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Preclinical Assessment of *Solanum trilobatum* Leaf Extracts as DNA Damaging Anti Cancer Agent in the Management of Breast Cancer

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Abstract: Nature become a great source of medicinal treatment for millions of years. Much of the world's biological diversity remains unexplored as a source of novel biological compounds and the search for new bio-active agents from natural sources, including extreme environmental niches is expanding. Unique bioactive compounds have many pharmacological activities. Drug from these compounds used to treat deadly diseases like cancer, AIDS, diabetes, arthritis, etc. The current study is based on these bioactive compounds for curing breast cancer called ductal carcinoma of the breast. The Solanum trilobatum leaves collected from Namakkal. Solanum trilobatum leaf extract of chloroform yielded a total amount of 4g of crude extract from 500g. Similarly aqueous extract yielded a total amount of 5.1g of crude extract. The protein content is found in 0.92mg/ml in chloroform extract and 1.50mg/ml in aqueous extract. Hemolytic assay results on Human erythrocyte using crude chloroform and aqueous extracts from Solanum trilobatum. The crude of chloroform and aqueous extract at different concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against 5 species of bacteria and 3 species of fungus. The extracts exhibited anticancer activity and hemolytic activity against the MCF-7 and HEp G2 cell lines with the inhibitory effect increased as the concentration of the solvent extract increased. Thus the extract become used for the treatment of Breast cancer as it has anticanceral activity.
Key words: Breast cancer, Hemolytic activity, Solanum trilobatum.

I. Introduction

Plants are playing an important role in the health of millions of people's life in India.. *S. trilobatum* is reported to treat many diseases viz., respiratory problems and bronchial asthma. Many pharmacological activities are found in *S. trilobatum* like hepatoprotective activity, antimicrobial activity, larvicidal activity, antidiabetic activity, cytotoxic activity and anticancer activity [1]. The leaves and stem of *S. trilobatum* are reported to possess antimitotic, anti-inflammatory and anti-ulcerogenic properties. The leaf extracts are used to increase male fertility and to cure snake poison. [2].

The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs. *Solanum trilobatum* Linn (Family: Solanaceae), a thorny creeper. The flower is bluish white and grows as a climbing under shrub [3]. It is a important medicinal plant available in southern India. This herbal medicine used to treat more diseases like tuberculosis, bronchial asthma and respiratory problems. The plant well known in ayurveda and siddh systems. Other language known that Sanskrit in 'Alarka', in Telugu 'Alarkapatramu', in Tamil 'Tuduvalai' and in Malayalam 'Tutuvalam'. The plant roots, berries and flowers are used for cough [4].

Many synthetic drugs are susceptible to various infectious microorganisms, so alternative therapy is very important. *Solanum trilobatum*, a thorny creeper with bluish violet flower, which is commonly available in Southern India has been used traditionally in Siddha system of medicines to treat various diseases [5]. Various chemical compounds are identified in solanum species they are flavanoides, sterols, saponins alkaloids, phenolics, and their glycosides. The secondary compound of alkaloids from soladunalinidine and tomatidine were isolated from leaf and stem of *Solanum* species [6]. Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. *Solanum trilobatum* leaf extracts posse's anticancer activity [7]. Therefore, the present was evaluate the antimicrobial activity, hemolytic activity and antiproliferative potential of *Solanum trilobatum.*

II. Materials and Methods:

2.1Collection and Preservation of Plant materials

Leaves of *Solanum trilobatum* are used throughout the study and they were collected from in and around Namakkal, Tamil Nadu. They were taken to the laboratory for drying under sun shadow and portion of

specimens preserved in 10% neutralized formalin for further identification. The sample *Solanum trilobatum was* identified based on the Taxonomical characteristic studies.

2.2Preparation of Extraction

Chloroform and water are used by the following method of Asirvatham Doss *et al.,* (2008) [8] with certain modifications.

2.3Partial Purification of Crude Protein

DEAE Cellulose Anion Exchange chromatography was used for the purification of crude protein according to the procedure of Stempion *et al.,* (1970) [9].

2.4Protein Estimation

Protein estimation was done as described by Lowry *et al.,* (1946) [10], using Bovine serum Albumin the standard rate of 1mg/ml. Different concentrations of the standard ranging from 0.1 to 1mg/ml were taken and made up to 1 mg/ml. Then alkaline copper reagent 5ml was added, thoroughly mixed and allowed to stand for 10 minutes at room temperature. Then 0.5ml of diluted Folin's phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature. The absorbance at 650nm was read Spectrophotometricaly. The protein concentrations of *Solanum trilobatum* extracts were estimated.

2.5Hemolytic Activity

Human blood was procured from EASMA Institute of technology, Aravakurichi, Karur Dt. The micro hemolytic test was performed in 96 well 'V' bottom micro titer plates. Various rows were selected for chick, goat and human blood. Serial two fold dilutions of the crude toxin were made in 100ml of Normal saline. This process was repeated up to the last well. Then 100 µl of RBC was added to all the wells. Appropriate controls were included in the test. To the 1% RBC suspension 100µl was added normal saline that served as negative control. The plate was gently shaken and then allowed to stand for two hours at room temperature and the results were recorded. Positive hemolysis indicates all the wells having uniform red color suspension. Lack of hemolysis indicates the wells having button formation in the bottom. Reciprocal of the highest dilution of the crude toxin showing pattern was taken as 1 Hemolytic Unit (HU).

2.6Antimicrobial Activity

The crude extract of *Solanum trilobatum* tested for antibacterial and antifungal activity. For antibacterial activity, *Pseudomonas sp, Streptococcus aureus, Vibrio cholerae,* and *Vibrio parahaemlyticus, Bacillus substillus* were used. For antifungal activity, *A.flavus, A.niger, Candida albicans* were used.

2.7Cell Line Culture

All the cells were detached from the culture flasks by addition of 1 ml of 0.25% trypsin-0.1% EDTA. Trypsin was then inactivated by the addition of 10 ml of experimental medium (RPMI-1640 or DMEM). Cells were separated into a single cell suspension by a gentle pipetting out and add 1 ml of the cell suspension to the culture flask containing 20 ml of culture medium. The balance cell suspension was centrifuged at 1000 rpm (Sorvall® T6000D) for 3 minutes and the supernatant discarded. 20 ml of experimental medium was added. By repeatedly pipetting out leads to cells dispersion in to the medium. An aliquot of the trypsinised cell suspension was stained with 2 mg/ml trypan blue (1:1 ratio) in order to obtain a more quantitative analysis of the cell suspension. Trypan blue is a water-soluble dye, which is insoluble in the cell membrane lipids.

The crude samples are used for cell line studies as experimental samples. After treating the samples with breast cancer cells, the cell viability were analyzed. It will thus only cross cell membranes of dead/non-viable cells. On visualization with the light microscope (Nikon), transparent appearance indicates viable cells, while dark blue stained cells are nonviable. The haemocytometer was used to determine the total number of viable and non-viable cells by counting cells in the 25 squares (each square is subdivided into 16 smaller squares of 0.1 mm2) at the top and bottom of the haemocytometer and the average number of cells per unit volume (millimeter, ml) of medium calculated. The cell suspension was adjusted with experimental medium to approximately 1.5 x 105 cells/ml. The experiment was carried out only when there was at least 95% cell viability.

III. Results and Discussion:

The *Solanum trilobatum* leaves collected from in and around Namakkal, Tamil Nadu, was identified based on Taxonomical character.

Solanum torvum, aqueous fruit extract contains maximum crude protein (0.73±0.011 mg BSAE) where as water extract of leaf contains maximum free phenol content (0.282±0.009 mg GAE). *Solanum trilobatum* gave maximum crude protein content in its aqueous extract of fruit (0.68±0.007 mg BSAE), maximum free phenol was found out in methanolic leaf extract [11].

S. trilobatum seed extract by using acetone, petroleum ether and chloroform were calculated. The result showed that the acetone extract has highest extractive value of 10.5 (%w/w) and the extractive values of petroleum ether and chloroform are 8.7and 9.2 (%w/w) [12].

In the present study chloroform extract of *Solanum trilobatum* yielded a total amount of 4g of crude extract from 500 .Similarly aqueous extract yielded a total amount of 5.1g of crude extract**.** The protein content in crude extract of *Solanum trilobatum was* found to be 0.92±0.006 mg/ml in case of chloroform extract and 1.50±0.002 mg/ml in case of aqueous extract.

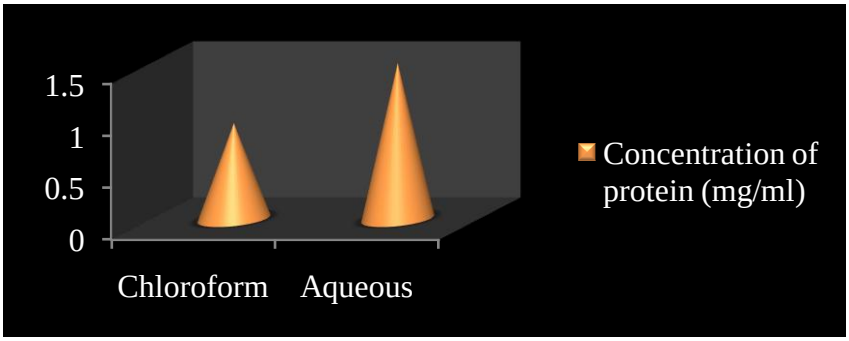


Figure: 1 Concentration of Protein

The acetone extract of *A. indicum* at a concentration of 0.2 to 1 mg/ml significantly saved the lysis of human erythrocyte membrane by a temperature induced condition .This is similar to the standard drug aspirin as positive control (1mg). The maximum hemolysis was observed in acetone extract of pod of *A. indicum* at the concentration 1000µg/ml**.** The minimum red blood cell destruction was showed in leaf (42.85±1.85) and flower (43.47±3.54) extracts of *A. indicum* which was compared to standard drug aspirin [13].

Table :1 Hemolytic Activity

S. No	Type of extract	Protein (mg)	Total Hemolysis up to Dilution	Hemolytic titre	Specific Hemolytic Activity (µg/ml)
1	Chloroform	0.92±0.006	14	14	12
2	Aqueous	1.50±0.002	14	9	8

In the present study the hemolytic assay results showed the crude Chloroform extract induced pronounced hemolysis on human blood. The hemolytic titer in case of chloroform extract of *Solanum trilobatum was* found to be 14 and its specific hemolytic activity was estimated to be 12 HT/mg of protein. *Solanum trilobatum* aqueous extract of hemolytic titer value was found to be 9 and its hemolytic activity was found to be 8 HT/mg of protein.

Ethanolic extract of *S.trilobatum* leaves showed antibacterial activity against tested bacterial strains in the order *Bacillus subtilis*(13mm), *Bacillus cereu* (11mm),*Pseudomonas aeruginosa* (9mm)*Staphylococcus aureus(* 8mm) *Escherichiacoli* (*6mm).*The MIC concentration was found in *Bacillus substilis* with the concentration 30mg/ml15, 16. The concentration of 30mg/ml showed highest *zone* of inhibition. *Solanum trilobatum* extractshaving the effective antibacterial activity of against the many organism indicates the medicinal value. The plant is used as a traditional healer which cure various diseases like asthma, liver disorder, cancer, cough and cold.(Priya et al., 2014) [14]

In the current study antibacterial activity showed that chloroform extract expresses higher activity against *Pseudomonus* sp (9mm).In case of *Streptococcus aureus* chloroform extract showed(5mm)and aqueous showed (6mm) activity. But the other species didn't express higher zonal activity showed in table 2.

Table.: 2 Anti Bacterial Activity

S.NO	Bacterial culture	Chloroform	Aqueous
1	*Pseudomonus* sp	9mm	No zone
2	*Streptococcus aureus*	5mm	6mm
3	*Vibrio cholera*	7mm	No zone
4	*Vibrio parahaemlyticus*	No zone	4mm
5	*Bacilus subtilus*	No zone	No zone

The crude of chloroform and water extract at various concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against 3 species of fungai. Aqueous extract showed better activity against *Candida albicans* (8mm). Chloroform extract showed (7mm) aqueous extract showed (6mm) against *A.flavus*.

Table :3 Antifungal activity

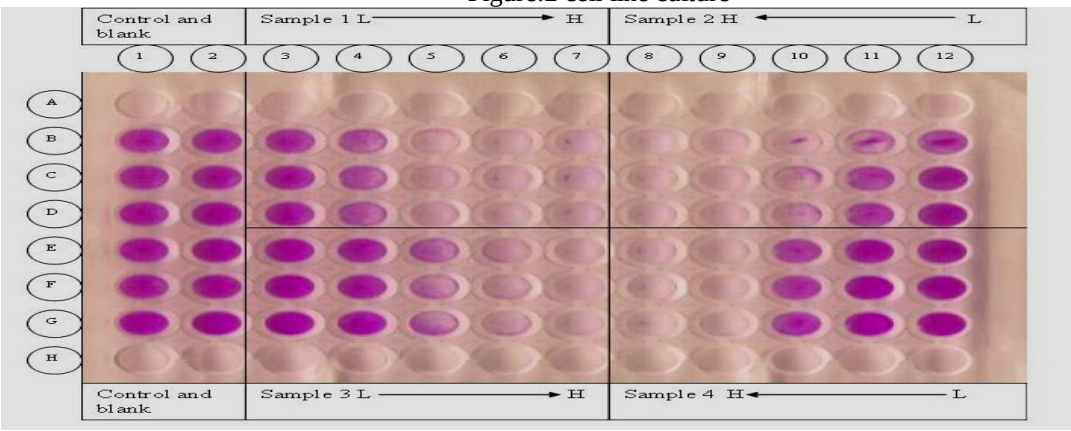
S. No	**Fungal culture**	**Chloroform**	**Aqueous**
1	*A. Niger*	5mm	No zone
2	*A. Flavus*	7mm	6mm
3	*Candida albicans*	No zone	8mm

The cytotoxicity assay (MTT assay) for SFST against HEp 2 cell lines at different concentrations determined the IC50 (50% growth inhibition) values. An augment in the % growth inhibition with increasing concentration of MEST (400, 800 and 1000 µg/ml) on HEp2 cell lines was determined. Among this concentrations1000 µg/ml of SFSTshowed maximum growth inhibition on HEp 2 cell lines [15].

The cytotoxic effect of fruit and leaf extracts were evaluated using MCF-7 tumor cell lines. Both the extract of fruit and leaf were active on MCF-7 cell lines. Different concentrations of the extracts were used and dose dependent growth inhibition of cancerous cells was observed. IC50 value for fruit extract was 154.9 µg.ml-1 and a maximum inhibition of cell growth was obtained at 300 µg.ml-1. The ethanol extract of *A.Bilimbi* leaf showed an IC50 value 668.3 µg.ml-1. The fruit extract showed a greater potential of cytotoxic activity as compared to leaf extract [16].

The present study agreed with above results *Solanum* leaf extracts exhibited anticancer activity against the HEp G2 and MCF-7 and cell lines with the inhibitory effect increased as the concentration of the solvent extract increased.In this assay, cell death and cell viability was estimated. The crude extract of chloroform showed antiproliferative activity 0.626 mg/ml for HEp G2 cell line and 1.24 mg/ml MCF-7 cell line. The inhibition was time and dose dependent manner. It indicates *Solanum* chloroform leaf extract has better antiproliferative activity towards HEp G2 cell line at minimum concentration. The various bioactive compounds such as, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, etc. might be responsible for the antiproliferative activity of *Solanum trilobatum.*

Figure:2 cell line culture



IV. Conclusion:

As the pharmacologist are looking forward to produce new drugs from medicinal plant sources. From based on the studies *Solanum trilobatum* leaf crude chloroform extract showed higher hemolytic activity and exhibited good anti microbial activity. *Solanum* leaf extracts exhibited promising anticancer activity against the

MCF-7 and HEp G2 cell lines with the inhibitory effect increased as the concentration of the solvent extract increased. These studies revealed that bioactive compounds of *Solanum* leaf extract having the good anticancer activity.

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