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In vitro antioxidant and cytotoxic activity of ethanol bark extract of *Madhuca longifolia* on MCF-7 and Vero cell lines

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Abstract
Antioxidant and cytotoxic activity of ethanol bark extract was evaluated in the present study. Breast cancer cell line MCF-7 and normal Vero cell line were exposed to various concentrations of extracts ranging from 7.8 µg/ml to 1000 µg/ml. The cytotoxicity study was carried out with MTT assay and free radical scavenging activity was evaluated using DPPH assay with ascorbic acid as standard. The results obtained in the present study reveals a potential cytotoxic activity especially against the MCF-7 cells where the IC_{50} amounts to 32.4µg/ml. However, the normal VERO cell lines do not show much variation where as 69%of cell viability was reported with the 1000µg/ml dosage. The results were in correlation with the presence of antioxidant activity of the extract, where 94.45% scavenging of free radicals was observed with the presence of flavonoids, polyphenols and anthocyanins in the extracts. To conclude the ethanol bark extract imparts a target specific cytotoxicity with reference to MCF-7 cell lines and there is positive correlation was found to exist between the presence of antioxidant compounds and cytotoxicity against cancer cells.

Keywords: Ethanol bark, *Madhuca longifolia*

Introduction
Plants are the chief source of natural products that are used in the field of pharmaceutical industries. Vast numbers of novel bioactive compound were isolated from the plant sources [1]. WHO stated that around 80% of the people living in developing countries depend on traditional medicine for their primary health care needs [2]. The plant derived bioactive compounds were more specific on their target [3]. Research on medicinal plants gain momentum around the world due to its potential therapeutic efficacy as well as their non-toxic nature to the normal cells [4]. Plant derived compounds therapeutic value depends on the presence or absence of various secondary metabolites that exist in the extracts. More than 40% of the drugs in the modern medical system were derived from natural products [5], which are considered safe when compared with synthetic derivatives [6].

Cancer is of more than 100 different diseases which can develop almost anywhere in the body, where the abnormal cells divide without control due to various genetic changes which interfere with the orderly process of generation and death of the cells in the body. In the year 2016, about 14 lakh cases of cancer were recorded, and this is going to be one of the leading causes of death among adults in India. Breast cancer a malignant tumour causes significant morbidity and mortality among women [7]. Indian Council of Medical Research (ICMR) has projected that India might report 17 lakh new cases of cancer and over 8 lakh death because of this disease by 2020. The breast cancer cell lines MCF-7, T-47D and MDA.MB231 were studied to analyse the anticancer activity *in vivo*. MCF-7 is a widely studied epithelial cancer cell line derived from adenocarcinoma with the characteristics of differentiated mammary epithelium.

Oxidative stress is one of the major risk factors for the onset of chronic diseases. Antioxidant compounds will inhibit the oxidative mechanism by which it protects the tissues from degenerative diseases [8]. An antioxidant is substance that delays or inhibits oxidative damage to a target molecule [9]. The toxicity and side effects resulting due to the continuous usage of conventional pharmacological agents necessitated the search for the non-toxic, eco-friendly compounds without any side effects. Around 70% of anticancer compounds were either natural products or natural product derived compounds [10]. Antioxidant potential of the bioactive compounds of the plant material is closely linked to their ability to suppress the growth of cancer cells through reduced oxidative stress. Antioxidant supplementation may reduce breast cancer recurrence and mortalities [11].

Madhuca longifolia commonly known as Iluppai in Tamil, is highly regarded as an universal panacea in ayurvedic medicine. The bark is recommended for phlegm and in rheumatism. The bark is used to treat itches, swelling, fractures and snake bite poisoning [12]. The stem bark is used to treat chronic tonsillitis, leprosy and fever [13]. The aim of the present study was to evaluate the antioxidant activity and anticancer potential of ethanol bark extract of *Madhuca longifolia* against MCF-7 breast cancer cell lines and VERO cell lines *in vivo*.

Materials and Methods

Chemical and Reagents

DMEM (Dulbecco's Modified Eagle Medium), FBS (Fetal Bovine Serum), 2, 2 Diphenyl-1-picryl hydrazyl and Nitro Blue Tetrazolium Chloride (NBT) and Ascorbic acid were purchased from Hi-Media laboratories, Mumbai. Trypsin, Methyl Thiazolyl Diphenyl Tetrazolium Bromide (MTT) and Dimethyl Sulfoxide (DMSO) were purchased from SISCO Laboratory Chemicals, Mumbai. All the other reagents used were of analytical grade.

Plant Collection

Madhuca longifolia belongs to the family *Sapotaceae* called as illuppai in Tamil are collected from Medicinal plant garden, Presidency College (Autonomous) Chennai, India and was identified taxonomically and authenticated at the PG and Research Department of Plant Biology and Biotechnology, Presidency College, Chennai, India. The bark material was cleaned and air dried at room temperature for a period of fifteen days. The bark was grinded into coarse powder using mixer blender.

The *Madhuca longifolia* bark powder was subjected to extraction using Soxhlet apparatus with ethanol as solvent. The solvent extracts were collected and concentrated by distilling the solvent using vacuum rotary evaporator [14]. The concentrated extracts were refrigerated and used to test the Antioxidant and Cytotoxic activity.

Phytochemical analysis

Qualitative analysis of the presence or absence of secondary metabolite compounds were analysed using standard protocols [15].

MTT Assay

Cell lines

MCF-7 and VERO cell lines were obtained from NCCS, Pune, India. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose supplemented with 10%FBS and Antibiotics at 37˚C in a humidified of 5% CO_2 incubator. The bark extracts of *Madhuca longifolia* was tested for *in vitro* cytotoxicity using Vero and MCF-7 cells by 3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Cells (1×10^5/well) were placed in 96 well plate and incubated at 37˚C with 5% CO_2 condition. Once the cells attained confluence the different concentration of samples was added and incubated for 24 hrs each sample was replicated 3times. After the process of incubation along with the sample 100µl/well (5mg/ml of 0.5 % of MTT was added and incubated for 4 hours. Until purple precipitate were clearly visible under a microscope. 100µl of DMSO was added and the plates were shaken for 5minutes. The absorbance of each well was measured at 540nm in a microtiter plate reader using DMSO as blank.

The percentage of cell viability was calculated using the formula,

$$\text{Percentage of cell viability} = \frac{\text{Absorbance of extract treated wells}}{\text{Absorbanc of Untreated wells}} \text{ X } 100$$

Graphs were plotted using the % of cell viability to calculate the IC_{50} concentrates that kills 50% of MCF7 and Vero cell lines.

Antioxidant Activity

DPPH assay (2,2-diphenyl-1-picrylhydrazyl).

The radical scavenging activity of different extract was absorbed using DPPH assay according to [16] with slight modifications. Forty µl of different concentration of extracts in DSMO was mixed with 2.96 ml of 0.1mM DPPH in ethanol. The mixture was mixed thoroughly and incubated in the dark for 30 minutes. Absorbance of the resulting solute was measured at 517nm (Thermo-UV-10-UV-Visible Spectrophotometer). All results were carried out in triplicate using ascorbic acid as reference.

The percentage of DPPH radical scavenging activity was calculated using the equation.

$$\text{DPPH Scavenging Activity} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \text{ X } 100$$

Absorbance of 0.1mM DPPH in ethanol was treated as control.

Results

The free radical scavenging activity of ethanol bark extract of *Madhuca longifolia* was observed using DPPH assay. The bark extract shows a significant inhibitory activity against the DPPH radicals at an absorption maximum on 517nm. DPPH assay is considered to be an appropriate method to evaluate the free radical scavenging activity because any substance that scavenges DPPH can decrease the absorbance at 517nm [17]. Ethanol extract shows potential scavenging activity of 81.704% to 94.33% with reference to the concentration ranging from 20 µg/ml to 200 µg/ml.

Cytotoxic Activity

Cytotoxic activity of ethanol bark extract of *Madhuca longifolia* was tested against MCF-7 breast cancer cell lines and normal Vero cell lines through MTT assay. A dose dependent cytotoxicity was observed with reference to MCF-7 cell lines. Ethanol extract was tested with the varying concentration of 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml concentration and recorded the cytotoxicity of 37.04, 44.45, 50.00, 55.56, 64.82, 77.78, 87.04 and 94.45 % respectively. The extract treated cells correspondingly results in the inhibition concentration value (IC_{50}) of 31.2 µg/ml. The cytotoxicity effect of the extract is due to its potential antioxidant activity where it shows 94.83% of free radical scavenging activity with reference to 200µg/ml concentration. *Madhuca longifolia* bark extract shows minimal cytotoxicity against the positive control Vero cell lines, whereas the cytotoxicity amounts to only 30.72% with reference to 1mg/ml concentration, which is very meagre when compared to cytotoxicity against MCF-7 cell lines where the percentage of cytotoxicity amounts to 94.45 with reference to 1mg/ml concentration.

Table 1: Antioxidant activity of ethanol extract of *Madhuca longifolia*

Extract	Concentration									
	20	**40**	**60**	**80**	**100**	**120**	**140**	**160**	**180**	**200**
Ethanol	81.704 ± 1.1074	87.744 ± 0.296	90.682 ± 0.224	91.552 ± 0.089	92.196 ± 0.249	92.336 ± 0.102	92.676 ± 0.102	92.798 ± 0.115	93.318 ± 0.246	94.332 ± 0.209

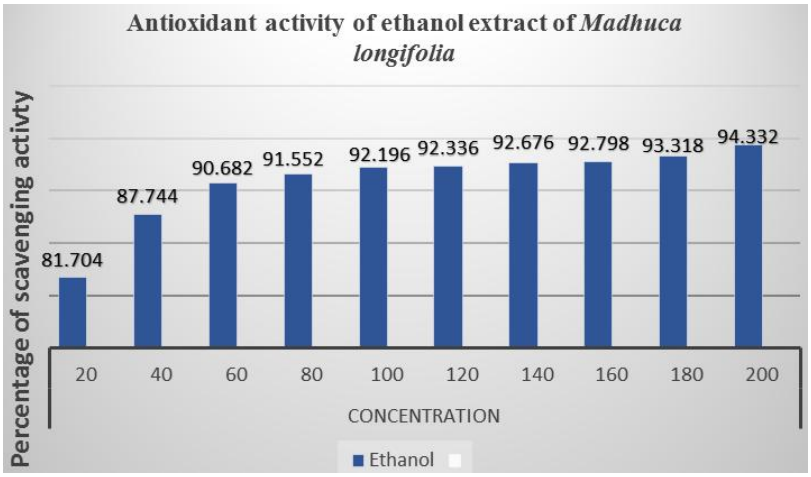

Fig 1: Free radical scavenging activity of ethanol bark extract of *Madhuca longifolia*

Table 2: Cytotoxic activity of *Madhuca longifolia* ethanol extract on MCF -7 cell lines

S. No	Concentration (µg/ml)	Cell Viability (%)	Cell Death (%)
1	1000	5.55	94.45
2	500	12.96	87.04
3	250	22.22	77.78
4	125	35.18	64.82
5	62.5	44.44	55.56
6	31.2	50.00	50.00
7	15.6	55.55	44.45
8	7.8	62.96	37.04
9	Cell control	100	00.00

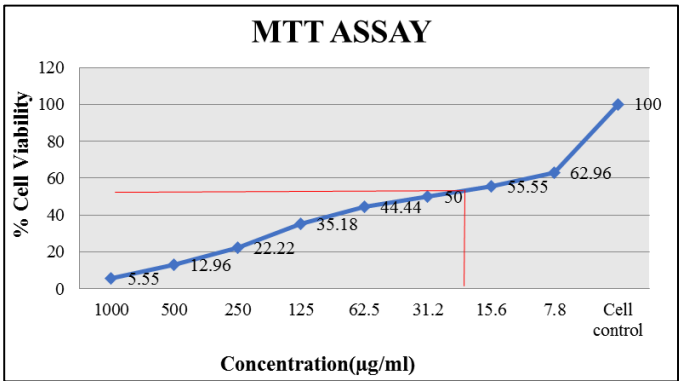

Fig 2: Cytotoxic activity of Bark extract of *Madhuca longifolia* on MCF 7 cell lines

Table 3: Cytotoxic activity of *Madhuca longifolia* ethanol extract on Vero cell lines

S. No	Concentration (µg/ml)	Cell Viability (%)	Cell Death (%)
1	1000	69.38	30.72
2	500	75.51	24.49
3	250	81.63	18.37
4	125	85.71	14.29
5	62.5	87.75	12.25
6	31.2	89.79	10.21
7	15.6	93.87	6.13
8	7.8	95.91	4.09
9	Cell control	100	0.00

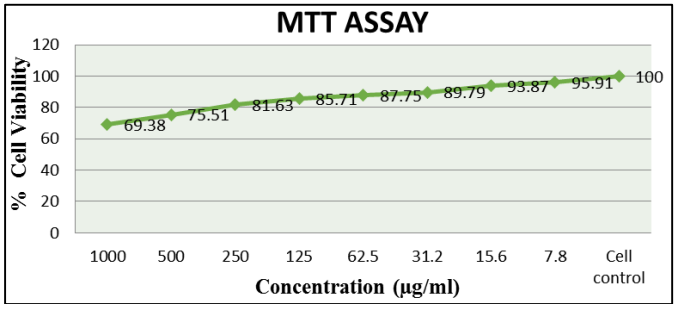

Fig 3: Cytotoxic activity of Bark extract of *Madhuca longifolia* on Vero cell lines

Discussion

Plants used in traditional medicine has contributed novel bio-active compounds to the field of preventive and curative medicines. Plant materials constitute a common alternative for the prevention of cancer around the world. Plant based compounds are the major source for the development of clinically useful anti-cancer drugs [18]. The cytotoxicity of *Madhuca longifolia* bark extract was evaluated against MCF-7 breast cancer cell lines and Vero cell lines, where the Vero cells were treated as positive control and untreated MCF-7 cells were considered as negative control.

The ethanol bark extract shows a potential cytotoxicity against MCF-7 as the IC_{50} amounts to 31.20 µg/ml l. Based on theIC_{50} values the extract can be defined as a strong cytotoxic agent (<100mg/ml) against MCF-7 cell lines. The extract shows a potential cytotoxic effect against breast cancer cells when compared to the normal Vero cells. The ethanol extract shows selective cytotoxicity when compared to positive control. Hence this can be a potential candidate for the identification of novel anti-cancer compound.

United States National Cancer Institute has declared that crude extracts with an IC_{50}<30-40 µg/ml l with reference to cytotoxicity are considered to be a promising *in vitro* cytotoxic agent [19]. Progression of cancer cells were inhibited by the plant metabolites by blocking and preventing the initiation of carcinogenic process as [20]. The use of food and medicinal herbs as a best source for the effective control of cancer [21].

Foods and beverages rich in poly phenols and flavones, will lower the occurrence of cancer [22]. There antioxidant compounds play a key role in the process of controlling cancer cell proliferation [23]. Antioxidant activity of ethanol bark extract amounts to more than 95% of antioxidant activity and the presence of high amount of flavonoids Poly phenols such as Anthocyanin in the extracts suggests the potential free radical scavenging of cancer cell lines [24, 25]. Flavonids interfere with the signal transduction and cell proliferation process by which it acts against cancer cells [26]. Hence, it is inferred from the present findings that a correlation exhibit between the potential antioxidant compounds and their potential role in the inhibition of breast cancer cell lines. To conclude the ethanol bark extract of *Madhuca longifolia* possess antioxidant potential with selective cytotoxicity against breast cancer cell lines not on the normal Vero cell lines. Further studies are to be performed the isolation of novel compounds with anticancer potential and the specific mode of action.

Reference

1. Ayoub Z, Mehta A. Medicinal plants of potential source of antioxidant agents: A review. Asian J Pharma. and Clinical Res. 2018; 11(6):50-56.
2. Criag WJ. Health – Promoting Properties of Common herbs. Am J Clin Nutr. 1999; 70:49I-499.
3. Jain JB, Kumane SC, Bhattacharya S. Medicinal flora of Madhya Pradesh and Chhattisgarh-A review. Ind J Traditional Knowledge. 2006; 5(2):237-242.
4. Shakya AK. Medicinal plants: future source of new drugs. Int J Herbal Medicine. 2016; 4:59-64.
5. Jassim SAA, Naji MA. Novel antiviral agents: A medicinal plant perspective. J Appl Microbiol. 2003; 95:412-427.
6. Hsieh MJ, Yen ZS, Chen SC, Fang CC. Acute cholinergic syndrome following ingestion of contaminated herbal extract, 2008.
7. Argelopoulos N, Barbounis V, Livadas S, Kaltsas D, Tolis G. Effects of estrogen deprivation due to breast cancer treatment. Endocr Relat Cancer. 2004; 11:523-535.
8. Wu YY, Li W, Xu Y, Jin EH, Tu YY. Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analysis. J Zhejiang Univ Sci B. 2011; 12:744-751.
9. Yaamagishi S, Matsui T. Nitric oxide a janus-faced therapeutic target for diabetic microangiopathy-Friend or foe. Pharmacol Res. 2011; 64:187-194.
10. Chen MS, Chen D, Dou OP. Inhibition of proteasome activity by various fruits and vegetables is associated with cancer cell death. *In Vivo*. 2004; 18:73-80.
11. Fleischauer AT, Simonsen N, Arab L. Antioxidant supplements and risk of breast cancer recurrence and breast cancer-related mortality among postmenopausal women. Nutr Cancer. 2003; 46:15-22.
12. Priyanka Y, Anurabha M, Nayak S. Microscopic studies of *Madhuca longifolia*, Scholars Res Lib. 2012; 1:66-72.
13. Kirtikar KR. Basu BD. Indian Medicinal Plants. Vol VII. Oriental enterprises. 2001; 2058-2061.
14. Verma GK, Jana S, Sen R, Chakrabothy, S, Sachan, Mishra A. Pharmacological Evalution of *saracaindica* leaves for central nervous system depressant activity in mice. J pharm Sci Res. 2010; 2(6):338-343.
15. Harborne JB. Phytochemical Methods, Chapman and Hall, Ltd., London. 1973, 49-188.
16. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Accacia confusa* bark and heartwood. J Agri Food Chem. 2001; 49:3420-24.
17. Motamed SM, Motlagh SS, Baghorzadeh H, Forouz SA, Tafazoli H. Evaluation of antioxidant activity of *Rutagraveolus*. L, extract on inhibition of lipid peroxidation and DPPH radicals and the effects of some external factors on plant extracts potency. Res. J Phcog. 2014; 1:45-50.
18. Shoeb M. Anticancer agents from medicinal plants. Bangladesh Journal of Pharmacology. 2006; 1(2):35-41.
19. Oskoueian, E, Abdullah N, Saad WZ, Omar AR, Kuan WB, Zolkifli NA, Hendra R, Ho YW. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. J Med Plants Res. 2011; 5:49-57.
20. Mauryaa DK, Nandakumar N, Devasagayam TPA. Anticancer property of gallic acid in A549, a human lung adenocarcinoma cell line and possible mechanisms. Journal of Clinical Biochemistry and Nutrition. 2010; 48(1):85-90.
21. Ferguson PE, Kurowska DJ, Freeman AF, Chambers, Koropatnick DJ. A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. J Natr. 2004; 134:1529-35.
22. Naasani I, Oh-Hara T, Feng WY, Johnston J, Chan K, Tsuruo T. Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells *in vitro* and *in vivo*. Cancer Res. 2003; 63:824-830.
23. Chinery R, Beauchamp RD, Shyr Y, Kirkland SC, Coffey RJ, Morrow JD. Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production and proliferation in colorectal cancer cells. Cancer Research. 1998; 58(11):2323-2327.
24. Matsuo M, Sasaki N, Saga K, Kaneka T. Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull. 2005; 28(2):253-259.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983; 65(1):55-63.
26. Heinonen MI, Ollilainen V, Linkola EK, Varo PT Koivistoinen PE. Carotenoids in Finnish foods: vegetables, fruits, and berries. J Agric Food Chem. 1989; 37(3):655-659.