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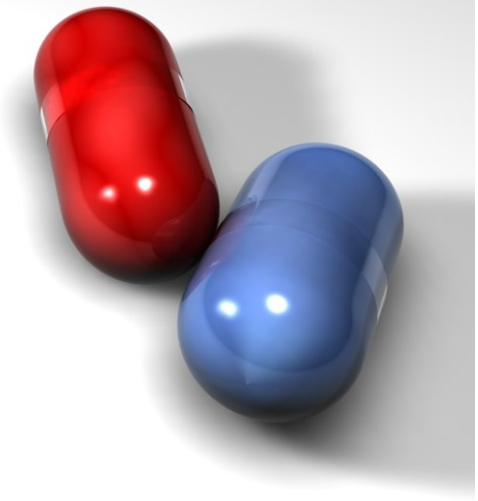

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Letter to the Editor

Antiproliferative activity of *Tribulus terrestris* stem bark extract against A431 cells

Dear Editor,

Human epidermoid carcinoma can cause tissue damage, metastasis, and destruction of lymph nodes. To treat this, radiation therapy, surgery, chemotherapy, and targeted delivery of EGFR inhibitors have been utilized (Johnston et al., 2006). However, these treatments may lead to severe side effects such as skin damage, fatigue, intense pain, and immune suppression (Zheng et al., 2017).

Preclinical, *in silico*, and clinical studies corroborate the usefulness of medicinal plants in managing cancers with fewer adverse effects (Gaikwad and Shinde, 2022).

Tribulus terrestris has been utilized in ancient systems, such as Ayurveda and Unani, to treat a variety of diseases, including reproductive and urinary system issues (Amanullah et al., 2021). Research on *Tribulus terestris* has confirmed the presence of diverse phytochemicals, including steroidal saponins, protodioscin, flavonoids, alkaloids, and polyphenolic compounds, thereby validating its therapeutic potential (Chhatre et al., 2014).

These phytoconstituents are known to exert their effects by interacting with cell signaling pathways, inhibiting cell proliferation, and inducing apoptosis. Notably, *Tribulus terestris* exhibits lesser toxicity to normal skin fibroblast cells, confirming its selectivity (Neychev et al., 2022). Previous research validates the cytotoxic potential of *T. terestris* on MCF-7 cells. This could be attributed to the presence of diverse secondary metabolites (Alshabi et al. 2022). In another study, the stem bark extract of *T. terestris* demonstrated cytotoxic effects on prostate cancer cells (Dabaghkar et al., 2025). Furthermore, the cytotoxic, antiproliferative, and apoptotic effects of the hydroalcoholic extract of the fruit of *T. terestris* have been reported on different cell lines, such as prostate, colon and fibroblast-like cells (Pourali et al. 2017). The antiglycation, antioxidant, and antiproliferative potential of saponin-rich plant extracts of *T. terestris* have also been observed against human tumor cell lines (Figueiredo et al. 2021).

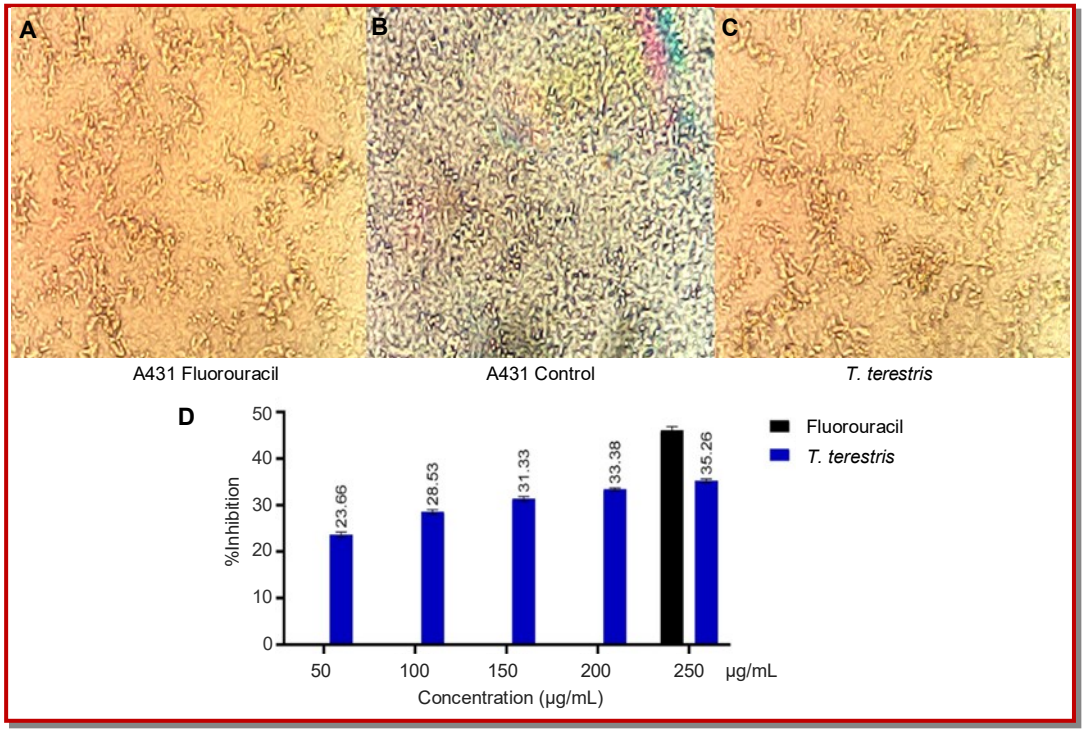

Figure 1: Comparative cell viability of A431 cells on treatment with 5-fluorouracil (standard), untreated control, and *T. terestris* stem bark methanolic extract. Bar graph demonstrating comparative cell viability inhibition

Thus, in line with the previous reports, we investigated the cytotoxic potential of the methanolic stem bark extract of *T. terestris* against A431 human epidermoid carcinoma cells. Cytotoxicity was evaluated using the MTT assay (Bahuguna et al., 2017). Cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated at 37°C with 5% CO_2 for 24 hours. After 24 hours of treatment with different extract concentrations, each well received 20 µL of MTT reagent (5 mg/mL). After 4-hours incubition period, formazan crystals generated by live cells were dissolved in DMSO, and the absorbance was measured at 570 nm. IC_{50} value was determined from the dose-response curve (Gai-kwad and Shinde, 2022).

As shown in Figure 1, the stem bark methanolic extract of *T. terrestris* inhibited the viability of 35.26 ± 0.95% A431 cells, corroborating the cytotoxic effect following a 24-hours treatment at the tested dose. In comparison, the standard 5-fluorouracil inhibited the viability of 46.15 ± 0.95% cells. These results indicate that the methanolic extract of the stem bark of *T. terrestris* could be used as a viable alternative to standard chemotherapy, even though the extract exhibited a marginally lesser effect than the standard drug.

The moderate cytotoxicity of *T. terrestris* extract showed increased interest in plant-based treatments. The secondary metabolites present in the plant *T. terrestris* may offer tailored, multi-modal responses with fewer adverse effects than traditional chemotherapeutics, which frequently have wide cytotoxic effects that result in systemic toxicity. This has prompted scientists to think about these substances' potential as chemopreventive or adjunctive treatments. Using MTT-based assessments and molecular docking, recent studies have shown the anti-cancer potential of medicinal plant extracts, such *Terminalia citrine* (Das et al., 2016).

The use of A431 cells in our work is relevant given the rising prevalence of skin malignancies other than melanoma and the shortcomings of existing treatments. Research on natural medicines is appealing, since there is a demand for safer and more economical alternatives. Given its ethnobotanical basis, *T. terrestris* is a strong contender for more preclinical and clinical investigations.

In summary, the methanolic extract of the stem bark of *T. terrestris* demonstrated moderate antiproliferative activity against A431 human epidermoid carcinoma cells. The consistency of these results with prior literature, coupled with the known phytochemical profile of the plant, suggests its strong potential as a source of anti-cancer agents. We recommend further fractionation, isolation of active components, and mechanistic studies to fully explore the therapeutic benefits of *T. terrestris*.

Conflict of interest: The authors declare that they have no conflicts of interest.

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