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RESEARCH ARTICLE

ANTICANCER STUDIES ON THE LEAVES OF *TINOSPORA CORDIFOLIA* (WILD) MIERS

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Abstract

The aim of the study was to evaluate anticancer activity of the methanol and aqueous extracts of leaves of *Tinosporacordifolia* Wild Miers against a Human Breast Carcinoma Cell line(MDAMB231) by MTT assay. The coarse powder of leaf were subjected to Soxhletextraction and microwave assisted extraction using methanol and water as solvent. The IC_{50} values of aqueous extract of leaf followed by soxhlation and methanol extract of leaf by microwave extraction were found to be 59.85µg/mL and 249µg/mLrespectively in MDAMB231 cells in comparison to Standard Vincristine 14.41µM whereas methanol extract of leaf by soxhlet and aqueous extract by microwave extraction did not possess any anticancer activity. This study suggests that *Tinosporacordifolia* can be useful in the treatment of breast cancer.

Introduction:-

Now a days, breast cancer is the most frequently diagnosed life-threatening cancer in women and the leading cause of cancer death among women. Since last two decades, research related to the breast cancer has led to extraordinary progress in our understanding of the disease, resulting in more efficient and less toxic treatments, increased public awareness and improved screening have led to earlier diagnosis at stages amenable to complete surgical resection and curative therapies[1].

Cancer cells are formed from normal cells due to a modification/mutation of DNA and/or RNA. These modifications/mutations can occur spontaneously, or they may be induced by other factors such as nuclear radiation, electromagnetic radiation, microorganisms, heat, chemicals in the air, water and food, mechanical cell-level injury, free radicals, evolution, ageing of DNA and RNA, etc. All these can produce mutations that may start cancer.

In traditional system of medicine, *Tinosporacordifolia* Wild Miers commonly named as “Guduchi” belonging to family Menispermaceae is known for its immense application in the treatment of various diseases.A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant, including root, stem, and whole plant. The plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, anti-neoplastic and antitubercularactivities [2-5].

The present aim of the study is to evaluate the anticancer activity of *T.cordifolia* against Human Breast Carcinoma Cell line(MDAMB231).

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Material And Methods:-

Plant material

The leaves of *Tinosporacordifolia* was collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by CCRAS. A voucher specimen (TOCOP/01/2020-21-TC) was deposited in The Oxford College of Pharmacy, Bangalore. The leaves were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in airtight container for further use.

Preparation of extract by soxhlet technique

Coarsely powdered leaves of *T.cordifolia* 20 g, each were subjected to extraction in soxhlet extractor with methanol and boiled with distilled water (150 ml) each respectively. The two extracts of leaves were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 9.79 and 14.52 % w/w respectively with reference to the air-dried leaf sample. [6]

Preparation of extract by Microwave assisted extraction(MAE)technique

Coarsely powdered leaves of *T.cordifolia* 20 g, each were subjected to extraction in Microwave extractor (Ragatech Company Microwave extractor with Power 3, set at temperature 75^0C for 15 minutes with continuous stirring) with methanol and water [150 ml] respectively. The two extracts of leaves were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 4.76 and 10.92 % w/w respectively with reference to the air-dried leaf samples. [7]

Chemicals

All chemicals and reagents used in this study were at least of analytical grade.

Phytochemical Screening of different extracts of *T.cordifolia*

Chemical tests for the screening and identification of bioactive chemical constituents (such as carbohydrates, alkaloids, glycosides, flavonoids, saponins, tannins and steroid) in different extracts were carried out using the standard procedures[8].

Determination of Total Phenolic Content

The phenolic contents of methanol extract of leaf were determined as follows: 0.5 ml aliquot of extracts or gallic acid (standard) was added with 5 ml of Folin–Ciocalteu reagent and 4 ml of aqueous sodium carbonate (1 M). After incubation for 15 min at room temperature, the absorbance was read at 765 nm. The total phenolic contents were expressed in terms of µg/mlofgallicacid[9].

Determination of Total Flavonoid Content

Flavonoid contents of methanol extract of leaf were determined as follows: 0.5 mL of extracts diluted in 1.5 ml of 95% methanol solution, 0.1 ml of 10% $AlCl_3$(w/v), 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were added. After 30 min at room temperature, the absorbance was measured at 415 nm. Quercetinwas used as a positive standard for preparing the standard calibration curve. The total flavonoid contents were measured in terms of µg/ml of Quercetin[9]

Cytotoxicity studies against MDAMB231 cell line

Preparation of test solutions:

For cytotoxicity studies, 32mg/ml stocks were prepared using DMSO. Serial two-fold dilutions were prepared from 320µg/ml to 10µg/ml using DMEM media for treatment.

Cell lines and culture medium:

MDAMB231 cells were procured from ATCC, stock cells were cultured in DMEM supplemented with 10% inactivated Foetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37^oC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs at 37^oC, 5 % CO_2 incubator.

Determination of anticancer activity

The cells were trypsinized and the cell count was adjusted to $5x10^5$ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added.

After 24 hrs, the supernatant was removed, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 0.05mg MTT was added to each well. The plates were incubated for 4hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO 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 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line[10-17].
% Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100.

IC_{50} Value
The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e., an enzyme, cell, cell receptor or microorganism) by half. The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

Statistical evaluation:
IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, San Diego, CA, USA).

Result AndDissusion:-

Percentage yield of extract:
The percentage yield of methanol and aqueous extract of leaves of T.cordifolia was calculated and given in the Table No 1. Aqueous extract of leaf after soxhlation extraction gave the maximum yield when compared to all extracts.

Preliminary Phytochemical screening:
Preliminary phytochemical screening revealed the presence of alkaloids, amino acid, flavonoids, saponins and triterpenoids which is given inTableNo 2.

Total phenol content:
The total phenolic content of methanolic and aqueous extract of leaf by soxhlation and microwave assisted extraction method was found to be 0.3812, 0.4726, 0.5902 and 0.6925 µg/ml respectively as obtained from the standard gallic acid graph (Fig No 1).

Total flavonoid content
The total flavonoid content of methanolic and aqueous extract of leaf by soxhlation and microwave assisted extraction methodwas found to be 9.0566, 0.8844, 4.7523 and 0.8140 µg/ml respectively as obtained from the standard quercetin(Fig No 2).

Cytotoxicity studies against MDAMB231 cell line
Traditionally, the *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3- [4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water-soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazancan be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of effects caused by the test material[13,17].

The anti-cancer activity was determined by MTT assay using methanolic and aqueous extract of leaf by soxhlation and microwave assisted extraction methodof *T.cordifolia*. Anticancer activity of breast cancer is reported from polysaccharide isolated from the methanol extract of *Tinosporacordifolia* stem[18]. IC_{50} values was found to be 59.85µg/mL, 249µg/mLfor aqueous extract of leaf by soxhlation and methanol extract of leaf by microwave assisted extraction respectively in MDAMB231 cells in comparison to Standard Vincristine 14.41µM(Table No. 3; Fig No 3).Images also show that the cell morphology is disrupted which confirms the cytotoxicity effect. Methanol extract of leaf by soxhlet and aqueous extract by microwave assisted extraction did not possess any anticancer activity.

Conclusion:-

The result of the present study revealed that *T.cordifolia* showed anticancer activity against breast cancer. The IC_{50} values of aqueous extract of leaf followed by soxhlation and methanol extract of leaf by microwave extraction were found to be 59.85µg/mL and 249µg/mLrespectively in MDAMB231 cells in comparison to Standard Vincristine 14.41µM whereas methanol extract of leaf by soxhlet and aqueous extract by microwave assisted extraction did not possess any anticancer activity. Thus *T.coordifolia* is a promising agent for nano chemoprevention of breast cancer cells used for the study.

Fig No. 1:- Determination of total phenol content of standard gallic acid.

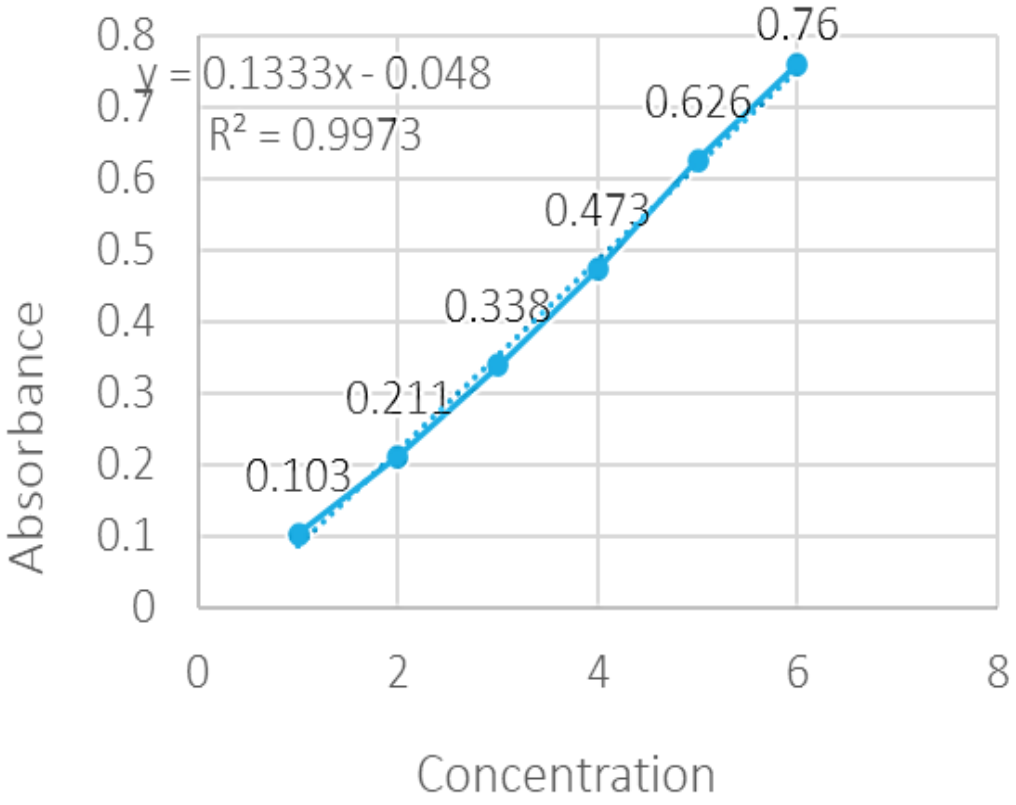


Fig No. 2:- Determination of total flavonoid content of standard quercetin.

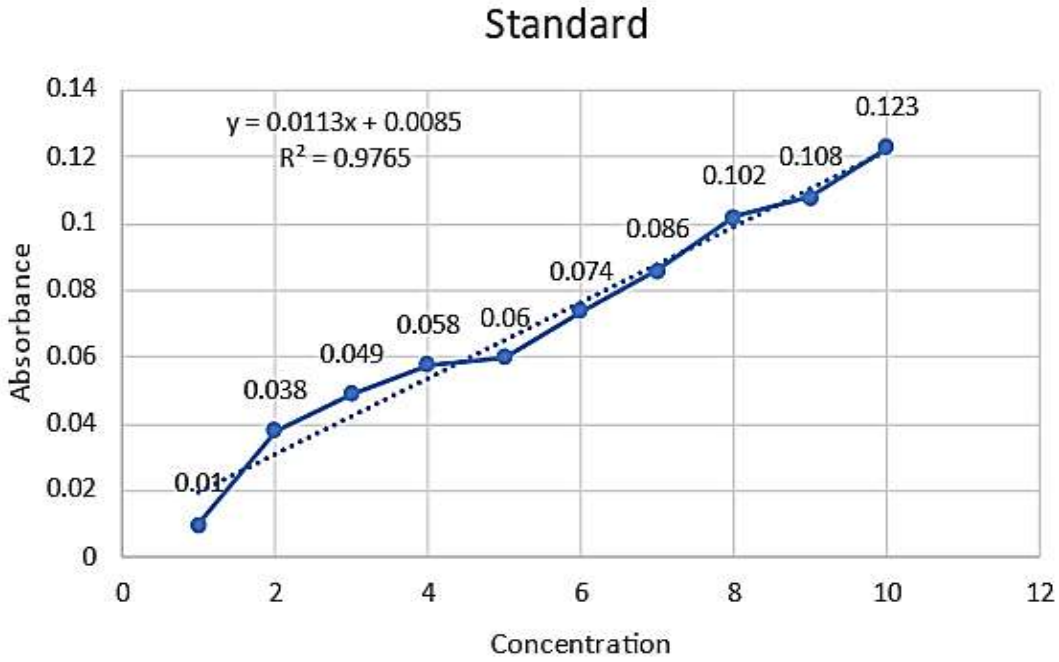
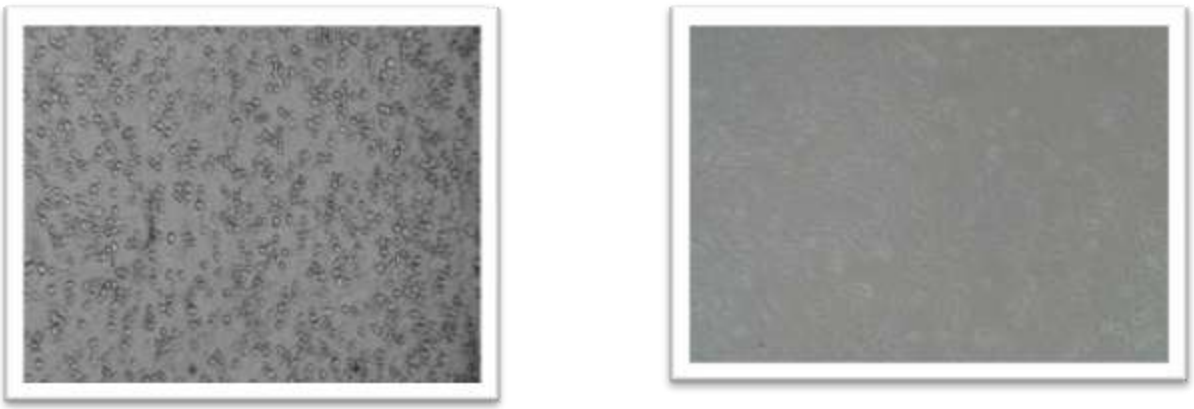


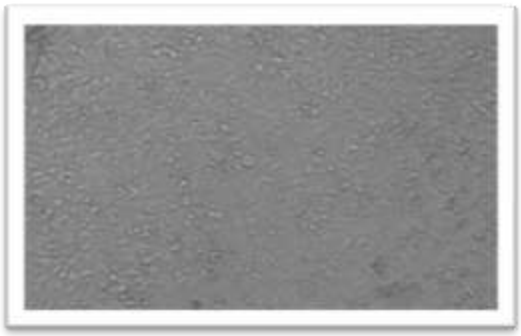
Fig No 3:- Anticancer effect of different extracts of *T.cordifolia* and vincristine.

Control Vincristine(3.125µM)



Vincristine(100 µM)Methanolic extract of leaf by MAE 10µgml

Methanolic extract of leaf by MAE 320µg/mlWater extract of leaf by soxhletmethod 10µgml



Water extract of leaf by soxhlet method – 320µgml

Table No.1:- Percentage yield of different extract of *T.cordifolia.*

Sl No	Extract details	Percentage yield (%w/w)
1	Methanolic extract of leaf by Soxhlet method	9.79%
2	Water extract of leaf after Soxhlet method	14.52%
3	Methanolic extract of leaf by microwave assisted method	4.76%
4	Water extract of leaf by microwave assisted method	10.92%

Table No.2:- Preliminary phytochemical screening of different extract of *T.cordifolia.*

Sl No	Phytochemicals	MEOH EXT OF LEAF(SOX)	WATER EXT OF LEAF(SOX)	MEOH EXT OF LEAF (MAE)	WATER EXT OF LEAF (MAE)
1	Carbohydrate	_	_	_	_
2	Alkaloids	+	+	+	+
3	Amino acid	_	_	_	_
4	Glycosides	_	_	_	_
5	Flavonoids	_	_	_	_
6	Tannins	+	+	+	+
7	Saponins	_	_	_	_
8	Steroids and Triterpenoids	+	+	+	+

Table No.3:- IC_{50} value of methanol and aqueous extracts of leaf by soxhlet and microwave assisted extraction of *T.cordifolia* on MDAMB231 cell line.

Sl No	Extract details	IC_{50} value(µg/ml)
1	Methanol extract of leaf by soxhlation	No inhibition at higher dose
2	Water extract of leaf by soxhlation	59.85
3	Methanol extract of leaf by MAE	249
4	Water extract of leaf by MAE	No inhibition at higher dose

5	Standard Vincristine	14.41

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Ethical Issues
There is none to be applied.

Conflict Of Interest
None to be declared.

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