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PHARMACOLOGICAL SCREENING OF *TABERNAEMONTANA DIVARICATA*: A PROMISING NATURAL SOURCE FOR CYTOTOXIC AND ANTIOXIDANT AGENTS

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Tabernaemontana divaricata is a medicinal plant belonging to the Apocynaceae family. Native to South and Southeast Asia, it is widely cultivated as an ornamental shrub due to its attractive white, pinwheel-like flowers. In Sinhalese, it is called "Wathusudda". The leaves of this plant have traditionally been used for medicinal purposes, particularly in the treatment of inflammation and pain. Recently, a few studies have investigated the antioxidant, anti-inflammatory, and anticancer properties of related species within the same family. Therefore, this study aimed to evaluate the bioactivity of *T. divaricata,* with a particular focus on its potential cytotoxic effects. The leaves of *T. divaricata* were subjected to ethanol extraction before analysis. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while antioxidant activity was evaluated via DPPH (1,1-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging assays *in vitro*. Anti-inflammatory activity was assessed through the human red blood cell (HRBC) membrane stabilization assay and the protein denaturation assay. Cytotoxicity was analyzed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay against the HeLa (cervical cancer) cell line. The extract exhibited a TPC of 114.32 ± 5.76 mg GAE/g, while the flavonoid content was 21.66 ± 1.68 mg QE/g. The leaf extract demonstrated high antioxidant activity, with an IC_{50} = 0.01 mg/mL in the DPPH assay (standard ascorbic acid IC_{50} < 0.06 mg/mL). However, in the ABTS assay, the extract showed a higher IC_{50} value of 0.7 mg/mL (standard ascorbic acid IC_{50} = 0.06 mg/mL). In anti-inflammatory assays, the leaf extract exhibited better activity in the HRBC assay (IC_{50} = 0.1 mg/mL) (standard ibuprofen IC_{50} < 0.06 mg/mL) compared to the protein denaturation assay (IC_{50} > 1 mg/mL) (standard ibuprofen IC_{50} < 0.06 mg/mL). Cytotoxicity evaluation revealed that the leaf extract inhibited 98.82% of HeLa cell viability at a concentration of 1 mg/mL, with an IC_{50} value of 0.1 mg/mL. *T. divaricata* possesses considerable therapeutic potential, particularly as a natural source of antioxidant and anticancer agents, warranting further mechanistic and *in vivo* investigations.

Keywords: *Tabernaemontana divaricata,* medicinal plant, antioxidant activity, anti-inflammatory activity, cytotoxicity

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INTRODUCTION

Medicinal plants are a rich source of bioactive compounds, many of which have been developed into therapeutic agents. *Tabernaemontana divaricata* (Apocynaceae) is an evergreen ornamental shrub widely distributed across Asia, Africa, and America. In Sri Lanka, it is known as "Wathusudda", and in India as "Tagar" or "Kath Mallika." Traditionally, various parts of the plant have been used in Ayurvedic and folk medicine to treat a range of conditions, including inflammation, pain, epilepsy, fever, wounds, and infections. Its therapeutic effects are attributed to the presence of diverse phytochemicals such as alkaloids, flavonoids, terpenoids, phenolic acids, and steroids. Although several studies have reported the pharmacological potential of species within this genus, scientific investigations into *T. divaricata* remain limited. This study aims to evaluate and compare the antioxidant and anti-inflammatory and cytotoxic properties of ethanol extracts of *T. divaricata* leaves.

METHODOLOGY

Plant Extraction

A botanical curator from the Bandaranaike Memorial Ayurveda Research Institute verified the plant's authenticity. Leaves and bark were collected, washed, and dried for ten days. The dried materials were ground, sieved, and extracted with ethanol for 72 hours. The extracts were concentrated using a rotary evaporator and stored at 4 °C.

Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu method. Absorbance was measured at 765 nm, and results were expressed as mg gallic acid equivalent (GAE)/g of dry weight.

Total Flavonoid Content (TFC)

TFC was assessed using the aluminum chloride colorimetric method. Absorbance was read at 415 nm and results were expressed as mg quercetin equivalent (QE)/g of dry weight.

Antioxidant Assays

- **DPPH Radical Scavenging Assay-** Various concentrations of extracts were reacted with DPPH solution. Absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as the standard.
- **ABTS Radical Scavenging Assay-** ABTS radicals were generated using $K_2S_2O_8$. Extracts were incubated with ABTS solution, and absorbance was measured at 734 nm. Results from both assays were calculated as % scavenging activity and expressed as IC_{50} values.

Anti-inflammatory Assays

- **HRBC Membrane Stabilization Assay-** Human red blood cells were treated with extract dilutions and incubated at 56 °C. Absorbance was measured at 560 nm to assess membrane stabilization.
- **Protein Denaturation (Egg Albumin) Assay-** Extracts were mixed with egg albumin and incubated at 37 °C and then at 70 °C. Absorbance at 560 nm was used to calculate percentage inhibition.

Cytotoxicity Assay (MTT Assay)

HeLa cells were seeded in 96-well plates (1×10^4 cells/well) and treated with different concentrations of *T. divaricata* leaf for 24 hours. After treatment, 20 µL of MTT solution (5 mg/mL) was added and incubated for 2 hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability (%) was calculated, and IC_{50} values were determined from the dose-response curve.

Data analysis

All results were expressed as mean values and analyzed using standard IC_{50} determination methods. The analysis was done using Excel.

RESULTS AND DISCUSSION

TPC/TFC

The total phenolic content of leaf extracts of the *Tabernaemontana divaricata* was measured by the Folin Ciocalteu method the value was 114.32± 5.76 mg GAE/g. The total flavonoid content (TFC) of leaves extract of the *Tabernaemontana divaricata* was determined as 21.66 ± 1.68 QE/g of extract.

Table 01 – Anti oxidant and anti-inflammatory potential of leaf extract.

	Leaf extract IC_{50} Values	*Standard drug* IC_{50} Values
DPPH Assay	0.01 mg/mL	< 0.06 mg/mL
ABTS Assay	0.7 mg/mL	0.06 mg/mL
HRBC Membrane stabilization assay	0.1 mg/mL	< 0.06 mg/mL
Protein denaturation assay	IC_{50} > 1 mg/mL	< 0.06 mg/mL

The cytotoxicity was evaluated by the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay using the Hela (cervical cancer) cell line. The leaf extract demonstrated IC_{50} value of 0.1 mg/ml suggesting a good cytotoxic activity against the Hela cell line. The highest inhibition was 98.82% at 1mg/ml of the leaf extract.

CONCLUSION

The ethanol leaf extract of *Tabernaemontana divaricata* exhibited promising bioactive properties, including high phenolic and flavonoid content, potent antioxidant activity (notably in the DPPH assay), and significant cytotoxic effects against HeLa cells, with an IC_{50} of 0.1 mg/mL. Moderate anti-inflammatory activity was also observed, particularly in the HRBC membrane stabilization assay. These findings suggest that *T. divaricata* possesses considerable therapeutic potential, particularly as a natural source of antioxidant and anticancer agents, warranting further mechanistic and *in vivo* investigations.

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