



Spectro Chemical Analysis And Evaluation Of Anticancer Activity Of Ethanolic Leaf Extract Of *Solanum Virginianum*

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ABSTRACT

Cancer is an inclusive term for a herd of diseases that can affect any part of the body. *SolanumVirginianum* has many herbal formulations, habitually used by an Indian population for the disease management. These herbal habituations demand exact scientific authentication to enhance their effectiveness and popularity. In this study, we endeavour scientific validation to the leaves of *SolanumVirginianum* through preliminary phytochemical investigation, characterization of efficient phytochemicals using UV Spectrophotometer, FTIR analysis and Gas chromatography – Mass spectrometry and Anti-cancer activity of identified phytochemical compounds. The Qualitative phytochemical screening of the ethanolic extract of leaves of *SolanumVirginianum* identifying twenty five compounds. The high proportion phytochemical compounds including Diethyl phthalate, Cyclohexasiloxane, dodecamethyl and lowest proportions including Furanylhexahydro1,3-Dioxo-4-Phenyl-, Methyl ester Dimethoxy-2-(2-Oxopropyl)-6-Dimethyl-1,3"-Dioxolan-anthraquinone. Eventually, the high proportion phytochemical compounds exhibited significant in vitro cytotoxicity against K562 tumor cell lines (IC₅₀ 54.9 µg). The all-inclusive study showed that the presented herbal formulations are enriched with bio-active phytochemical compounds with favoured pharmaceutical values.

Keywords : *SolanumVirginianum*, Anti-cancer Activity, Ethanol extraction, IC₅₀

INTRODUCTION

In the worldwide, many plants have been extensively used by rural population and tribal communities as an indispensable source of medicine¹. *Solanum virginianum* L, a diffuse and spiky shrub belongs to the family Solanaceae which grows commonly in the intercontinental on sandy soils and is distributed throughout India². The Solanaceae is a huge plant family with 96 genera and 2,300 species, about one half of which belong to the genus *Solanum*. *Solanum* is one of the biggest genera of flowering plants³, relatively woody at the base, zigzag stem, compressed spikes exceeding 1.3 cm long, leaves 5-10 cm long, ovate or elliptic, sinuate or sub-pinnatifid, obtuse or subacute, armed on the midrib and nerves with long yellow sharp spikes. Flowers are in extra-axillary few flowered cymes; corolla white, 2 cm long. Berry 1.3-2 cm diameter, yellow or white⁴. *Solanum virginianum* Linn has various phytochemicals that are medically valuable and are important sources of pharmacology. It has antispermatic, hepatoprotective and anticancer properties⁵. Phytochemical profile of *S. surattense* reported the presence of alkaloids, flavonoids, phenols, and steroids. The clinical studies of *S. surattense* revealed that it has potential anti-asthmatic activity and dental anti-inflammation⁶.



Fig : 1 The whole plant of *Solanum virginianum*

MATERIALS AND METHODS

Plant collection

Solanum virginianum was identified and mature plants were collected in the Garden of Arulmigu Palaniandavar College of Arts and Culture, Palani in the month of March. The plants were washed thoroughly with tap water to remove dust. Then the plants were shade dried to avoid the loss of bio-active compounds. After complete drying, each part of the plant were subjected to mechanical grinding and collected in an air tight container.

Extract Preparation

100g of each plant part were extracted using different organic and aqueous solvents which have varying polarity (Petroleum ether, EtOH, water). Each 250ml of the solvent used, with the help of the Soxhlet apparatus the extract was prepared and stored in clean beakers.

Gas Chromatography Mass Spectrometry Analysis

The ethanolic extract of *Solanum Virginianum* underwent gas chromatography-mass spectrometry analysis (GC-MS - QP-2020) with the thermal desorption (TD) system.

Experimental conditions of the GC-MS system were as follows:

The Trace -5 Mass spectrometry consists of capillary standard non-polar column whose dimension -30 meters; internal diameter-0.25 mm; and film thickness- 0.25 μm . The flow rate of the mobile phase (carrier gas: helium) is 1.2 ml/min. In the gas chromatography phase, the temperature programme (oven temperature) was 50°C, which was raised to 250°C at 10°C/min, and the injection volume was 1 μl . Samples dissolved in chloroform were run fully at a range of 50-500 mass-to-charge ratio (m/z).

In-vitro evaluation of anti-cancer activity by MTT assay

Cell culture

Chronic Myelogenous leukemia cell line (CML) was provided by National Centre for Cell line (NCCS), Pune and was grown in Minimum essential medium (MEM) supplemented with fetal bovine serum (FEM). All cells were maintained at 37 °C, with 5% CO₂, 95% air.

MTT assay

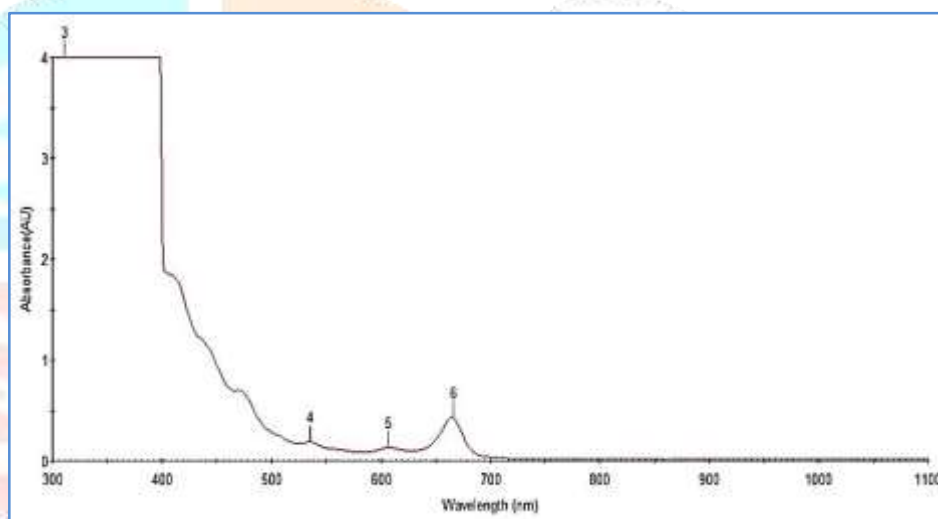
After 24 hours of incubation of each well, 10 μl of MTT (1mg /ml) in Phosphate- buffered saline (PBS) (p^H 7.2) was added and incubated at 37 °C for 3-4 h. The medium with MTT was snapped and the formazan crystals were formed which was solubilized in 100% of DMSO. Using micro-plate reader the absorbance was measured at 570 nm. The images were done using Inverted Phase Contrast Microscope.

UV – Ultraviolet Visible spectroscopy

The UV – Visible spectrum of the samples was recorded by using spectrophotometer (model UV-1800) in the range of 664 cm⁻¹ and 607 cm⁻¹. The peak at 665 cm⁻¹ is due to absorption in blue green (N=O), 607 cm⁻¹ is due to the absorption of Green blue (N=O), 200 cm⁻¹ – 400 cm⁻¹ is due to the Poly- unsaturated and aromatic carbonyl compounds (C=O, H-CH=O).

Table : 1 Description of UV – Visible Spectrum

S.No	Wave number	Description
1	200 nm to 400 nm	Poly- unsaturated and aromatic (C=O, H-CH=O)
2	665 nm	Blue- green (N=O)
3	607nm	Green – blue (N=O)

**Figure : 2 UV – Visible spectrum of *Solanum Virginianum***

FTIR- Fourier Transform Infrared Spectroscopy

FTIR spectral measurements were performed to find out the potential functional group in *Solanum Virginianum* leaf extract showed stretching vibrations of the bond.

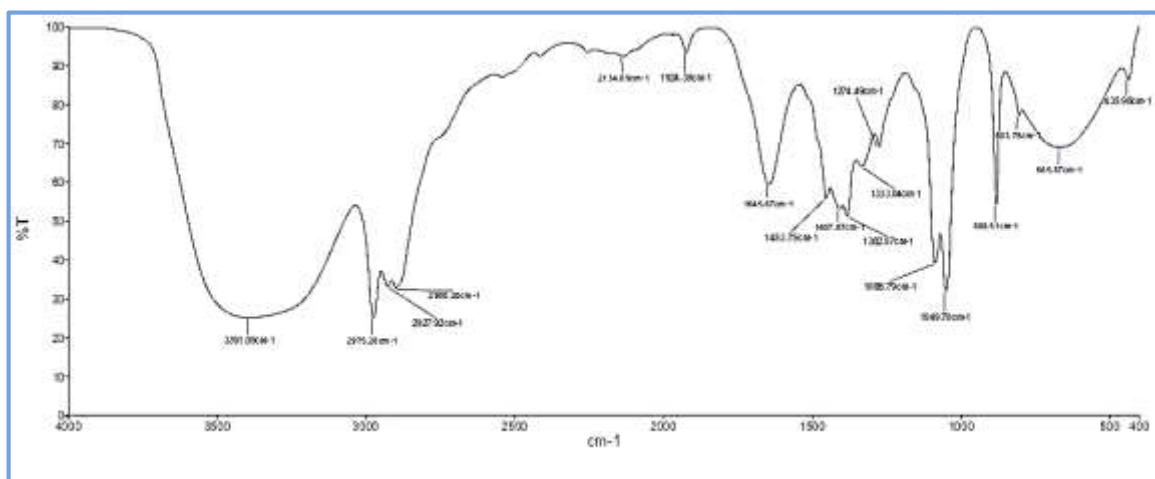


Table : 2 Functional group present in leaf extract

S.No	Wave number (cm ⁻¹)	Functional group
1	2975.20	C-H stretching of alkanes
2	3391.05	O-H stretching of carboxylic acids
3	2134.09	C≡C stretching
4	2927.92	C-H stretching of alkanes
5	2134.09	C≡C stretching

Gas chromatography- Mass spectrometry analysis

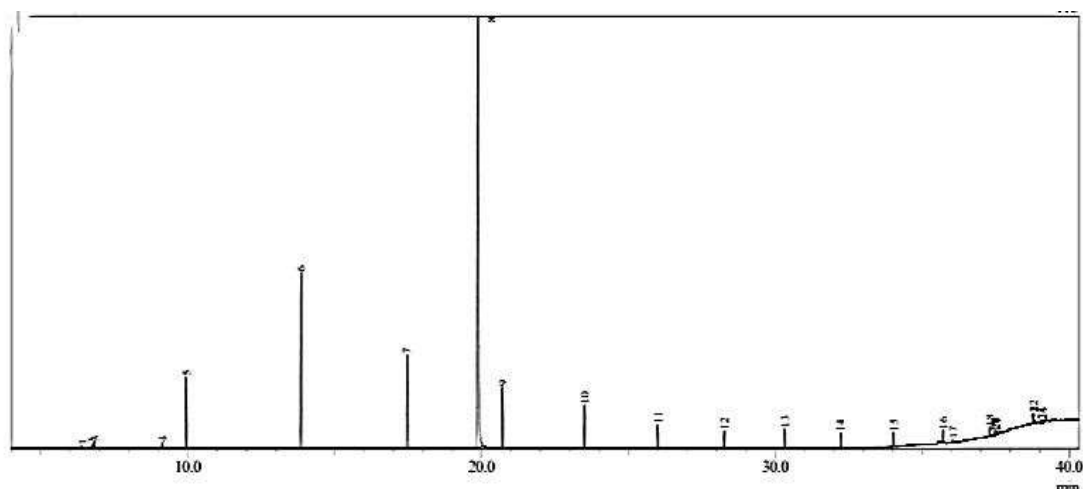


Figure : 4Chromotogram of ethanolic extract of *SolanumVirginianum*

The bioactive compounds present in the ethanolic extract of *SolanumVirginianum* coupled with massspectroscopy (GC-MS) reports are given in fig 5.

In the ethanolic sample of *SolanmVirginianum* 25 compounds were identified and the highest percentage compound content a peak area 41.18 (Diethyl phthalate with RT 19.876), peak area of 15.24 (Cyclohexasiloxane , dodecamethyl with 13,868 RT) and lowest percentage of content area of 0.15 Furanyl-hexahydro1,3-dioxo-4-phenyl-methylester with RT 37.455 and peak area of 0.14(1,5-Dimethoxy-2-(2-oxopropyl)-6-dimethyl-1"3"-dioxolan-anthraquinone with RT 36.04). Phytochemical with their retention time (RT), molecular formula and molecular weight(MW) in the ethanolic extract present in table 5.

Table : 3 Phytochemical constituents in ethanolic extract of *SolanumVirginianum*

S.No	RT	Name of the compound	Molecular formula	MW	Area%
1	6.392	Octahydro-4A,7,7-Trimethyl-5-(1,1-Dimethylene)	C ₁₉ H ₃₄ O ₃ Si	338	0.25
2	6.745	2-(2-Oxo-2-Phenyl-Ethyl)-1,3-Dioxolane	C ₁₀ H ₉ ClO ₄	228	0.31
3	6.825	3-Ethyl-4-Methyltetrahydrofuran-3-OL	C ₇ H ₁₄ O ₂	130	0.55
4	9.139	Pentadecane	C ₁₅ H ₃₂	212	0.43

5	9.946	Cyclopentasiloxane,Decamethyl	C ₁₀ H ₃₀ O ₅ Si ₅	370	5.73
6	13.868	Cyclohexasiloxane,dodecamethyl	C ₁₂ H ₃₆ O ₆ Si ₆	444	15.24
7	17.478	Cycloheptasiloxane,tetradecamethyl	C ₁₄ H ₄₂ O ₇ Si ₇	518	8.05
8	19.876	DiethylPhthalate	C ₁₂ H ₁₄ O ₄	212	47.49
9	20.74	Cyclooctasiloxane,hexadecamethyl	C ₁₆ H ₄₈ O ₈ Si ₈	592	5.21
10	23.509	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	3.6
11	25.994	1cosmethylcyclodecasiloxane#	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	740	1.91
12	34.498	Benzoic Acid, 2,4-Bis(Trimethylsiloxy},TrimethylsilylEster	C ₁₆ H ₃₀ O ₄ Si ₃	370	1.56
13	34.575	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	1.56
14	36.314	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	1.36
15	38.12	3,4-Dihydroxymandelicacid,4TMSderivative	C ₂₀ H ₄₀ O ₅ Si ₄	472	1.24
16	35.715	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	1.27
17	36.04	1,5-Dimethoxy-2-(2-Oxopropyl)-6Dimethyl-1"3"-Dioxolan-anthraquinone	C ₂₄ H ₂₄ O ₇	424	0.14
18	37.297	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	1.08
19	37.455	1H-Furo[3,4-C]Purrole-4CarboxylicAcid, Furanylhexasahydro1,3-Dioxo-4-Phenyl-,MethylEster.	C ₁₈ H ₁₅ NO ₆	341	0.15
20	37.48	Pentanenitrile,4,4-Dimethyl	C ₇ H ₁₃ N	111	0.59
21	37.531	1H-Indole,1-Acetyl-3-Tetrahydro-1-Methyl-4-Pyridinyl)Carbonyl	C ₁₇ H ₁₈ N ₂ O ₂	282	0.29
22	38.789	Cyclononasiloxane,octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	1.17

23	38.835	Silane,[[4{1,2Bis[(Trimethylsilyl)oxy]ethyl]-1,2-Phenylenebis(Oxy)]Bis[Trimethyl	C ₂₀ H ₄₂ O ₄ Si ₄	458	0.15
24	39.076	1-Aza-4,5-(2,3B-5-[4Methoxyphenyl]Furyl))Bicyclo[4.3.0]Nonan-9-One	C ₁₇ H ₁₇ NO ₃	283	0.42
25	39.125	3-Hydroxy-2-Adamantylisoindolin-1-One	C ₁₈ H ₂₁ NO ₂	283	0.22

Table : 4 Highest percentage of compound in the ethanolic leaf extract of *S. Virginianum*

S.No	RT	Name of the compound	Molecular formula	MW	Area %
1	19.876	DiethylPhthalate	C ₁₂ H ₁₄ O ₄	212	47.49
2	13.868	Cyclohexasiloxane,dodecamethyl	C ₁₂ H ₃₆ O ₆ Si ₆	444	15.24
3	17.478	Cycloheptasiloxane,tetradecamethyl	C ₁₄ H ₄₂ O ₇ Si ₇	518	8.05

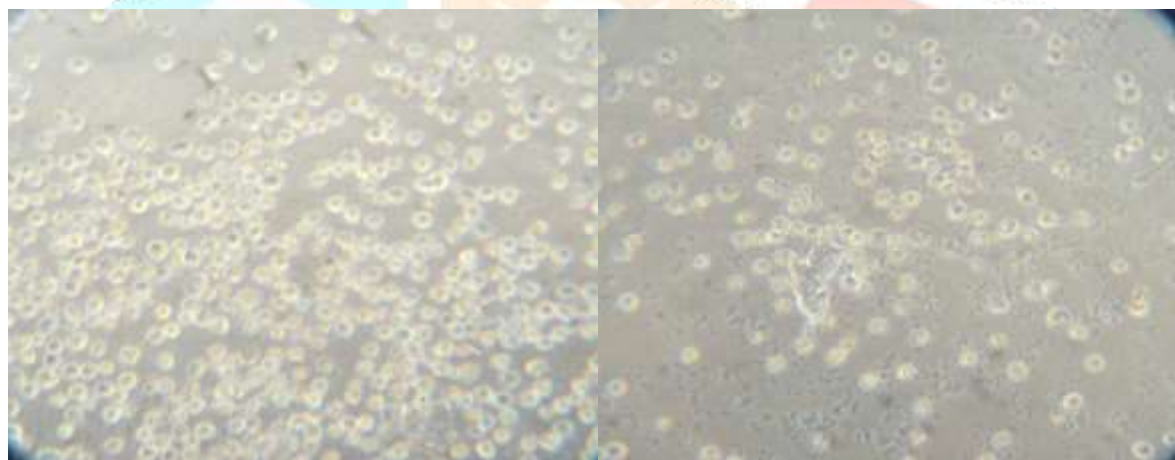
In-vitro Anticancer Activity

Table : 5 Percentage of Cytotoxicity of ethanolic leaf extract of *Solanum Virginianum* on K562 cell lines by MTT assay

Concentration	Cytotoxicity (%)	Cell Viability(%)	Cytotoxic Reactivity	With IC50 value
10	32	68	Mild	
20	38	62	Mild	

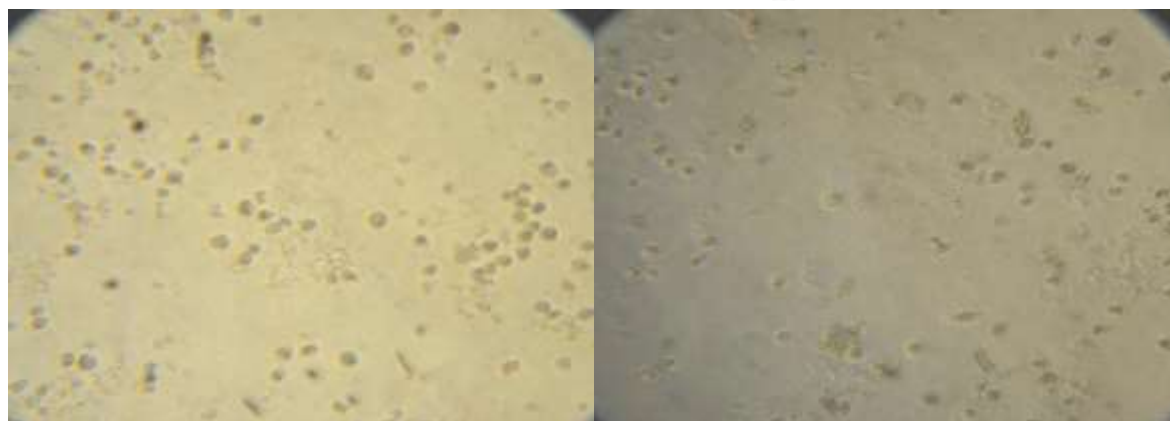
30	41	59	Mild	54.9 μ g
40	45	55	Mild	
50	50	50	Mild	
60	54	46	Moderate	
70	58	42	Moderate	
80	64	36	Moderate	
90	67	33	Moderate	
100	72	28	Severe	

The results for cytotoxicity by the extract against K562 lines for various concentration shown in table .As the concentration increases with the increases of cytotoxicity increases. The ethanolic extract of *Solanum Virginianum* showed Mild to Severe cytotoxicity to K562 cells after 24 hrs. The obtained IC₅₀ values 54.9 μ . Control showed none cytotoxicity.



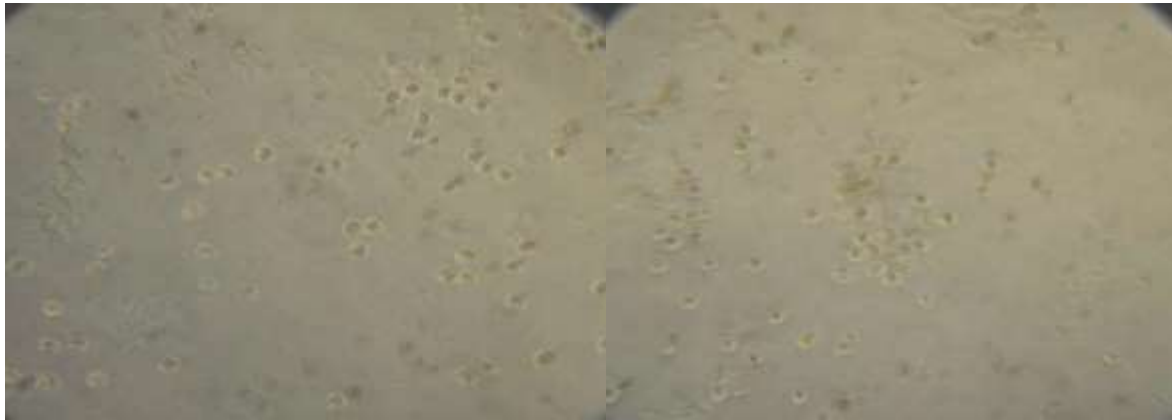
(A)

(B)



(C)

(D)



(E)

(F)



(G)

(H)



(I)

(J)



(K)

Fig. : 4 Anticancer Activity of *S. Virgianum* extract against K562 Cell lines from leaf extract of *Solanum Virginianum* with various concentrations.

(A) Control , (B) 10 μg , (C) 20 μg , (D) 30 μg , (E) 40 μg , (F) 50 μg , (G) 60 μg , (H) 70 μg , (I) 80 μg , (J) 90 μg , (K) 100 μg .

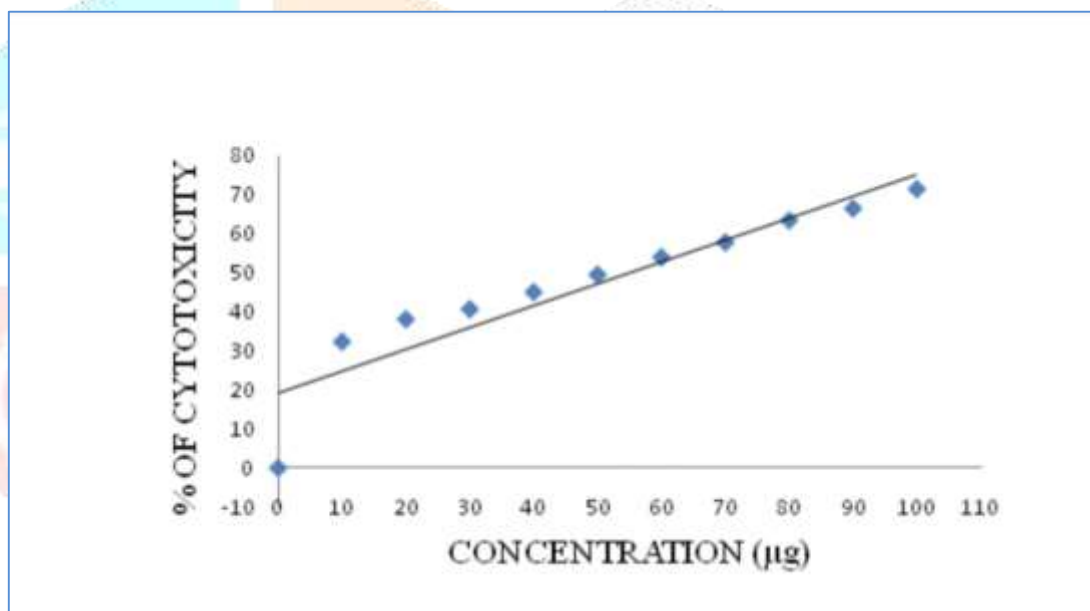


Fig. : 5 Concentration Vs % of cytotoxicity

An indigenous medicine comprises medical knowledge systems that developed over generations with in various societies before the era of modern medicine. Traditional medicines are prepared from the single plant or combination of more than one plant. Indian contribution of herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species. Hence the present study phytochemical compounds in ethanolic extract of *Solanum Virginianum* highest percentage compound content a peak area 41.26 acid, diethyl Phthalate with RT 13.876), followed by the peak area of 13.868(Cyclohexasiloxane,dodecamethyl with 15.24 RT) and and peak area of 8. 05 (Cycloheptasiloxane,tetradecamethyl with RT 17.467.) and the lowest percentage of content area of 0.14(1,5-

Dimethoxy-2-(2-Oxopropyl)-6Dimethyl-1"3"-Dioxolan-anthraquinone RT 36.04).The ethanolic extract of *S. Virginianum* showed Mild to Severe cytotoxicity to K562 cells after 24 hrs. The obtained IC₅₀ values 54.9 µg. Control showed none cytotoxicity.

CONCLUSION

The ethanolic leaf extract of *Solanum Virginianum* having numerous pharmaceutical properties. Diethyl Phthalate, Cyclohexasiloxane, dodecamethyl component are present in leaf extract. Hence the leaf extract of *S. Virginianum* emphatically used in the various ailments for the Herbal Medicine Industry.

The in-vitro studies against K562 lines revealed that the ethanolic leaf extract of *Solanum Virginianum* has a moderate anticancer activity. However, there was increase in the cytotoxicity when concentration of sample is increased, the IC₅₀ value is 54.9 µg. Hence the level of cytotoxicity increases with the increase of volume. In future, these components can be isolated and test the pharmacological activity in various metabolites.

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