AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Inhibition of cancer cell growth by crude ethanolic extract of *Tinospora malabarica* (Miers) Ann. An endangered medicinal plant

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ABSTRACT

An ethanolic extract of *Tinospora malabarica* (Menispermaceae) was studied for its effects on growth in two malignant cell lines including a Human Melanoma cancer cell lines (A 375) and Skin cancer cell lines (A 431) using MTT assay. In these cell lines studied, the extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. The ethanolic extract of *T. malabarica* showed good cytotoxicity and IC50 value of 49.87μg/mL and 112.54 µg/mL respectively. It could be a reliable source of potent pharmacophore for treatment of disease like cancer.

Keywords: *Tinospora malabarica,* Ethanol extract, Melanoma cancer and Skin cancer.

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Received 13 August 2012, Accepted 25 August 2012

Please cite this article in press as:Punitha *et al.*, Inhibition of cancer cell growth by crude ethanolic extract of *tinospora malabarica* (miers) ann. An endangered medicinal plant. American Journal of PharmTech Research 2012.

INTRODUCTION

According to WHO, 80% of the rural population in developing countries are depending on medicinal plants for their primary health care [1]. Apart from other diseases, the first US recognized drug for the treatment of cancer is from *Catharanthus roseus*. The other plants like *Angelica gigas, Podophyllum peltatum, Taxus brevifolia, Podophyllum emodii, Ocrosia elliptica*, and *Campototheca acuminata* have been used in the treatment of advanced stages of various malignancies [2]. There are also various medicinal plants reported to have anti-cancer in the Ayurvedic system of medicine.

Tinospora malabarica Miers in Ann. (Menispermaceae) is a woody climber with smooth shining stem, bark light grey and papery. Leaves broadly ovate or orbicular, deeply cordate at the base, thickened and twisted at the base. Flowers greenish yellow in racemes 7-14 cm long, usually solitary in the female, clustered in the male. It contains a bitter substance, Berberine, and also yields Giloin and crude Giloinin. The watery extract *"Sat Giloe"* is sold in the bazar and is often used as a febrifuge under the name of *"Indian quinine"*. An infusion is prepared from the stem and the root which is considered to be a valuable tonic, and a cure for intermittent fever and dyspepsia[3].

MATERIAL AND METHOD

Cytotoxicity Study:

Cell line used:

Human Melanoma cancer cell line (A 375) and Skin cancer cell line (A 431).

Cell proliferation kit:

MTT assay kit

Media:

DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum)

Glass wares and plastic wares:

96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Equipments:

Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet class II (Esco), cytotoxic safety cabinet (Esco), CO_2 incubator (RS Biotech, mini galaxy A), Deep freezer, ELISA plate reader (Thermo), Micropipettes (Eppendorff), RO water system (Millipore).

Preparation of plant extract:

Tinospora malabarica was collected from Western Ghats, Kerala and authenticated by Dr. M. Murugaesan, Scientist, SACON, Coimbatore, Tamilnadu. Accurately weighed 5 gms of aerial parts of *Tinospora malabarica* powder was extracted with 25 ml ethanol by mechanical shaking for 1 week. The filtered extract was concentrated under reduced pressure to remove the solvent. The extract was obtained by drying the concentrated pooled extract under vacuum.

Microculture tetrazolium (MTT) assay

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is an indication of the viability of the cells[4].

Procedure

This MTT assay was performed according to a slight modification of the procedure reported by Mosman[4]. The monolayer cell culture was trypsinized and the cell count was adjusted to 3 lakh cells/ ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37º C in 5% CO_2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm. Then the percentage growth inhibition was calculated as follows.

$$\%\text{cell inhibition} = 100-\{(At-Ab)/(Ac- Ab)\}x100$$

Where, At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

RESULTS AND DISCUSSION

In vitro confirmation of this cytotoxicity of the *T. malabarica* on Human Melanoma cancer cell lines (A 375), Skin cancer cell lines (A 431) cell lines were reported. This extract was screened

for its cytotoxicity against two cell lines at different concentrations and determined the IC50 values (50% growth inhibition) by MTT assay. The present results are tabulated (Table 1) and graphically represented (Figure. 1and 2). The dose dependent responses for the Human Melanoma cancer (A 375) and Skin cancer cell lines (A 431) are also shown in the Figure 1. Upon treating A375 cancer cells and A 431 cancer cells, the ethanolic extract of *T. malabarica* shows good cytotoxicity and IC50 value of 49.87 μg/mL and 112.54 µg/mL respectively.

Table 1: *In vitro* cytotoxicity effect of *Tinospora malabarica* on melanoma and skin cancer cell lines.

S.No	Concentration (µg/mL)	Dilution	*Tinospora malabarica* A 431	A 375
1	1000	Neat	18.63±0.42	3.71±1.13
2	500	1:1	28.27±0.56	11.41±0.72
3	250	1:2	37.08±0.96	14.23±0.99
4	125	1:4	45.71±0.82	36.97±1.10
5	100	1:8	53.81±1.05	46.58±1.17
6	50	1:16	59.81±0.71	49.02±1.10
7	25	1:32	64.87±0.28	54.67±0.75
8	12.5	1:64	69.72±0.59	68.49±0.66
9	10	1:128	77.64±0.55	74.32±0.64
10	5	1:264	88.33±0.29	81.07±1.0
11	**Cell control**	-	100	100
Inhibitory Concentration IC 50			112.54 µg/mL	49.87 µg/mL

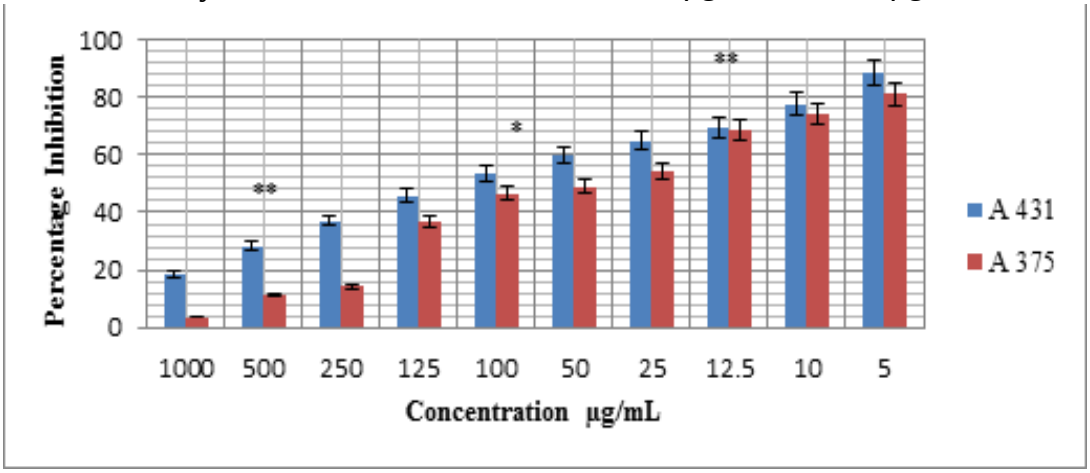

Figure 1: *In vitro* cytotoxicity of different concentrations of ethanolic extract of *Tinospora malabarica.*

The Preliminary evidences from Moongkarndi[5] and Jongsomboonkusol[6] have demonstrated that the crude extract of *Stephania venosa* was inhibited cell proliferation in breast cancer cell lines and induce apoptosis and cell cycle arrest in ovarian and breast cancer cell lines. The previous reports on *S. venosa* showed highly potential for cancer therapy for ovarian cancer.

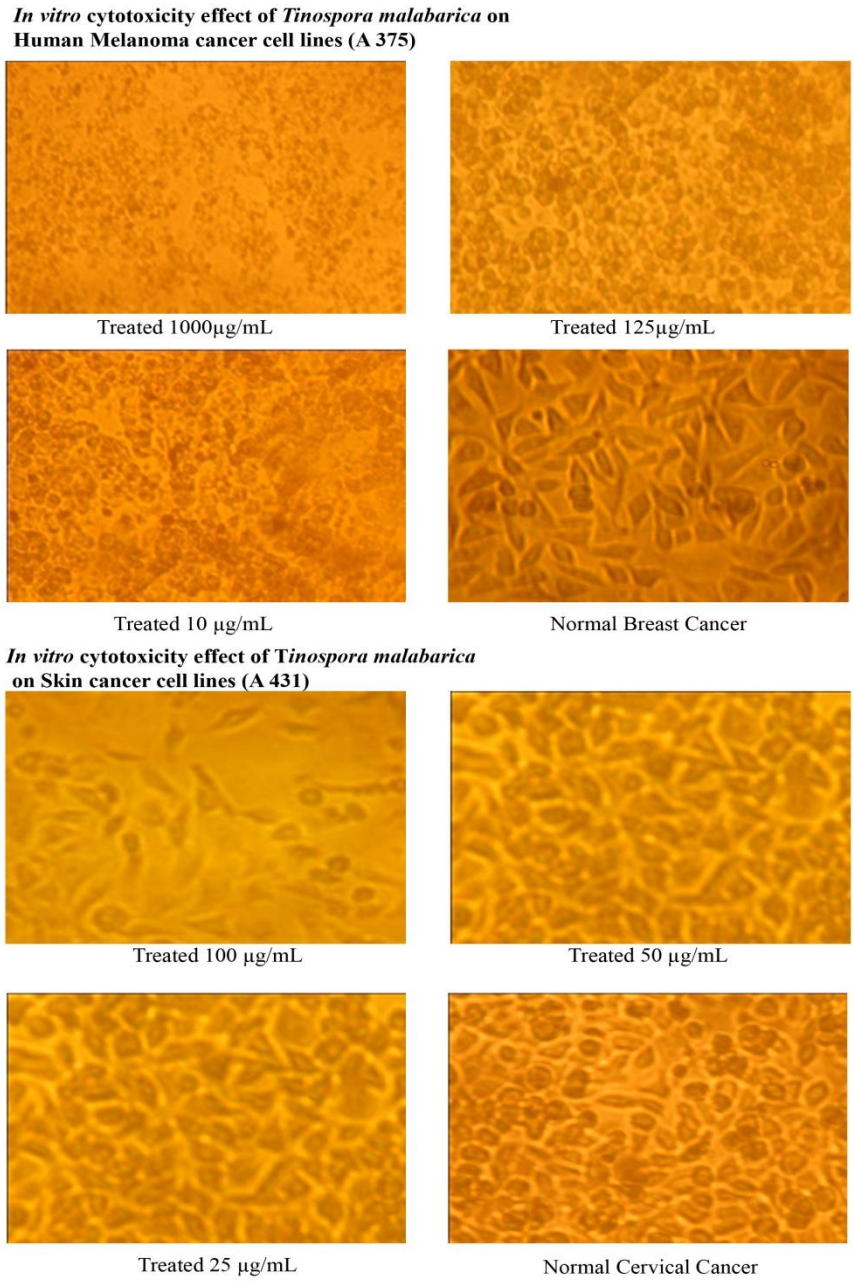

Figure 2: Photomicrograph of Human melanoma (A 375) and skin cancer (A 431) cell lines treated with various concentrations of ethanolic extract of *Tinospora malabarica.*

The anticancer activity of dichloromethane extract of *Tinospora cordifolia* of Menispermaceae in the mice transplanted with Ehrlich ascites carcinoma (EAC) was investigated[7]. The EAC mice received 25, 30, 40, 50 and 100 mg/kg *T. cordifolia* extract which showed a dose dependent elevation in tumor-free survival. A highest number of survivors were observed at 50 mg/kg plant extract, which was considered as an optimum dose for its neoplastic action. The average survival time (AST) and median survival time (MST) for this dose were approximately 56 and 55 d, respectively when compared with 19 d of non-drug treated controls. Administration of 50 mg/kg

T. cordifolia extract resulted in 100% long-term survivors (up to 90 d). An attempt was also made to evaluate the effectiveness of *T. cordifolia* extract in the various stages of tumor development, where 50 mg/kg *T. cordifolia* extract was administered intraperitoneally after 1, 3, 6, 9, 12 or 15 d of tumor inoculation and these days have been arbitrarily designated as stage I, II, III, IV or V, respectively for reasons of clarity. The maximum anticancer activity was recorded for stage I, II and III where number of long term survivors (LTS) was approximately 33, 25 and 17%, respectively.

CONCLUSION

Nature continues to be the most prolific source of biologically active and diverse chemotypes. Although relatively few of the actual isolated compounds advance to become clinically effective drugs in their own right, these unique molecules often serve as models for the preparation of analogues using chemical methodology such as total or combinatorial synthesis, or manipulation of biosynthetic pathways. This study results show that ethanolic extracts of *Tinospora malabarica* is promisingly cytotoxic against human melanoma and human skin cancer cell lines.

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