Phytochemical, *In-silico* Analysis and Anticancer Activity of a Bioactive Principle isolated from *Amaranthus tricolor* (L)

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Abstract
Cancer is a serious concern at present. Development of affective and side effects lacking anticancer therapy is the trending research direction in healthcare. Bioactive phytochemicals are preferred as they pretend differentially on cancer cells only without altering normal cells. The present study is aimed to isolate, characterize and to evaluate anticancer activity of a lead phytochemical from leaves of Amaranthus tricolor (L). The methanolic extract of A. tricolor (L) leaves was prepared by cold maceration and labelled as ATME. The ATME extract was fractionated with equal volume of chloroform and water. The chloroform extract was further fractionated with n-hexane:ethyl acetate (6:4 v/v) as mobile phase suggested by HPTLC.

Based on bioautography the third fraction was selected for further in-silico analysis and anticancer activity. The structural interpretation of isolated compound SOWIS-III was determined as a flavonol glycoside 24-methylene cycloartanol. It was docked with human oestrogen receptor and confirmed as anticancer lead molecules. The antioxidant property for 24-methylene cycloartanol was determined by DPPH method. It showed strong radical scavenging property in dose dependent manner. The IC_{50} values of 24-methylene cycloartanol and standard ascorbic acid was found to be 31.03 and 14.29 µg/mL respectively. The compound 24-methylene cycloartanol inhibited the growth of MCF-7 cells from human breast cancer with IC_{50} value 16.93 µg/mL and cisplatin with IC_{50} value 4.586 µg/mL determined by MTT assay. It was observed that the tested phytochemical showed promising anticancer property towards the selected cancer cell lines.

Keywords: *A.tricolor*(L), 24-methylene cycloartanol, anticancer property.

Introduction
Cancer is a group of diseases with abnormal growth of cells and multiplying with the inability to be controlled and gradually expands throughout body and leads to death by invading and killing the cells. According to recent survey, it is one of the major leading causes of death. Globally, a constant battle has been going on with lot of research and development in preventive and curative therapies of cancer. Current therapies such as chemotherapy and radiotherapy put the patients under a lot of stress and further damage their health. Therefore, there is a focus on using new or alternative treatments with less or nil side effects.

World is endowed with a rich wealth of medicinal plants. A good number of drugs that are being used today are isolated from plant sources. They hold great promise for the discovery and development of new pharmaceuticals in diverse human ailments. Medicinal plants have received increasing attention over the past 3 decades for their potential therapeutic anticancer activity[1,2]. It was evidently proven that phyto-pharmaceuticals act as inhibitors at various stages of tumorigenesis. Currently the drugs used for the treatment of cancer have been isolated from natural products[3].They are less injurious with reduced side effects when compared to current drugs used in chemotherapy. Many medicinal plants are still not explored scientifically for their medicinal potential.

In the current study we tried to isolate an anticancer bioactive principle from leaves of *Amaranthus tricolor* (L). It is an ornamental plant belongs to the family Amaranthaceae. The leaves are highly nutritious and possess fiber, protein, vitamin A, vitamin C, thiamin (Vit B_1), riboflavin (Vit B_2), niacin, minerals like carbohydrates, iron and calcium. Because of huge nutritional value; the plant received considerable attention. The plant was scientifically proven for diuretic and astringent[4], control haemorrhage following abortion[5], antibacterial[6,7], hepatoprotective[8], antioxidant[9], antihyperglycaemic and antinociceptive activities[10].

Material and Methods
Plant Selection: The plant *Amaranthus tricolor* was collected from fields in and around Guntur andhra Pradesh, India. The plants were identified and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Ref. No. BSI/SRC/5.23/2016/Tech./197).

Phytochemical Analysis: Methanol, Chloroform, n-hexane, ethyl acetate.

***In-silico* Analysis:** 24-Methylene cycloartanol, oestrogen receptor.

Antioxidant and Anticancer Activity: Ascorbic acid, DPPH (1, 1-diphenyl-2-picryl hydrazyl), MTT (3- (4, 5-

dimethylthiazol-2-yl) -2, 5- diphenyl tetrazolium), tryphan blue, trypsin, EDTA, phosphate buffered saline (PBS) were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) was purchased from Gibco.

Extraction of phytoconstituents: Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The healthy leaves were shade dried and powdered to get coarse powder. The powdered plant material of *A.tricolor* was extracted by cold maceration process[11]. The powder of 1kg each plant material was imbibed with 1 L of methanol and incubated on shaker for 3 days. The extract was filtered using muslin cloth and the process was repeated twice. The collected extracts were pooled together and solvent was evaporated under reduced pressure and labelled as ATME. The percentage yield of extract was calculated and preserved in desiccator for further study.

Preliminary phytochemical screening: The phytochemical screening for the prepared extracts was carried using standard methods[12,13]

Fractionation of phytoconstituents from ATME: 2 g of ATME was dissolved by adding 100 mL of chloroform. The extract was taken in separating funnel and mixed with equal volume of water. The separating funnel was shaken thoroughly to separate chloroform and aqueous fractions. The two fractions were collected separately into china dishes. The chloroform fraction was evaporated and further used for separation of phytoconstituents. The fraction was subjected to HPTLC to optimize mobile phase.

Fingerprint analysis of chloroform fraction of ATME by HPTLC

Instrumentation and Chromatographic conditions: A precoated silica gel aluminium plate 60 F254 (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) was spotted with 10 µL of sample using a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) and CAMAG Linomat 5 sample applicator (Switzerland).

The linear ascending development of TLC plate was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using n-hexane: ethyl acetate (6:4 v/v) as mobile phase. The chamber was saturated with mobile phase for 15 min. The chromatogram was run for 15 min to a length of 8 cm, dried with using hair dryer. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 350 nm operated by WINCATS software version 1.4.2.

Bioautography screening: The fractions of extract with good sensitivity against microorganisms were determined by contact bioautography. The TLC plates after development were air-dried till complete removal of traces of solvent. The chromatogram was placed onto the inoculated agar medium facing down and allowed to diffuse the fractions for 3-4 hours. The TLC plates were removed before incubating and then incubated at 37°C for 24 hours. After incubation clear inhibition zones were observed on the agar surface where the antibacterial spots contacted the agar. The fractions I, III, V, VI, VIII with R_f values 0.07, 0.18, 0.29, 0.48, 0.67 respectively were observed for inhibition of growth of bacteria. Among these the third fraction (SOWIS-III) was selected for further study because of higher inhibition rate of bacteria.

Isolation of Phytoconstituents: The separation and isolation of chloroform fraction of ATME was done by column chromatography as per the HPTLC report compounds present in $CHCl_3$ fraction of ATME. Wet packing mount method was used to pack the column and porosity was enhanced by mixing extract with silica gel G (Mesh 60-120). The separation and isolation of phytoconstituents was done with the help of mobile phase n-hexane: ethyl acetate (6:4 v/v). Similar fractions were pooled together and further purified by preparative TLC and confirmed by HPLC.

Structural Interpretation of Isolated Compounds: The results of bioautography suggested that fractions I, III, V, VI and VIII may act as antibacterial agents. Among these the III fraction, SOWIS-III was further studied for structural interpretation, *in silico* analysis and biological activities evaluation. The purity of the fraction was confirmed by HPLC and the structure was elucidated by NMR and Mass spectroscopical studies. The compound was identified as a flavonol glycoside 24-Methylene cycloartanol (figure 1).

***In silico* analysis:**

Drug target details*:* Oestrogens are involved in the growth, development and homeostasis of a number of tissues. The physiological effects of these steroids are mediated by a ligand-inducible nuclear transcription factor, the oestrogen receptor (ER)[14]. Hormone binding to the ligand-binding domain (LBD) of the oestrogen receptor initiates a series of molecular events culminating in the activation or repression of target genes. Transcriptional regulation arises from the direct interaction of the ER with components of the cellular transcription machinery. The expression of Oestrogens is elevated in many cancers. Hence, it was taken as attractive drug target protein for docking study of 24-Methylene cycloartanol.

Methodology

Ligand preparation: The ligands were initially drawn using chemsketch tool and the chemical language format is converted into protein data bank format for the docking.

Protein preparation: The human oestrogen receptor was prepared with auto dock 4.02v software. The polar hydrogen atoms were added along with kollmann charges and the respective drug target protein was saved in current mode of protein data bank. The protein after adding the polar hydrogen atom was shown in figure 2.

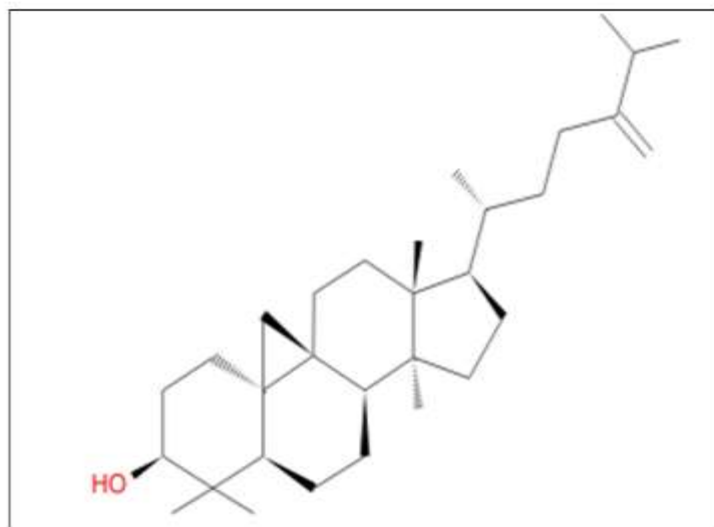


Figure 1: Structure of 24-Methylene cycloartanol

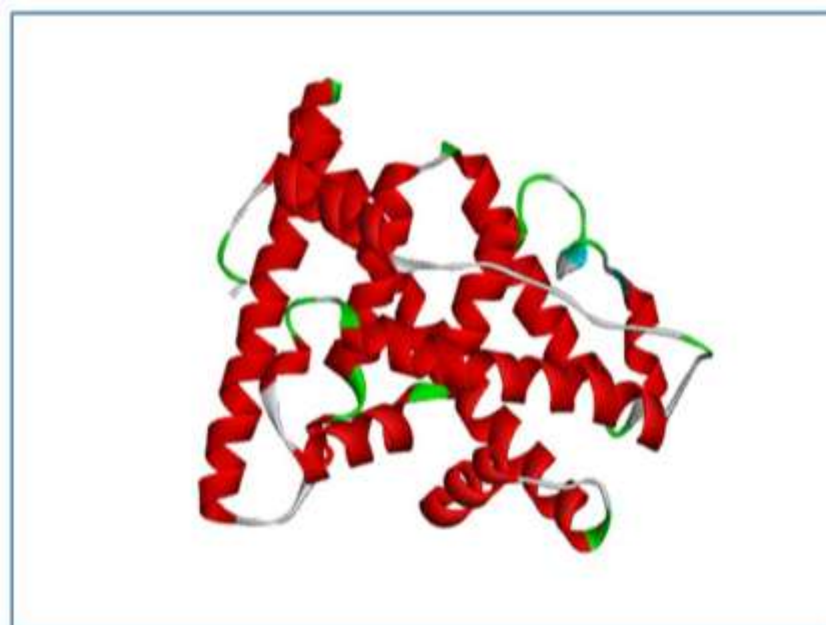


Figure 2: Secondary structure of Human oestrogen receptor

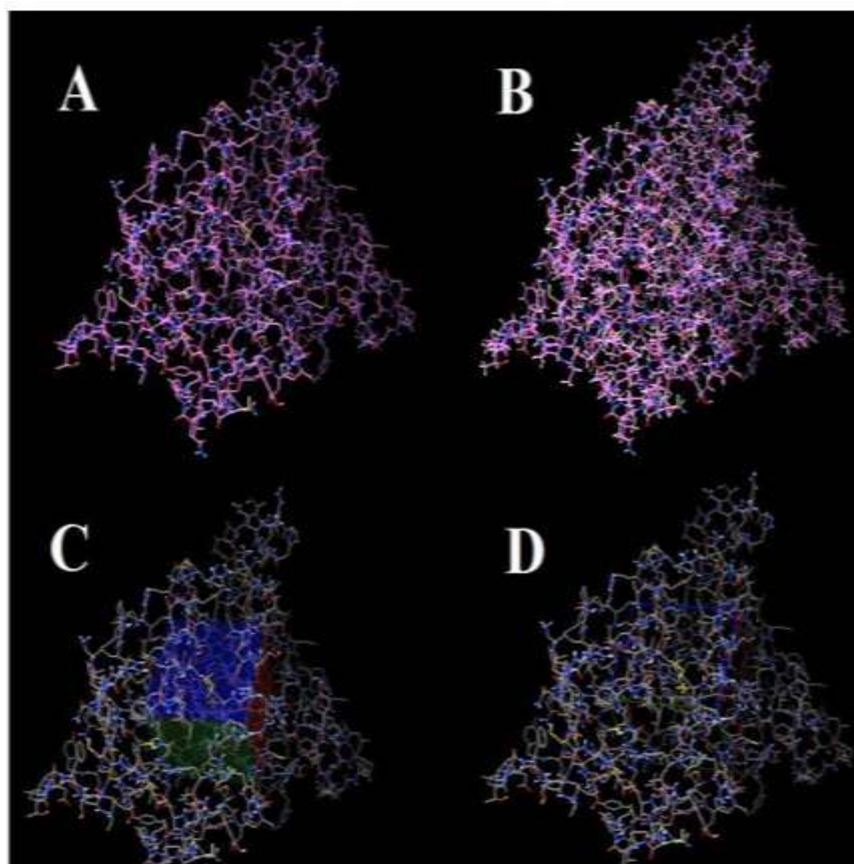
Auto Grid calculation: The grid maps were generated and then used by AutoDock docking calculations to know the total interaction energy for a ligand with macromolecule.

Grid dimensions: Grid is placed in equal dimensions 40X40X40 in XYZ dimensions with 0.4 spacing in angstroms along box placed in 13.215 X, 55.676 Y and 127.735 Z directions respectively (figure 3).

Molecular docking: Human oestrogen receptor ligand-binding domain of PDB:1ERE was chosen and docked with 24-Methylene cycloartanol.

Evaluation of Antioxidant Activity: The reagent 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used to assess the free radical-scavenging (antioxidant) activity. The test samples and standard ascorbic acid (vitamin C) were prepared in varied concentration such as 5, 10, 25, 50, 75 and 100 (μg/mL). The test and standard preparations were incubated for 20 min and readings were noted at 517 nm. The percent inhibition of antioxidant activity was calculated by using the formula and readings of test sample are compared with that of ascorbic acid (Vitamin C) (Positive control).

$$\text{DPPH Scavenged (\%)} = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \text{ X } 100$$



A:Before grid calculation ,B:After grid calculation , C&D: Grid map

Figure 3: Grid calculation for 24-Methylene cycloartanol against oestrogen receptor

Evaluation of Cytotoxic Activity

Maintenance of Cell Line: The MCF 7 cells were purchased from NCCS, Pune and the cells were maintained in RPMI –1640 medium supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}) in atmosphere of 5% CO_2 /95% air at 37^0C.

Preparation of Sample: The test compounds were weighed separately and dissolved in DMSO to get a concentration of 1 mg/mL. The cells were treated with series of concentrations from 10 to 100 µg/ mL.

MCF 7 Cell Viability by MTT Assay:

Principle: MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to insoluble, dark purple coloured formazan crystals. The cells are then solubilized with DMSO and then released solubilized formazan reagent is measured spectrophotometrically at 570 nm.

Procedure: Cytotoxic property of 24-methylene cycloartanol and standard (cisplatin) was evaluated by the MTT Assay in triplicates. MCF 7 cells were trypsinized and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells /well in 100 µL media in 96 well micro-titre plate and incubated overnight at 37^0C. After incubation, discard the old media and add fresh media 100 µl with different concentrations of test compound in respective wells in 96 plates. Discard the drug solution and add the media with MTT solution and plates were incubated at 37^0C for 3 hrs.

After incubation chromophore formazan crystals are formed with the reduction of the MTT salt metabolically active mitochondria. The absorbance was measured at 570 nm on a micro plate reader. The percentage of cell viability was calculated using the following formula:

$$\% \text{ cell viability} = \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

where At = Absorbance of Test, Ab= Absorbance of Blank (Media) and Ac= Absorbance of control (cells).

Statistical Analysis: Microsoft excel was used to enter and capture data. The graphs and tables of the study were prepared from this data.

Table 1
Preliminary Phytochemical Screening of ATME

Name of the test	ATME
Alkaloids	+
Glycosides	+
Aminoacids	+
Proteins	+
Carbohydrates	+
Steroids	+
Flavonoids	+
Tannins and Phenolic compounds	+

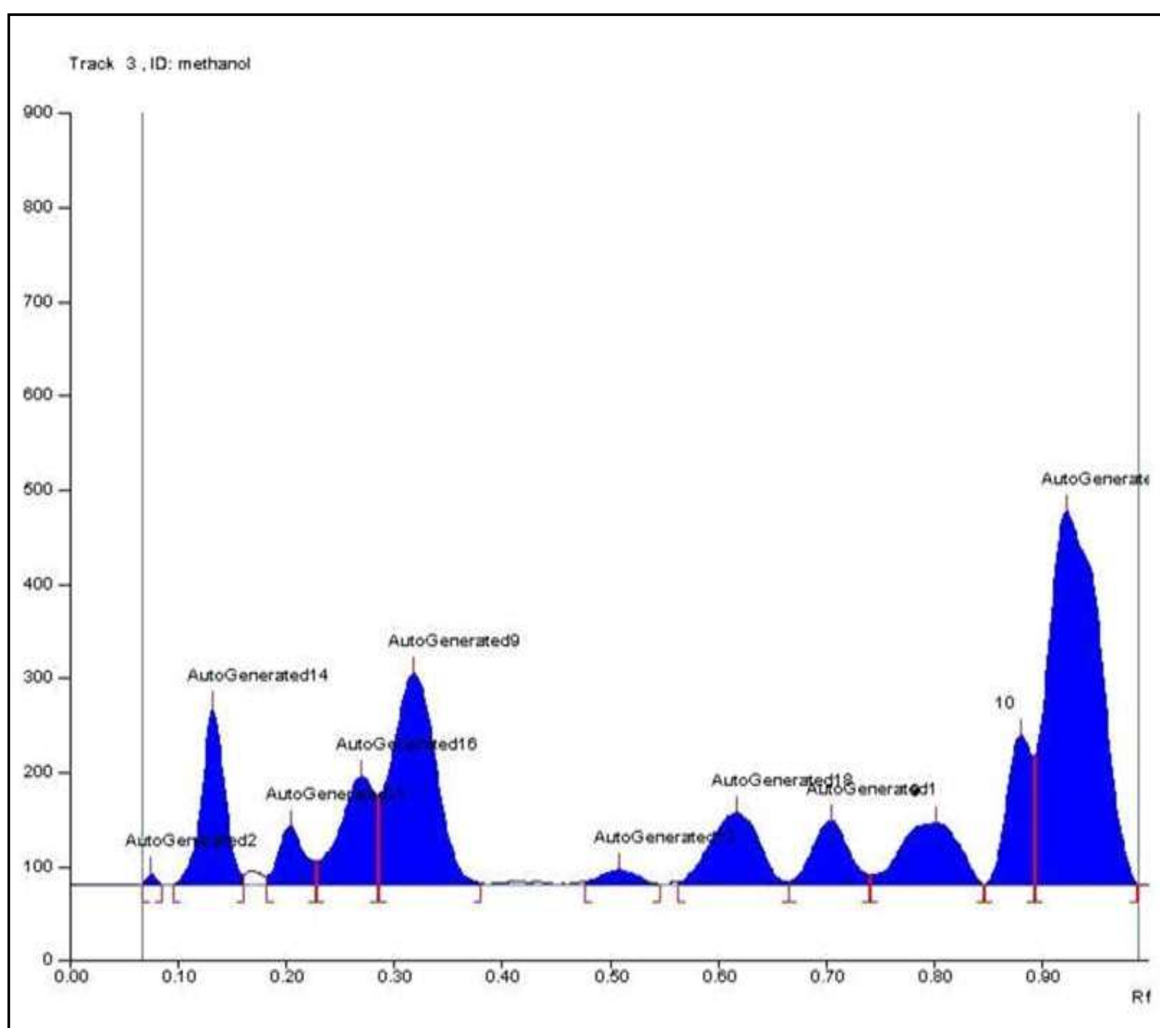


Figure 4: Representative densitogram of chloroform fraction of ATME

Results and Discussion

^{13}C NMR spectra: 169.294(C=O), 133.573-129.845 (double bonds), 72.881 (C-O), 66.650(OCH_3), 40.26-29.773 (cyclic CH_2), 14.053-28.979 (all methyls and methelene).
^{1}H NMR spectra: 7.736-7.618(aryl protons), 3.2-4.314(all C-O protons), 1.468-2.337(all cyclic protons), 0.9-1.2(terminal methyls).

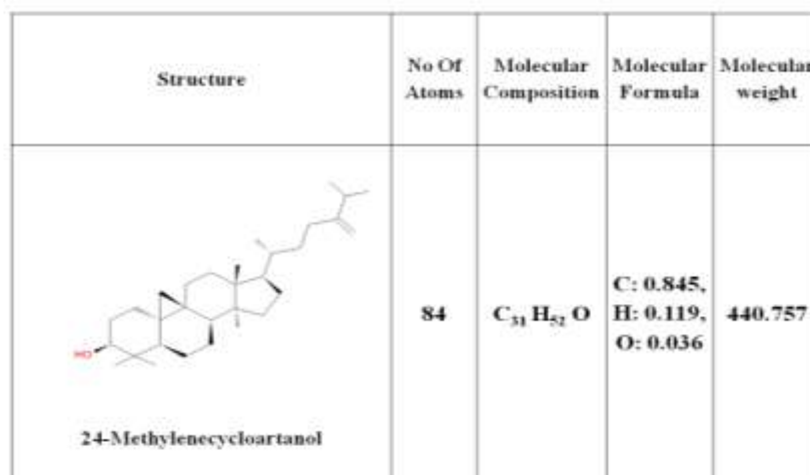
The compound was identified as a flavonol glycoside 24-methylene cycloartanol.

The preliminary phytochemical screening of ATME revealed the presence of various constituents such as carbohydrates, amino acids, proteins, alkaloids, glycosides, steroids, flavonoids, tannins and phenolic compounds and they are shown in table 1. The presence of these secondary metabolites lead to further study on fractionation, isolation, structural elucidation, *in silico* analysis and cytotoxic activity evaluation of ATME. The ATME was further fractionated into chloroform and aqueous fractions. The HPTLC fingerprint analysis of chloroform fraction of ATME suggested the presence of 11 compounds.

Table 2
R_f values of HPTLC analysis of chloroform fraction of ATME

Peak S.N.	**Peak** Strat	**Peak** Position	**Max** Position	**Max** Height	Max %	**End** Position	**End** Height	Area	**Area** %	**Assigned** Substance
1	0.07 Rf	1.2 AU	0.08 Rf	11.9 AU	0.86%	0.09 Rf	0.5 AU	100.3 AU	0.21%	AutoGenerated2
2	0.10 Rf	0.2 AU	0.13 Rf	187.5 AU	13.55%	0.16 Rf	10.5 AU	3674.4 AU	7.78%	AutoGenerated14
3	0.18 Rf	8.8 AU	0.20 Rf	62.8 AU	4.54%	0.23 Rf	24.7 AU	1381.5 AU	2.93%	AutoGenerated21
4	0.23 Rf	25.1 AU	0.27 Rf	114.8 AU	8.29%	0.29 Rf	95.5 AU	3468.9 AU	7.35%	AutoGenerated16
									17.91	
5	0.29 Rf	95.6 AU	0.32 Rf	224.6 AU	16.23%	0.38 Rf	2.4 AU	8456.8 AU	%	AutoGenerated9
6	0.48 Rf	3.2 AU	0.51 Rf	15.8 AU	1.14%	0.55 Rf	0.0 AU	495.0 AU	1.05%	AutoGenerated13
7	0.56 Rf	3.0 AU	0.62 Rf	76.3 AU	5.51%	0.67 Rf	3.1 AU	3207.8 AU	6.79%	AutoGenerated18
8	0.67 Rf	3.5 AU	0.71 Rf	68.4 AU	4.94%	0.74 Rf	10.2 AU	2115.1 AU	4.48%	AutoGenerated1
9	0.74 Rf	10.3 AU	0.80 Rf	66.4 AU	4.80%	0.85 Rf	0.4 AU	3317.7 AU	7.03%	unknown *
10	0.85 Rf	0.2 AU	0.88 Rf	159.1 AU	11.50%	0.89 Rf	136.6 AU	3378.1 AU	7.16%	unknown *
								17612.8AU	37.31%	
11	0.90 Rf	138.1 AU	0.92 Rf	396.3 AU	28.63%	0.99 Rf	1.8 AU			AutoGenerated5

Table 3
General properties of 24-Methylene cycloartanol



Structure	No Of Atoms	Molecular Composition	Molecular Formula	Molecular weight
24-Methylenecycloartanol	84	$C_{31}H_{52}O$	C: 0.845, H: 0.119, O: 0.036	440.757

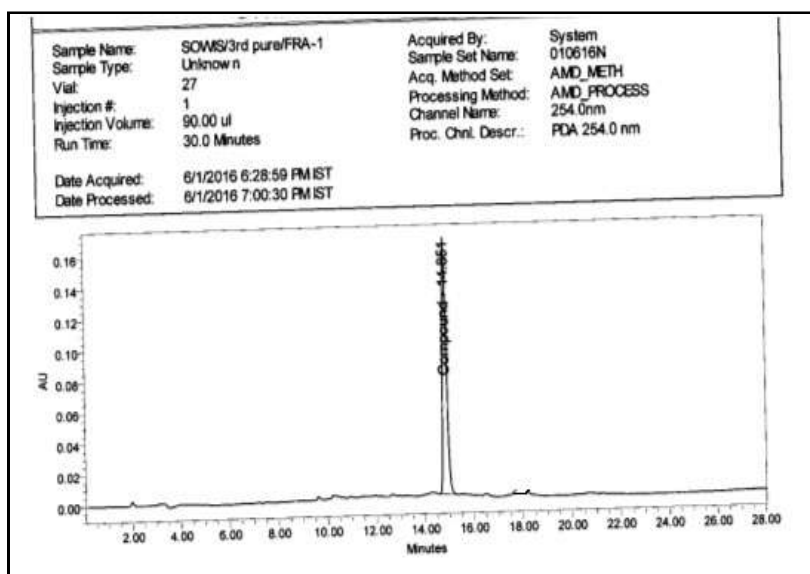


Figure 5: HPLC of isolated compound SOWIS-III

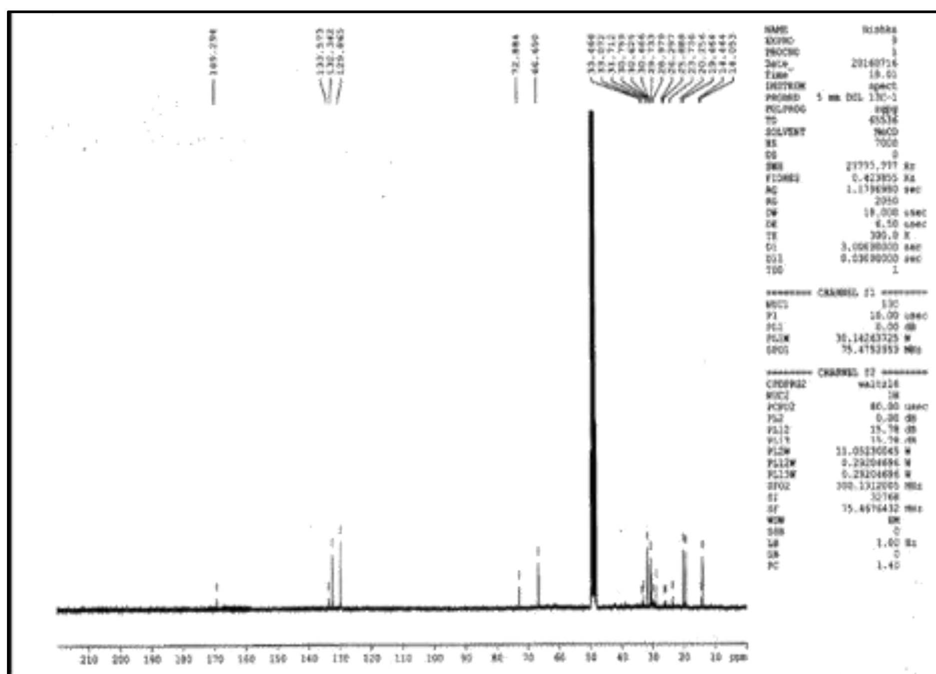


Figure 6: ^{13}C NMR spectral data of SOWIS-III

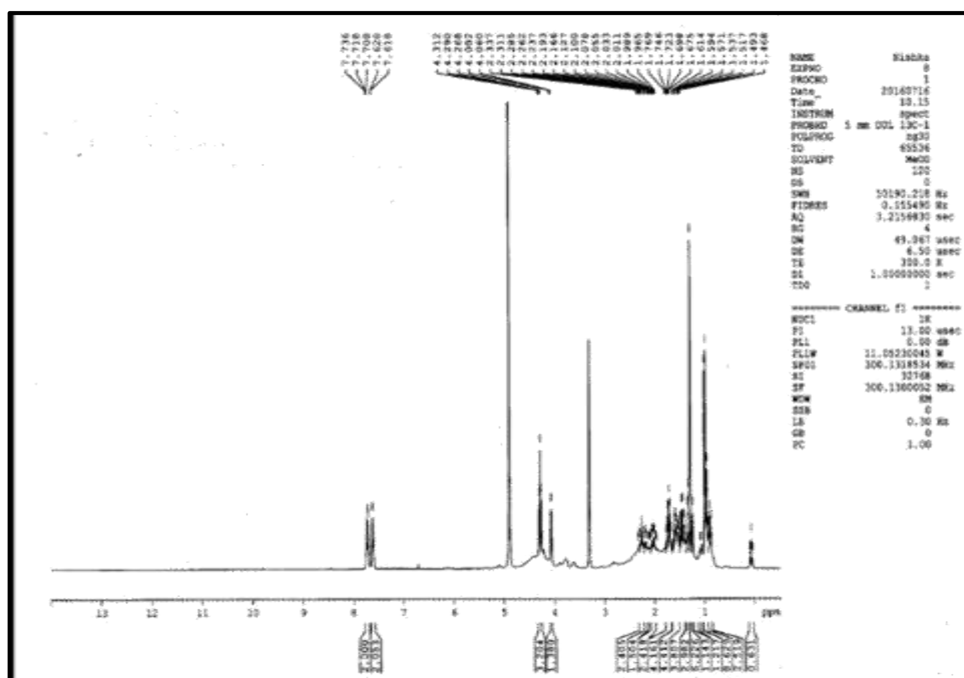


Figure 7: ^{1}H NMR spectral data of SOWIS-III

Table 4
Docking results of 24-Methylene cycloartanol and oestrogen receptor

Estimated Free Energy of Binding	-1.17 kcal/mol [=(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, Ki	345.24 mM (millimolar) at temperature = 298.15 K
(1) Final Intermolecular Energy	-0.09 kcal/mol
vdW + Hbond + desolv Energy	-0.09 kcal/mol
Electrostatic Energy	0.00 kcal/mol
(2) Final Total Internal Energy	0.00 kcal/mol
(3) Torsional Free Energy	+0.90 kcal/mol
(4) Unbound System's Energy [=(2)]	0.00 kcal/mol

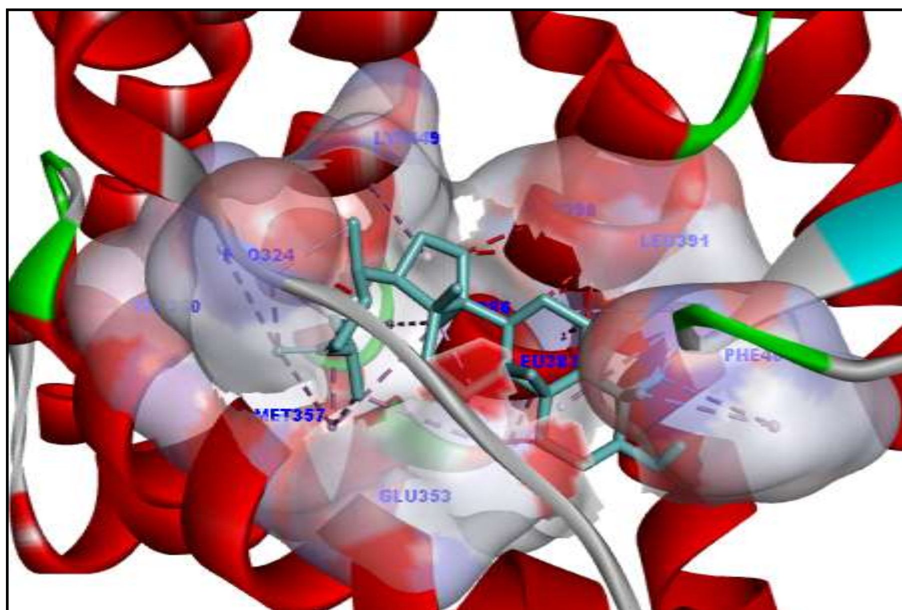


Figure 8: The binding of 24-Methylene cycloartanol to active site of Oestrogen receptor

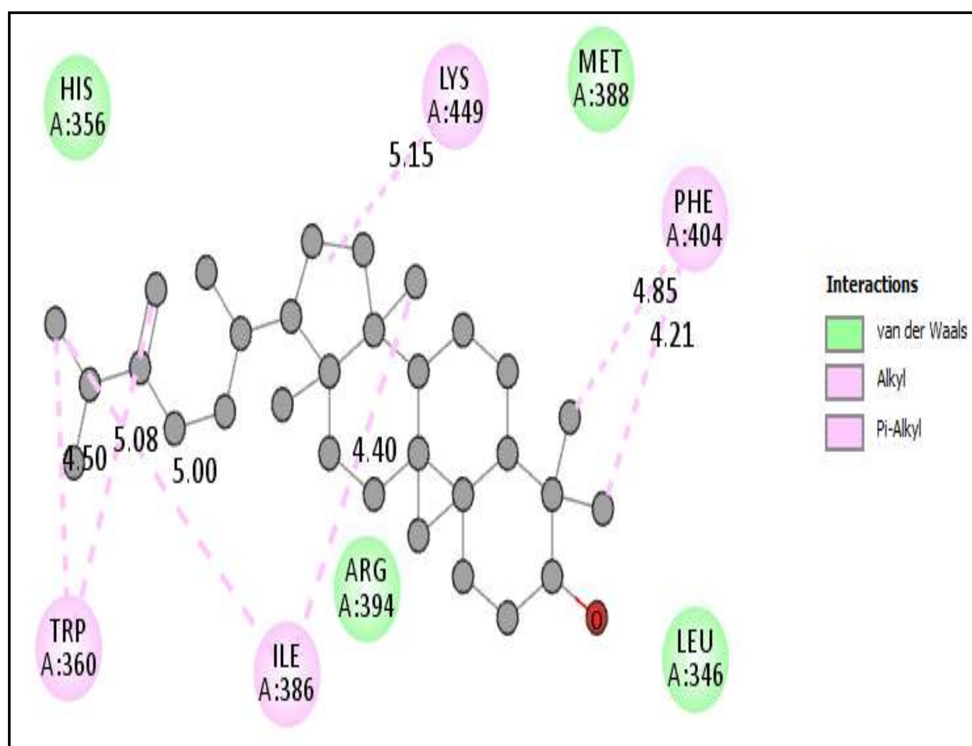


Figure 9: Active site amino acid and 24-Methylene cycloartanol binding interaction (Oestrogen receptor)

Table 5
The interaction and binding energy of 24-Methylene cycloartanol with Oestrogen receptor

RECEPTOR and LIGAND	Amnio acid Binding	Distance in Å	Free Energy of Binding kcal/mol
Drug target protein (Oestrogen receptor)- 24-Methylene cycloartanol	TRP 360	4.50	-1.17
		5.08	
	ILE 386	4.40	
		5.00	
	PHE 404	4.85	
		4.21	
	LYS 449	5.15	

Table 6
Antioxidant property of 24-Methylene cycloartenol

Conc (µg/mL)	Absorbance at 517 nm			Average	% Inhibition	IC_{50} (µg/mL)
5	0.734	0.735	0.737	0.735	34.37	31.03
10	0.686	0.687	0.689	0.687	38.66	
25	0.563	0.565	0.567	0.565	49.55	
50	0.426	0.427	0.428	0.427	61.87	
75	0.303	0.304	0.306	0.304	72.85	
100	0.203	0.205	0.206	0.204	81.78	
Control	1.11	1.12	1.13	1.12	-	

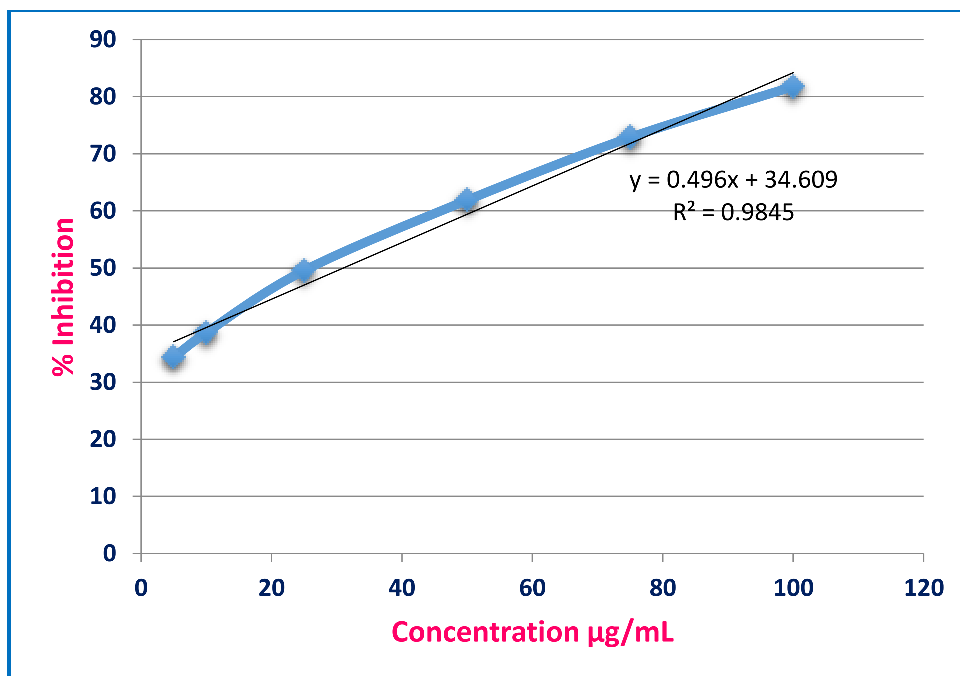


Figure 10: Antioxidant property of 24-Methylene cycloartanol

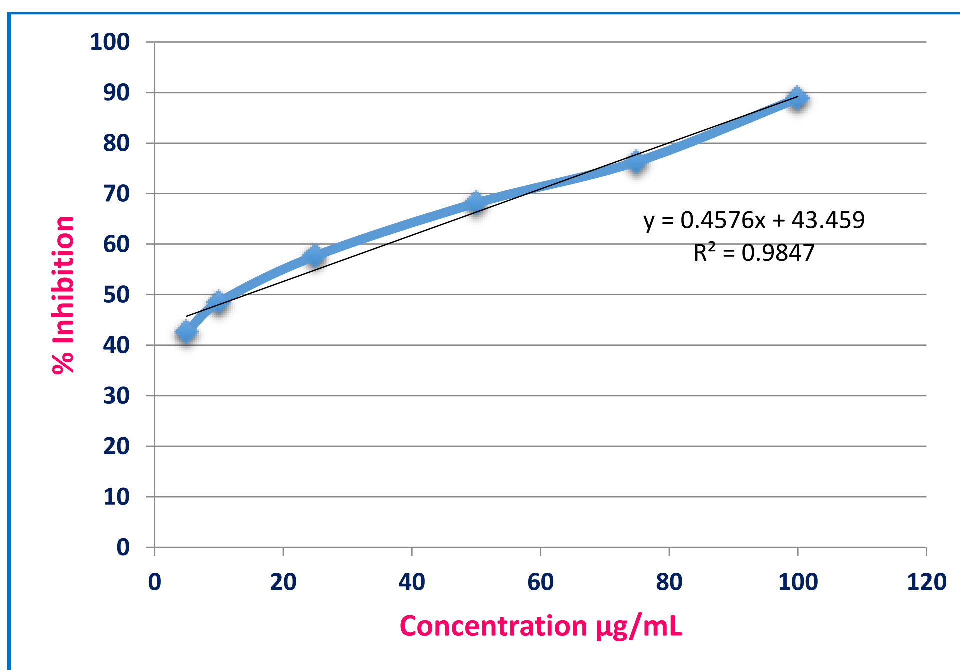


Figure 11: Antioxidant property of Ascorbic acid

Table 7
Antioxidant property of Ascorbic acid

Conc (µg/mL)	Absorbance at 517 nm			Average	% Inhibition	IC_{50} (µg/mL)
5	0.643	0.644	0.644	0.643	42.58	14.29
10	0.576	0.577	0.579	0.577	48.48	
25	0.472	0.474	0.477	0.474	57.67	
50	0.356	0.358	0.359	0.357	68.12	
75	0.264	0.265	0.266	0.265	76.33	
100	0.124	0.125	0.127	0.125	88.83	
Control	1.11	1.12	1.13	1.12		

Table 8
***In-vitro* cytotoxic activity of 24-Methylene cycloartenol**

Conc (µg/ml)	Absorbance at 570 nm			Avg	Avg-Blank	% Cell Viability	IC_{50} (µg/ml)
100	0.338	0.340	0.341	0.339	0.335	28.36	16.93
75	0.392	0.393	0.395	0.393	0.389	32.99	
50	0.499	0.499	0.501	0.499	0.495	42.07	
25	0.573	0.575	0.576	0.574	0.570	48.50	
10	0.594	0.596	0.598	0.596	0.592	50.38	
5	0.638	0.638	0.639	0.638	0.634	53.98	
Untreated	1.175	1.176	1.175	1.175	1.171	100	
Blank	0.005	0.004	0.005	0.004	0	-	

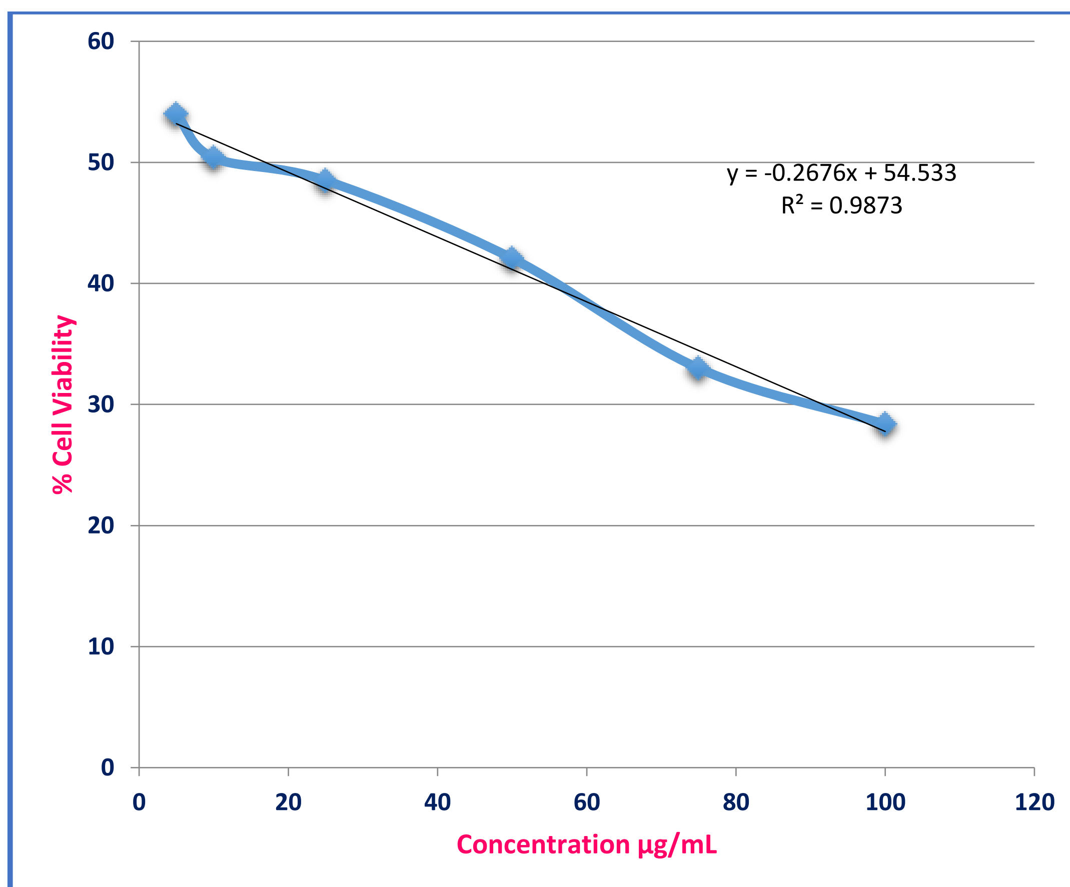


Figure 12: *In-vitro* cytotoxic activity of 24-Methylene cycloartanol

Table 9
***In-vitro* cytotoxic activity of Cisplatin**

Conc (µg/mL)	Absorbance at 570nm			Avg	Avg-Blank	% Viability	IC_{50} (µg/mL)
100	0.319	0.32	0.322	0.32	0.316	26.985	4.586
75	0.378	0.379	0.381	0.379	0.375	32.023	
50	0.475	0.476	0.479	0.476	0.472	40.307	
25	0.529	0.53	0.531	0.53	0.526	44.918	
10	0.551	0.552	0.554	0.552	0.548	46.797	
5	0.604	0.605	0.607	0.605	0.601	51.323	
Untreated	1.175	1.176	1.175	1.175	1.171	100	
Blank	0.005	0.004	0.005	0.004	0	-	

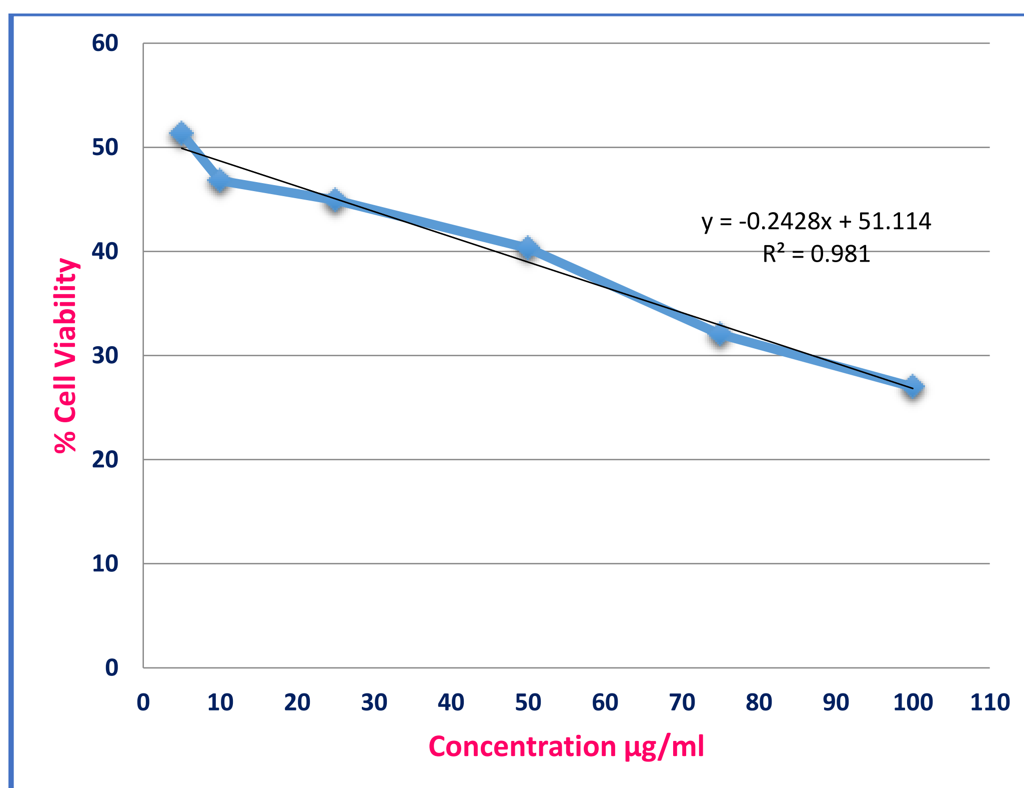


Figure 13: *In-vitro* Cytotoxic Activity of Cisplatin

The separation was aided by using mobile phase n-hexane:ethyl acetate (6:4 v/v). Bioautography screening for antibacterial analysis was performed by contact bioautography method. Based on bioautography the third fraction (SOWIS-III) was selected for further isolation and identification of the fraction. The structural interpretation of isolated compound SOWIS-III was determined as a flavonol glycoside 24-methylene cycloartanol (Table 2, 3 and figure 1-7).

In-silico analysis of 24-methylene cycloartanol was performed by molecular docking. The compound was docked with human oestrogen receptor and they showed four amino acid interactions with estimated free energy of binding of -1.17 kcal/mol. The amino acid Trp, Ile, Phe and Lys residues are present in the active site of the drug target protein favors, the binding of 24-methylenecycloartanol. Both pi-alkyl and alkyl interactions were seen. Also, few vanderwaals interactions were present. The strong bonding and interaction of the compounds depicted bioactivity of compound as 345.24 mM. Hence it was confirmed as drug candidate for cancer activity (Table 4, 5 and figure 8, 9).

The antioxidant property of 24-methylene cycloartanol was detected by DPPH assay method. It showed strong radical scavenging property in dose dependent manner. The IC_{50} values of 24-methylene cycloartanol and standard ascorbic acid were found to be 31.03 and 14.29 µg/mL respectively (Tables 6, 7 and figures 10, 11).

The crude chloroform, methanolic and aqueous leaf extracts of *Amaranthus tricolor* (L) showed good antioxidant activity. It was reported for strong free radical scavenging in dose dependent manner. IC_{50} values for methanol, chloroform, aqueous and ascorbic acid were found to be 290, 657, 830 and 130 µg/mL respectively[9].

The cytotoxic activity of 24-methylene cycloartanol was performed by MTT assay using MCF 7 breast cell lines. The compound showed significant cytotoxic property and the results are comparable with cisplatin. It inhibited the growth of cancer cells with IC_{50} value 16.93 µg/mL whereas cisplatin inhibited cancer cells with IC_{50} value 4.586 µg/mL (Table 8, 9 and figure 12, 13).

Conclusion

Innumerable types of bioactive compounds exhibiting various pharmacological activities have been isolated from plant sources. A lot of them are currently in clinical and preclinical trials and undergoing further appraisal. But still a large portion has to be explored phytochemically. It is anticipated that plants could be a potential source for the development of new leads to combat varied types of diseases. The findings from the study indicated that the methanolic extract of *Amaranthus tricolor* (L) leaf possesses vast potential as a medicinal drug in the treatment of cancer.

Based on the results obtained in the study still it needs further investigations on the isolation of compounds from *Amaranthus tricolor* (L) plant and cross checking of their other biological activities. It is also necessary to experimentally validate the other pharmacological effects and pharmacokinetics *in vivo* model of the isolated compound 24-methylene cycloartanol.

Acknowledgement

The authors wish to thank the management of Vignan Pharmacy College, Vadlamudi, Guntur (Dt) for providing laboratory facilities for conducting this work. Our special thanks go to Dr. Menaga Magendran, Director, BioNeem Tech India Pvt. Ltd., Chennai, Tamil Nadu, India for providing services to *in-silico* analysis studies.

References

1. Newman D.J., Natural products as leads to potential drugs: an old process or the new hope for drug discovery?, *Journal of Medicinal Chemistry*, **51(9),** 2589–2599 **(2008)**

2. Newman D.J., Cragg G.M. and Snader K.M., Natural products as sources of new drugs over the period 1981–2002, *Journal of Natural Products*, **66(7),** 1022–1037 **(2003)**

3. Gordaliza M., Natural products as leads to anticancer drugs, *Clinical and Translational Oncology*, **9(12),** 767–776 **(2007)**

4. Chopra R.N., Nayar S.L. and Chopra I.C., Glossary of Indian Medicinal Plants **(1986)**

5. Duke J.A. and Ayensu E.S., Medicinal Plants of China Reference Publications, Inc., 20-24 **(1985)**

6. Sowjanya Pulipati, Srinivasa Babu P. and Lakshmi Narasu M., Quantitative determination of tannin content and Evaluation of antibacterial activity of A*maranthus tricolor* (L), *International Journal of Biological and Pharmaceutical Research,* **5(7),** 623-626 **(2014)**

7. Sowjanya Pulipati, Srinivasa Babu P. and Lakshmi Narasu M., Phytochemical analysis and antibacterial efficacy of *Amaranthus tricolor* (L) methanolic leaf extract against clinical isolates of urinary tract pathogens, *Afr. J. Microbiol. Res.,* **9**, 1381-1385 **(2015)**

8. Simran Aneja, Manisha Vats, Sushma Aggarwal and Satish Sardana, Phytochemistry and hepatoprotective activity of aqueous extract of *Amaranthus tricolor* (Linn.) roots, *Journal of Ayurveda and Integrative medicine,* **4(4),** 211-215 **(2013)**

9. Sowjanya Pulipati, Srinivasa Babu P., Naveena U., Rafeeka Parveen S.K., Sumaya Nausheen S.K. and Tanmai Naga Sai M., Determination of Total Phenolic, Tannin, Flavonoid Contents and Evaluation of Antioxidant Property of *Amaranthus tricolor* (L), *International Journal of Pharmacognosy and Phytochemical Research,* **9(6),** 814-819 **(2017)**

10. Mohammed Rahmatullah, Mobasser Hosain, Shahnaz Rahman, Shiblur Rahman, Mahfuza Akter, Farhana Rahman, Fatema Rehana, Mahmuda Munmun and Marjina Akter Kalpana, Antihyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of *Amaranthus tricolor* L. (Amaranthaceae), *Afr J Tradit Complement Altern Me,* **10(5),** 408-411 **(2013)**

11. Handa S.S., Khanuja S.P.S., Longo G. and Rakesh D.D., Extraction Technologies for Medicinal and Aromatic plants, 1st edition, no. 66, Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology **(2008)**

12. Evans W.C., Trease and Evans Pharmacognosy, Elsevier Pub., New Delhi, India, 15th edition, 538-547 **(2006)**

13. Kokate C.K., Purohit A.P. and Gokhale SB, Pharmacognosy, 7th edition, 105-144 **(1997)**

14. Brzozowski A.M., Pike A.C., Dauter Z., Hubbard R.E., Bonn T., Engstrom O., Ohman L., Greene G.L., Gustafsson J.A. and Carlquist M., Molecular basis of agonism and antagonism in the oestrogen receptor, *Nature,* **389**, 753-758 **(1997)**.

(Received 18th March 2020, accepted 19th May 2020)