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Der Pharmacia Lettre, 2017, 9 [6]:82-95
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USA CODEN: DPLEB4

Di Preliminary evaluation of In vitro Anti-proliferative Activity of Tinospora cordifolia (willd) Miers and Estimation of Berberine content by HPLC

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ABSTRACT

An indigenous drug, Tinospora cordifolia Miers. (Fam: Menispermaceae) has been defined for its use in cancer in various traditional systems of medicine. In sight of its folklore uses, \in breast and colorectal carcinoma cell lines its anticancer activity was evaluated and to estimate the berberine content an attempt was done. Ethanol extract (TCE) and subsequent petroleum ether (TCP), dichloromethane (TCD), n-Butanol (TCB) and aqueous (TCA) fractions of stems of T.cordifolia were prepared. Anti-proliferative activity was assessed breast cancer (MCF-7) and colorectal cancer (HCT-116) cell lines by MTT and SRB assay. By using HPLC berberine content in extract and fractions was estimated.The results demonstrates that the dichloromethane fraction and ethanol extract of T.cordifolia exerts strong cytotoxicity against HCT-116 with an IC_{50} of 54.24 µg/ml and 101.26 µg/ml; MCF-7 with an IC_{50} of 59.59 µg/ml and 107.2 µg/ml respectively by MTT assay; and HCT-116 with an IC_{50} of 48.92 µg/ml and 87.54 µg/ml; MCF-7 with an IC_{50} of 51.98 µg/ml and 94.23 µg/ml respectively through SRB method. The results of HPLC demonstrates that TCD contain the highest concentration of berberine (1.84 % w/w) and it was done by using berberine as a marker. Our outcomes proposed that TCE/fractions have major anti-proliferative activity which can be accredited to the presence of berberine. Besides, TCD emerged as the most effective fraction and the molecular mechanism along with the isolation of phyto-constituents responsible for its anti-cancer activity will be doing in further studies along with the screening for cytotoxicity in other available cancer cell lines.

Key words: *T.cordifolia*, Anti-proliferative activity, Berberine, HCT-116, Breast cancer, MCF-7.

INTRODUCTION

Cancer is known to be the second most common cause of death, surpassed only by cardiovascular disease. The ACS report 2014 establishes, nearly 1 in every 4 deaths can be attributed to cancer with a possibility of 585,720 deaths due to cancer this year. There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) worldwide, reported by IARC in 2012. Breast and ovarian cancer are the major cause of cancer death in American women. In India about 5.55 Lakh cancer deaths have been reported in 2010. Despite tremendous advances in the Cancer chemotherapy, search for new and better chemotherapeutic agent is relentlessly pursued. Compounds of natural origin have provided potential leads for cancer chemotherapy in the past; many of them are drugs of choice in cancer treatment. Taxol for breast cancer, Vinca alkaloids for leukemia, Podophyllo toxin, etoposides and campototheca etc. are some examples of the natural products used in cancer treatment. Herbs these days are also being used as chemo-protectant against severe adverse effects caused due to cytotoxicity of anticancer drugs. The available chemotherapeutic agents including herbal medicines such as Paclitaxel, Etoposide and Vincristine etc. are highly expensive due to less abundance of plants and very less amount of active constituents.

An indigenous drug, *Tinospora cordifolia* Miers. (Fam: Menispermaceae) is an important medicinal plant cultivated throughout the Indian subcontinent. It has been extensively used for the treatment of various ailments [1]. *T. cordifolia* contains several classes of secondary metabolites such as alkaloids, glycosides, diterpenoids, lactones, steroids, sesquiterpenoids, aliphatics, and phenolic compounds. Among the alkaloids, the roots and stems of the plants are reported to contain berberine, palmatine, tembatarine, magniflorine, choline, tinosporine, isocolumbine, and minor amount of jatrorhizine [2]. The major isolated compounds include the norditerpene furan glycosides such as cordifolioside-A, B, C, D, and E [3], the ducan type sesquiterpenes tinocordifolin and tinocordifolioside [4] and the furanoids diterpene glycoside palamatoside C and F and amritosides [5]. The clerodane diterpenoides, cordioside, tinosponone and tinocordiside were also present in this plant [6]. Other constituents includes phenolic lignane, octacosanol, heptacosane, beta sitosterol, tinosporidine, cordifolin A and siringine were also reported in *T.cordifolia* [7].

Literature survey and ethnopharmacological background of *T. cordifolia* suggested, its manifold pharmacological responses such as immune-stimulant [1, 8], hepato-protective, diuretic, anti-inflammatory, radioprotective [9], antioxidant [10] and hypoglycemic [11]. Among the above described activities, immune-stimulating, hepato-protective, and antioxidant activities may be responsible for protection against radiation and alkylating chemicals.

Hence we aimed to investigate the anti-proliferative effect of stem extract and it's fractions from *Tinospora cordifolia* against human colorectal cancer cells HCT 116 and breast cancer cells MCF-7 and this is the first preliminary study which employed the

stems of this plant as potential source of cytotoxic agent and to estimate the berberine content in initial extract and its subsequent most active fraction and correlate it's content to cytotoxic potential, an attempt was made.

MATERIALS AND METHODS

Plant collection

The stems of *Tinospora cordifolia* were procured from local market of Udupi, Karnataka and the identification of the plant was done by Dr. Gopala krishna Bhat, Taxonomist, Udupi. The plant was matched with voucher specimen (PP 614) in the institute and for its further validation; the microscopic characteristics of this plant were studied and matched with available literature. The fresh plant material collected was air-dried and grinded to fine powder and stored in air-tight containers for further studies.

Reagents and Chemicals

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) reagent and SRB (Sulforhodamine B) were procured from Sigma Aldrich, USA. Methanol, ethanol (95%), dichloromethane, acetonitrile, petroleum ether and DMSO were obtained from usual source and all were of analytical grade. The standard berberine marker was purchased from Sigma Aldrich, USA.

Preparation of extract and its fractionation

The shade-dried and coarsely powdered stems of *T.cordifolia* were exhaustively extracted with absolute ethanol by conventional soxhlet extraction. After completion of extraction, the extract was concentrated under reduced pressure and controlled temperature and stored in desiccator for further use. The ethanol (TCE) extract was dissolved in water and partitioned with 3times each of petroleum ether (TCP), Dichloromethane (TCD), *n*-butanol (TCB) and the last fraction was aqueous (TCA). All the fractions were dried by using rotary evaporator under reduced pressure and controlled temperature and stored for further use. Percentage yield of extract/fractions was calculated and reported.

Cell culture

Human colorectal cancer cells (HCT 116); breast cancer cells (MCF-7) were obtained from National Centre for Cell Science, Pune, India. By using Dulbecco's Minimum Essential Medium (DMEM) with 10% fetal bovine serum (FBS) and 50µg/mL gentamicin cells were cultured. The cells were grown by incubating at 37°C with humidified atmosphere by providing 5% CO_2 and 95% air in CO_2 incubator. By using inversion microscope to see the morphology and cell growth, while ensuring that no contamination occurs in a culture flask the cultured cells were observed and checked daily. The cells were sub-cultured up to 70% - 90% collisions between the cells. For cell viability assay, cells which are in their exponential phase were used.

Cell viability by MTT assay

MTT assay

[12] From 75 cm^2 tissue culture flasks HCT-116 (colorectal carcinoma) and MCF-7 (Breast cancer) cells were collected and a stock cell suspension ($1x10^5$ cells/ml) was prepared. A 96-well plate was seeded with 0.1 mL of DMEM medium and supplemented by adding 10% FBS and allowed to attach for 24hrs. Just prior to the experiment, test compounds were dissolved in 0.1% DMSO. Cells were treated with 20 µL of test solutions from respective stocks (25, 50, 100 and 200µg/ mL) after 24hours of incubation, a fresh medium of 80 µL was added and incubated for 48 hrs. The medium containing 0.1% DMSO alone served as the control. Doxorubicin was used as standard. After the treatment drug containing media was removed and washed with 200µL of PBS. After adding 100µL of MTT reagent, cells were incubated for 4hours at 37° C. After incubation, MTT reagent was removed by inverting the plate and formazan crystals were solubilized by adding 100µL of 100% DMSO. An ELISA plate reader at 540 nm was used to measure the optical density (O.D). Percentage viability of the each extract was calculated using the formula: Percent viability = ((Control-Blank) – (Test-Blank)/ (Control-Blank)) x 100. Experiment was done in triplicates; Results were expressed as Mean ± SEM values (proportional to cell survival) and graphs were plotted against the drug concentrations which were tested for cytotoxicity.

SRB assay [13]

100 µL of cell suspension (HCT-116 and MCF-7) were introduced into each well of 96-well tissue culture plate. Cells were treated with 100 µL of various concentrations (25, 50, 100 and 200 µg/mL) of the test solution and incubated for 48hrs. The medium containing 0.1% DMSO only served as control and Doxorubicin was used as standard. After incubation, cells were fixed by treating with ice cold TCA for 1 h at 40° C. Plates were washed and allowed for drying. Cells were subjected for staining at room temperature for 30min by adding 50 µL of SRB solution. 1% v/v acetic acid was added to remove unbound SRB and allowed to dry. 100 µL of 10 mM unbuffered Tris Base (pH 10.5) was added to solubilize the bound SRB and the plate was kept on a shaker platform for 5 min. An ELISA plate reader at 570 nm was used to measure the optical density (O.D). The IC_{50} values were determined by plotting O.D values against the tested concentrations of the drug. Percentage viability of the each extract was calculated using the formula: Percent viability = ((Control-Blank) – (Test-Blank)/ (Control-Blank)) x 100. Experiment was done in triplicates; Results were expressed as Mean ± SEM values (proportional to cell survival) were plotted against the drug concentrations which were tested.

HPLC estimation of Berberine [14]

HPLC test samples were prepared by dissolving 10 mg of ethanol extract and dichloromethane fractions in HPLC grade methanol. Samples were shifted to 2 mL eppendorf tubes after filtering with 0.2 µm syringe filter. To obtain 5 mg/mL concentration volume was made up to 2 mL with methanol and used for analysis. 0.1 mg/mL of standard berberine was weighed and dissolved in 1 mL of methanol (HPLC grade). For the analysis, Shimadzu (Kyoto, Japan) HPLC system consisted of pump

(LC-10AT VP), photodiode array detector (SPD-M10A VP), CLASS-VP 6.12 SP5 integration software and a Rheodyne injection valve fitted with a 20 µl injection loop, was used. By using a Phenomenex Luna C-18 column (250 × 4.6 mm; 5 µm) and an isocratic solvent system consisting of acetonitrile-water in the ratio 10:90 (v/v). Baseline resolution of berberine was obtained at 25 ± 2°. By using 0.45 µm PVDF filter mobile phase was filtered and which was degassed before using. The flow rate was kept constant at 0.6 mL/min and the detection was at 266 nm. The standard solution was injected in triplicate and the average detector response was measured. The stem extracts were assayed in triplicate and then by comparing with the reference standard berberine retention time and peak area extract and its fractions were identified.

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM) of three replicate determinations and then analyzed by Graph pad prism 5. One way analysis of variance (ANOVA) and post-hoc Tukeys test were used to determine the differences among the means. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Extraction and fractionation

Percentage yield of ethanol extract and fractions were reported in (Table 1)

Table 1: Percentage yield (% w/w) of extract and its subsequent fractions of *T.Cordifolia*

S.No	Extract/Fraction	% yield (w/w)
1	Ethanol (TCE)	7.4
2	Petroleum ether (TCP)	19.08
3	Dichloromethane (TCD)	17.9
4	n-Butanol (TCB)	16.21
5	Aqueous (TCA)	42.56

Anti-proliferative activity

Phyto-compounds have been used as an outstanding source of drug leads from several decades due to their unique nature and their wide range of structural diversity and are "biologically friendly" by Mother Nature [15]. A study reported recently by the European anticancer drug market states that 155 anti-tumor drugs accepted clinically of which 47% were obtained from natural source or derivative products from them [16].

MTT assay - In vitro

T.cordifolia ethanol extract and its fractions were evaluated for anti-proliferative activity against breast carcinoma cell lines MCF-7 and colorectal carcinoma HCT-116 using MTT (3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Based on the principle involved in MTT assay which is the conversion of yellow colored tetrazolium (MTT) to the purple colored formazan dye by reduction reactions in the presence of mitochondrial dehydrogenase enzyme, which is present only in viable cells and the quantity of fromazan is inversely proportional to the number of dead cells [17]. The cytotoxic potential results visibly demonstrates that the dichloromethane fraction and ethanol extract of *T.cordifolia* exerts strong cytotoxicity against HCT-116 with an IC_{50} of 54.24 µg/mL and 101.26 µg/mL; MCF-7 with an IC_{50} of 59.59 µg/mL and 107.2 µg/mL respectively. Inhibition of tumor cell growth was in dose-dependent manner by both ethanol extract and dichloromethane fractions of *T.cordifolia*. On the other hand successive fractions of ethanol extract which includes, petroleum ether, *n*-butanol and water fractions, shown relatively distinct cytotoxic effect (Table 2 and Figure 1(A and B)). Similar cytotoxic activity between extract and its fractions was obtained by Tukey's post hoc tests study; and doxorubicin as indicated in (Table 2).

Table 2: Cytotoxicity results of *T.cordifolia* by MTT assay

S.No	Concentration (µg/mL)	% Cell Death					
		TCE	**TCP**	**TCD**	**TCB**	**TCA**	**Doxorubicin**
1	0.01	--	--	--	--	--	40.95±0.69*
2	0.1	--	--	--	--	--	49.27±0.31*
3	1	--	--	--	--	--	67.04±0.90*
4	10	--	--	--	--	--	81.28±1.04*
5	25	39.29±0.36*	29.24±0.60*	55.02±0.68**	3.63±1.51**	28.58±2.51**	--

6	50	56.06±0.73*	53.43±0.48*	55.95±043**	29.79±1.73	25.84±1.98	--
7	100	66.07±0.51*	66.00±1.12*	73.90±0.59**	40.95±0.69*	13.47±2.88	--
8	200	80.97±1.71*	77.30±0.33*	81.60±2.25**	76.54±0.58**	84.02±0.46*	--
9	IC_{50}	101.26±1.42[a]	131.59±1.23[c]	54.24±1.32[a,b]	276.06±2.00	366.05±1.74	1.89±0.02[b]

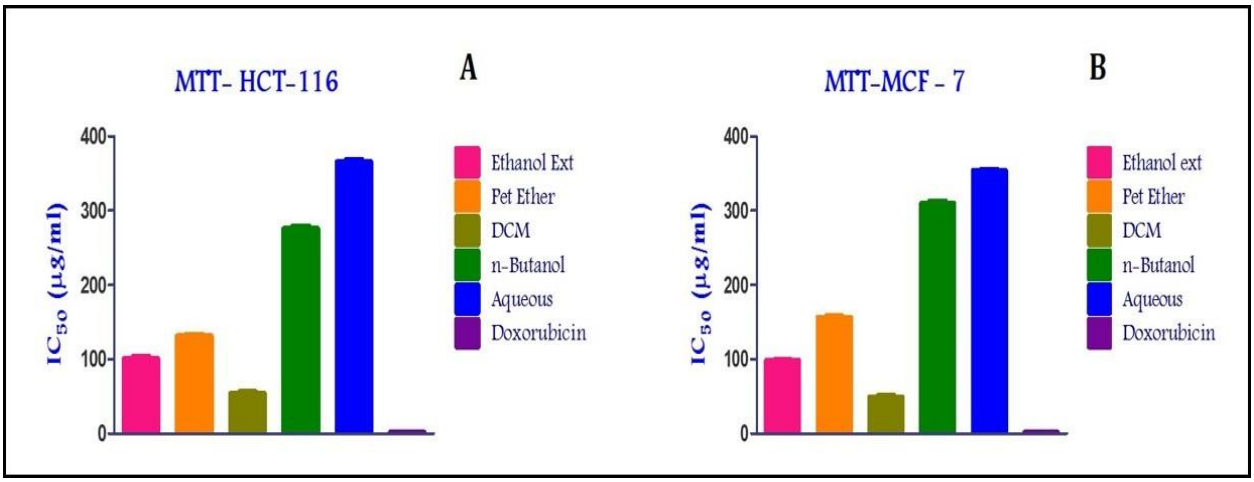


Figure 1: *Invitro* MTT assay on HCT-116 and MCF-7 cells

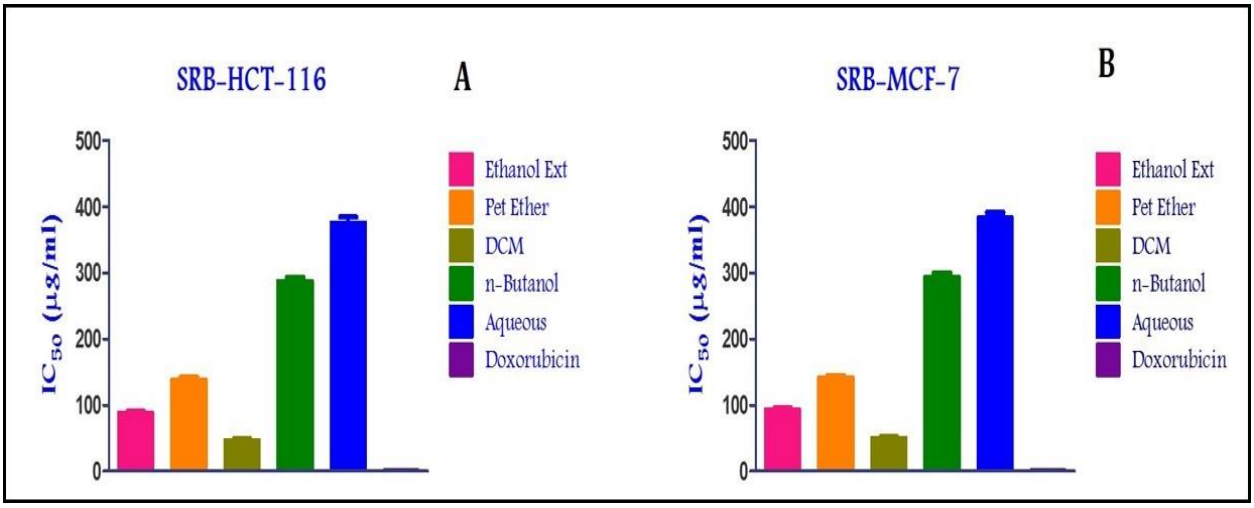
SRB assay - In vitro

T.cordifolia ethanol extract and its fractions were evaluated for anti-proliferative activity against breast carcinoma cell lines MCF-7 and colorectal carcinoma HCT-116 using SRB assay. Based on the measurement of cellular protein content, a unique method called Sulforhodamine B (SRB) assay to determine the cytotoxicity was developed by Skehan and his coworkers [18]. The results of SRB assay clearly demonstrate that the dichloromethane fraction and ethanol extract of *T.cordifolia* exerts strong cytotoxicity against HCT-116 with an IC_{50} of 48.92 µg/mL and 87.54 µg/mL; MCF-7 with an IC_{50} of 51.98 µg/mL and 94.23 µg/mL respectively similarly like MTT assay. Inhibition of tumor cell growth was in dose-dependent manner by both ethanol extract and dichloromethane fractions of *T.cordifolia*. On the other hand successive fractions of ethanol extract which includes, petroleum ether, *n*-Butanol and water fractions, shown relatively distinct cytotoxic effect (Table 3 and Figure 2). Similar cytotoxic activity between extract and its fractions was obtained by Tukey's post hoc tests study; and doxorubicin as indicated in (Table 3).

Table 3: Cytotoxicity results of T.cordifolia by SRB assay

S.No	Concentration	% Cell Death					
	(µg/mL)	TCE	TCP	TCD	TCB	TCA	Doxorubicin
1	0.01	--	--	--	--	--	40.95±0.69**
2	0.1	--	--	--	--	--	49.27±0.31**
3	1	--	--	--	--	--	67.04±0.90**
4	10	--	--	--	--	--	81.28±1.04**
5	25	37.67±0.36**	27.35±0.62*	53.82±0.70**	6.40±2.56**	32.01±6.73	--
6	50	54.89±0.75**	52.18±0.49*	54.78±0.57**	27.92±0.89	29.20±3.14	--
7	100	65.17±0.53**	65.10±1.15**	73.21±0.60**	39.38±0.17*	11.17±5.01	--
8	200	80.46±1.76*	76.69±0.93*	81.10±2.31**	75.91±0.59**	83.60±0.48*	--
9	IC_{50}	107.20±0.80[a]	138.70±1.05[c]	59.59±1.09[a,b]	287.14±1.98	374.09±1.25	1.89±0.02[b]

Figure 2: Invitro SRB assay on HCT-116 and MCF-7 cells



Overall, both MTT and SRB assay for anti-proliferative activity of *T.cordifolia* extracts against HCT-116 and MCF-7 cell line followed the similar trend. To some extent higher IC_{50} values by SRB assay was observed in aqueous and *n*-butanol fractions of *T.cordifolia* and on the other hand IC_{50} values of ethanol extract and dichloromethane fraction, were found to be somewhat lesser than MTT assay. There was good correlation between the IC_{50} values attained by both MTT and SRB assays, as a whole. Thus we conclude that *T.cordifolia* extract and fractions hold effective anti-proliferative activity.

We witnessed that aqueous and *n*-Butanol fractions of *T.cordifolia* demonstrated relatively less anti-proliferative activity against MCF-7 and HCT-116 cell lines than dichloromethane fraction and ethanol extract. Due to the presence of highly polar constituents such as carbohydrates, amino acids, glycosides, proteins and minerals any plant aqueous and *n*-butanol fractions express its cytotoxicity comparatively at higher concentration (>250 µg/mL) than the non-polar extracts/fractions attained from non-polar solvents like petroleum ether, acetone, chloroform, ethyl acetate etc. Additionally, the presence of constituents such as carbohydrates, glycosides, saponins and tannins in the water extract has been revealed by the phytochemical screening of *T.cordifolia*. Precisely, terpenes and terpenoids were not found in *T.cordifolia* aqueous extract because of its polar nature towards dissolving terpenes and terpenoids and these compounds are particularly soluble in non-polar solvent like hexane, chloroform and petroleum ether [19]. Previous studies reported that sesquiterpens from *T.cordifolia* shown potent cytotoxic activity [20]. So we conclude that the reason for less cytotoxic activity of aqueous fraction of *T.cordifolia* is due to the nonexistence of terpens and terpenoids.

Oxidative stress is among the major causative factor for producing free radicals and reactive oxygen species (ROS). ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH), as well as non-free radicals (H_2O_2) and singlet oxygen. These free radicals cause oxidative stress which leads to harm the cellular molecules like DNA and proteins which is associated with cancer initiation and progression.These free radicles involved in tumor growth by encouraging the transcription of c-fos, c-jun, c-myc (proto-oncogenes) and signaling pathways [21]. Antioxidants are compounds known to be active against these free radicles due to the presence of phenolic and flavonoid kind of phyto-constituents, thus these antioxidants play a major role in cancer prevention by scavenging the free radicles produced in the body [22]. The free radicle inhibition potential of *T.cordifolia* was studied as a part of our research and we found that the methanol, ethanol extracts and n-butanol fractions of *T.cordifolia* were shown powerful antioxidant activity. So we can accept as true that the potent cytotoxic effect of *T.cordifolia* was due to its powerful free radicle scavenging activity.

Estimation of berberine by HPLC

By using HPLC (High performance Liquid Chromatography) and berberine as a marker component content of berberine was estimated in ethanol extract and dichloromethane fractions. Both, in ethanol extract and dichloromethane fraction berberine was eluted at RT 5.51 (as per the chromatograms in Figure 3A-C).The dichloromethane fraction of *T.cordifolia* contains the highest concentration of berberine (1.84 % w/w) and low concentration of berberine in ethanol extract (0.86% w/w) which can be seen in (Table 4 and figure (3A-C)).

Table 3B: Cytotoxicity results of *T.cordifolia* by SRB assay on MCF-7 (Human breast carcinoma)

	Concentration	**% Cell Death**

S.No	(µg/mL)	TCE	TCP	TCD	TCB	TCA	Doxorubicin
1	0.01	--	--	--	--	--	42.47±0.91**
2	0.1	--	--	--	--	--	48.24±0.27**
3	1	--	--	--	--	--	68.43±1.02**
4	10	--	--	--	--	--	82.54±0.92**
5	25	45.92±0.26*	28.58±0.82	56.12±0.94**	6.83±1.54	14.57±1.52	--
6	50	55.38±0.61**	48.47±0.80**	58.98±0.31**	25.99±1.41	32.58±1.40	--
7	100	68.76±0.17**	68.07±1.01*	72.89±0.84**	39.45±0.71*	28.17±1.28	--
8	200	81.71±1.46**	78.21±0.38*	80.06±0.28**	71.28±0.40*	78.29±0.84**	--
9	IC_{50}	87.54±1.32[b]	137.44±1.47[c]	48.92±0.94[a]	283.91±1.00	377.39±1.87	1.85±0.02[a]

Table 3C: Cytotoxicity results of *T.cordifolia* by SRB assay on MCF-7 (Human breast carcinoma)

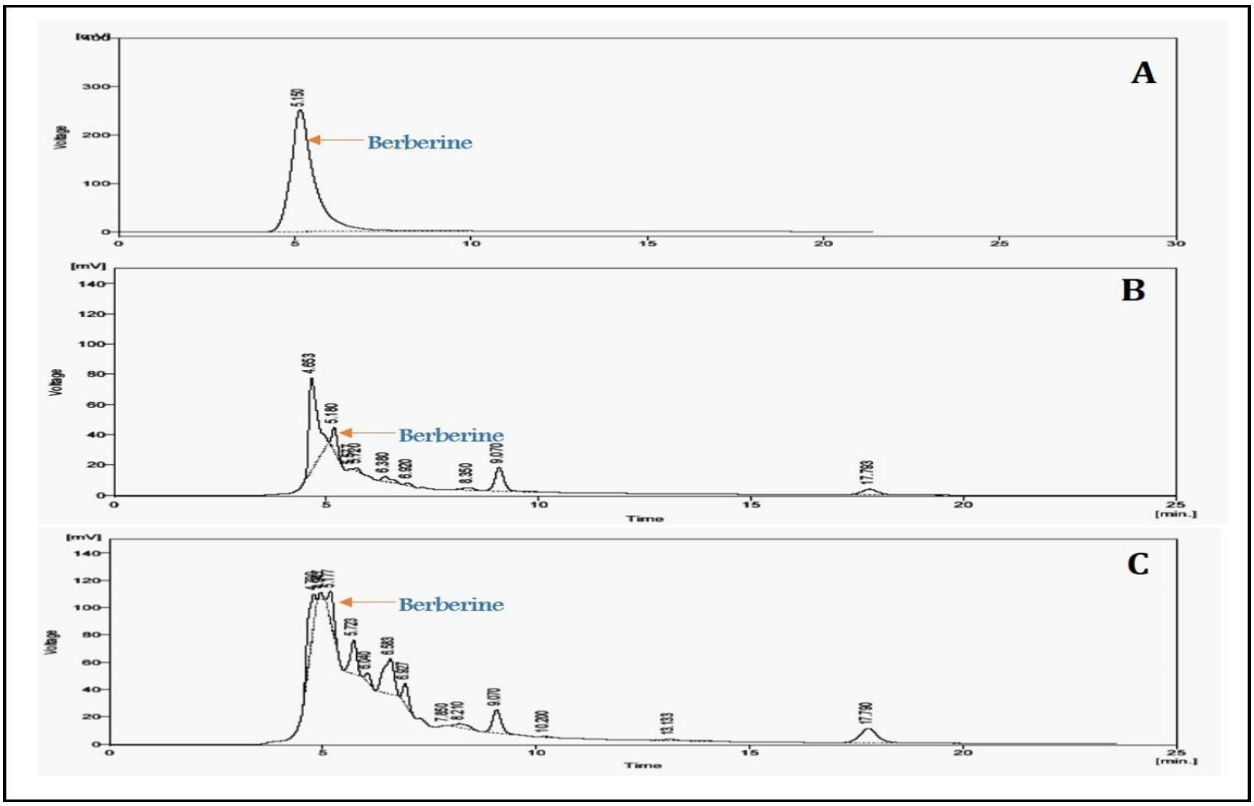
S.No	Concentration	% Cell Death					
	(µg/mL)	TCE	TCP	TCD	TCB	TCA	Doxorubicin
1	0.01	--	--	--	--	--	42.47±0.91**
2	0.1	--	--	--	--	--	48.24±0.27**
3	1	--	--	--	--	--	68.43±1.02**
4	10	--	--	--	--	--	82.54±0.92**
5	25	42.34±0.16*	29.68±0.68*	54.21±0.10**	5.19±3.54	12.01±4.01	--
6	50	56.94±0.47*	50.78±0.12	58.41±0.74**	26.62±0.14	18.47±3.96	--
7	100	68.25±0.54**	59.45±0.97*	74.24±0.25**	40.81±2.81*	31.17±5.00	--
8	200	80.05±0.92**	74.87±0.19**	82.99±1.01**	69.61±2.95*	81.09±0.43**	--

9	IC_{50}	94.23±0.38[b]	142.26±1.35[c]	51.98±0.82[a,b]	294.46±2.36	384.44±1.09	1.85±0.02[a]

Table 4: Estimation of berberine by HPLC

S.No	Sample	Retention time (RT)	Peak Area	Percentage Content (%w/w)
1	Berberine	5.15	1.2E+07	98
2	Ethanol Extract	5.18	5201656	0.86
3	TCD Fraction	5.177	1.1E+07	1.84

Figure 3: Estimation of berberine in T.cordifolia ethanol extract and its fractions



The anti-proliferative potential of ethanol extract and its dichloromethane fraction is possibly due to the existence of berberine in them. The results established through HPLC fingerprinting confirm that berberine is present in the (Figure 3) in dichloromethane fraction of *T.cordifolia.* Some studies reported that berberine is a natural isoquinoline alkaloid used for eras in different folklore medicines [23], has shown *in vitro* anti-proliferative and cytotoxic activity against human breast cancer (MCF-7), human cervical cancer (HeLa) and leukemia (L1210) cells [24], it also shown to be cytotoxic in human colorectal cancer cells (HCT-116), human gastric carcinoma (SNU-5), prostate cancer ((LNCaP) and human adenocarcinoma (HepG2) cell [25], Remarkably, it has shown not the same effects on the cell cycle, with cell cycle arrest G_1 phase [26-28] and arrest at G_2/M phase of the cell cycle along with the no effect on cell cycle, depending on the cell line type used for the study [29-30]. In A431cell line (human epithelial carcinoma) by up regulating the proteins like Kip1/p27 and Cip1/p21, it induced cell cycle arrest at G1 phase by the formation of heterotrimeric complexes and it also has been reported that it also arrest cell cycle by inhibiting Cdk4 and Cdk6, in addition to decrease in the expression of cyclins D1, D2 and E. Some studies reported berberine as anti-cancer compound by cell cycle arrest at S-phase by inhibiting the DNA synthesis [31]. Kettmann V *et al*., reported that in cervical cancer (HeLa) and leukemia (L1210) cell lines berberine induces the cell death by DNA topoisomerase I poisoning [32-33]. Even though cell cycle components may not be intricate in all forms of cell death, in several instances apoptosis and cell proliferation can share mutual pathways which are related closely [34]. Those proteins which regulate the apoptotic pathway have directly control on the cell cycle [35, 36], as it can be proved when anti-apoptotic action withholding the proliferative action caused during earlier tumor management [37].

CONCLUSION

The ethanol extract and dichloromethane fractions of *T.cordifola* shown noteworthy anti-proliferative activity in MCF-7 (breast carcinoma) and HCT-116 (human colorectal carcinoma) cell lines. So, it can be concluded that *T.cordifoila* has the potential to be established as a chemo-preventive option for cancer. Though, it shown cytotoxicity at this stage it is very difficult to accomplish the possible mechanism. So, the molecular mechanism along with the isolation of phyto-constituents responsible for its anti-cancer activity will be doing in further studies along with the screening for cytotoxicity in other available cancer cell lines.

Acknowledgements: The authors thank Manipal University, Manipal College of Pharmaceutical Sciences, Manipal, India for providing the facilities to carry out this study.

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