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Preliminary Phytochemical Investigation of Mangifera indica leaves and screening of Antioxidant and Anticancer activity

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ABSTRACT

Mango (Mangifera indica) is one of the most important tropical plants. 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 using methanol and were tested for its antioxidant and anticancer activity. The result showed significant antioxidant activity of methanolic extract. And it also showed high cytotoxicity on adenocarcinoma cell lines. In conclusion, some natural products from Mangifera indica leave have potential for use as therapeutic for disease such as cancer.

Keywords: Mangifera indica, Phytochemicals, Antioxidant, Anticancer, Adeno carcinoma cells.

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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 of mortality and morbidity in the world. Cancerlls are characterized by unregulated growth as well as unsufficient and unappropriate vascular supply. Biological active components from plants are significant and important sourse of new drugs that are likely lead to new and better treatments for cancer. Phenolic and flavavonoid contents provide antioxidant activities that may underlie the anticancer potential [5]. Traditionally Mangifera indica has medicinal application. Many phenolic compounds have beentected in mango peelsl , mango barks, mango puree concentrate, mango leaves, mango pulps and seed kernels. Several pharmacological activities of mango leaves extract have been reported including anti-inflammatory, anti-oxidant, anti-allergic and anthelmintic [8]. There were no reports on the ability of Mangifera indica leaves on anticancer activity. The present investigation aims to study the analysis of phytochemicals and antioxidant and anticancer effect of Mangifera indica against AGS cell lines.

MATERIALS AND METHODS

Plant materials and preparation

Mango leaves were collected from Kannur district, Kerala, India. Mangifera indica leaves were used in the form of crude 90% methanolic extract and this extract was prepared according to the traditional system of medicine. One kilogram of the shade dried and coarsely powdered plant was extracted with 90% methanol in cold for 72 hours. The erxtract was filtered and distilled on water bath, the syrupy mass obtained was dried at low temperature under reduced pressure in a rotator evaporator and a crude residue was obtained [1].

Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analysis to different constituents such as alkaloids, flavonoids, saponins, phenols, steroid, glycosides, resins, tannins, thiols and terpenoids [3].

Antioxidant activity

The antioxidant activity of the leaf plant extracts was evaluated by using the 1,1diphenylpicrylhydrazyl (DPPH) assay, FRAP assay, Superoxide dismutase assay.

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH)

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To 1ml of various concentration of test compound, 1.0ml of o.5mM DPPH was added. The test tubes were incubated at 37^0C for 30 minutes. The absorbance was read at 517nm. It was calculated as

Ferric Reducing Ability of plant (FRAP) Assay [7]

The FRAP reagent was prepared by adding 200ml of acetate buffer,20ml TPTZ; 20ml $FeCl_3$; and 24ml of distilled water. To 1ml of various concentrations of test compound added 1ml of FRAP reagent, mixed the contents thoroughly and incubated at 73^0C for 5 minutes. Read the absorbance at 593nm.

Superoxide dismutase activity

The reaction mixture contained 50Mm phosphate buffer (Ph 7.6), 20mg riboflavin, 12mM EDTA, NBT 0.1mg/3ml, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 15minutes. Immediately after illumination, the absorbance was measured at 590nm.

Anticancer activity

MTT (Methyl trizolyl tetrazolium) reduction assay

Cell viability was measured with blue formazan that was metabolized from MTT by mitochondrial dehydrogenase, which is active only in live cells. AGS cells were seeded in 96-well plate at a density of 1.0×10^5 cells per cells, cultured overnight and pretreated with various concentrations of GBA. After incubation for 24h, the MTT(5mg/ml) colorimetric viability of cells. The absorbency of each well was measured at 540nm using an ELISA reader, and the percentage viability was calculated [6].

RESULTS AND DISCUSSION

Phytochemical analysis

Prliminary phytochemical analysis revealed that the plant possessedthe phytoconstituents flavonoids, terpenoids, saponins, tannins and glycosides.

Table 1: Phytochemical studies

TESTS	OBSERVATION
Alkaloids	
1.Drogendroff's test	-
2. Wagner's test	-
3.mayer's test	-
Flavanoids	+
Saponins	+

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Carbohydrates	
1. Fehlings test	+
2. Benedicts test	+
3. Mollischs test	+
Proteins	
1. Millions test	+
Phenols	
1. Ferric chloride test	+
2. Lead acetate test	+
3. Liebermanns test	+
Steroid	
1. Libermanns-Burchards test	+
2. Salkowsi reaction	+
Glycosides	+
Resins	-
Tannins	
1. Ferric chloride test	+
2. Lead acetate test	+
Thiols	-
Terpenoids	+

(+)……. Positive **(-)..... Negative**

Antioxidant activity

Table 2: DPPH Activity

S.NO	Concentration of extract (µg)	% of DPPH scavenged
1	20	41.8
2	40	42.5
3	60	44.0
4	80	45.0
5	100	49.0

$$\text{Rate of inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The table shows increased concentrations of extract possess increased scavenging activity. The radical scavenging activity and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert into Diphenyl picryl hydrazine. The degree of decolourization from purple to yellow colour was measured spectrometrically at 517nm. The methanolic extract of Mangifera indica leaves contains high antioxidant activity.

Table 3: FRAP Assay

Sl.no	Concentration of standard (µg)	Absorbance at 593nm
1.	20	0.17

2.	40	0.34
3.	60	0.51
4.	80	0.68
5.	100	0.85

The absorbance test is 0.75. From the table we get the concentration 95µg/ml. So the methanolic extract of Mangifera indica leaves revealed the ferric reducing concentration of 95µg/ml. So the methanolic extract of Mangifera indica leaves shows high ferric reducing power.The results showed that the methanolic extract of Mangifera indica leaves possess ferric reducing antioxidant power.

Superoxide Dismutase Activity

The enzymatic antioxidant activity was determined by Superoxide dismutase and the following result was obtained.

Table 4: Estimation of Superoxide dismutase

Sl.no	Concentration of extract in µg	Inhibition rate % of superoxide dismutase
1.	20	18.2
2.	40	21.6
3.	60	27.7
4.	80	46.2
5.	100	66.0

$$\text{Rate of inhibition(\%)} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Superoxide radical is a highly toxic species,which is generated by numerous biological and photochemical actions. The methanolic extract of Mangifera indica leaves showed 66% of superoxide dismutase antioxidant activity. In the present study showed that the methanolic extract of Mangifera indica leaves possess high enzymatic antioxidant activity.

Table 5: Anticancer activity

Concentration (µg)	% Cell Inhibition
31.25	0.63091
62.5	7.8233
125	31.230
250	73.690
500	99.747

The % cell inhibition was determined using the following formula:
% cell Inhibition = 100- Abs (sample)/Abs (control) x100.
Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using Graph Pad Prism software.

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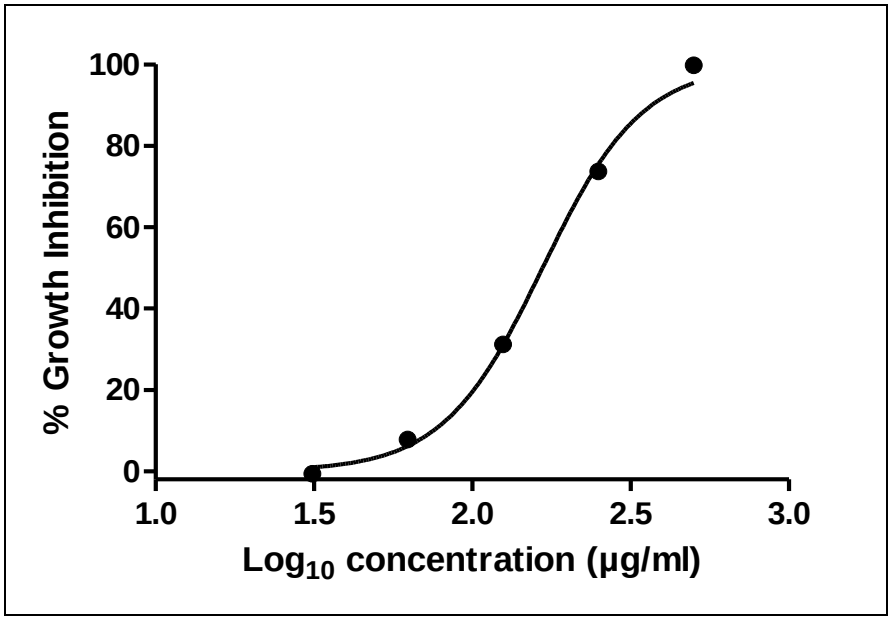
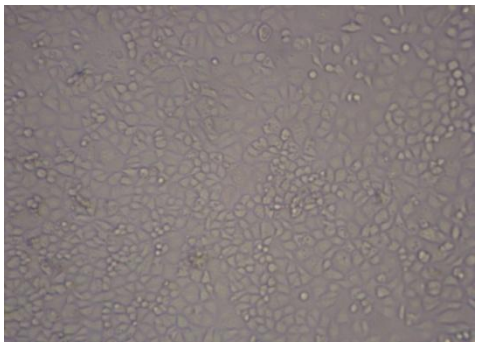
50% of inhibition concentration =**166.9µg/ml**



The antiproliferative effect was preceded by accumulation of cells in G2/M phase of cell cycles. The leaf extracts will be inhibiting AGS cancer cell proliferation in vitro mainly by accumulating cells in G2/M phase.

Eleven human colorectal AGS cell lines established in the lab were classified into three groups based on morphological features (Light & Electron microscopy). Modal chromosome number and ability to synthesize carcino embryonic cell lines G1 cell lines contains both dedifferentiated and differentiated cells growing in tight clusters. G2 cell lines were more dedifferentiated were hyper diploid.G3 cell line were morphologically similar to those of G1. Thus the results showed that the methanolic extract of Mangifera indica leaves possess antitumor activity.



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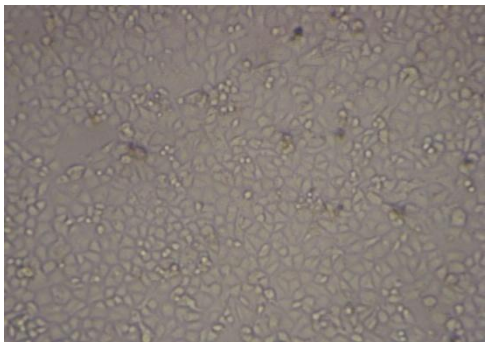

31.25µg/ml

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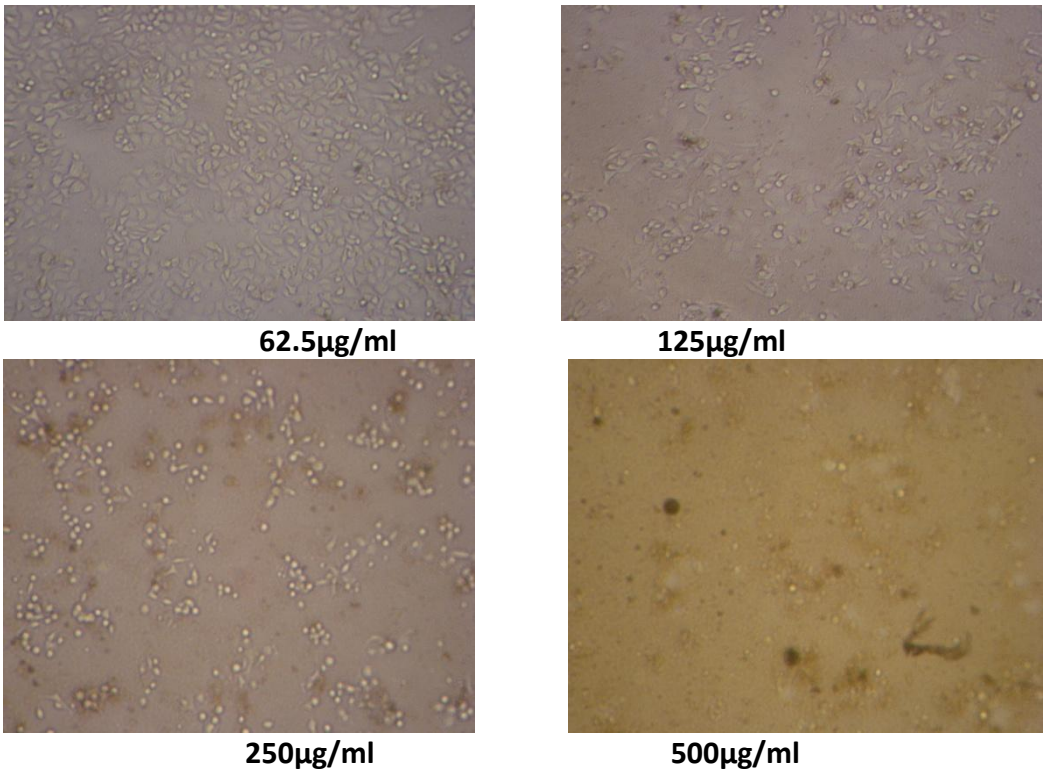

Fig 1: anticancer activity of Mangifera indica leaves extract

CONCLUSION

The result obtained from present study reveal that 90% methanolic leaf extract of Mangifera indica exhibit significant antioxidant and anticancer activity due to the increased flavonoids and terpenoids level. The phytochemical analysisl of leaf extract indicate the constituents present is responsible for pharmacological effects. The investigation validate used of Mangifera indica as herbal drug for anticancer and antioxidant activity.

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