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# Cytotoxicity and Anticancer activity of Aqueous Leaf Extract of *Solanum torvum* on Normal Vero and Human Breast Adenocarcinoma MCF-7 Cell Line

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*Solanum torvum* leaves; Phytochemical analysis, Normal Vero cell line, Human breast adenocarcinoma cell line, Cell proliferation studies, Tryphan Blue, Selectivity index.

**ABSTRACT:** Natural herbal products are formulated with a combination of phytoconstituents from plants which play a pivotal role because of their diverse medicinal properties. Limited plants have been screened for their complete biological and pharmacological nature. In the present research work an attempt was made to infer the medicinal value of aqueous extract of Solanum *torvum* (*S. torvum*) leaves. Phytochemical analysis of the aqueous extracts of *S. torvum* leaf in the present study ascertain the presence of flavonoids, phenols, saponins, alkaloids, coumarins, sterols, proteins and reducing sugars. Further, the potentiality of aqueous extract of *S. torvum* leaves was assessed for its cytotoxic effect on Normal Vero cell line and anticancer activity on Human breast adenocarcinoma cell line by 3- (4, 5 dimethyl thiazole-2-yl) -2, 5-diphenyl tetrazolium bromide assay. A 24-hour incubation cell proliferation study reduced the cell viability of MCF-7 breast cancer cell lines. *In vitro* studies on cytotoxicity analysis on Vero cell line revealed that the aqueous leaf extract of *S. torvum* has no toxicity and further it was found to be effective in the prevention of cell proliferation by MCF-7 cell lines. 

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## INTRODUCTION

Cancer is a disease characterized by uncontrolled cell proliferation in the body, leading to impaired functions and high mortality. In 2012, it was estimated that there were 14 million new cases and 8.2 million cancer related deaths and by the year 2050; this could increase to 27 million cases, 17 million deaths and 75 million persons living with cancer [1]. Breast cancer is generally treated in recent days by radiotherapy, hormonal or chemotherapy. In most of the case, the patients experience adverse side effects from intensive therapy. Chemotherapeutical agents usually results in nausea and failure of bone marrow function [2]. Cancer cells are resistant to action of chemical drugs [3].

According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. Herbals are natural store house of phytochemicals with immense therapeutic quality. The medicinal value of these plants lies in bioactive substances called phytochemicals that produce definite physiological action of human body. The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significances in therapeutic treatment. Animal studies indicate that many phytochemicals in plants are potential antioxidants and possess anticancer properties [4].

Over the past 30 years, plants belonging to genus *Solanum* have received considerable attention in chemical and biological studies. *Solanum* is the largest genus in the family Solanaceae, comprising of about 2000 species distributed in the subtropical and tropical regions of Africa, Australia, and parts of Asia (China, India and Japan). Many plants of this family are economically significant species. Many species belonging to this genus present wide range of pharmacological activities such as cytotoxicity to different

tumors as breast cancer (4T1 and EMT), colorectal cancer (HCT116, HT29, and SW480), and prostate cancer (DU145) cell lines. Previous phytochemical investigations on *Solanum* species led to the identification of steroidal saponins, steroidal alkaloids, terpenes, flavonoids, lignans, sterols, phenolic compounds, coumarins and their biological activities associated with their pharmacological nature have been attributed these compounds [5].

*S. torvum* belongs to the family Solanaceae and is distributed throughout the Southern parts of India. This is a shrub with a growth upto 5 mt tall, cultivated in the tropics for its tasty immature fruits. [6] Several pharmacologically active potential chemical compounds which include flavonoids, sterols, saponins, alkaloids, phenolics and glycosides have been identified. Secondary metabolite compounds like alkaloids, sapogenin, chlorogenin, solasodine, solamargine, solanine and tomatidine were isolated from leaf and stem of *Solanum* species [7 8].

Pharmacological studies reveal that the stem, roots and fruits of *S. torvum* have cytotoxic, anti-tumor, anti-bacterial, anti-viral and anti- inflammatory properties[9]. Anticancer phenolic compounds have also been isolated from leaves and seeds of *S. torvum* [10]. Solvent extracts of *S. torvum was* found to be extremely effective in the prevention of cell proliferation of the mammary gland breast adenocarcinoma cell lines [11].

Callus extracts of *S. torvum* and isolated glycoalkaloids show increased cytotoxicity against human breast carcinoma cell lines [12]. Aqueous extract of *S. nigrum* whole plant possess antitumor activity, inhibitory effect on cell migration and suppression of aerobic glycolysis towards breast cancer MCF-7 cells [13]. Methanolic leaf extracts of *Datura stramonium* possesses *in vitro* immunostimulatory potential that boosts the immune cells and enhances their anticancer abilities against human breast (MCF-7) carcinoma cell lines[14].

Breast cancer is the second leading cause of mortality among women. MCF-7 breast cancer cells always serves as an excellent source of *in vitro* model for studying the mechanism of tumor response as well as complex relationships between binding and biological systems. Studies reported earlier on *S. torvum* highlights extensively on the pharmacologic properties of its fruits using various solvent systems on cancer cell lines. Studies pertaining to the pharmacologic action of aqueous extracts of leaves of *S. torvum* on Vero and MCF 7 cell lines are scanty. Many studies need to be undertaken to arrive at a meta-analysis by medical practioners for its use in the field of modern medicine. Information on ethnopharmacognosy is to gain knowledge for an effective method in the discovery of new anti-infective molecules from these plants. In this study an attempt was made to find the effect of aqueous extract of *S. torvum* leaves on normal Vero and MCF-7 cell lines to unravel the cytotoxic effect and the anticancer activity respectively.

# MATERIALS AND METHODS

### Collection and Identification of Plant Material

Leaves of *S. torvum* used for the study were collected from in and around Kanchipuram District, Tamil Nadu during the months of February and March, 2018. Fresh plant specimen collected was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Tambaram, Chennai. Registration No. (PARC/2018/3855).

### Processing and Preservation of Plant Material

The plants were freshly collected, the leaves were separated from the stem. Leaves were washed with running tap water and rinsed in distilled water. The leaves were shade dried for two week for complete dryness. The dried leaves were powdered, using mechanical grinder. They were ground well to fine powder and then transferred into air-tight containers until further use.

### Cold Water Extraction

5 g of the dried leaf powdered samples was soaked and dissolved in 50 ml of distilled water in a 250 ml conical flask. The flask was plugged with cotton wool and aluminium foil and was placed in a shaker for 24h. The filtrate was concentrated in a Soxhlet apparatus to get the crude plant extracts. The extract was filtered using Whatman filter paper No 1. The filtered extracts in the form of concentrated paste were used for the study. The aqueous extract was evaluated for preliminary phytochemical screening.

### Procurement and Maintenance of Cell lines

Normal Vero and MCF-7 cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines procured were maintained at Life Teck Research Centre, Arumbakkam, Chennai, Tamilnadu, India. The cells were maintained in Minimal Essential Media (MEM) and was supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 U/ml) and Streptomycin (100µg/ ml) in a humidified atmosphere of 5% $CO_2$ at 37°C.

### Cell Proliferation Assay: *In vitro* studies

Aqueous leaf extracts of *S. torvum* was analyzed for cytotoxicity of Normal Vero cell line and Anticancer activity of MCF-7 cell line based on the principle of MTT assay.[15]

### Incubation of Vero and MCF-7 cell lines

Cells ($1 \times 10^5$/well) were plated in 24-well plates and incubated in 37°C with 5% $CO_2$ condition. After the cell reaches the confluence, the prepared sample plant extract was added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) 100 µl/well without serum. 5 mg/ml of 0.5% MTT was added and incubated for 4h. After incubation, 1ml of DMSO was added to all the wells. The absorbance at 570 nm was measured with UV-spectrophotometer using DMSO as the blank in triplicates. Measurements were performed and the concentration required for a 50% inhibition ($IC_{50}$) was determined graphically. The % cell viability was calculated using the following formula:

% Cell Viability= $A_{570}$ of treated cells/$A_{570}$ of control cells X 100

**Morphological studies of Vero and MCF-7 cells after 24 hr incubation**

The aqueous leaf extract treated cell lines (Normal Vero and MCF-7) were observed and photographed under inverted animal cell culture microscope (LABOVERT-FS) under10x objective.

**Cell Viability Analysis - Tryphan Blue Dye Exclusion method**

A simple cell count method was performed to assess live and dead cells using hemocytometer and Tryphan blue, a vital dye. This assay was based on the assumption that the dead cells will take up the dye and viable cells will not take up dye.[16] Cell count was performed for MCF-7 cell lines treated with aqueous leaf extracts by staining with tryphan blue dye. The dead and live cells counted were obtained from the $IC_{50}$ concentration at the end of 24h incubation. The percentage of viable cells (live cells) and non-viable cells (dead cells) from the aqueous extracts treated cell line were calculated. The percentage growth inhibition was calculated as

% Growth Inhibition (Dead Cells) = 100 – (Total Cells – Dead Cells / Total Cells) x 100

**Determination of Selectivity index (SI)**

The degree of selectivity of the aqueous extract of leaf of *S. torvum* is expressed by its SI value. The SI value was calculated based on the effect of the extracts on Normal Vero cell line and MCF- 7 cell line. [17] The SI value of the extract was calculated using the formula:

$$SI = CC_{50} \text{ normal cell line} / IC_{50} \text{ cancer cell}$$

Cytotoxic concentration of ($CC_{50}$) of normal vero cell line and inhibitory concentration ($IC_{50}$) of MCF-7 cell line

# RESULTS AND DISCUSSION

Phytochemical analysis of the aqueous extracts of *S.torvum* leaf showed the presence of various specific phytoconstituents such as flavonoids, alkaloids, phenols, saponins, coumarins, sterols, proteins and reducing sugars (**Table 1**).

**Cytotoxicity and Anti-cancer activity of aqueous leaf extract of *S. torvum* on normal Vero cell line and MCF -7 cell line**

The percentage cell viability shown by Vero cell line treated with aqueous leaf extracts was 88.96 % at 7.8 µg/ml and 50.32 % at 1000 µg/ml. The $IC_{50}$ was also recorded at 1000 µg /ml during 24h of incubation. The MCF 7 cells treated with the leaf extracts showed percentage cell viability of 80.37% at 7.8 µg /ml and 31.02% at 1000 µg /ml. The $IC_{50}$ is reported at 125 µg /ml at 24h of incubation (**Figure 1**).

**Table 1: Phytochemical screening of aqueous extracts of S. *torvum* leaf**

| S.No | Phytochemical Compounds | *S. torvum* leaf extract |
|---|---|---|
| 1 | Flavanoids | + |
| 2 | Alkaloids | + |
| 3 | Phenols | + |
| 4 | Coumarin | + |
| 5 | Triterpenes | + |
| 6 | Saponins | + |
| 7 | Steroids | + |
| 8 | Proteins | + |
| 9 | Reducing Sugars | + |
| 10 | Anthraquinones | - |
| 11 | Anthocyanins | - |
| 12 | Tannins | - |

Presence of the compound (+) and absence of the compound(-)

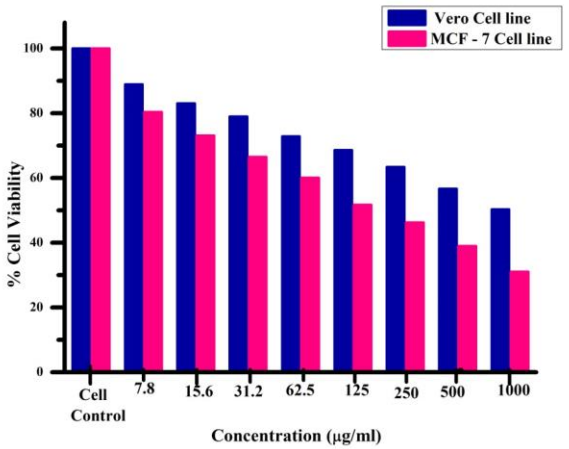


**Fig.1: Graph representing the cytotoxicity and anticancer activity of of *S. torvum* aqueous leaf extract on normal Vero cell line and MCF-7 cell line at 24h incubation.**

**Morphological studies of Vero cells treated with aqueous extracts of *S. torvum* leaf**

Vero cells are uniformly spread in confluent layer with long and elongated shape in appearance. At highest concentration of 1000 µg /ml of leaf extracts the treated cells lose their normal cytostructure and show polygonal shape with shrunken cells at the end of 24h incubation (**Figure 2**).

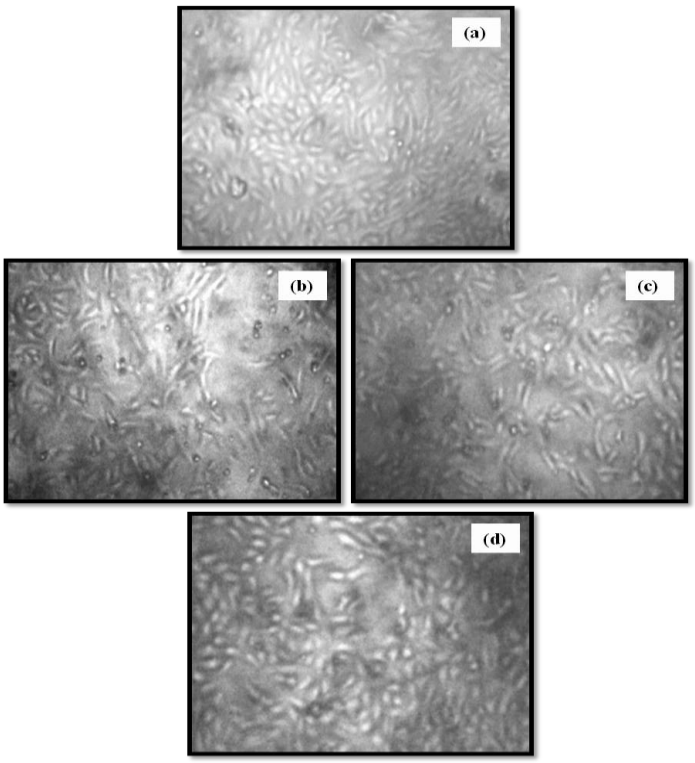


**Fig. 2 : Photomicrograph of *S. torvum* aqueous extract of leaf on the morphology of normal vero cell line at different concentrations (a) Vero Cell line (b) 1000 µg/ml (c)125 µg/ml (d) 7.8 µg/ml**

**Morphological studies of MCF-7 cells treated with aqueous extracts of leaf**

MCF-7 cells treated with different concentrations of the extracts show reduction in number of cells as the concentration increases. Cells show loss of regular shape and size. Majority of cells are with flattened structures with cell to cell contact disappearing (**Figure 3**).

**Cell count of live and dead cells by Tryphan blue dye**

MCF-7 breast cancer cell line treated with aqueous extract of leaf show variation in the number of live and dead cells. The cell viability was 53.84% and cell death was 46.16% at $IC_{50}$ concentration of 125 µg /ml (**Table 2**).

**Table 2**: **Cell count to observe live and dead cells of MCF-7 cell line treated with aqueous extract of *S. torvum* leaf by Tryphan blue dye at $IC_{50}$ concentration**

| Aqueous extract | Live cells observed | Dead cells observed | Viability (%) | Cell death (%) |
|---|---|---|---|---|
| Leaf | 142 | 123 | 53.84 | 46.16 |

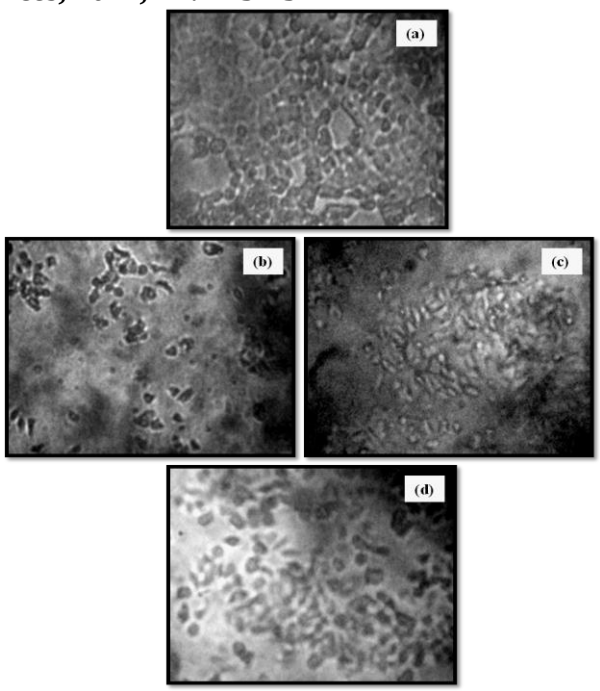


**Fig. 3: Photomicrograph of *S.torvum* aqueous extract of leaf on the morphology of MCF-7 cell line at various concentrations (a) Normal MCF-7 Cell line (b) 1000 µg/ml (c)125 µg/ml (d) 7.8 µg/ml**

**Selectivity Index (SI)**

The SI for ($CC_{50}$) of Vero cell line and ($IC_{50}$) of MCF 7 cell line for cells treated with aqueous extracts of leaf was 8 after 24 hr of incubation study (**Table 3**).

**Table 3**: **SI of ($CC_{50}$) of normal Vero cell line and ($IC_{50}$) of MCF-7 cell line treated with aqueous extract of leaf of *S. torvum* at 24 hrs of incubation**

| Aqueous extract | Vero cell line (µg/ml) | MCF-7 cell line (µg/ml) | SI ($CC_{50}$) / ($IC_{50}$) |
|---|---|---|---|
| Leaf | 1000 | 125 | 8 |

Medicinal plants are the paramount sources for drug discovery in the modern era and can be an alternative for treatment of cancer. In the present study cytotoxicity analysis on Vero cell line revealed that the aqueous leaf extract of *S. torvum* has no toxicity. The $CC_{50}$ was found at 1000 µg/ml for African green monkey kidney (Normal Vero) cell line when treated with the aqueous extract of *S. torvum* leaves at 24 h of incubation showed 50.32 % of cell viability and 49.68% cell death or cell inhibition respectively.

The $IC_{50}$ was found at 125 µg/ml for human breast cancer MCF-7 cell line when treated with the aqueous extract of *S.*

*torvum* leaves after 24 h of incubation. The MCF-7 cell line treated with aqueous extracts of leaves show 51.70 % of cell viability and 48.30% cell death or cell inhibition respectively during cell proliferation studies.The extracts was effective in reducing the cell viability of MCF-7 breast cancer cell lines. The $IC_{50}$ was determined based on inhibitory concentration that induced 50% inhibition on the growth of the treated cells as compared to the untreated cells.

The phytochemical analysis in the present study shows presence of phytoconstituents such as flavonoids, alkaloids, phenols and saponins in the aqueous extract of leaves which may have contributed to inhibition in cell proliferation. The observed cytotoxic and anticancer action of the aqueous extracts of *S. torvum* leaves *are* in accordance with earlier studies carried out by several researchers on the properties of *Solanum* phytoconstituents.[18]. The phytocompounds isolated from Solanaceae family have been reported to possess several medicinal values, antioxidant and anticancer properties. [19, 20].

Flavonoids which prevent oxidative cell damage have shown to possess anti-proliferative role in cancer cells. This compound has profound effects on signal transduction mechanisms in cell proliferation and angiogenesis.[21] Polyphenol constituents like kaempferol, quercetin, rutin and gallic acid isolated from *Zingiber officinale* inhibited the growth of human breast cancer cell lines such as MCF-7 and 3,4-methylenedioxyamphetamine (MDA)MB-231.[22]

*Solanum* genus have also been shown to contain steroidal glycoalkaloids and steroidal saponins with significant cytotoxic and anti-tumour activities that have a close structural relationships.[23] Saponins react with cholesterol rich plasma membrane of cancer cells and inhibit their proliferation. Studies have demonstrated that several classes of compound including phenolic compounds, terpenes and coumaric acid may possibly act synergistically to inhibit cell proliferation and induce apoptosis in cancer cells. [24]The anti-proliferative effect observed in MCF-7 cell line in the present study can be attributed to the combined integrated mechanism of action of the phytochemicals present in *S. torvum* leaf aqueous extracts. The qualitative analysis of these phytochemicals from the extracts also substantiates this role.

Considerable amount of sterol content in aqueous extracts of *S. torvum* leaf have been reported. The anticancer activity of *S. torvum may* be attributed, at least partially, to steroidal alkaloid and steroidal saponin substances present in the leaves.[25] Similar results were observed on mutagenic and cytotoxic studies in mice induced with cancer and treated with ethanolic leaf and fruit extracts of *S.paniculatum*. [26]

The cytotoxic action indicates that the aqueous leaf extracts probably contain secondary metabolites or novel compounds which may inhibit cellular division in cancer cells.[27,28] Studies on *S. erianthum* revealed high cytotoxic and anticancer activity which is attributed to presence of compounds like flavonoids and phenols.[29] Aqueous extract of *S. macranthum* fruit was found to be very potent to inhibit the growth of MDA-MB-231 breast cancer cell line at low concentration by regulating carcinogen metabolism, oncogenesis expression, inhibiting DNA binding and cell adhesion.[30,31]

Aqueous extracts of *S. torvum* leaf was found to be extremely effective in the prevention of cell proliferation of breast adenocarcinoma cell lines. The extract if tested in animal models or administered in human may prevent cell proliferation by possible mechanism which could directly combine with cell receptors and elicit cellular apoptosis. Plant derived phytochemicals coupled with chemotherapy has gained much importance now-a-days in alleviating the proliferation of various carcinomas with minimal side effects. [32]

Based on our results of cell proliferation studies, cell viability was also performed by staining the cells treated with tryphan blue. The dead and live cells were counted from $IC_{50}$ concentrations of 24 h incubation represented about 53.84% live cells and 46.16% dead cells were identified. It is reported from other studies that this phenomenon is hormesis, which is characterized by the fact that low dose of test compound stimulates cell growth and high dose causes a reduction in cell viability. [33]

Tryphan blue is water soluble dye and it is insoluble in membrane lipids. Chromophores is negatively charged and does not interact with cells unless the membrane is damaged. This could have been the possible mechanism in the present study when dead or non-viable cells show membrane damage and take up the dye whereas the viable cells do not take up the dye and are transparent as revealed from the cell count of the study. [34] Similar results on Trypan blue dye exclusion is reported in MCF-7 cell lines treated with aqueous and hydro-alcoholic extracts of stem in *Tinospora cordifolia* [35] and in A549 cell lines treated with acetone and ethanolic leaf extracts of *Tridax procumbens.* [36]

Morphological alterations in Vero and MCF-7 cell lines clearly validated that extracts of *S. torvum* induced drug dosage response in the present study. Microscopic observations clearly revealed Vero cells with regular shape and size at lower concentrations of the extract and with loss of their normal cytostructure at higher concentration. MCF-7 cells are seen in clusters, spindle shaped at lower concentrations. Cells with flattened structures are seen with increase in concentration of the extract at 24 h of incubation. The progressive changes seen are in a dose dependent manner.

Cell morphological analysis indicated a significant loss of cell structure and disruption of cellular organelle integrity following treatment of the MCF-7 cell lines with aqueous extracts of leaf which can be correlated to non-viable cells exposed to tryphan blue. Concentration and time dependent changes were revealed in morphology of MCF-7 cell line treated with solasodine and caulophyllumine. Similar morphological changes were observed in MCF-7 cells treated with *Pongamia glabra* seed oil extract [37] and alkaloid

extracts of leaf from *Excoecaria agallocha*. [38] Loss of cell adhesion, shrinkage in cytoplasmic area, rounding up of cells and nuclear condensation were identified in liver and lung cancer cell lines treated with aqueous extracts of turbinol, a sterol isolated from *Turbinaria conoides* [39]

The degree of selectivity of the compounds is expressed by its SI value . The greater SI value (above 2) of a compound suggests selective toxicity and differential action against the target cells, while a compound with SI value (less than 2) indicates general toxicity of the pure compound which can also cause cytotoxicity in normal cells. [40] The $IC_{50}$ values are used to determine the SI of each extract which represents the overall activity between normal cell line and cancer cell line. The SI value for the present study on Normal Vero cell lines and MCF-7 breast cancer cell lines is calculated as 8 for cells treated with leaf aqueous extracts.

Based on SI it is indicated that the *Boehmeria virgata ethanolic* leaf extract could be evaluated as a very strong anticancer agent against cancer cell line, HeLa, WiDr and T47D suggesting its general toxicity to the cell. [41] Methanolic extracts of *Centaurea antiochia* aerial parts (SI > 227), *Cotinus coggygria* leaves (SI >3.4) and *Rosa damascene* flowers (SI >3.8) had effective and stronger anticancer potential potential against the HeLa cancer cell line according to the SI values.[42] 'Therapeutic index' is an important parameter to select samples for developing drugs.[43]

The results of the present study suggest that the uptake of the bioactive compounds from aqueous extract of *S.torvum* leaf may suppress growth of cancer cells *in vitro* in breast cancer cell line MCF-7 and may prevent breast cancer development and proliferation induced by carcinogens. This is promising for its further development as an anticancer drug.

Medicinal properties of herbals are pivotal because of their diverse phytochemicals and nutrients which are potent in them. The raw materials form the base for drug production in pharmaceuticals. Proper understanding of the interaction of various constituents of anticancer herbs, would help in formulating and designing drug to attack the cancerous cells without harming the normal cells of the body.[44]

# CONCLUSION

This medicinal plant *Solanum torvum* can be considered for drug development and synthesis. In this scenario, secondary metabolites from herbals may be the best remedy and alternative for cancer therapy. The present information related with these secondary biomolecules will lead to new sources in search of lead molecules for curing communicable diseases which are infectious and diseases occurring due to lifestyle modification such as diabetes, cardiovascular diseases and cancer.

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# CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interests

# DATA AVAILABILITY

The datasets supporting the conclusions of this research article are included within the article

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