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EVALUATION OF ANTICANCER ACTIVITY OF MANGIFERA INDICA (LEAVES)

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ABSTRACT Mango *(Mangifera indica)* is one of the most important tropical plants. It is cultivated on an area of approximately 3.7 million ha worldwide and conquers the second position as a tropical crop, in terms of production. Much of the spread and naturalization has occurred in conjunction with the spread of human populations, and as such, the mango plays an important part in the diet and cuisine of many diverse cultures. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. Most studies on the exploitation of mango have been dealing with mango peels, juices and stem bark, however a little attention has been given to mango leaves. In this study the active components of leaves of *Mangifera indica* were extracted and were tested for its anticancer activity. The result showed significant anticancer activity of leaves extract on HeLa cell lines.

KEYWORDS : Mango, Mangifera indica leaves extract, anticancer activity and HeLa Cells.

INTRODUCTION

Cancer is a disease that knows no geographic boundaries. In virtually every country of the world it is a major growth health problem and is a primary cause of mortality and morbidity in the world. Cancer cells are characterized by unregulated growth as well as unsufficient and unappropriate vascular supply. Biological active components from plants are significant and important source of new drugs that are likely lead to new and better treatments for cancer. Phenolic and flavonoid contents provide antioxidant activities that may underlie the anticancer potential [1].

Mangifera indica also known as mango, it has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years. Mangoes belong to genus Mangifera which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae [2]. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter [3].

The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world [4]. At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly form plants [5]. It grows in the tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies [6]. Many phenolic compounds have been detected in mango peels [7], mango bark [8], mango puree concentrate [9], mango pulps and seed kernels [10]. Several pharmacological activities of mango extracts have been reported including anti-inflammatory [6], antioxidant [11], antiallergic and antihelmintic [12] and antiamoebic [13].

It has been well documented that mango fruits are an important source of micronutrients, vitamins and other phytochemicals.

Moreover, mango

fruits provide energy, dietary fibre, carbohydrates, proteins, fats and phenolic compounds [14], which are vital to normal human growth, development and health [15]. There were no reports on the ability of Mangifera indica leaves on anticancer activity. The present investigation aims to study the analysis of anticancer effect of Mangifera indica against HeLa cell lines (cervical cancer cell lines).

ANTICANCER STUDIES ALREADY REVEALED ON MANGO (MANGIFERA INDICA)

Antiproliferative effect was preceded by accumulation of cells in G2/M phase of cell cycles with 90% methanolic extract of mango leaves. The the leaves extract of mangifera indica on different concentrations range (62.5-500µg/ml) showed anticancer activity .The leaf extracts inhibit cancer cell proliferation in vitro mainly by accumulating cells in G2/M phase[16]. The potential anticancer effects of the ethanolic kernel extract on breast cancer cells were evaluated using MTT, anti-proliferation, neutral red (NR) uptake and lactate dehydrogenase (LDH) release assaysshowed that the extract is significantly cytotoxic to these cell lines in a dose-dependent manner, and considerably less towards normal breast cells MCF-10A [17]. The cells treated with different concentrations of ethanolic extract of the M. indica kernel(10-1000 µg/mL) .M. indica extract appears to be more cytoxic to both estrogen positive and negative breast cancer cell lines than to normal breast cells. The extract of M. indica, therefore, has potential anticancer activity against breast cancer cells. Antiproliferative activities of mango peel might be due to the synergistic actions of bioactive compounds present in them [18].

MATERIALS AND METHODS

COLLECTION OF LEAVES OF MANGIFERA INDICA

The leaves of Mangifera indica (Anacardiaceae) were selected on the basis of ethnopharmacological and ethnobotanical literature survey. The plant materials were collected from the tropical region of Jawadhu Hills, Tiruvannamalai district (12°36'10''N, 78°53'07''E, and altitude 705 m), Tamil Nadu, India. The taxonomic identification was made through Mrs. S. Isabella Rosaline, Associate Professor, Department of Botany, Auxilium College, Katpadi, Vellore District and Tamil Nadu. The voucher specimen was numbered and kept in our research laboratory for further reference.

PREPARATION OF PLANT EXTRACTS

The leaves of Mangifera indica were air-dried for 7-15 days in the shade at the environmental temperatures (27-37°C day time) and the dried leaves were powdered mechanically using commercial electrical stainless steel blender. Dry powder (250 g) was macerated in 1 litre of deionized water then kept for 24 h at room temperature. The resulting aqueous extract was filtered with Whatman filter paper no.1. The filtrate was concentrated in a drying-room at 40°C for 24 h. The extract was stored at -20°C [19].

ANTICANCER ACTIVITY

MEDIA PREPARATION (SIGMA)

1. Co2 incubator- Thermo Fisher, USA
2. Multimode microplate reader- BioTek, USA
3. Refrigerated centrifuge- Eppendorf Germany
4. Cell: HeLa - NCCS Pune
5. MTT,(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))
6. Fetal bovine serum
7. Trypsin
8. Penicillin
9. RPMI1640 medium
10. DMSO

The sachet (12.0g) was dissolved in 800 ml of sterile distilled water to which 2.5g of sodium bicarbonate was added. The beaker was covered with aluminum foil and stirred using magnetic stirrer for 10 minutes. The medium pH was adjusted to 7.2 using 0.1M NaOH. The volume of the medium was made to 1000 ml and filtered through sterile 0.2µ membrane filter unit. The medium quality control was checked by incubating 5 ml of filtered medium in the CO2 incubator for 2 days. The antibiotics and serum was added before it was used for cell culture.

CELL CULTURE AND MTT ASSAY PROCEDURE

The HeLa human cancer cell line was purchased from NCCS Pune. The cells were grown in a RPMI1640 medium supplemented with 10% fetal bovine serum and antibiotics as mentioned earlier[20], [21]. Cell proliferation (MTT) assay was performed following the method described by and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones[22].

For the MTT assay, the cells were grown in 25 cm ×25 cm ×25 cm tissue culture flasks containing RPMI1640 medium as culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2. Cells were regularly passaged and maintained before including for the experiment. When a cell density in a culture flask reached 70-80% confluence, they were trypsinized and seeded in 96-well plates at varying cell number according to the size and shape of the cells were seeded in the density of 3000 cells per well in 100 µL and incubated for 24 hours at CO2 incubator. (Biorad, 680).

Test items were prepared as 20 mg/ml stocks by adding DMSO. The working stock of 2X (2000, 200. 20, 2.0 and 0.2 µg) concentration to the cell in 100 µL volume and the final concentration range were: 1000, 100, 10, 1.0 and 0.1 µg/ml. The plates were further incubated for 48 in the CO2 incubator. MTT solution was composed of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in phosphate buffered saline (1.5 mM KH2PO4, 6.5 mM Na2HPO4, 137 mM NaCl, 2.7 mM KCl; pH 7.4), from this solution 50 µl was pipette out into each well to achive 1 mg/mL as final concentration. The plate was further incubated for 2.30 hours in incubator and the medium was carefully decanted. The formazan crystals were air dried in dark place and dissolved in 100 µL DMSO and the plates were mildly shaked at room temperature and the OD was measured using Synergy HT microplate reader at 570nm[23].

From the optical densities the percentage growths were calculated using the following formula:

Percentage growth= 100× [(T-T0)/(C-T0)]
If T is greater than or equal to T0, and if T is less than T0,
Percentage growth = 100× [(T-T0)/T0)],
Where T is optical density of test,
C is the optical density of control,
T0 is the optical density at time zero.
From the percentage growths a dose response curve was generated and GI50 values were interpolated from the growth curves.

CELL IMAGING

The end of 48 hour's time point the images were captured before adding the MTT. Different concentration treated cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

RESULTS

CELL GROWTH INHIBITION PROPERTY

The leaves of *Mangifera indica* exhibited good growth inhibition in tested HeLA human cell line with 52.9 µg as GI50. The *Mangifera indica* leaves extract of each concentration was performed in quadruplicate and cumulative variation were maintained less than 20% between the data points. Three set of cell lines were tested in a 96 well plate as described in the below 96 well format.

TABLES AND FIGURES

Table 1: Percentage growth of HeLa cells against the *Mangifera indica* leaves extracts (SMIT)

Compound			Percentage Growth			Growth Inhibition in µg		
Mangifera indica leaves extract(SMIT)	1000 µg	100 µg	10 µg	1.0 µg	0.1 µg	GI50	TGI	LC50
	31	103	101	103	90	52.9	1000.0	1000.0

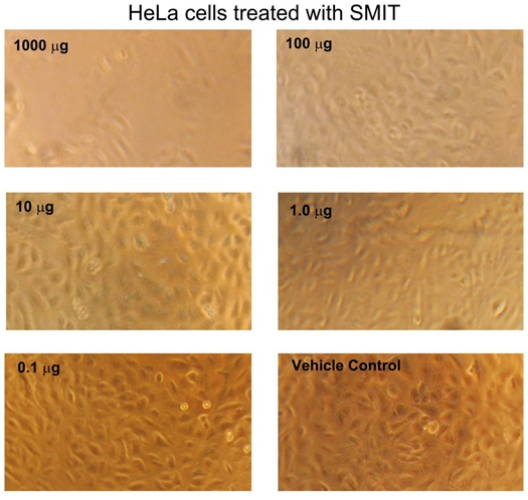

Figure 1: HeLa cells treated with *Mangifera indica* leaves extracts (SMIT) for 48 hours

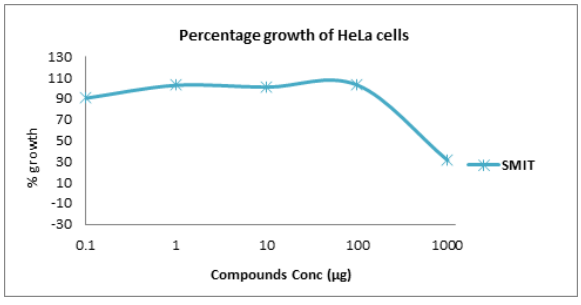

Figure 2: Percentage growth curve of HeLa cells against the *Mangifera indica* (SMIT) leaves extracts

CONCLUSION

To treat various diseases, different types of herbs are used as a remedy. Traditionally, large number of plant materials is used as herbal drugs without knowing their pharmacological efficacy. Great attention has been paid to phytochemicals present in different fractions of mango that can exert their beneficial potential counteracting either the action of pro-inflammatory molecules or

reactive oxygen species associated to human pathologies such as cancer, cardiovascular diseases, aging and neurodegenerative diseases. The result obtained from present study reveal that the leaves extract of Mangifera indica exhibit significant potential anticancer activity due to the increased flavonoids and terpenoids level. The investigation validate used of Mangifera indica as herbal drug for anticancer activity.

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