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PHYTOCHEMISTRY AND ANTICANCER ACTIVITY OF MADHUCA LONGIFOLIA LEAVES

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ABSTRACT

Cancer is a broad term. It describes the disease that results when cellular changes cause uncontrolled growth and division of cell treatment of cancer with synthetic drugs is associated with various side effects. Due to this reason researchers are manufacturing of naturally derived anticancer agents from herbal plants. The last is the subject of this analysis and ranges a wide scope of exercises. The hereditary heterogeneity and intricacy of cutting edge malignant growths unequivocally bolster the method of reasoning for early interference of the cancer-causing process and an improved spotlight on counteractive action as a need system to diminish the weight of malignant growth; be that as it may, the focal point of malignant growth avoidance the executives ought to be on people at high hazard and on essential limited sickness in which screening and recognition ought to likewise assume an imperative job. The planning and portion of (chemo) preventive mediation additionally influences reaction. The mediation might be inadequate if the objective populace is extremely high hazard or previously giving preneoplastic sores with cell changes that can't be switched. This study consists of phytochemistry and anticancer activity of madhuca Longifolia leaves. The result indicates that, it consists of flavanoids (Quercetin) in acetone extract.

KEYWORDS: Madhuca Longifolia, flavanoids, quercetin, acetone, anticancer activity.

INTRODUCTION

Cancer is an immense and developing challenge, with the number of individuals around the globe who get an analysis every year expected to rise drastically, from 15 million out of 2015

to 24 million out of 2035.[1] Socio-economic factors are successively determined cancer burden of India's, as poor people are bound to pass on from melanoma earlier the time life of 70 years in comparison to prosperous people. However, estimated 600000–700000 passings in India were brought about by cancer in 2012. This partly shows late stage location and poor treatment results and other avoidable causes such as tobacco use, infections etc.[2] Ways to deal with lessen the worldwide weight of malignancy incorporate two significant systems: screening and early identification and dynamic preventive mediation. The last is the subject of this analysis and ranges a wide scope of exercises. The hereditary heterogeneity and intricacy of cutting edge malignant growths unequivocally bolster the method of reasoning for early interference of the cancer-causing process and an improved spotlight on counteractive action as a need system to diminish the weight of malignant growth; be that as it may, the focal point of malignant growth avoidance the executives ought to be on people at high hazard and on essential limited sickness in which screening and recognition ought to likewise assume an imperative job. The planning and portion of (chemo) preventive mediation additionally influences reaction. The mediation might be inadequate if the objective populace is extremely high hazard or previously giving preneoplastic sores with cell changes that can't be switched. The field needs to move past general ideas of carcinogenesis to focused organ site aversion approaches in patients at high hazard, as is as of now being accomplished for bosom and colorectal malignant growths. Setting up the advantage of new malignant growth preventive mediations will take years and potentially decades, contingent upon the result being assessed.[3] The malignant transformation is a multistep procedure related with the accumulation of numerous molecular alterations. These molecular changes impact cellular function within the tumor and its microenvironment, and culminate in the hallmarks of cancer: sustained proliferative signaling, resistance to apoptosis, senescence, angiogenesis, invasion and metastasis, deregulating cellular energetics, avoiding immune destruction, tumor-promoting inflammation, and genome insecurity and transformation.[4] Proliferation is an important part of cancer development and progression. This is manifest by altered expression and/or activity of cell cycle related proteins. Constitutive activation of many signal transduction pathways also stimulates cell growth. Early strides in tumor improvement are related with a fibrogenic reaction and the advancement of a hypoxic situation which supports the endurance and expansion of malignancy undeveloped cells. Some portion of the endurance procedure of malignant growth undifferentiated organisms may manifested by adjustments in cell digestion. When tumors show up, development and metastasis might be bolstered by overproduction of fitting hormones (in hormonally

subordinate malignant growths), by advancing angiogenesis, by experiencing epithelial to mesenchymal change, by activating autophagy, and by submitting general direction to encompassing stromal cells genome shakiness and transformation.[5]

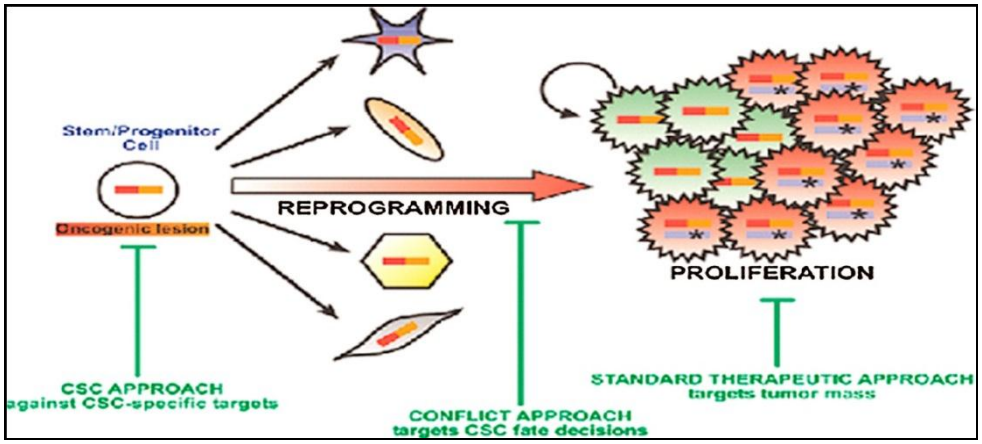

Figure no 1: Proliferation of cell in Cancer.

MATERIALS AND METHODS

The leaves sample of M. longifolia were washed in water to remove the dirt and was air dried at room temperature (27 ± 2°C) for 30 days. The samples were powdered using mortar and pestle. The fine powdered leaves sample was macerated in distilled water (dH2O) in the ratio of 1:2 (w/v). They were soaked for 24 h and were filtrated by Whatman filter paper number 1.The filtrate was dried on the water bath to give the yield of 10% (w/w) of the aqueous leaves extract which was later used for the study.[6]

Phytochemical analysis

Qualitative Phytochemical Analysis

Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

a. Test for Flavonoids

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour was observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicated the presence of flavonoids in the sample extract.[7]

b. Test for Alkaloids

To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicated the presence of alkaloids.[8]

c. Test for Saponins

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicated the presence of saponins.[7]

d. Test for Tannins

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicated the presence of tannins.[9]

e. Test for Phenols

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicated the presence of phenols in the sample extract.[9]

f. Test for Proteins

To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicated the presence of peptide linkage molecules in the sample extract.[7]

g. Test for Cardiac Glycosides

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicated the presence of cardiac glycosides in the sample extract.[8]

h. Test for Terpenoids

1 ml of extract of each solvent was taken and added 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicated the presence of terpenoids in the extract.[10]

i. Test for Carbohydrates

1 ml of extract was taken, added few drops of Molisch's reagent and then 1 ml of concentrated sulphuric acid was add ed at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicated the presence of

carbohydrates in the sample extract.[10] The extract was filtered (preferably 10 mm pore size filter) into a suitable sized vessel. The leaves were extracted, two to three times and the extract were filtered into the same vessel. The combined filtrate was concentrated to a syrupy consistency and dried under vacuum (between 400 and 600 mm of Hg) at a temperature not exceeding 80 0C till the moisture was below 5%. The mass was milled and the powder was sieved through 500 mm mesh to obtain the extract and packed. The yield obtained is about 25%.

TLC plate consists of 20×10 cm, precoated with silica gel 60 F254 TLC plates (E. Merck) of uniform thickness 0.2 mm with aluminium sheet support. The spotting device was CAMAG Linomat V Automatic Sample Spotter. The Syringe, 100 ml (from Hamilton), the developing chamber was a CAMAG glass twin trough chamber (20 10 cm). The Densitometer consists of a CAMAG TLC Scanner 3 linked to WINCATS software. Stationary phase: Silica Gel.60 F254, Mobile phase: Toluene:Ethyl acetate:Formic acid (5:4:1).[11]

Table no 1: Qualitative TLC analysis of *Madhuca Longifolia.*

Plate No.	Solvent system	Visualizing Reagent	Target phytoconstituents
1	SS-7	VR-1	Terpenoids, Phenylpropanoids, Steroids, Saponins, Bitter principles
2	SS-7	VR-6	Flavonoids, Phenolic compounds
3	SS-7	VR-3	Anthraquinones, Flavoinoids
4	SS-3	VR-5	Anthraquinones, Anthrone, Coumarins
5	SS-3b	VR-7	Terpenoids, Saponins, Phenylpropanoids

Anticancer activity of *Madhuca Longifolia Leaves*

Study Set up

Anticancer Activity of Madhuca Longifolia was performed to explore the protective effect of acetone extract of Madhuca longifolia leaves (AEML) against Ehrlich Ascites Carcinoma (EAC) in mice. The activity was assessed using mean survival time (MST), tumor volume, tumor weight, tumor cell count, body weight, haematological studies and In-vitro cytotoxicity. Results found that oral administration of AEML increased the mean survival time. tumor volume, tumor weight, body weight and tumor cell count were also significantly reduced. haematological parameters including protein and PCV, which were altered by tumor inoculation, were restored. The effects of AEML was comparable with standard drug 5-Flourourasil.[12]

Animal Grouping and Study

Swiss Albino mice (20-25gm) of either sex and of approximately the same age, were used for Anticancer study and Wistar albino rats (150-200 g) were used for acute toxicity studies. Animals were housed in polypropylene cages and fed with standard rodent pellet diet and water ad libitum. The animals are exposed to alternate cycle of 12 hrs of darkness and light each. Before each test, the animals are fasted for at least 12 hrs. The experimental protocols were subjected to the securitization of the Institutional Animals Ethical Committee and were cleared by the same. All experiments were performed during morning according to CPCSEA guidelines for care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The animals (Swiss albino mice weighing 20-25 g) were divided into 4 groups consisting of 12 animals. Animals were fed with basal diet and water throughout the experimental period. All the groups were injected with EAC cells except the group I. This was taken as day zero. From day 1st, normal saline (5 ml/kg) was given in group I, 5-fluorouracil (20mg/kg) and AEML (500 mg/kg) were given to group III and group IV respectively, for 14 consecutive days, whereas group II was serve as a tumor control group and normal saline (5 ml/kg) was given to this group also, on day 15th half of the mice from each group were sacrificed, 24h after last dose, for the determination of tumor volume, tumor weight, haematological parameters etc, and rest were kept with food and water ad libitum to check the increase in the life span of the tumor hosts and body weight.[12]

Effect of AEML on mean survival time

Animals were inoculated with 1×106 cells/mouse on day ‘0’ and treatment with AEML was started 24 h after inoculation, at a dose of 500 mg/kg/day, p.o. The control group was treated with the same volume of 0.9% sodium chloride solution. All the treatments were given for 14 days. The mean survival time (MST) of each group was noted. The anticancer efficacy of AEML was compared with that of 5- fluorouracil. The MST of the treated groups was compared with that of the control group using the following calculation (results are given in table 1):

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1]x100

Mean survival time = [1st Death + Last Death] / 2

Table no 2: Effect of AEML on mean survival time.

Sr no	Treatment	Mean Survival Time (Days)	Increase in life span (%)
1	Tumor Control	21.50 ± 2.73	-
2	5- FU (20mg/kg, i.p)	40.16 ± 2.13*	86.79 %
3	AEML (500 mg/kgp.o)	30.33± 4.7*	

n=6 animals in each group, *P<0.01 Vs control. Days of treatment = 14, Values are expressed as mean ± SEM

Effect of AEML on tumor volume and tumor weight

On 15th day, after 24h of dose, 6 mice from each group were dissected and the ascites fluid was collected from peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. The tumor weight was measured by taking the weight of mice before and after collection of ascites fluid from peritoneal cavity.

Effect of AEML on tumor cell count

To determine the effect of AEML on tumor cell count, the ascites fluid withdrawn from the peritoneal cavity of the mice was taken in WBC pipette and diluted 100 times with normal saline. A drop of a diluted cell suspension was placed on the neubauers chamber and the number of cells in the 64 square was counted. The viability and non viability of cells was checked by tryphan blue method. On staining viable cells did not take the dye whereas the non viable cells were stained blue.[13]

Table no 3: Effect of AEML on tumor cell count.

Sr no	Treatment	Tumor Volume(ml)	Tumor weight (gm)	Tumor cell count	
				Viable cells[1]	Nonviable cells[2]
1	Tumor Control	6.70 ± 0.16	6.87 ±0.21	9.83 ±0.3	0.33 ±0.21
2	5- FU (20mg/kg, i.p)	1.01 ± 0.10*	1.1 ±0.06*	0.83 ±0.3*	1.67 ±0.33**
3	AEML (500 mg/kg, p.o)	3.46 ± 0.07*	3.54 ±0.31*	3.66 ±0.21*	2.5 ±0.22*

n=6 animals in each group, *P<0.01 Vs control. Days of treatment = 14, Values are expressed as mean ± SEM, Viable

cells1x 107/ml, 2Nonviable cells2 x 107/ml

Effect of AEML on body weight

Body weights were recorded every 7th day till 40 days of treatment or till the death of the animal.

Effect of AEML on haematological parameters

On the 15th day, blood was drawn by retro orbital plexus method. WBC count, RBC count, haemoglobin, protein and packed cell volume were determined Cells smear was prepared in slide and stained with Lieshman stain solution. Red blood cells (RBC), White blood cells (WBC) and Heamoglobin (Hb) were estimated with the help of MS-09 heamatology analyzer (France).

Statistical analysis

All the values were expressed as mean + SEM (standard error of mean) for six rats.

Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparision Test. The value of probability less than 5% ($P < 0.05$) was considered statistically significant.[14]

RESULT AND DISCUSSION

It was revealed from the phytochemical studies that chemical constituents viz., Volatile oils, Glycosides and Saponins are absent in all the extracts. Tannins and Phenolic compounds, Flavonoids are significantly present in 70% Hydroalcoholic and Methanolic extract whereas the alkaloids, Triterpenoids, Sterols, Amino acids and Proteins presence was found negligible by the colour reactions. The phytochemical investigations of all the extracts is summarized in following table.

Table no 3: The phytochemical investigations of *Madhuca Longifolia.*

Sr. No	Chemical Constituents	Methonolic
1	Alkaloids	+++
2	Flavonoids	+++
3	Tannins	++
4	Terpenoids	+
5	Saponins	+++
6	Cardiac glycosides	+
7	Proteins	+
8	Carbohydrates	+
9	Phenols	++

Keywords: '-' absent., '+' presence, '++' more clarity, '+++' highly significant.

Anticancer Activity of Madhuca Longifolia was performed to explore the protective effect of acetone extract of Madhuca longifolia leaves (AEML) against Ehrlich Ascites Carcinoma

(EAC) in mice.The chemical Constituent responsilble for anticancer activity is quercetin. The result of antitumor activity are.

Table no 4: Antitumor activity of Madhuca Longifolia.

Treatment	Tumor Volume(ml)	Tumor weight (gm)	Tumor cell count	
			Viable cells[1]	Nonviable cells[2]
Tumor Control	6.70 ± 0.16	6.87 ±0.21	9.83 ±0.3	0.33 ±0.21
5- FU (20mg/kg, i.p)	1.01 ± 0.10*	1.1 ±0.06*	0.83 ±0.3*	1.67 ±0.33**
AEML (500 mg/kg, p.o)	3.46 ± 0.07*	3.54 ±0.31*	3.66 ±0.21*	2.5 ±0.22*

The results of the present study show a protective effect of AEML against EAC in Swiss albino mice. There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioural changes at a dose level of 5000 mg/kg. This finding suggests that the AEML was safe in or non-toxic to rats and hence doses of 500 mg/kg, po were selected for the study.

Table no 5: protective effect of AEML against EAC in Swiss albino mice.

Treatment/dose	7th Day	14th Day	21st Day	28st Day	35th Day
Normal	22.00±0.77	23.00±0.51	24.16±0.54	27.66±0.66	31.83±0.83
Tumor Control	27.83±0.79*	40.33±0.76*	50.16±0.65*	-	-
5-FU (20mg/kg, i.p)	23.33±0.61	24.50±0.34$	26.83±0.6 $	-	32.83±0.47
AEML(500 mg/kg, p.o)	24.33±0.33**	29.50±0.76#	30.5±0.88$,#	31.00±0.25#	35.83±0.30#

n= 6 in each group, * P< 0.001 Vs Normal control, $ P< 0.001 Vs Tumour control, # P<0.001 Vs Standard, ** P<0.01Vs

Tumour control, Values were expressed as mean± SEM.

Table no 6: protective effect of EAC in Swiss albino mice treated with AEML.

Parameter	Normal	Tumor control	5 FU (20 mg/kg)	AEML	Parameter
Hb(g/dl)	14.3±0.10	8.35±0.09*	14.0±0.05*,$	12.4±0.4*,$	Hb(g/dl)
RBC (million/mm3)	4.68±0.06	2.6±0.07*	4.11±0.04*,$	3.18±0.3*,$	RBC (million/mm3)
WBC(million/mm3)	7.48±0.03	27.19±0.07*	8.23±0.02*,$	9.6±0.7*,$	WBC(million/mm3)
Proteing %	8.21±0.06	13.95±0.2*	8.65±0.04*,$	9.2±0.1*,$	Proteing %
PCV (mm)	16.5±0.42	31.5±0.42*	19.5±0.42*,$	26.2±0.1*,$	PCV (mm)
Neutrophils %	30.83±0.60	68.83±0.60*	31.83±0.47*,$	38.1±2.2*,$	Neutrophils %
Lymphocytes %	68.5±0.42	30±0.57*	64.66±0.42*,$	50.3±2.1*,$	Lymphocytes %
Monocytes %	1.16±0.16	2.16±0.16#	1.33±0.21 ^{ns}	1.8±0.3^{ns}	Monocytes %

n= 6 in each group, * P< 0.001 Vs Normal control, $ P< 0.001 Vs Tumour control, # P<0.05Vs Normal Control, ns – not

significant, Values are expressed as Mean ±SEM.

A significant enhancement of MST was observed. The MST for the control group was 21.50±2.73 days, whereas it was 30.33± 4.7 and 40.16 ± 2.13 days for the groups treated with AEML (500 mg/kg/day, p.o.) and 5-FU (20 mg/kg/day, i.p.) respectively. The % increase se in the lifespan of tumour-bearing mice treated with AEML and 5-FU was found to be 41.06 and 86.79% respectively (P< 0.01) as compared to the control group. The result is showed in table .There was reduction in the tumour volume, tumour weight and tumour cell count of mice treated with AEML (P<0.001). Tumour volume of control animals was 6.70 ± 0.16, whereas for the extract-treated group it was 3.46 ± 0.07 for AEML. Tumour weight of control animals was 6.87 ±0.21 g, whereas for the extract-treated group it was 3.54±0.31g. There was a significant decrease in the weight gain by AEML treated mice when compare with tumour control as showed in table. The analysis of the haematological parameters showed minimum toxic effect in mice treated with AEML. After 14 days of transplantation, AEML were able to reverse the changes in the haematological parameters consequent to tumour inoculation. The total WBC count, proteins and PCV were found to increase with a reduction in the haemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased (P<0.001) while that of lymphocytes decreased(P<0.001). At the same time interval, AEML (500 mg/kg/day, p.o.) treatment could change these altered parameters to near normal.

^{1}HNMR Spectrum of Quercetin

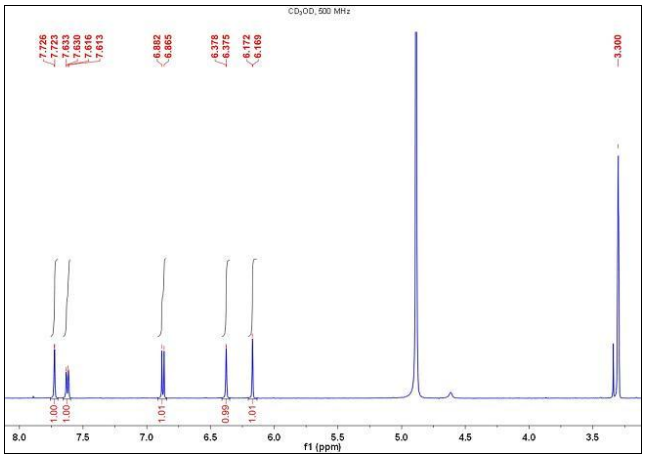

Figure no 2: ^{1}HNMR Spectrum of Quercetin.

CONCLUSION

Madhuca Longifolia have many pharmacological activities in different types of diseases and disorders. These different medicinal properties are due to the presence of different types of phytoconstitutents which are described in results. Madhuca Longifolia rich in alkaloids,

flavanoids, saponins. Aprt from these flavanoids like quercetin is abundantly present. Due to this, madhuca Longifolia is having Anticancer activity.

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