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Research Article

BREAST CANCER ACTIVITY OF ISOLATED COMPOUND FROM *TINOSPORA CORDIFOLIA* AERIAL

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ABSTRACT

Objectives: The present study undertaken to explore antioxidant and the cell line study of isolated compound from ethanol extract from *Tinospora cordifolia* belongs to the family *Menispermaceae*.

Methods: The air dried powdered sample of aerial parts of T. cordifolia was extracted in a Soxhlet using five different solvents. Most active ethanol extracts were purified using silica gel column chromatography. Characterized the structure of the isolated compound using Fourier transform infrared spectrum, 1H nuclear magnetic resonance, and liquid chromatography–mass spectrometry studies. Antioxidant and anticancer activity of isolated compound was determined using 2,2-diphenyl-1-picrylhydrazyl and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Results: Magnoflorine was isolated from most active ethanol extract from aerial parts of *T. cordifolia* which shows maximum antioxidant activity 64% at the tested concentration 500 µg/ml. The percentage of cell viability varied from 53.3% at the minimum tested concentration 3.12 µg/ml to 1.9% at the maximum tested concentration 100 µg/ml.

Conclusion: The isolated characterized compounds would be useful to prepare plant-based pharmaceutical preparation to treat various diseases linked with human diseases.

Keywords: Column chromatography, Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Michigan Cancer Foundation-7 cell line, Breast cancer, *Tinospora cordifolia*.

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INTRODUCTION

Novel chemical compounds are synthesized from the plant active constituents, which are used in medicine and other useful applications [1]. Purification and isolation of bioactive compounds from plant is a technique that has undergone new development in recent years [2,3]. Chromatographic techniques have significant role in natural products chemistry as well as contribute dramatically in the discovery of novel and innovative compounds of pharmaceutical and biomedical importance.

Oxidative stresses induced by reactive oxygen species have been considered the main cause of induction and progression of a number of chronic diseases. In the guidelines, it is recommended that one should take fruits and vegetables that have enough carotenoids and pharmacologically active phytochemicals [4]. Carotenoids are mainly synthesized by plants and microorganisms but not animals. These are important dietary sources of Vitamin A and are considered valuable in preventing human diseases [5]. Over the centuries, plants have been used in the treatment of cancer. Out of an estimated 2,50,000 plant species worldwide more than 3000 have reported to significant anticancer potential [6,7]. Plant-based drug discovery has contributed and is contributing to the development of anticancer drugs as well as new anticancer lead compounds in clinical trials [8].

METHODS

Collection of plant material

The aerial parts of *Tinospora cordifolia* were collected from wastelands in Colachel, Kanyakumari district, Tamil Nadu, India (8°17'86"N and 77°25'61"E) during July 2016. This plant is identified and authenticated by the Department of Botany, Scott Christian College (Autonomous), Nagercoil, Kanyakumari, Tamil Nadu, India. Voucher specimen of this plant was deposited at herbarium of this institute (Voucher no. SCCN 4401).

Preparation of extract

The aerial parts of *T. cordifolia* were washed and air dried over a period of 1 month. The dried samples were milled into a fine powder by pounding manually on a clean, sterile mortar, and stored in sterile cellophane bags in a cool dry place. The air dried powdered sample of 100 g was extracted in a Soxhlet sequentially in 1000 ml of petroleum ether, chloroform, ethyl acetate, ethanol, and water. The process was run for 24 h after which the sample was concentrated using reduced pressure distillation under vacuum pump and freeze dried to powdered form. The dried extracts were weighed and kept in labeled sterile specimen bottles.

Column chromatography

Based upon the antioxidant and anticancer study, the most active ethanol extract from *T. cordifolia* aerial was purified using silica gel column chromatography. The column was packed with a solution of silica gel (60–120 mesh) with methanol by wet slurry method [9]. The concentrated crude extract was mixed with methanol in a beaker and loaded into a column into a silica gel column packed in a methanol. Here, silica gel is act as a stationary phase. The solvents methanol and dichloromethane (DCM) (5:95) act as a mobile phase.

Spectroscopic study

Fourier transform infrared spectrum (FTIR)

The FTIR of the purified compound was recorded in the range of 500–4000 cm^{-1} using the instrument of Thermo Nicolet, Avatar 370.

^{1}H nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR of purified compounds was recorded in dimethyl sulfoxide (DMSO) as internal standard solution using the instrument model Bruker Avance III, 400 MHz.

Liquid chromatography–mass spectrometry (LC-MS)

The LC-MS spectrum was recorded using single quad detector and electrospray ionization as ion source.

Bioactive properties

Antioxidant activity-2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of different extracts was determined using DPPH assay. Antioxidant was determined in test tube containing 200 µl of DPPH reagent in 3.7 ml methanol is added to the 100–500 µl of plant extract including blank and incubated at the room temperature for 30 min. Ascorbic acid was used as a standard. The absorbance of the sample was read at 517 nm [5].

%Antioxidant activity = (Absorbance of blank–Absorbance of test solution/Absorbance of blank) × 100

***In-vitro* anticancer activity**

Sub-cell culture

Bring the medium and trypsin, phosphate, versene, and glucose solution (TPVG) to room temperature. The tissue cultures flask for growth, cell generation, pH (7.2–7.4), and turbidity are observed. Select the flask for splitting.

The following procedure is followed in sequence, mouth of the flask was wiped with cotton soaked in spirit. Discard the medium and wash the cells with minimum essential medium (MEM) for twice. Then, 4 ml of TPVG (pre-warmed to 37°C) was added over the cells and allowed TPVG to react for 15 s-1 min. After that discard the TPVG and add 5 ml of 10% MEM. Break for the cell clusters by gently pipetting back. Then, 20 ml of growth medium is added to tissue cluster flask and transfer the cells into 96 well plates.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cancer activity of samples of Michigan Cancer Foundation–7 (MCF-7) cell was determined by MTT assay. Cells (1 × 105/well) were plated in 0.2 ml of medium/well in 96 well plates. Incubate at 5% CO_2 incubator for 72 h, then added various concentration of the sample of 0.1% DMSO for 48 h at 5% CO_2 incubator. View the image under Inverted Microscope 40× and take photos. After removal of sample solution, 20 µl/well MTT reagents were added. Viability cells were determined by the absorbance of 540 nm. About 50% inhibition of cell viability (IC_{50}) values was determined graphically. The effect of the samples of the proliferation of MCF-7 cells was expressed as the % cell viability [6].

% cell viability = A540 of treated cells/A540 of control cells × 100%.

Cell death = 100–cell viability %.

RESULTS

Isolation of bioactive compound from ethanol extract of *T. cordifolia* aerial

Five grams of ethanol extract of aerial part of *T. cordifolia* were loaded into a column packed with a solution of silica gel (60–120 mesh) with DCM using wet slurry method. The fractions are eluted with 5% methanol and 95% DCM. The fractions are collected from the column were subjected to thin-layer chromatography. Then concentrated under vacuum at 50°C and it was dried by high vacuum at 60°C for 15 min to get brown solid. The isolated bioactive compound was subjected to various spectroscopic methods, namely, FTIR, ^{1}H NMR, and LC-MS to elucidate the structure.

FTIR spectroscopic analysis

The FTIR spectrum of purified isolated compound from ethanol extract from aerial part of *T. cordifolia* is shown in Fig. 1.

The FTIR spectrum of compound shows a broad band of 3250 cm^{-1} corresponding to the phenolic OH group. The peak at 1217 cm^{-1} corresponds to O-C stretching of aryl ether. Methyl group connected with oxygen was seen at 2848 cm^{-1}. The peak at 1450 cm^{-1} corresponds to $N(CH_3)_2$ group. Aromatic C=C stretching bands were observed in the range of 1600–1400 cm^{-1}.

^{1}H NMR spectroscopic analysis

The ^{1}H NMR spectrum of isolated compound from ethanol extract from aerial parts of *T. cordifolia* is shown in Fig. 2.

The ^{1}H NMR spectrum was recorded using DMSO as the solvent. The peak at 2.272 ppm, 2.273 ppm, 3.775 ppm, and 3.834 ppm corresponds to methyl proton. The peak in the region of 2.64–3.08 ppm corresponding to methylene proton and the peak at 2.513 ppm implies DMSO. The resonance peaks at aromatic protons found in the region of 6.778 ppm–6.956 ppm. The peak at 10.447 ppm corresponds to phenolic proton.

LC-MS spectroscopic analysis

The LC-MS spectrum of pure isolated compound from ethanol extract of *T. cordifolia* aerial was shown in the Fig. 3.

The m/z value of the isolated compound is 342.7. The exact mass of the compound is 342.41 g/mol. The molecular formula for the isolated compound is $C_{20}H_{24}NO_4$+. Based on the above result, the purified compound was identified as Magnoflorine and its structure is depicted in Fig. 4. The International Union of Pure and Applied Chemistry (IUPAC) name of the compound is found to be 1,11-dihydroxy-2,10-dimethoxy-6,6-dimethyl-5,6-dihydro-4H-dibenzo[de.g]quinolin-6-ium.

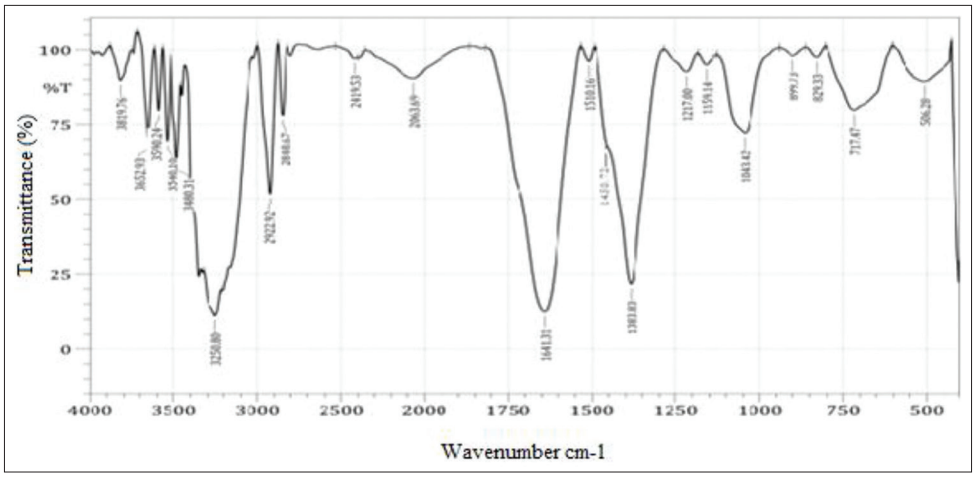

Fig. 1: Fourier transform infrared spectrum of isolated compound from *Tinospora cordifolia* aerial

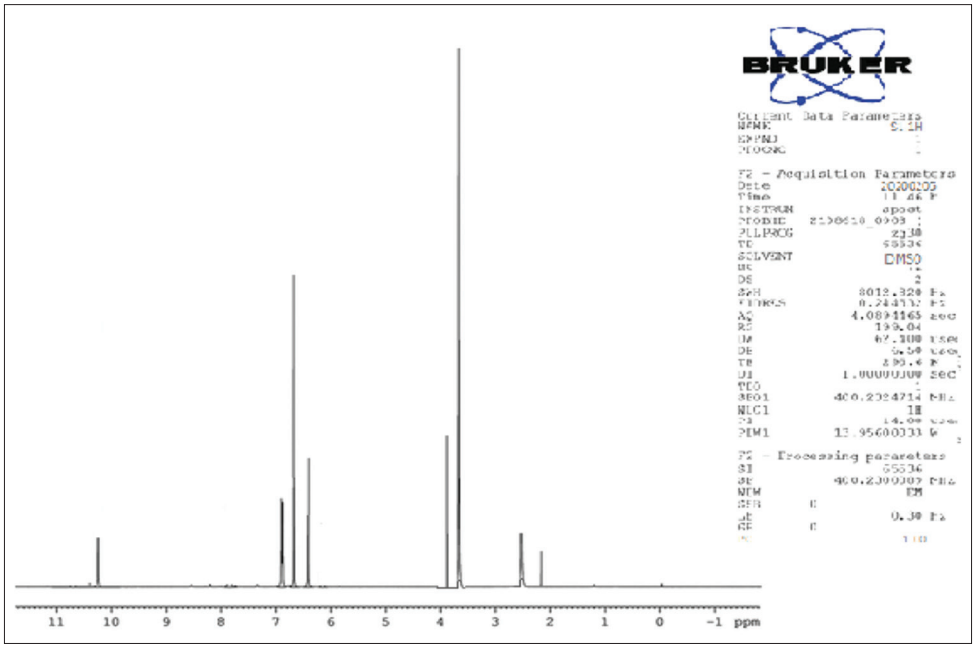

Fig. 2: ^{1}H nuclear magnetic resonance of isolated compound from *Tinospora cordifolia* aerial

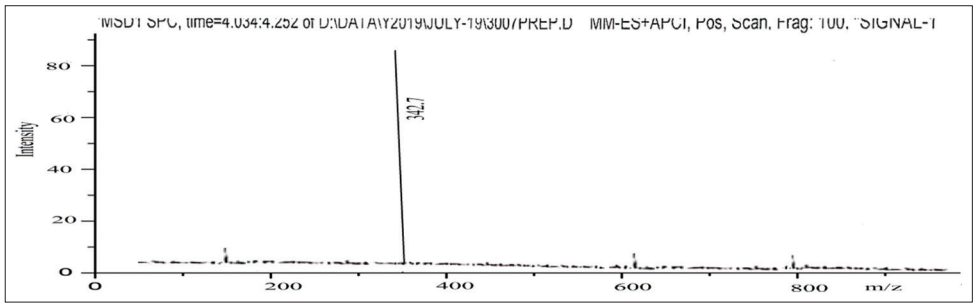

Fig. 3: Liquid chromatography–mass spectrometry spectrum of isolated compound from *Tinospora cordifolia* aerial

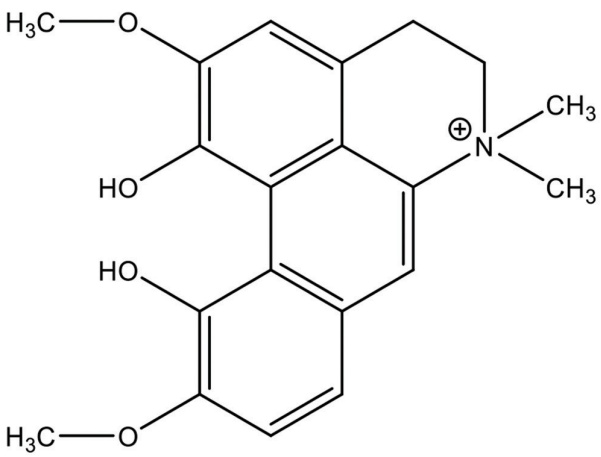

Fig. 4: Structure of Magnoflorine isolated from aerial parts of *Tinospora cordifolia*

Bioactive properties of isolated compound from *T. cordifolia* aerial

Antioxidant activity

DPPH method is used to determine radical scavenging activity of isolated compound Magnoflorine. The result presented in Table 1. Magnoflorine shows minimum activity 52% at the tested concentration 100 µg/ml and shows maximum activity 64% at the tested concentration 500 µg/ml.

Table 1: Radical scavenging activity of isolated Magnoflorine

Concentration(µg/ml)	RSA (%) *T. cordifolia*	Standard (%)
100	52	38.9
200	54	54.2
300	60	71.1
400	62	74.5
500	64	99.8

T. cordifolia: Tinospora cordifolia; RSA: Radical scavenging activity

Anticancer activity

MCF-7 is a breast cancer cell line isolated in 1970 from a 69 year old Caucasian woman. MCF-7 is the acronym of MCF-7, referring to the institute of Detroit where the cell line was estimated in 1973 by Herbert Soule *et al*.

The anticancer activity of isolated Magnoflorine from T. cordifolia aerial proved that the cancer cell line inhibited their activity significantly with the increase in drug concentration. It was observed that MCF-7 cell line more cytotoxicity effect was observed in drug for 24 h treatment. The cell viability assay was conducted to assess the effect of cell growth of A540 cells that were treated with isolated compound of Magnoflorine from T. cordifolia aerial are presented in Table 2 and Fig. 5.

MCF-7 MTT assay

The percentage of cell viability varied from 53.3% at the minimum tested concentration 3.12 µg/ml to 1.9% at the maximum tested concentration

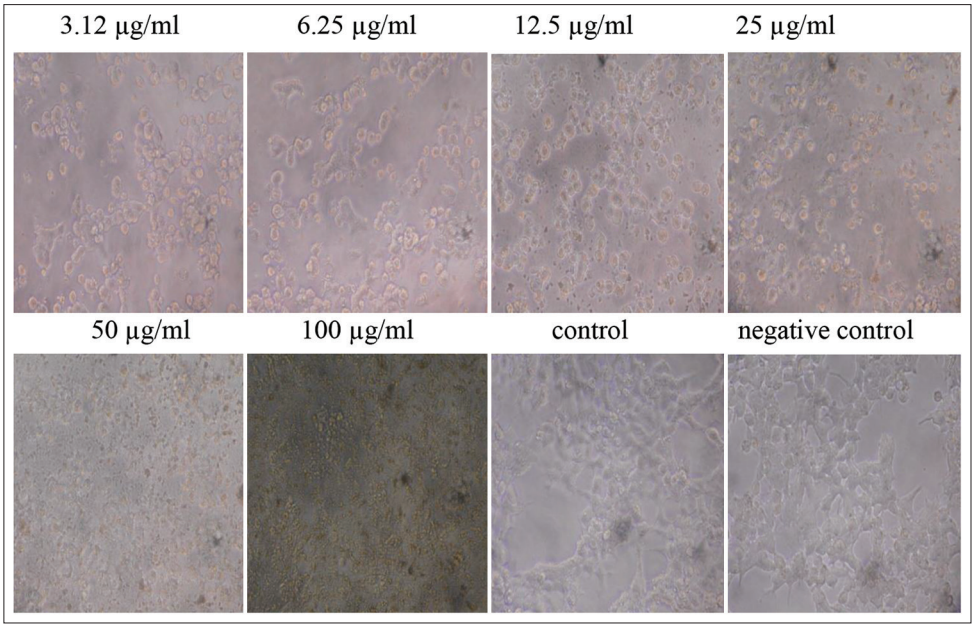

Fig. 5: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay–isolated Magnoflorine from *Tinospora cordifolia* aerial

Table 2: Anticancer activity of isolated compound Magnoflorine from *T. cordifolia*

Concentration (µg/ml)	MCF 7-MTT Assay
	Cell viability (%)
100	1.9
50	4.7
25	14.2
12.5	24.7
6.25	38.0
3.12	53.3
DMSO	98
Control cells	100

MCF-7: Michigan Cancer Foundation-7;
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;
DMSO: Dimethyl sulfoxide

100 µg/ml. This result reveals the increasing concentration decreases the cell viability. That means to increase the cell death. IC_{50} values of this compound are 3.74 µg. The percentage of cell death is 97.9.

DISCUSSION

Magnoflorine is a quaternary benzylisoquinoline alkaloid of the aporphine structural subgroup which has been isolated from various species of *Menispermaceae* family. It has a role as plant metabolites. In recent year, Magnoflorine has received increasing attention due to its multiple pharmacological activities. Magnoflorine is expected to be a potential drug candidate for the treatment of diabetes, depression, and Alzheimer's disease. Magnoflorine is practically insoluble in water and a very weak acidic compound. Magnoflorine is an inhibitor of nuclear factor kappa B activation and to be an agonist at the β2–adrenergic receptor [10].

CONCLUSION

Bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problem. The chromatographic technique is the most valuable in the identification of phytochemicals. The isolated bioactive compounds are identified using standard spectroscopic studies, namely, FTIR, ^{1}H NMR, and LC-MS. Based upon the spectroscopic analysis, the structure of the bioactive compound is Magnoflorine. Cancer is a complex multifractional disease. Many cancers cannot be cured and some are still very hard to treat. The conventional treatment causes several side effects that can affect a person's quality of life. Magnoflorine isolated from *T. cordifolia* aerial inhibits the growth of MCF-7 breast cancer cell with less side effects and it may be used in the production of new anticancer drug in future.

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AUTHORS' CONTRIBUTIONS

The authors declared that there is no contribution related to this work.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest related to this study.

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