# In Vitro Evaluation of the Anti-Inflammatory, Antioxidant, Cytotoxic, and Anti-Cancer Activities of *Tabernaemontana divaricata* Extracts

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**ABSTRACT:**

**Ovarian cancer is one of the most serious gynecological cancers and remains a significant cause of cancer-related deaths among women worldwide. The limitations associated with conventional therapies, including side effects and drug resistance, have increased interest in plant-derived compounds as alternative therapeutic agents. In the current research, the antioxidant, anti-inflammatory, cytotoxic, and anticancer activities of *Tabernaemontana divaricata* plant extracts were assessed using in vitro models.**

**The antioxidant potential of the extracts was assessed through free radical scavenging assays, while anti-inflammatory activity was resolved using the protein denaturation inhibition method. Cytotoxicity studies were conducted on normal L929 fibroblast cell lines to assess the biocompatibility of the extracts. The anticancer activity against the human ovarian cancer cell line SKOV3 was evaluated using the MTT assay at different concentrations of the plant extracts.**

**The study findings revealed that the extracts exhibited appreciable antioxidant activity by effectively neutralizing free radicals in a dose-dependent manner. Remarkable anti-inflammatory activity was also observed through the inhibition of protein denaturation. Cytotoxicity analysis indicates minimal toxicity to L929 normal cell lines, suggesting relative safety toward non-cancerous cells. In contrast, treatment of SKOV3 ovarian cancer cells with the extracts resulted in a substantial reduction in cell viability, indicating strong anticancer potential.**

**Overall, the outcome indicates that *Tabernaemontana divaricata* possesses promising bioactive properties with potential therapeutic relevance for ovarian cancer. The study supports the potential use of this medicinal plant as a natural source for developing safer, more effective anticancer agents, warranting further molecular and in vivo investigations.**

***KEY WORDS:* *Tabernaemontana divaricata*, Methanol extracts, MTT assay, Anti-cancer, Anti-oxidant, Anti-inflammatory, Cytotoxicity.**

## 1. INTRODUCTION:

Globally, Ovarian cancer is one of the main causes of morbidity and death in females, posing a significant public health concern. Due to a lack of screening approaches in the early detection of ovarian cancer. According to worldwide cancer statistics, millions of new cases and deaths occur each year, highlighting the critical need for better prevention, early diagnosis, and effective therapy techniques [1,2]. Cancer's clinical symptoms vary depending on the afflicted organ system, but they often include unexplained weight loss, persistent tiredness, chronic discomfort, abnormal swelling or tumours, changes in skin appearance, and increasing organ malfunction. Early-stage malignancies may be asymptomatic, which can delay detection and reduce treatment effectiveness. As a result, public health measures centered on screening, risk awareness, and early intervention are essential for improving patient outcomes and survival rates [3,4,5].

Over the last few decades, medical science has made considerable advances in cancer treatment. Traditional therapeutic procedures such as surgery, chemotherapy, and radiation remain essential. However, modern approaches such as targeted medicines (e.g., tyrosine kinase inhibitors), immunotherapy (e.g., immune checkpoint inhibitors), and precision medicine have enhanced therapeutic specificity while reducing systemic toxicity [6,7,8]. Despite these advancements, cancer remains a complicated and diverse illness that needs multidisciplinary treatment, continuous research, and comprehensive public health policies [9,10].

*Tabernaemontana divaricata (L.)* R. Br. ex Roem. & Schult., commonly known as crepe jasmine, is an evergreen shrub from the family Apocynaceae. It is widely found in tropical and subtropical regions, especially in India, where it is appreciated not only for its ornamental beauty but also for its medicinal value. For many years, different parts of the plant, such as leaves, roots, and latex, have been used in traditional medicine to manage conditions like fever, inflammation, pain, and certain neurological disorders, as noted by Khongsombat *et al.* [11]. Over the past decade, there has been a noticeable increase in scientific interest in this plant, mainly because of its rich phytochemical composition. Researchers, including Traxler *et al.* [12], have identified several important bioactive compounds, including monoterpene indole alkaloids, flavonoids, phenolic compounds, and terpenoids. These compounds are believed to play a key role in the plant's wide range of pharmacological activities and have attracted attention for their potential therapeutic applications.

Recent studies have particularly focused on the antioxidant and neuroprotective properties of Tabernaemontana divaricata. According to Khongsombat *et al.* [11], plant extracts can help reduce oxidative stress and protect neuronal cells, suggesting possible benefits in managing neurodegenerative disorders. Supporting this, Lu *et al.* [13] reported the presence of newly identified alkaloids that show promising neuroprotective effects. Aside from this, the plant has also demonstrated significant antimicrobial activity. John and Cheriyan [14] highlighted its effectiveness against oral pathogens, which supports its traditional use in treating infections. In addition, various studies suggest that the plant may possess anti-inflammatory, analgesic, and even anticancer properties. Although these findings are encouraging, more detailed studies are required to standardize its extracts and confirm its safety and effectiveness through clinical trials. All in all, Tabernaemontana divaricata remains an important subject of research due to its diverse biological properties and potential role in future drug development.

### 1.1 Medicinal uses:

*Tabernaemontana divaricata* is widely recognized for its medicinal importance, and several recent studies have supported its traditional uses. Different parts of the plant, such as leaves, roots, and latex, are known to contain bioactive compounds that contribute to its therapeutic effects. The plant has shown significant antifungal and antimicrobial properties. Zhang *et al.* [15] reported that alkaloids isolated from T. divaricata enhanced the activity of antifungal drugs against Candida albicans strains resistant to antifungal drugs, indicating their potential role in infection control. It also possesses strong analgesic and anti-inflammatory activity. Khan *et al.* [16] observed that plant extracts exhibited notable pain-relieving effects, supporting their traditional use in treating inflammation and body pain. In addition, the plant is known for its antioxidant potential. Traxler *et al.* [17] discussed the presence of monoterpene indole alkaloids and other phytochemicals that contribute to its ability to neutralize free radicals and protect cells from oxidative damage. The plant has also been explored for its anticancer properties. Deng *et al.* [18] identified several novel alkaloids with potential biological activity, underscoring their importance in cancer research and drug development. Furthermore, Singh *et al.* [19] conducted molecular docking studies, which demonstrated that phytochemicals from T. divaricata can effectively interact with microbial targets, supporting its antimicrobial applications.

Another important aspect is its diversity of bioactive compounds, highlighted in phytochemical studies. Recent research by Wang et al. [20] reported new compounds from the plant with potential pharmacological relevance. These studies indicate that *Tabernaemontana divaricata* possesses a wide range of medicinal properties, including antimicrobial, antifungal, antioxidant, analgesic, and anticancer activities. Even so, further studies are still needed to fully understand its clinical applications and safety.

## 2. MATERIAL AND METHODS:

### 2.1. Plant materials collection and preparation of plant extracts:

The Tabernaemontana divaricata plant leaves and flowers were collected from the local area near Arran Scientific Lab in Madanapalle, Andhra Pradesh, India. The collected leaves and flowers were cleaned with water, air-dried, cut into small pieces and ground into a fine paste using a mixer grinder. For the methanol extract, the paste (50 g) was weighed and mixed with methanol in a 1:2 ratio (50 g plant material to 100 mL solvent). For water extract, the paste (50 g) was weighed and mixed with distilled water in a 1:3 ratio (50 g plant material to 150 mL solvent). The mixture was kept in a water bath at 70 °C for 1 hour and then allowed to cool to room temperature. The extracts were first strained through muslin cloth and then followed by Whatman No. 1 filter paper to produce a clear filtrate. The 10 mL methanol extracts were concentrated by evaporation on a hot plate at 70°C until dry. The dried residue was dissolved in 10 mL of distilled water and stored in the refrigerator (2-8°C) [41].

### 2.2. Anti-oxidant activity:

Antioxidant assay measures a substance's ability to scavenge free radicals and inhibit oxidation. Bring all reagents and samples to room temperature for 30 minutes before starting the assay. 200 µl of test samples were taken in test tubes, to which 1.8 ml of methanol was added. All the test tubes were vortexed. Stock solutions were prepared by dissolving 100mg of ascorbic acid was dissolved in10 ml of distilled water and vortexing the samples. Different concentrations were prepared in the test tubes, to which 2000 µl of methanol was added to make standards. The samples were then covered with parafilm and vortexed. 3.9mg of DPPH was weighed and dissolved in 100ml of methanol. Later, 1 mL of plant extract and 1 mL of DPPH were added to the cuvette. Thereafter, readings were taken with a UV-visible spectrophotometer at 660 nm [42].

### 2.3. Anti-Inflammatory assay:

The plant extracts can increase or decrease inflammation, as determined by an anti-inflammatory assay. All reagents and samples were kept at room temperature for 30 min before the assay began. To prepare the Aspirin standard solution, 5% egg albumin, and 1x PBS. Take ten test tubes, one of which was filled with 2 mL of drug,2.6 mL of 1x PBS, and 0.4 mL of egg albumin to serve as the positive control. Aspirin served as the standard reference medication. Another test tube was filled with 4.6 mL of 1x PBS and 0.4 mL of egg albumin to serve as the negative control. For the test samples, 2 mL of the reconstituted plant and water extracts were combined with 2.6 mL of 1x PBS and 0.4 mL of egg albumin at the appropriate concentrations. To ensure appropriate mixing, all test tubes were vigorously vortexed. The reaction mixtures were first incubated at 37°C in a water bath for 15 minutes, then at 70°C for 5 minutes. Following incubation, the samples were cooled to room temperature. Finally, 2 mL of each reaction mixture was transferred to a cuvette and measured at 660 nm with a UV-Visible spectrophotometer [43].

### 2.4. Cell culture and plant extracts exposure:

SKOV3 (Sloan Kettering ovarian cancer 3) and L929 (NCTC clone 929) cell lines were bought from the National Centre for Cell Science (NCCS) in Pune, India. SKOV3 cells were cultured in RPMI 1640 media with 10 % fetal bovine serum (FBS) from HIMEDIA and 1% antibiotic (Penicillin and Streptomycin) solution at 37°C with 5% CO2 in an incubator. SKOV3

(Sloan Kettering ovarian cancer 3) and L929 (NCTC clone 929) cell lines were subcultured for 24 hr before treatment with water and methanol extracts of Tabernaemontana divaricata plant. The plant extracts are diluted in RPMI 1640 media.

**2.5. MTT Assay:**

The anticancer potential of plant extracts is assessed using the MTT assay, which measures their ability to inhibit cancer cell proliferation. In brief, SKOV3 cells were seeded in a 96-well plate and incubated in a 5% CO2 atmosphere at 37 °C for 24 hr. After incubation, the cells were exposed to water and methanol extracts of the tabernaemontana divaricata plant's flowers and leaves and incubated in a CO2 incubator at 37°C for 24 hr. After 24 hours, the Media was carefully removed, MTT reagent was added to each well, and the plate was incubated for 4 hrs at 37°C in a $CO_2$ incubator. After incubation, the solubilization solution (DMSO) was added to the wells, wrapped in Aluminum foil, and placed on an orbital shaker for 15 minutes. The plate was read at 540 nm using a multiwell microplate reader (Thermoscientific Multiskan FC, USA). The untreated well served as a control [44].

**2.6. Neutral Red Uptake (NRU) assay:**

Cytotoxicity of L929 cells due to exposure to water and methanol extracts of flowers and leaves of the *Tabernaemontana divaricata* plant was performed by using the NRU assay. In brief, L929 cells were seeded in a 96-well plate and incubated in a CO2 incubator at 37 °C for 24 hr. After incubation, the cells were exposed to water and methanol extracts of the tabernaemontana divaricata plant's flowers and leaves and incubated in a $CO_2$ incubator at 37°C for 24 hr. After 24 hours, the Medium was carefully removed, Neutral red reagent was added to each well, and the plate was incubated for 2 hrs at 37°C in a $CO_2$ incubator. Neutral red solution was removed from the wells, washed with 1x PBS, and then removed. Add destain solution to the wells, wrap them in Aluminium foil, and place them on the orbital shaker for 10 minutes. The plate was read at 540 nm using a multiwell microplate reader (Thermoscientific Multiskan FC, USA). The untreated well served as a control [45].

**3. RESULTS AND DISCUSSION:**

**3.1. Anti-Inflammatory Assay:**

The anti-inflammatory potential of *Tabernaemontana divaricata* was assessed using the protein denaturation assay. Aspirin (1 mg/mL) served as the standard, producing 75–80% inhibition. Among all samples, the methanolic leaf extract showed the strongest activity, with 80–85% inhibition, slightly exceeding the standard. The aqueous leaf extract also demonstrated high inhibition, reaching approximately 70–75%. These results indicate that leaf extracts contain a higher concentration of anti-inflammatory compounds. The methanolic flower extract showed moderate activity, with 50–55% inhibition. The aqueous flower extract displayed the lowest activity, with only 25–30% inhibition. Overall, leaf extracts were considerably more effective than flower extracts. Methanol proved to be a better solvent for extracting anti-inflammatory constituents. These findings confirm that *T. divaricata* leaves are a promising source of natural anti-inflammatory agents **Fig. 3.1**.

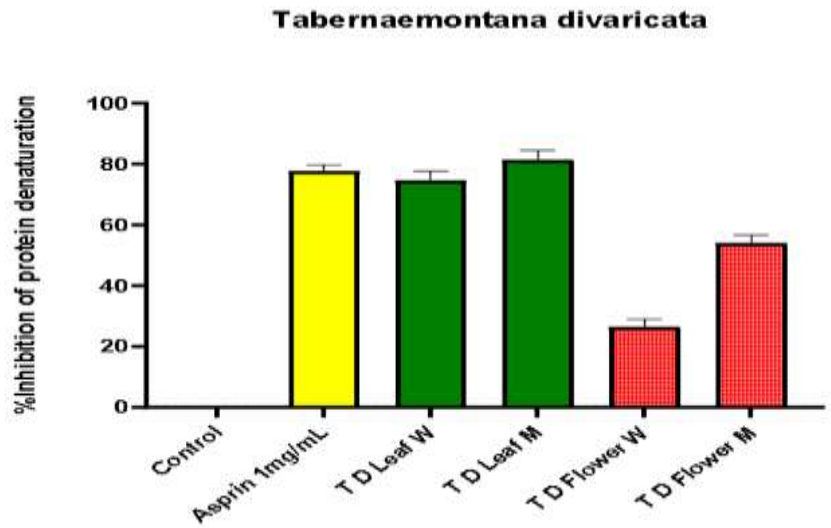


**Fig. 3.1: Anti-inflammatory Activity of Tabernaemontana divaricata extracts**

**3.2. Anti-oxidant Assay:** The antioxidant activity of *Tabernaemontana divaricata* plant extracts was evaluated by using the DPPH radical scavenging assay. The standard antioxidant compound used here shows strong dose-dependent antioxidant activity. This provides a benchmark for comparing plant extracts (like the ones shown in **Fig. 3.2**). Any plant extract that performs equal to or better than these standards can be considered highly effective. Methanol extracts of leaf, Methanol extracts of flowers and Water extract of flowers have higher antioxidant activity when compared to the standards. Leaf water has lower antioxidant activity than the standards.

The water and Methanol extracts of flowers and the methanol extracts of leaves have high antioxidant activity when compared to the water extracts of leaves.

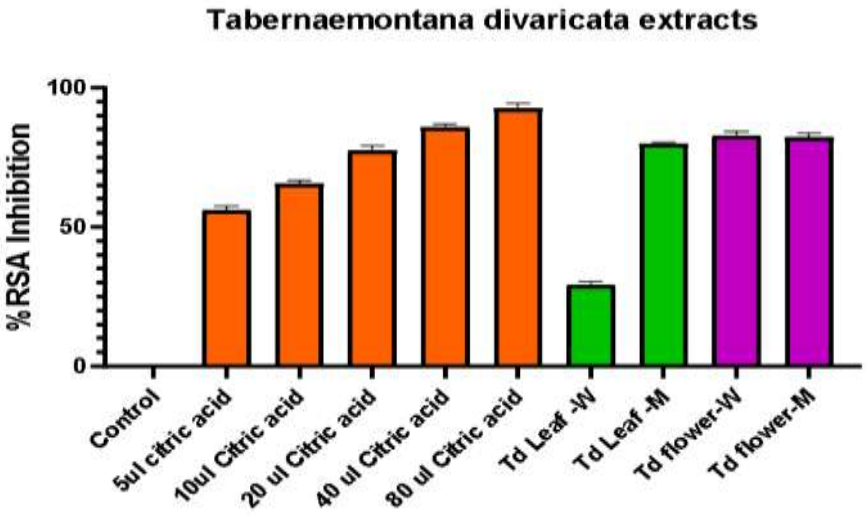


**Fig.3.2: Anti-oxidant Activity of Tabernaemontana divaricata extracts**

### 3.3. Cytotoxicity assay:

The effects of *Tabernaemontana divaricata* extracts on L929 viability showed that all samples were generally safe to normal cells. The untreated control was considered as 100% viable cells. Most extracts maintained or slightly increased cell viability, indicating low toxicity and good cytocompatibility. The aqueous and methanolic flower extracts produced the highest viability, with the aqueous flower extract showing the strongest supportive effect on cell growth. Leaf extracts displayed moderate viability and did not significantly affect normal cell survival. These results suggest that the extracts do not damage healthy fibroblast cells. In some cases, the bioactive compounds may even enhance cellular metabolic activity. Overall, *T. divaricata* extracts exhibited excellent compatibility with normal cells, supporting their potential as safe natural therapeutic agents **Fig. 3.3.a and 3.4.b**.

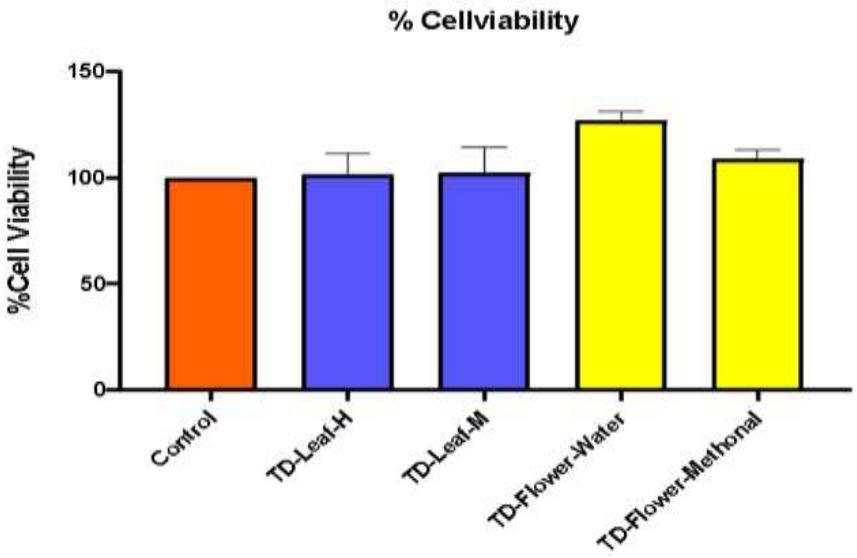


**Fig. 3.3.a: cell viability of Tabernaemontana divaricata plant extracts on L929 Cells**

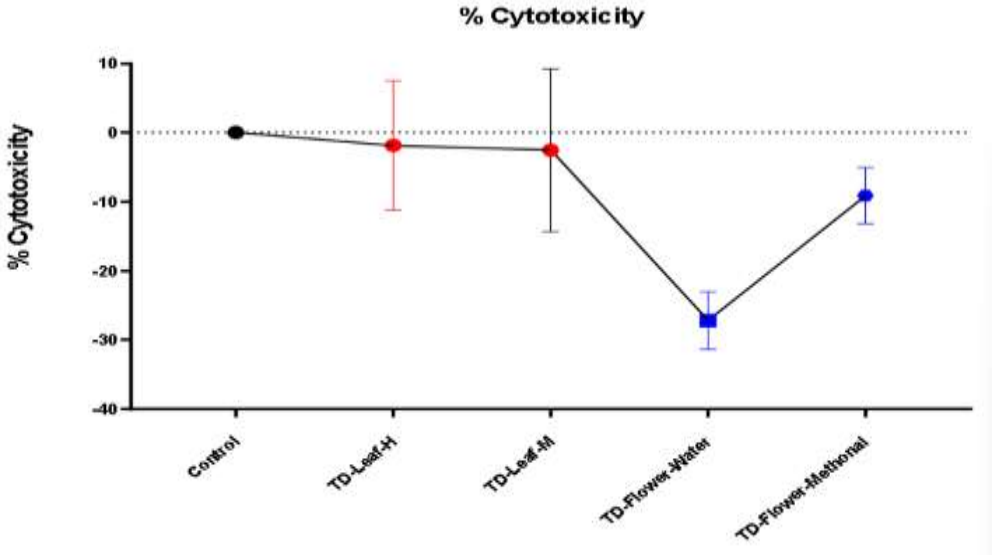


**Fig. 3.3.b: cytotoxicity of Tabernaemontana divaricata extracts on L929 cells**

### 3.4. Anti-Cancer (MTT) Assay:

The present study examines the effects of different extracts of *Tabernaemontana divaricata* on cell viability and cytotoxicity in SKOV3 cells using standard in vitro methods. The results shown in **Fig. 3.4.a** (cellular viability of SKOV3 cell lines) and **Fig. 3.4.b** (cytotoxicity of SKOV3 cell lines) provide a comparative understanding of the biological activity of different plant extracts. 5FU is taken as a known drug (control), and the plant extracts are compared with 5FU. Leaf water extract is the most effective among all plant samples. It shows the Negative cell proliferation and the highest cytotoxicity, performing even better than the chemotherapy drug in this specific assay. Flower water extract shows the least proliferation when compared to the leaf methanol and flower methanol extract. Among the tested samples, flower extract and leaf extract show the strongest inhibition of cell growth, even compared with 5FU, while the control shows maximum growth. This suggests the plant extracts may possess potential anticancer activity.

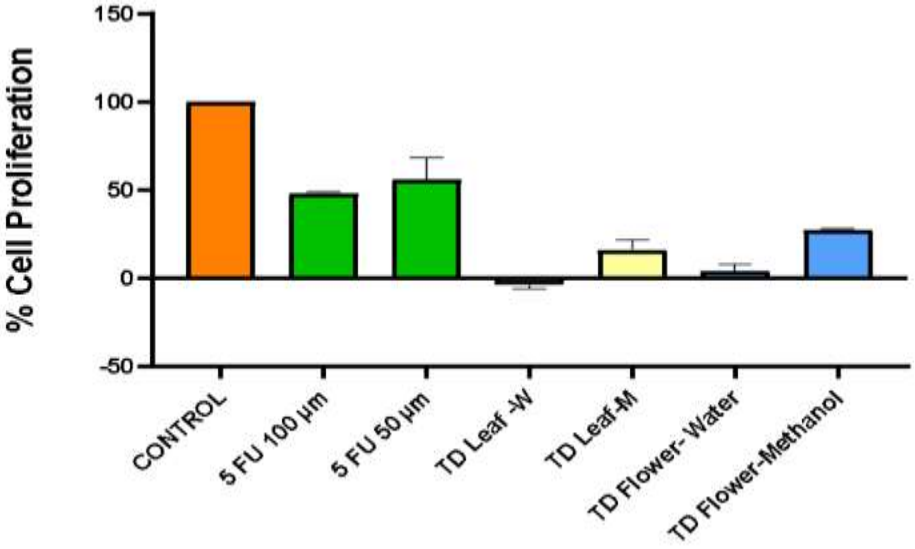


**Fig. 3.4.a: cell viability of Tabernaemontana divaricata plant extracts on SKOV3 Cells**

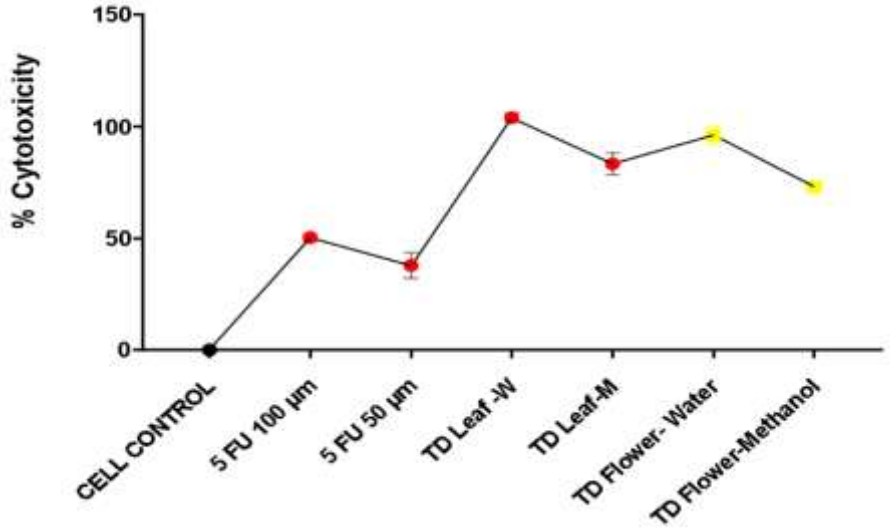


**Fig. 3.4.b: cytotoxicity of Tabernaemontana divaricata extracts on SKOV3 cells**

**4. Discussion:**

The present investigation demonstrates that *Tabernaemontana divaricata* exhibits a broad spectrum of biological activities, including antioxidant, anti-inflammatory, cytocompatibility, and anticancer effects. These findings reinforce the pharmacological importance of this medicinal plant and are consistent with recent advances in plant-based therapeutic research and drug discovery from natural sources [21,22].

In the DPPH assay, both aqueous and methanolic extracts effectively scavenged free radicals, indicating the presence of phenolics, flavonoids, tannins, and indole alkaloids that protect cells from oxidative damage [23,24]. The methanolic extract exhibited higher antioxidant activity, suggesting that methanol is more efficient at extracting bioactive compounds. Previous studies have reported that *Tabernaemontana divaricata* extracts possess strong antioxidant activity across multiple assays, including DPPH and FRAP [25,26]. Additionally, nanoparticle-mediated extracts of *T. divaricata* have demonstrated enhanced antioxidant efficiency due to increased surface reactivity [26]. These findings are consistent with the present study, in which methanolic extracts exhibited superior antioxidant activity compared to aqueous extracts.

In the protein denaturation assay, the extracts significantly inhibited albumin denaturation, confirming their anti-inflammatory potential [27,28]. This effect is likely due to phytochemicals that stabilize proteins and suppress inflammatory mediators. Similar anti-inflammatory effects have been reported for *T. divaricata* in both in vitro and in vivo studies [29,30]. The activity is primarily attributed to bioactive compounds such as alkaloids, flavonoids, and terpenoids, which inhibit pro-inflammatory mediators and enzymes [31,32]. Furthermore, natural antioxidants can suppress inflammatory pathways by reducing oxidative stress and modulating cytokine production [33,31]. Thus, the combined antioxidant and anti-inflammatory activities observed in this study highlight the therapeutic relevance of *T. divaricata*.

Cytocompatibility studies showed that the extracts were well tolerated by normal cells at lower concentrations, indicating a favorable safety profile. In contrast, treatment of the SKOV-3 caused a concentration-dependent reduction in cell viability, demonstrating potent activity against Ovarian Cancer cells. The anticancer effect may involve apoptosis induction, mitochondrial dysfunction, oxidative stress, and cell-cycle arrest [34,21]. *T. divaricata* contains bioactive compounds such as coronaridine, voacangine, flavonoids, and phenolic acids, which are known for their antioxidant, anti-inflammatory, and cytotoxic properties [35]. The anticancer activity of *T. divaricata* is attributed to the presence of monoterpenoid indole alkaloids, which are known for their potent cytotoxic effects against cancer cells [36]. These compounds can induce apoptosis, inhibit cell proliferation, and interfere with multiple signaling pathways involved in tumor progression [37]. Previous studies have also demonstrated the cytotoxic potential of *T. divaricata* extracts against various cancer cell lines [38,39].

Natural products have been widely recognized as important sources of anticancer agents, with many clinically used drugs being derived from plant metabolites [21,22]. The results of this study support this concept, highlighting the potential of T. divaricata as a source of novel anticancer compounds.

A strong correlation between antioxidant activity and anticancer potential was observed in this study. This relationship is well-established, as oxidative stress plays a critical role in cancer initiation and progression. Phytochemicals with antioxidant properties can modulate cellular signaling pathways involved in apoptosis, proliferation, and metastasis [32]. Flavonoids, in particular, have been shown to exert anticancer effects by scavenging free radicals and regulating gene expression [32,40]. Therefore, the dual activity of *T. divaricata* extracts as antioxidants and anticancer agents enhances their therapeutic significance.

Overall, the findings of this study suggest that *T. divaricata* possesses significant pharmacological potential and may be a promising candidate for the development of natural therapeutic agents. However, further studies focusing on the isolation of active compounds, elucidation of mechanistic pathways, and in vivo validation are necessary to fully explore its clinical applicability. Despite the promising results, the study has certain limitations. The findings are based on in vitro assays, and further in vivo studies are required. Future research should focus on phytochemical analysis, elucidation of molecular mechanisms, and evaluation in animal models and clinical settings.

## 5. Conclusion:

*Tabernaemontana divaricata* exhibited strong anti-inflammatory, antioxidant, and anticancer activities, and the effects varied with the plant part and extraction solvent used. Leaf extracts were more effective in reducing inflammation and inhibiting cancer cell growth, while flower extracts exhibited slightly better antioxidant activity. In the protein denaturation assay, the methanolic leaf extract produced the highest anti-inflammatory effect, with activity close to that of Aspirin, indicating efficient extraction of flavonoids and alkaloids by methanol. In the DPPH assay, all extracts scavenged free radicals, but methanolic extracts generally performed better than aqueous extracts. The higher antioxidant activity observed in flower extracts suggests that floral tissues contain greater amounts of phenolic compounds. Anticancer studies using SKOV-3 cells demonstrated a concentration-dependent reduction in cell viability across all extracts. Among them, the aqueous leaf extract showed the strongest cytotoxic effect, followed by the methanolic leaf extract, with some responses approaching those of Fluorouracil. Importantly, the extracts showed little to no toxicity toward L929 normal fibroblasts, indicating selective action against ovarian cancer cells. The biological effects are likely due to the combined action of flavonoids, phenolics, and indole alkaloids. Overall, the study confirms that *T. divaricata* is a promising medicinal plant with potential for the development of natural therapies, particularly for the treatment of Ovarian Cancer.

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