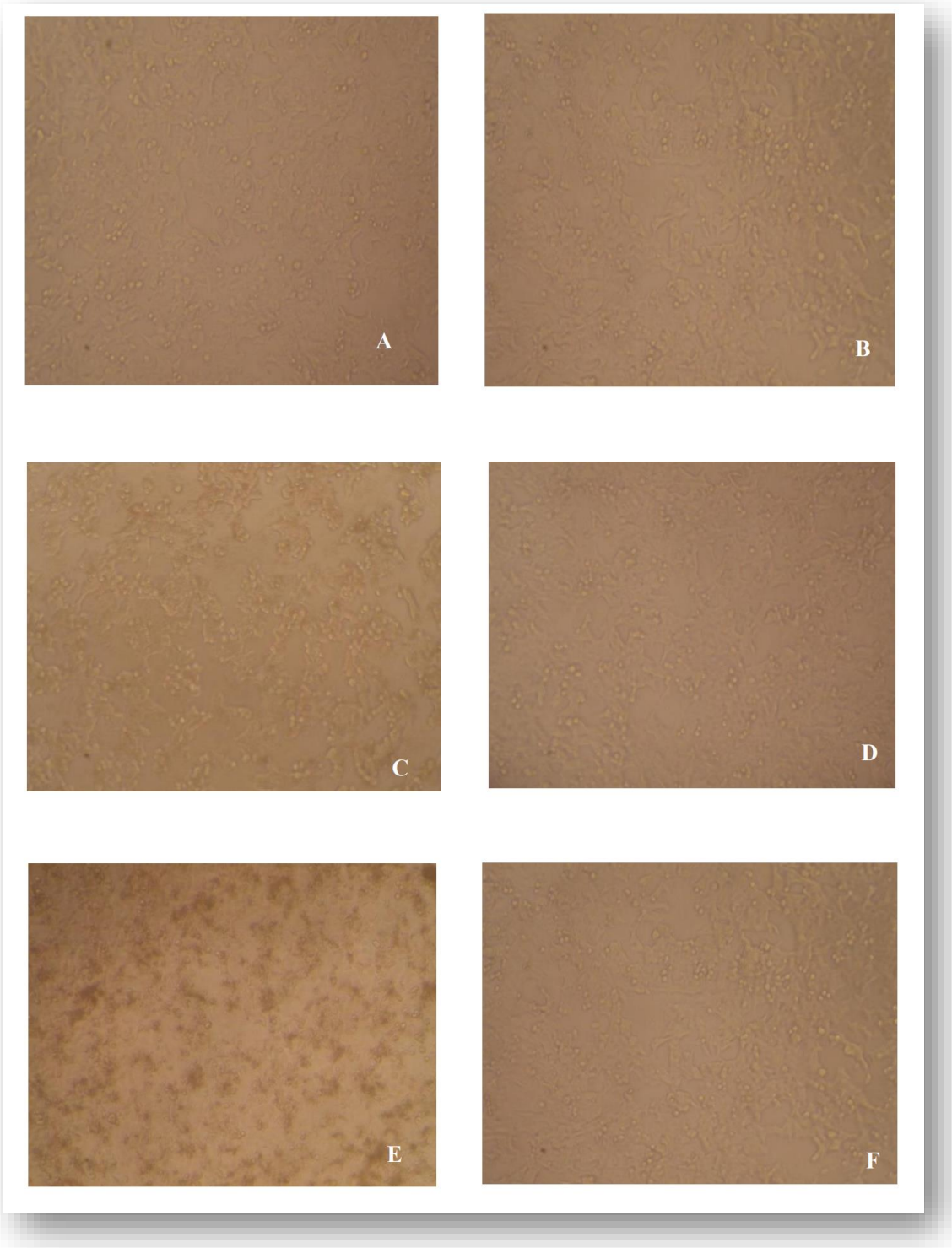


Figure 4. Showing cytotoxicity studies for MCF-7 cell line by MTT assay

A- Control, B- *C. zedoaria* 10µg/ml, C- *C. zedoaria* 320µg/ml D- *S. travancorium* 10µg/ml, E- *S. travancorium* 320µg/ml, F- *P. vettiveroides* 10µg/ml

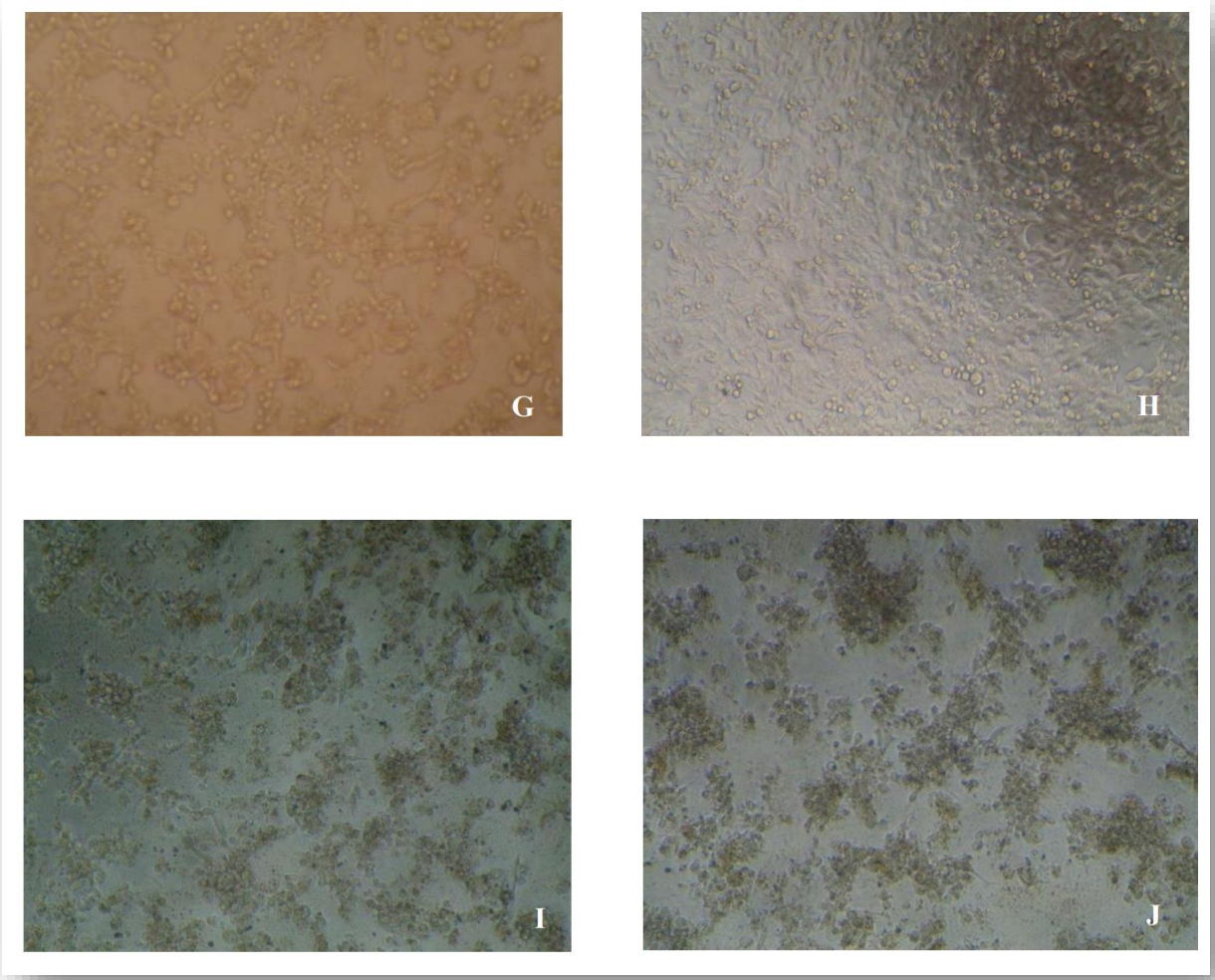


Figure 5. Showing cytotoxicity studies for MCF-7 cell line by MTT assay

G- *P. vettiveroides* 320µg/ml, H- *P. vettiveroides* 320µg/ml, I- *U. salicifolia* 320µg/ml, J- *U. salicifolia* 320µg/ml

DISCUSSION

Chemicals originating from plants are widely employed in oncology due to their vast therapeutic properties and minimal toxicity. Various biological and molecular characteristics of cancer cells are the focus of natural substances. Furthermore, several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. The greatest bioresource for pharmaceutical intermediates, modern and traditional medicine, nutraceuticals, food supplements, folk remedies, and chemical entities for synthetic drugs is found in medicinal plants.[5,12]

Anticancerous compounds bring about direct absorption of reactive oxygen species or the promotion of enzymes with antioxidant properties such as glutathione, catalase, superoxide dismutase etc... in a transformed cell. A phytochemical can restrain the cancerous transformation of an initiated pre-neoplastic cell or arrest the metabolic conversion of the pro-carcinogen. They can also regulate events relating to cellular growth, invasion and metastasis of cancerous cells. Therefore, in this study we aimed for evaluation of anticarcinogenic activities of endangered plant species of western ghats in cytotoxicity studies for MCF-7 cell line by MTT assay method.

Results of our study delineated that all the tested extracts of endangered plant species viz. *C. zedoaria, S. travancorium, P. vettiveroides*, and *U. salcifolia* have shown the good anticarcinogenic activity. However, among the four endangered plant species of western ghats leaf extract of *U. salicifolia* have shown low IC_{50} value, and hence leaf extract of *U. salicifolia* could be the very efficient source for anticarcinogenic activity.

Studies in the literature have reported anticarcinogenic activities of various plant species. For instances Rai et al., tested the chloroform extract of *Pogostemon heyneanus* and ethanol extract of *Plectranthus amboinicus* for cytotoxicity by the method of MTT assay for MCF-7 and MDA-MB-231 cell lines of breast cancer cells. Both extracts showed effective activity against MCF-7 cells. Whereas, chloroform extract of *P. heyneanus* showed more activity against MDA-MB-231 cells.[13] Lakshmi et al., showed that isocurcumenol, an active compound extracted from *Cyclanthera pedata* by spectroscopy successfully inhibited the multiplication of cancerous cells unaccompanied by toxic effects on non-cancerous cells.[14] Hamdi et al., conducted a study to analyse the cytotoxic compounds present in the hexane extract of *C. zedoaria* against Ca Ski, HT-29, MCF-7 and PC-3 cell lines. It was observed that the compounds curcumenol and curcumenone significantly inhibited the cancer cell proliferation in the MCF-7 cell line by inducing apoptosis.[15]
Moreover, Keshamma et al., reported that use of herbs and plants in cooking and medicinal dates back thousands of years. Many plant species that still have immune-boosting and cancer-fighting properties. Carotenoids, flavonoids, ligands, polyphenolics, terpenoids, sulphides, lignans, and plant sterols are some of the many active phytochemicals found in different types of herbs. There are a number of mechanisms through which these phytochemicals exert their effects. They either prevent cell division or stimulate the synthesis of a protective enzyme such as glutathione transferase. The cancer-fighting and cholesterol-lowering effects of mevalonate are counteracted by the volatile oils and plant extracts from various herbs and plants.[5]

CONCLUSION

In conclusion, results of this study demonstrated that all the four endangered plant species of Western ghats *viz. C. zedoaria, S. travancorium, P. vettiveroides,* and *U. salcifolia* possesses potent anticarcinogenic properties. However, stem extract of *U. salicifolia* exhibited IC_{50} value, and therefore it showed highly efficient cytotoxic effect than other tested endangered plant species of western ghats *viz. C. zedoaria, S. travancorium,* and *P. vettiveroides*. Hence, stem extract of *U. salicifolia* an endangered plant species of western ghat could be considered for the development of natural anticarcinogenic drugs.

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