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IN-VITRO ANTICANCER ACTIVITY OF *MICHELIA CHAMPACA* L. FLOWERS AGAINST EHRLICH ASCITES CARCINOMA CELL LINE

T.ANANTHI*, M.CHITRA AND B.ARUNA

PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi-614016, Tamil Nadu, India.

ABSTRACT

Cancer is a disease in which there is an uncontrolled multiplication and spread, within the body, of abnormal forms of the body's own cells. Assessment of *In-vitro* cytotoxicity has been recently become popular as a primary screening method for evaluating anti-cancer activities of various natural source. This study was carried out to characterize anti-cancer activity of methanolic extract of *Michelia champaca* L. flower on Ehrlich Ascites carcinoma cell line [EAC] by Trypan Blue Dye exclusion method and MTT [(3-(4,5 dimethyl thiazol-2-yl) 2,5-di phenyl tetrazolium bromide] assay. The preliminary phytochemical analysis was also carried out. Cell viability, inhibition was determined by Trypan blue dye exclusion method. The results showed that the methanolic extract of *Michelia champaca* L. decreased cell viability and increased growth inhibition in a concentration dependent manner and also altered the cell morphology. *Michelia champaca* L. methanolic extract has a significant anticancer effect against EAC cell line concentration range between 50µg to 150µg by using MTT assay. The minimum inhibition of plant extract showed 13.95% at 50µg/ml and maximum inhibition 54.46% was observed at 150µg/ml. IC50 value of *Michelia champaca* L. on EAC cell line was 147.5µg/ml by MTT assay. From the results, it showed that the methanolic extract of *Michelia champaca* L. has potent anti-cancer activity on EAC Cell line.

KEY WORDS: **Anti-cancer, MTT Assay , EAC Cell Line , *Michelia champaca* L.**

T.ANANTHI
PG and Research Department of Biochemistry,
Sengamala Thayaar Educational Trust Women's College,
Mannargudi-614016, Tamil Nadu, India.

***Corresponding author**

INTRODUCTION

Cancer is the second leading cause of death all over the world [1]. It is characterized by uncontrolled growth and spread of abnormal cells. World Health Organization (WHO) reported that there are 7.6 million deaths in 2008 and it is estimated up to 13.1 million deaths in 2030 [2].The term cancer is derived from the Latin word cancerene for "crab". It is a multifactorial, multifaceted and multimechanistic disease requiring a multidimensional approach for its treatment, control and prevention [3]. It is caused by internal factors (tobacco, chemical, radiation and infectious organism) and external factors (mutation, hormone and immune conditions) [4, 5]. Age is also a primary risk factor for most cancer, with about 77% of all cancer diagnosed among people aged 55 or older (NCI Research finding). Breast cancer is the most common form of cancer in women world-wide [6]. According to an estimate, 50% of breast cancer and 37% of prostate cancer patients used herbal products [7] and can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy. *Michelia champaca* L. (Magnoliaceae) commonly known as Yellow champaca. Medium sized evergreen tree with yellow fragrant blossoms. Leaves ovate-lanceolate, coriaceous, glabrescent. Flowers solitary, auxillary, pale yellow. Fruit an aggregate of follicles [8]. *M.champaca* is used in the treatment of fever, colic, leprosy, eye disorder**,** inflammation, cough rheumatism, gonorrhea, cephalagia and gout [9, 10]. It has been used in India for the treatment of abdominal tumour [11].The plant is also reported to have significant wound healing[12], antimicrobial[13], antidiabetic[14], antitumour[15] anti-inflammatory[16], antioxidant[17], and antiinfective[18] properties. The aim of the present study was to investigate the presence of secondary metabolites and cytotoxic activity of methanolic flower extract of *Michelia champaca* against EAC cell lines.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The *Michelia champaca* L. flowers were collected from the local areas of Udumalaipettai, Coimbatore District, Tamilnadu. The collected flowers were carefully stored in plastic box and used for the present studies. The collected plant material was botanically identified with the help of Flora of Presidency of Madras[19] and authenticated by Dr.S.John Britto, The Director, RAPINAT Herbarium, Department of Botany, St. Joseph's college, Thiruchirappalli, Tamilnadu. (Voucher no.001).

Preparation of the extract

The flowers were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powder was then subjected to successive extraction with methanol by Soxhlet method. The extract were then collected and distilled off on a water bath at atmospheric pressure and stored at 4º C.

Preliminary Phytochemical screening

Phytochemical screening of various extracts of *Michelia champaca* L. flowers were carried out using standard qualitative method[20].

In Vitro Anticancer Assay
Maintenance Of EAC Cells

Ehrlich Ascites Carcinoma cell lines were obtained from the Amla Cancer Research Centre, Thrissur and maintained by weekly intra-peritoneal inoculation of 1X106cells/mouse [21].

Tryphan blue dye exclusion assay

The trypan blue dye exclusion assay is the most commonly utilized test for cell viability. Trypan Blue is a blue acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan blue is an essential dye use in estimating the number of viable cells present in

a population [22]. In this assay, the cells are washed with HBSS (Hank's Buffered Salt Solution) and centrifuged for 10 - 15 min at 10,000 rpm. The procedure is repeated thrice. The cells are suspended in known quantity of HBSS and the cell count is adjusted to 2 x 106 cells /ml. The cell suspension is distributed into Eppendorf tubes (0.1 ml containing 2 lakhs cells). The cells are exposed to drug dilutions and incubated at 37 °C for 3 h. After 3 h, dye exclusion test, that is, equal quality of the drug treated cells are mixed with tryphan blue (0.4 %) and left for 1 min. It is then loaded in a haemocytometer and viable and non-viable count is recorded within 2 min. Viable cells do not take up colour, whereas dead cells take up colour. However, if kept longer, live cells also generate and take up colour [23].

MTT Assay

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of 2×105 mL-1 per well in 100 µL of RPMI 1640 and allowed to grow in CO2 incubator for 24 h (37 ˚C, 5 % CO2). The medium is then removed and replaced with fresh medium containing different concentrations of sample for 48 h. The cells are incubated for 24-48 h (37˚C, 5% CO2). Then, 20 µL MTT ([3-(4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560nm. Untreated cells (basal) are used as a control of viability (100 %) and the results are expressed as % viability (log) relative to the control [24].

RESULTS AND DISCUSSION

Cancer is one of the most dreaded disease of 20th century and spreading further continuously with increasing incidence in 21th century. It is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, growth, local tissue invasion and distant metastases [25].Over the past few years, cancer has remained a major cause of the death and number of individuals affected with cancer is continuing to expand. Hence a major portion of current pharmacological research is developed to anticancer drug design customized to fit new molecular targets [26]. Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical contituents with antitumor and cytotoxic activities [27, 28].

Preliminary phytochemical screening

The preliminary phytochemical analysis of the plant powder and various extracts (hexane, chloroform, ethyl acetate, alcohol, water) revealed the presence of tannins, steroid, flavonoid, coumarine, quinone, lignin, alkaloids and sugar were presented (Table 1). Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom [29]. Flavonoids have a chemo-preventive role in the cancer through their effect on signal transduction in cell proliferation and angiogenesis [30]. Tannins received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages, like green teas and red wines can cure or prevent a variety of ills [31]. Many human physiological activities, such as stimulation of phagocytic cells, host mediated tumour activity and a wide range of anti-infective actions have assigned to tannins [32].Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against disease and endurance against stress [33].

Table 1
Preliminary Phytochemical screening of Various extracts of Michelia champaca

S.No	Test	Hexane	Chloroform	Ethyl acetate	Alcohol	Water
1	Saponins	-	-	-	-	-
2	Tannins	+	-	-	+	+
3	Steroids	-	+	-	+	-
4	Terpenoids	-	-	-	-	-
5	Flavonoids	-	-	+	+	+
6	Coumarin	+	-	-	+	-
7	Quinone	+	+	-	-	-
8	Lignin	-	+	+	+	+
9	Alkaloids	+	-	+	+	-
10	Sugar	-	-	-	-	+

+ indicates presence; whereas – indicates absence

In vitro Anticancer Activity

The Ehrlich Tumour was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behaviour and is able to grow in almost all strains of mice [34]. The tumour implantation includes a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascetic fluid accumulation [35]. The ascetic fluid is essential for tumour growth, since it constitutes a direct nutritional source for tumour cells [36]. Hence EAC cell was used in the present study to screen the anticancer potential of the selected plant drug.

Trypan Blue Dye Exclusion Method

The methanolic extract of the *Michelia champaca* L.was tested against EAC cell lines. Different concentrations of the plant extract were inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive method. The test based on the principle that the dead cell accepts dye and stain with blue colour. The plant drug would have disturbed the membrane integrity and have caused the cell death, which is one of the hall marks of apoptosis. The methanolic extract showed 76.48% of cytotoxicity (500µg/ml) against EAC cell line. The loss of membrane integrity is considered as an indicator of apoptosis. The results are tabulated and graphically represented in Table 2

Table 2
***In vitro* Anticancer effect of *Michelia champaca* L. flowers against EAC cell line**

S. No	Concentration of plant extract (µg/ml)	No.of Viable cells	Viable cell (%)	No. of Dead cells	Dead cells in %
1	Control	65	90.28	7	9.72
2	100	48	78.69	13	21.31
3	200	15	36.59	26	63.41
4	300	35	35.71	63	64.29
5	400	23	34.32	44	65.68
6	500	20	23.52	65	76.48

MTT Assay

In-depth *In-vitro* cytotoxic study was carried out in methanolic extract of *Michelia champaca L.* against EAC cell lines employing MTT assay method. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4,5 dimethyl thiazolium -2)-2, 5 di phenyl tetrazolium bromide) is reduced by metabolically active of cells by the action of mitochondrial dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intra cellular purple formation can be solubilized and quantified by spectrophotometric method. From the result it was observed that the plant drug under study showed maximum cytotoxicity 54.4% for EAC at a concentration of 150µg/ml. The extract exhibit potent cytotoxicity against the cancer cell lines (IC50=147.5µg/ml) and it may be due to disturbance in the mitochondrial assembly which resulted in the increased cytotoxicity of EAC cell lines. The loss of mitochondrial activity is consider as one the hallmark of apoptosis. The result clearly indicated that the extract accelerate the apoptotic pathway in the EAC cell lines. (Table 3)

Table 3

In vitro Anticancer effect of Michelia champaca L. flowers against EAC cell line

Concentration (µg/ml)	OD-1	OD-2	OD-3	Average	Percentage	IC50 Value (µg/ml)
Control	0.431	0.437	0.444	0.437		147.5
50	0.381	0.376	0.371	0.376	13.95	
75	0.359	0.354	0.349	0.354	18.9	
100	0.311	0.316	0.309	0.312	28.60	
125	0.291	0.289	0.271	0.283	35.24	
150	0.201	0.207	0.187	0.218	54.46	

CONCLUSION

From the above findings it could be concluded that the minimum inhibition of 13.95% was found at 50µg/ml and maximum inhibition of 54.46% at 150µg/ml by MTT assay. Further in-depth studies have to be carried out to understand the molecular mechanisms of anticancer action of the methanol extract of *Michelia champaca* L. flower coupled with animal studies and clinical trials would result in the arrival of cost effective, safe, efficacious anticancer drug which is a boon for ailing human society.

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