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Assessment of Antioxidant, Antihemolytic and Cytotoxic Effect of *Tridax Procumbens* Linn. Methanol Extracts in HaCaT - Human Keratinocytes Cell Lines

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Abstract: *Tridax procumbens* Linn. is widely employed in the Ayurvedic system of medicine due to its diverse array of therapeutic properties. The present study emphases on the phytochemical screening, antioxidant study, hemolytic study and *in vitro* cytotoxic effects of *T. procumbens.* Phytochemical evaluation of the plant's aerial parts confirmed the presence of alkaloids, flavonoids, phenols, steroids and terpenoids in the methanol extract. The DPPH and reducing power assays demonstrated that the extract effectively scavenges free radicals in a concentration-dependent manner. Hemolytic activity exhibited by *T. procumbens* at the tested concentrations was within the permissible limit (< 5%). There was a mild reduction in clotting time, during *in vitro* condition. Cytotoxicity evaluation of *T. procumbens,* carried out using standard *in vitro* methods (HaCaT - Human keratinocytes cell line studies), indicated dose-dependent cytotoxic effects of the extracts (IC_{50} value of 142.275µg/mL) and cell viability (non-significant at 6.25 µg/mL, compared to standard ascorbic acid; $p < 0.0001$ for doses 12.5 to 100 µg/mL). The study reveals antihemolytic action of the methanol extract at low concentration and moderate cytotoxic activity at higher concentrations, suggesting safe and potential applications in anticancer research, although further detailed investigations are required.

Keywords: *Tridax procumbens; Hair Growth; Anti Hemolytic Activity; in Vitro Cytotoxicity; HaCaT-Cell Line Studies*

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I. INTRODUCTION

Medicinal plants have traditionally been acknowledged for their significant therapeutic potential due to abundant bioactive compounds within them. *Tridax procumbens* Linn. is a wild herb distributed throughout the world's tropics and subtropics, thriving in waste areas and agricultural fields. "It was determined to possess potential therapeutic activities like wound healing, antidiabetic, hypotensive, antimicrobial, insect repellent, anti-inflammatory, antioxidant, treat bronchial catarrh, dysentery, diarrhea, prevent hemorrhage, falling of hairs and leads to hair growth promotion" (Himanshu *et al*., 2022). "In folk medicines leaf extract were known to treat infectious skin diseases" (Amutha *et al*., 2019); Studies confirmed that the leaf juice extracts have been applied topically for wound healing by the native populations of Tamil Nadu, India (Mahima Anil Nikam, 2024). Reviews reveal that the plant has been used extensively for promoting hair growth and treating skin diseases. However, detailed report on the toxicity and effectiveness of the plant are still lacking. The current study focuses on the phytochemical screening, antioxidant study, hemolytic study and *in vitro* cytotoxic effects of *T. procumbens.*

II. MATERIALS AND METHODS

- *Plant Materials*

Fresh aerial parts of *Tridax procumbens* were gathered from Mulloorthurai, a coastal village in southern part of Tamil Nadu, India. It was packed immediately in polythene bags to avoid decomposition of bioactive compounds and was transported to the laboratory for further processing and analysis. The plant was taxonomically identified and authenticated with the help of standard botanical literature.

- *Chemicals and Standards*

All chemicals, solvents and standards utilized for the phytochemical screening and antioxidant assays were of analytical grade with high degree of purity, purchased from Nice, Cochin and Himedia Laboratories Pvt Ltd, Mumbai.

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➢ *Extraction of Plant Materials*

Fresh aerial parts of *T. procumbens* were washed, shade dried and powdered. About 50 g of powdered plant material was packed using muslin cloth into a Soxhlet apparatus. Methanol extraction was carried out over a duration of 3 hours. The extract obtained was then concentrated by solvent evaporation and the residue was weighed and stored at low temperature for further studies such as phytochemical analysis, antioxidant activity, hemolytic study and cytotoxicity study.

➢ *Phytochemical Analysis*

The crude methanol extract of *T. procumbens* obtained by Soxhlet extraction was evaluated to identify its various phytochemical components. The phytochemical analysis was performed according to the following methods.

- *Test for Carbohydrate*

✓ *Molisch's Test:*

An aliquot of 2 mL of the extract was treated with a few drops of Molisch's reagent, followed by the careful addition of a small amount of concentrated sulfuric acid. The presence of carbohydrates was indicated by the development of a characteristic purple ring at the junction of the two liquids.

- *Test for Reducing Sugar*

✓ *Fehling's Test:*

To 2ml of plant extract, added 5ml of distilled water and filtered. The resulting filtrate was then boiled with 3 to 4 drops of Fehling's solutions A and B for two minutes. The presence of reducing sugars was confirmed by the development of an orange-red precipitate.

✓ *Benedict's Test:*

To 2.5 ml of Benedict's reagent, 4 drops of the plant extract was added and the tube was kept in boiling water bath for 2 minutes. Formation of brick red precipitate indicated the presence of reducing sugar.

- *Test for Protein*

Taken 2 ml of plant extract and added 2 ml of Biuret reagent, shaken well, and allowed to stand at room temperature for 5 minutes. A violet colour formation showed the presence of protein.

- *Test for Amino Acids*

Taken 1ml of plant extract and added few drops of Ninhydrin reagent. Kept in boiling water bath for 5 minutes. Formation of deep blue or violet colour indicated the presence of amino acids.

- *Test for Tannins*

✓ *Braymer's Test:*

A 2ml aliquot of the plant extract was diluted with water and heated briefly in a water bath. After thorough mixing and filtration, the filtrate was treated with a 5% ferric chloride reagent. The appearance of an intense dark green hue confirmed the presence of tannins.

- *Test for Alkaloids*

✓ *Dragendroff's Test:*

Taken 1ml of Dragendorff's reagent and added 2 ml of plant extract. An orange red precipitate indicated the presence of alkaloids.

- *Test for Terpenoids*

2ml of extract was treated with 1ml of 2,4-dinitrophenyl hydrate dissolved in 100ml of 2M KCl. Formation of yellow-orange colouration confirmed the presence of terpenoids.

- *Test for Flavonoids*

Taken 2 to 3 drops of diluted NaOH and added 2ml of plant extract to form a deep yellow colour. To this, added a few drops of diluted HCl. Formation of a colourless solution indicated the presence of flavonoids.

- *Test for Steroids*

✓ *Salkowski Test:*

Taken 2ml of extract and 2 ml of chloroform in a test tube and added 1ml of concentrated H_2SO_4 carefully along the sides of the test tube. The development of red colour at the junction indicated the presence of steroids.

- *Test for Phenols*

A 2 ml aliquot of the plant extract was mixed with 2 ml of distilled water in a test tube, followed by the addition of 2 mL of 10% ferric chloride solution. A bluish black colour showed the presence of phenols.

- *Test for Saponins*

An aliquot of 2 ml of the plant extract was diluted with 5 ml of distilled water and heated to a boil. The formation of persistent frothing, characterized by the appearance of a creamy mass of small bubbles, indicated the presence of saponins.

- *Test for Glycosides*

✓ *Libermann's Test:*

To 2 ml of plant extract, added 2ml of chloroform and acetic acid. The change of violet to blue coloration indicated the presence of glycosides.

➢ *Antioxidant Assay*

- *Total Antioxidant Capacity*

The total antioxidant capacity of the methanol extract of *T. procumbens* was determined by phosphomolybdenum method (Asok Kumar *et al.*, 2008) using ascorbic acid as the standard. An aliquot of 1ml of extract (100µg) was mixed with 1ml of reagent (0.6M sulphuric acid, 28µM Sodium phosphate and 4 µM ammonium molybdate). The tubes were stoppered and incubated in a boiling water bath at 95ºC for 90 minutes. Allowed to cool to room temperature and the absorbance was measured at 695nm in a UV-Spectrophotometer. The blank solution contained 1ml of reagent and 1 ml of methanol, which was incubated under

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same conditions as the test sample. The total antioxidant capacity was expressed as equivalence of ascorbic acid.

- *Reducing Power Assay*

The reducing power of the extract was assessed by the method of Yen and Duh (1993). Varying concentrations of the extract (25 to 200µg/ml) were mixed with 2.5ml of Phosphate buffer (200 mM; pH 6.6). After addition of 2.5ml of 1% Potassium ferricyanide, the mixture was incubated for 20 minutes at 50ºC. Subsequently, 2.5ml of 10% TCA was added to this mixture, and centrifuged at 650xg for 10 minutes. The upper layer (5ml) was added to 5ml of distilled water and 1ml of 0.1% Ferric chloride, and the absorbance was read at 700 nm. A blank was also treated in a similar manner excluding sample. Varying concentrations of ascorbic acid served as the standard.

- *DPPH (2,2- Diphenyl-1-Picrylhydrazyl) Scavenging Activity*

The DPPH radical scavenging activity of the extracts was determined according to Chang *et al*., (2001). 40µl of varying concentration (12.5 to 200 µg/ml DMSO) of the extract was made up to 3ml by adding 2.96 ml DPPH (0.1 mM) solution. The reaction mixture was incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3 ml of DPPH was taken as control. Ascorbic acid (10mg/ml DMSO) was used as reference. Percentage inhibition was calculated using the formula,

$$100\%\ Inhibition = \frac{Control - Test}{Control} X\ 100$$

➢ *Hemolytic Study*

- *Determination of in-Vitro Hemolytic Activity*

Hemolytic activity was studied using blood collected from five healthy student volunteers. First, erythrocyte suspension was prepared by collecting blood in tubes containing 2 mg/mL K3-EDTA, followed by centrifugation at 1,300 g for 10 minutes. Plasma obtained and the top layer of cells were discarded. The plasma and upper buffy coat layer were discarded. The remaining erythrocytes were resuspended in 10% (v/v) phosphate-buffered saline (PBS), mixed thoroughly, and centrifuged again at 2500 g for 10 minutes. This washing process was repeated until a clear supernatant was obtained, after which the final erythrocyte pellet was resuspended in 10% (v/v) PBS.

In-vitro hemolytic activity was performed by spectrophotometer method (Yang et al., 2005). A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (125, 250, 500 and 1000 µg/ml concentrations in phosphate buffer saline). For control of 100% hemolysis, mixtures of 0.5ml erythrocyte suspension and 0.5ml pure distilled water were used. Tubes containing only the erythrocyte suspension and PBS served as the negative control. Following incubation at 37○C for 60 minutes, the tubes were centrifuged at 1300 g for 3 minutes. The absorbance of free hemoglobin in the resulting supernatant was then measured using a spectrophotometer at 540 nm. The percentage of hemolysis induced by the extracts was determined using the following formula:

$$Percentage\ hemolysis = \frac{(Absorbance\ of\ samples - Absorbance\ of\ blank)}{Absorbance\ of\ positive\ control} X\ 100$$

- *Clotting Time*

Two glass slides were marked as *Tridax procumbens (TD)* and Control (C). A healthy volunteer was randomly selected from the hemolytic study group and clotting time was recorded. The finger was pricked aseptically and blood was dropped to the glass slide marked as 'C'. Started the stopwatch, noted the clotting time after every 30 seconds. When the blood was clotted, stopwatch was stopped and the clotting time was recorded. To study the effect of *T. procumbens* on clotting time, 20µl of fresh extract was taken in the glass slide and a drop of blood was added (same blood), mixed gently and the clotting time was recorded. The effect was also noted in the same way after airdrying 20µl of extract (Table 8). The contents in each slide were added 20µl of 10% PBS, mixed gently and viewed under the microscope.

➢ *In Vitro Cytotoxic Effect*

- *Determination by MTT Assay*

The HaCaT (human keratinocyte) cells, originally obtained from the National Centre for Cell Sciences (NCCS), Pune, India, were maintained in Dulbecco's modified Eagles medium (DMEM; Sigma-Aldrich, USA).

The cell lines were grown in 25 cm^2 tissue culture flask containing DMEM, 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany). The culture medium was further supplemented with antibiotics including Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2 .5µg/ml). The cultured cell lines were incubated at 37 ºC in a humidified 5% CO_2 incubator (Eppendorf, Germany).

Morphological changes and cell viability were initially monitored under an inverted phase-contrast microscope, followed by a definitive MTT cell viability assay.

- *Cells Seeding in 96 Well Plate*

Two days old confluent monolayer of cells was trypsinized and the collected cells were suspended in 10% growth medium. Subsequently, 100µl of the cell suspension ($5x10^3$ cells/well) was transferred to a 96-well tissue culture plate, followed by incubation at standard conditions (37 ºC, humidified 5% CO_2 incubator).

- *Preparation of Stock Solution*

One milligram of the sample was dissolved in 1ml 0.1% DMSO using a vortex mixer. To ensure sterility, the sample solution was filtered through a 0.22 µm Millipore syringe filter.

- *Cytotoxicity Assessment*

The growth medium was removed following a 24 h incubation, and freshly prepared compounds were added at concentrations ranging from 6.25 µg/ml to 100 µg/ml in DMEM. Each treatment was performed in triplicate, and the

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cells were incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. Untreated cells served as the control.

- *Morphological Assessment of Cytotoxicity*

Following the 24 h treatment period, cellular morphology and viability were examined directly using an inverted phase-contrast microscope (Olympus CKX41). Digital images were recorded using an attached Optika Pro5 CCD camera. Detectable alterations in cell morphology, including cell rounding, shrinkage, cytoplasmic granulation, and vacuolization, were evaluated as indicators of cytotoxicity.

- *Cytotoxicity Assay Using the MTT Method*

An MTT stock solution was prepared by dissolving 15 mg of MTT (Sigma, M-5655) in 3 ml of PBS, followed by filter sterilization.

Subsequent to the 24 h treatment period, the medium was removed from the wells, and 30 µl of the reconstituted MTT solution was added to all the test and cell control wells. The plate was gently agitated and then incubated for 4 h at standard conditions (37 ºC, humidified 5% CO_2). The supernatant was carefully discarded after 4 h, and 100 µl of Dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) was added to each well. The formazan crystals were then dissolved completely by gentle pipetting. The absorbance of each well was measured using a microplate reader at a wavelength of 540 nm (Talarico et al., 2004). Cell growth inhibition (%) was then calculated as follows:

$$\%\ of\ Viability = \frac{Mean\ OD\ of\ samples}{Mean\ OD\ of\ Control\ group} X\ 100$$

III. RESULTS AND DISCUSSION

➢ *Dry Weight and Moisture Content of Selected Plants.*

The dry weight and moisture content in aerial parts of *T. procumbens* are shown in Table 1.

Table 1 Dry Weight and Moisture Content of *Tridax Procumbens*.

Parameters	g/100g
Dry weight	85.4
Moisture content	14.6

➢ *Percentage Yield of Methanolic Extracts of Tridax procumbens.*

The percentage yield obtained for the aerial parts of *T. procumbens* was found to be 25.76% (Table 2).

Table 2 Percentage Yield of Methanolic Extracts of *Tridax procumbens.*

Extract	Yield (%)
Methanol	25.76

➢ *Phytochemical Analysis of Tridax procumbens methanolic Extract.*

Table 3 displays the result of phytochemical screening of *T. procumbens* which showed the presence of carbohydrates, reducing sugar, amino acids, alkaloids, terpenoids, flavonoids, steroids and phenols in the methanol extract. These compounds are known for their diverse biological activities, particularly their antioxidant properties.

Table 3 Phytochemical Analysis of *Tridax procumbens* Methanolic Extract.

S.No.	Phytochemicals	*Tridax procumbens* (aerial part)
1	Carbohydrates	++
2	Reducing sugar	++
3	Amino acids	++
4	Protein	_
5	Tannins	_
6	Alkaloids	++
7	Terpenoids	++
8	Flavonoids	++
9	Steroids	+++
10	Phenols	++
11	Glycosides	_
12	Saponins	_

(+++), (++), (+) sign indicate presence of phytochemical content in high, moderate and low amount respectively; (-) sign indicates absence of phytochemical content.

➢ *Antioxidant Study*

- *Total Antioxidant Capacity of Tridax procumbens Methanolic Extracts.*

The total antioxidant capacity was expressed in terms of mg/g ascorbic acid equivalent. The results were interpreted in Table 4. The total antioxidant capacity in the methanolic extract of *T. procumbens* was found to be 42.85±1.06 mg/g ascorbic acid equivalent.

Table 4 Total Antioxidant Capacity of *Tridax procumbens* Methanolic Extracts.

Plant part used for study	Plant	Total antioxidant capacity (mg/g Ascorbic acid equivalent)
Aerial part	*Tridax procumbens*	42.85±1.06

- *Reducing Power Assay of Tridax procumbens Methanolic Extract.*

The reducing power activity was evaluated using various concentration (25 to 200 µg/ml) of *T. procumbens* methanolic extract. The increase in absorbance on increase in concentration indicated increased reducing power in the range of 0.065±0.003 to 0.483±0.012 OD in comparison with standard Ascorbic acid (0.085±0.002 to 0.627±0.003 OD). The results are interpreted in Table 5.

Table 5 Reducing Power Assay of *Tridax procumbens* Methanolic Extract.

Plant Extract	Concentration (µg/mL)	OD (700 nm)
Tridax procumbens (Aerial part)	25	0.065±0.003
	50	0.112±0.002
	100	0.243±0.002
	200	0.483±0.012

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Ascorbic acid (Standard)	25	0.085±0.002
	50	0.162±0.003
	100	0.338±0.007
	200	0.627±0.003

Values expressed are Mean±SD of triplicate analysis.

- *DPPH (2,2-Diphenyl-1-Picryl Hydrazyl) Radical Scavenging Activity of Tridax procumbens Methanolic Extract.*

Free radical scavenging activity was assayed using DPPH against various concentrations of methanolic extract. The percentage of inhibition was found to increase in the range of 42.31±1.21% to 81.40±2.31% (*T. procumbens*) and 51.54±1.75% to 88.46±2.49% (Ascorbic acid). The results are interpreted in Table 6. The results exhibited a dose dependent pattern and was comparable to that of standard ascorbic acid.

Table 6 DPPH (2,2-Diphenyl-1-Picryl Hydrazyl) Radical Scavenging Activity of *Tridax procumbens* Methanolic Extract.

Plant Extract	Concentration (µg/ml)	OD at 517 nm	% Inhibition
Tridax procumbens (Aerial part)	Control	0.130±0.001	
	12.5	0.185±0.003	42.31±1.21
	25	0.064±0.002	50.77±1.69
	50	0.056±0.001	56.92±1.47
	100	0.037±0.001	71.54±2.13
	200	0.024±0.001	81.40±2.31
Ascorbic acid (Standard)	12.5	0.063±0.004	51.54±1.75
	25	0.042±0.004	67.69±1.77
	50	0.031±0.001	76.10±2.06
	100	0.022±0.001	83.08±2.46
	200	0.015±0.002	88.46±2.49

Values expressed are Mean±SD of triplicate analysis.

The antioxidant study exhibited promising antioxidant activity of *T. procumbens* methanolic extract. This is attributed to the presence of abundant levels of phytoconstituents in the extracts. The plant extract demonstrated notable free radical scavenging activity, with increasing concentration leading to enhanced inhibition of DPPH radicals. The reducing power assay further supported these findings, indicating strong electron-donating ability of the extract.

➢ *Hemolytic Study*

- *Hemolytic Activity of Tridax procumbens Methanolic Extract.*

The hemolytic activity was done at concentrations ranging from 25 to 200 µg/ml of methanolic extract of the plant. The percentage hemolysis was found to be insignificant in *T. procumbens* and was between 1.428±0.008% and 5.714±0.165%). Under the microscopic view (40X), RBC cells were not ruptured in both *T. procumbens* and negative control. In Positive control, RBC cells were ruptured (Table 7 & Fig 1). The results presented minimal hemolysis at all concentrations, suggesting that the extract is relatively safe and biocompatible within a certain range. These results are reliable with the study of Junior *et al.* (2019) who reported that the absence of hemolysis or hemolysis within the minimum limits is a reliable indicator of hematologic safety and the possibility of using plant extracts in pharmaceutical and biological applications.

Table 7 Hemolytic Activity of *Tridax procumbens* Methanolic Extract.

Plant Extract	Concentration (µg/ml)	OD at 540 nm	% Hemolysis
Tridax procumbens (Aerial part)	25	0.01±0.002	1.428±0.008
	50	0.02±0.002	2.857±0.076
	100	0.03±0.001	4.285±0.171
	200	0.04±0.004	5.714±0.165

Values expressed are Mean±SD of triplicate analysis.

- *Effect of Tridax procumbens Methanolic Extract on Blood Clotting Time.*

The time required for the sample of blood to coagulate *in vitro* under standard condition is called clotting time. The effect of fresh solvent extracts and dried extract on the clotting time of healthy volunteers' blood was performed. The mean value of clotting time was 3.59 minutes in healthy volunteer's blood, while it was slightly reduced to 3.15 minutes by the plant extract (Table 8). Under the microscopic view (40X), in slides of *T. procumbens* and normal control, red cells were intact and not ruptured. The mild reduction in clotting time is suggestive of either activating coagulation factors or exhibiting coagulating property of phytoconstituents during *in vitro* condition. The present study is in correlation with the previous study of and Manjusha Borde *et al.*, (2014) and Dhivyavarshni *et al.* (2018) in various other extracts of *T. procumbens* leaves.

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Table 8 Effect on Blood Clotting Time by *Tridax procumbens* Methanolic Extract.

Clotting Time (minutes)	
Normal Control	3.59
Tridax procumbens	3.15

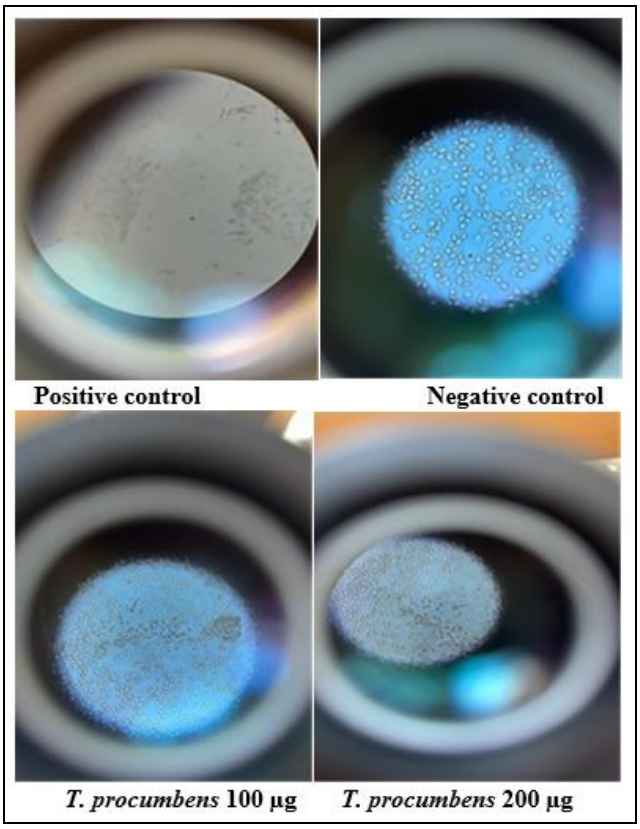

Fig 1 Microscopic Examination of RBC Showing Hemolytic Activity by *Tridax procumbens* Methanolic Extract.

➢ *In-Vitro* Cytotoxicity Study

- *Cytotoxicity Assay of T. procumbens by Direct Microscopic Observation*

The viability of HaCaT (Human Keratinocyte) cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. No significant changes in morphology of cells were observed at a concentration of 6.25 µg/ml. But at higher concentrations, considerable changes were observed in a concentration dependent manner (Fig 2).

- *Cytotoxicity Assay of T. Procumbens by MTT Method*

The viability of HaCaT (Human Keratinocyte) cells were further evaluated by MTT assay and is presented in Table 9 and Fig 3. Cell viability was highest (98.18%) at a concentration of 6.25 µg/ml. At higher concentrations, reduction in cell viability was exhibited in a dose dependent manner. The cytotoxicity was statistically insignificant p< 0.0001 compared to control group. *T. procumbens* methanol extract exhibited IC50 value of 142.275 µg/ml.

This study indicates that *T. procumbens* protects the normal cells at lower concentration (less than 6.25 µg/ml) and therefore may be safe and promotes normal keratinocytes growth. It is proven in the present study that *T. procumbens* have cytotoxic activity at higher concentrations and can therefore be used for destruction of cancerous cells.

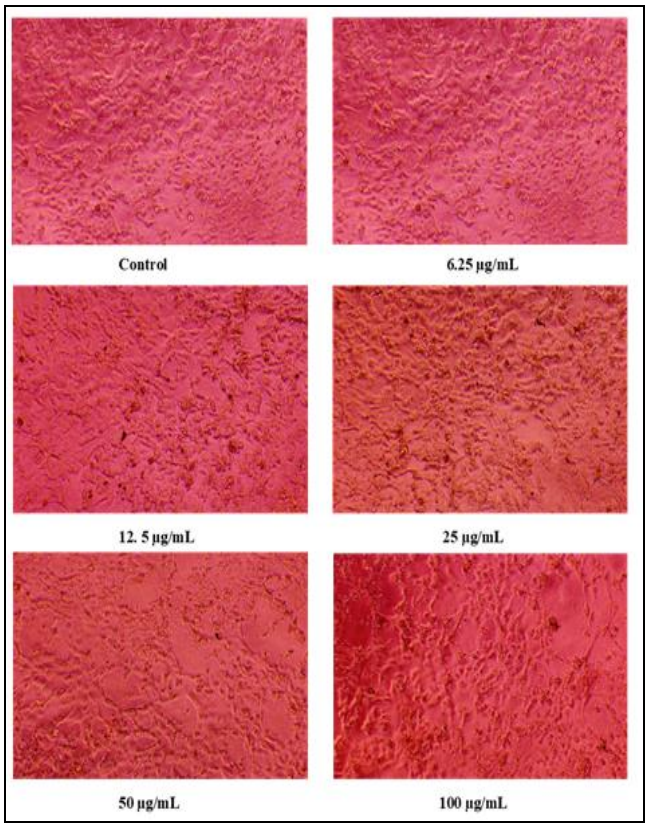

Fig 2 Cytotoxicity Assay by Direct Microscopic Observation

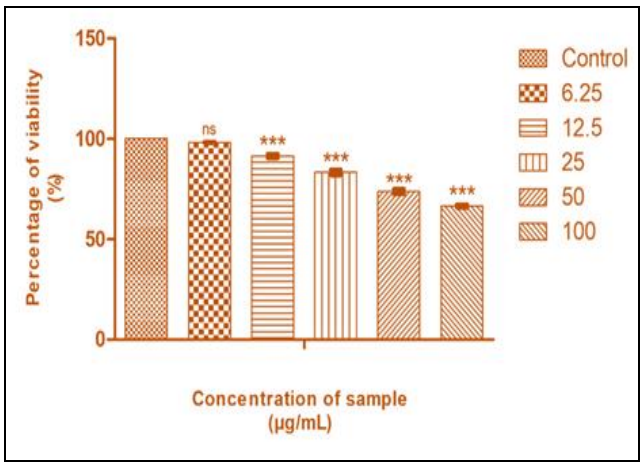

Fig 3 Graphical Representation Depicting the Cytotoxic Effect of Methanolic Extract by MTT Assay

All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***p< 0.0001 compared to control group, ns- non significant compared to control group.

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Table 9 Cytotoxicity Assay of *Tridax procumbens* Using MTT Method

Sample (µg/mL)	Absorbance value I	Absorbance value II	Absorbance value III	Average Absorbance	Percentage Viability
Control	0.5564	0.5629	0.5721	0.5638	100.00
Methanolic Extract of *Tridax procumbens*					
6.25	0.5492	0.5521	0.5593	0.5535	98.18
12.5	0.5128	0.5213	0.5114	0.5152	91.37
25	0.4756	0.4693	0.4629	0.4693	83.23
50	0.4226	0.4125	0.4118	0.4156	73.72
100	0.3722	0.3813	0.3715	0.3750	66.51

IC 50 VALUE (Hypothetical) of methanolic extract: 142.275 µg/mL (Calculated using ED50 PLUS V1.0 Software)

IV. CONCLUSION

Tridax procumbens confirmed the presence of key secondary metabolites and higher antioxidant activity, suggesting its strong potential as natural antioxidants. The hemolytic assay indicated low levels of erythrocyte membrane damage at lower concentrations, demonstrating that the relative safety and biocompatibility of the extracts are within permissible limits. Furthermore, cytotoxicity studies of *T. procumbens* on HaCaT Human keratinocytes cell lines showed moderate, dose-dependent cytotoxic effects, indicating possible applications in anticancer research, while emphasizing the need for controlled usage and further validation.

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