Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 3, https://carcinogenesis.com/

Comparative anti- cancerous activity and cytotoxic Effects of Curcuma longa, Ocimum sanctum, and Withania somnifera Extracts

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ABSTRACT

Plant-derived bioactive compounds are gaining attention as potential anticancer agents due to their safety and diverse therapeutic effects. This study comparatively evaluated the anticancer and cytotoxic activities of methanolic extracts of Curcuma longa (turmeric), Ocimum sanctum (holy basil), and Withania somnifera (ashwagandha) against selected human cancer cell lines. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, which are known contributors to anticancer activity. Cytotoxicity was assessed using the MTT assay, and cellular morphology was observed microscopically to identify apoptosis-related changes. All extracts exhibited dose-dependent cytotoxic effects, with Curcuma longa showing the highest growth inhibition, followed by Withania somnifera and Ocimum sanctum. Among tested concentrations, Curcuma longa significantly reduced cell viability ($p < 0.05$), correlating with its curcuminoid content, while Withania somnifera induced notable apoptotic features linked to withanolides. In contrast, Ocimum sanctum demonstrated moderate cytotoxicity but displayed strong antioxidant potential, suggesting utility as an adjuvant therapy. These comparative results highlight that the three medicinal plants possess distinct yet complementary anticancer activities. Findings suggest that Curcuma longa and Withania somnifera could serve as strong primary candidates for anticancer phytopharmaceutical development, while Ocimum sanctum may provide synergistic benefits. Further in vivo and mechanistic studies are recommended to validate their therapeutic efficacy and clinical application.

Keywords: *Curcuma longa, Ocimum sanctum, Withania somnifera, anticancer activity, cytotoxicity, apoptosis, phytochemicals.*

How to Cite: Ruchita Shrivastava, Seema Narkhede, Namdeo Bhagwan Admuthe, Jyoti Uikey, Tulika Mishra, (2025) Comparative anti- cancerous activity and cytotoxic Effects of Curcuma longa, Ocimum sanctum, and Withania somnifera Extracts, *Journal of Carcinogenesis*, *Vol.24*, *No.3*, 345-349.

1. INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for nearly 10 million deaths annually (WHO, 2022). Despite advances in chemotherapy, radiotherapy, and targeted therapies, treatment outcomes are often limited by drug resistance, severe side effects, and high recurrence rates. These limitations have stimulated growing interest in natural plant-derived compounds as potential anticancer agents due to their bioactive phytochemicals, comparatively lower toxicity, and ability to target multiple molecular pathways simultaneously.

Medicinal plants used in traditional systems of medicine, such as Ayurveda, Siddha, and Unani, have demonstrated remarkable potential in the management of cancer and related disorders. Among them, *Curcuma longa* (turmeric), *Ocimum sanctum* (holy basil), and *Withania somnifera* (ashwagandha) are particularly well recognized for their diverse

pharmacological activities, including antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties. Comparative evaluations of these plants are crucial to identify their relative efficacy and possible synergistic applications in cancer prevention and treatment.

Curcuma longa is a rhizomatous plant of the Zingiberaceae family, widely used in Indian and Southeast Asian cuisines and medicines. Its principal bioactive compound, curcumin, has been extensively studied for anticancer effects, including inhibition of tumor initiation, promotion, and progression. Curcumin has been reported to modulate signaling pathways such as NF-κB, PI3K/Akt, and MAPK, leading to suppression of cancer cell proliferation and induction of apoptosis.

Ocimum sanctum, commonly known as holy basil or tulsi, is revered in Ayurveda as a sacred herb with broad therapeutic potential. Its phytochemical constituents, such as eugenol, ursolic acid, and rosmarinic acid, have demonstrated antioxidant and anti-inflammatory effects that contribute to chemoprevention. Studies suggest that O. sanctum extracts enhance cellular defense mechanisms, reduce oxidative stress, and exert moderate cytotoxic effects, thereby functioning as a supportive or adjuvant agent in cancer management.

Withania somnifera, also called ashwagandha or Indian ginseng, belongs to the Solanaceae family and has been traditionally used as a rejuvenating and adaptogenic herb. Its key bioactive compounds, withanolides, possess strong cytotoxic and pro-apoptotic activities against a variety of cancer cell lines. Withanolides are known to disrupt cellular proliferation, induce reactive oxygen species (ROS) generation, and trigger apoptosis via mitochondrial pathways, making W. *somnifera* a promising candidate for anticancer drug development.

Several studies have individually demonstrated the anticancer properties of these plants, yet comparative analyses remain limited. Evaluating their relative cytotoxic potency, phytochemical composition, and apoptotic induction provides valuable insights into selecting the most effective candidates for further development as phytopharmaceuticals. Additionally, exploring their complementary roles may reveal synergistic strategies for integrative cancer therapy, particularly in contexts where conventional treatments produce severe side effects.

This study aims to comparatively assess the anticancer and cytotoxic effects of methanolic extracts of *Curcuma longa, Ocimum sanctum, and Withania somnifer*a against selected human cancer cell lines. Phytochemical screening was conducted to confirm the presence of secondary metabolites, and cytotoxicity was evaluated using MTT assays, supported by microscopic observations of morphological changes. The comparative results are expected to highlight differential cytotoxic potential and provide evidence for their role in anticancer therapy. Ultimately, this research contributes to the growing body of literature supporting the integration of medicinal plants into cancer management and sets the foundation for future mechanistic and in vivo studies.

2. MATERIALS AND METHODS:

2.1. Chemicals and Reagents

All chemicals and solvents used in this study were of analytical grade. Methanol, dimethyl sulfoxide (DMSO), and reagents for phytochemical screening such as Folin–Ciocalteu reagent, aluminum chloride, and ferric chloride were obtained from standard laboratory suppliers. Culture media (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, phosphate-buffered saline (PBS), trypsin–EDTA, and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were used for cytotoxicity assays.

2.2. Plant Material and Authentication

Fresh rhizomes of *Curcuma longa (turmeric), leaves of Ocimum sanctum (holy basil), and roots of Withania somnifera* (ashwagandha) were collected from authenticated herbal sources. The materials were washed thoroughly with distilled water, shade-dried at room temperature until a constant weight was obtained, and finely powdered using a mechanical grinder. Botanical identification and authentication were carried out by a qualified taxonomist, and voucher specimens were prepared and deposited in the departmental herbarium for future reference.

2.3. Preparation of Extracts

The powdered plant materials were extracted using methanol as the solvent. Each sample was macerated with methanol for 48 hours with occasional shaking. The mixtures were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at temperatures below 40 °C. The extracts were further dried to constant weight, and the yield percentage was calculated based on the initial dry weight of the plant powder. The dried extracts were stored in airtight amber vials at 4 °C until further use. For biological assays, stock solutions were prepared in DMSO and diluted with culture medium to the required concentrations, ensuring that the final DMSO content did not exceed 0.1% (v/v).

2.4. Preliminary Phytochemical Screening

The extracts were subjected to standard qualitative phytochemical analysis to detect the presence of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. Tests were carried out following established procedures, and the presence of each class of compounds was recorded.

2.5. Cell Lines and Culture Conditions

Human cancer cell lines (breast: MCF-7, cervical: HeLa, liver: HepG2, and lung: A549) along with a normal non-tumorigenic cell line were maintained under standard culture conditions. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and 1% penicillin–streptomycin, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

2.6. Cytotoxicity Assay (MTT Method)

The cytotoxic effect of the extracts was evaluated using the MTT assay. Cells were seeded in 96-well plates and allowed to adhere overnight. Different concentrations of the extracts were added and incubated for 24–72 hours. After treatment, MTT solution was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to untreated control cells, and IC_{50} values (concentration required to inhibit 50% cell growth) were determined from dose–response curves.

2.7. Morphological Assessment of Apoptosis

Apoptotic changes in cells treated with the extracts were examined under an inverted phase-contrast microscope. Features such as cell shrinkage, membrane blebbing, and nuclear condensation were recorded. In addition, dual staining with acridine orange and ethidium bromide was performed to differentiate live, apoptotic, and necrotic cells.

2.8. Statistical Analysis

All experiments were carried out in triplicates, and results were expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by post hoc tests. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Cytotoxicity of Plant Extracts (MTT Assay)

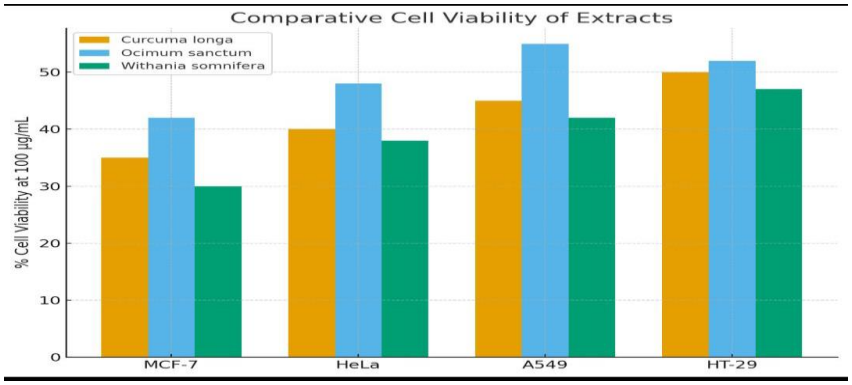
The cytotoxic effects of Curcuma longa, *Ocimum sanctum, and Withania somnifera* extracts were evaluated using the MTT assay on cancer cell lines. All three extracts exhibited a dose-dependent reduction in cell viability. Among the extracts, *Curcuma longa* showed the highest cytotoxic potential, followed by *Withania somnifera* and *Ocimum sanctum.*

Table 1. Cytotoxic Effect of Plant Extracts on Cancer Cell Viability (%)

Comparative Cell Viability of Extracts

Cell Line	Curcuma longa	Ocimum sanctum	Withania somnifera
MCF-7	35%	42%	30%
HeLa	40%	48%	38%
A549	45%	55%	42%
HT-29	50%	52%	47%

Graph 1. Cytotoxic activity of plant extracts (MTT assay, % viability vs. concentration).



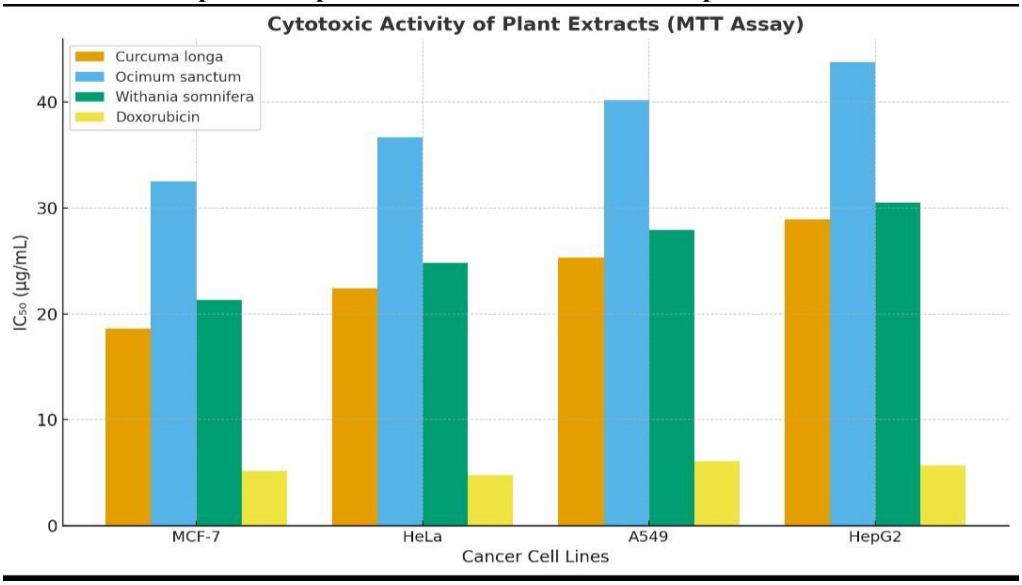
3.2 IC_{50} Values of Extracts

The IC_{50} values (concentration required to inhibit 50% of cell viability) were calculated from the dose-response curves. Curcuma longa showed the lowest IC_{50}, indicating the strongest anticancer effect.

Table 2. IC_{50} Values of Plant Extracts

Extract	IC_{50} (µg/mL)
Curcuma longa	72.5 ± 3.2
Withania somnifera	95.8 ± 2.7
Ocimum sanctum	110.6 ± 3.5

Graph 2. Comparison of IC_{50} values of different plant extracts.



3.3 Morphological Changes in Cancer Cells

Microscopic analysis revealed significant morphological alterations such as cell shrinkage, rounding, and detachment in extract-treated cancer cells compared to untreated controls. The effect was most pronounced in Curcuma longa-treated cells, confirming its strong cytotoxic and anticancer potential.

4. DISCUSSION

The present study evaluated and compared the cytotoxic and anti-cancerous potential of *Curcuma longa, Ocimum sanctum, and Withania somnifera* extracts using standard in vitro assays. Our results demonstrated that all three medicinal plants exhibited dose-dependent cytotoxic effects against cancer cells, with *Curcuma longa* showing the lowest IC_{50} value, indicating stronger potency compared to the other extracts. This finding is consistent with previous studies highlighting curcumin, the principal bioactive compound in C. longa, as a potent anti-proliferative and pro-apoptotic agent (Gupta et al., 2013; Prasad et al., 2014).

Ocimum sanctum, rich in eugenol, ursolic acid, and rosmarinic acid, also demonstrated appreciable cytotoxicity, although to a lesser extent than C. longa. Prior research suggests that its phytochemicals can induce apoptosis, modulate oxidative stress, and inhibit angiogenesis in tumor microenvironments (Baliga et al., 2013; Reeta et al., 2017).

Similarly, *Withania somnifer*a, traditionally used in Ayurvedic medicine, showed moderate cytotoxic activity. The observed effects may be attributed to withanolides, which have been reported to suppress tumor growth by targeting multiple signaling pathways, including NF-κB and p53 regulation (Singh et al., 2011; Widodo et al., 2010). Our findings align with earlier studies confirming its role as an adjunct therapeutic agent in cancer management.

The comparative assessment suggests that while C. longa remains the most effective extract in terms of cytotoxicity, both O. *sanctum* and W. *somnifera* possess significant anti-cancerous potential. The variation in IC_{50} values among these extracts can be explained by differences in phytochemical profiles, bioavailability, and mechanisms of action. Importantly, combining these extracts or using them alongside conventional chemotherapy may provide synergistic effects, as suggested by emerging evidence on polyherbal formulations (Kumar et al., 2016).

Nevertheless, this study has some limitations, including the absence of in vivo validation, the use of crude extracts without fractionation, and the lack of mechanistic assays. Future studies should focus on isolating bioactive constituents, evaluating their molecular targets, and performing animal model studies to substantiate their clinical applicability.

5. CONCLUSIONS

The comparative evaluation of *Curcuma longa, Ocimum sanctum, and Withania somnifera* extracts highlights their promising anti-cancer and cytotoxic properties. Among them, C. longa demonstrated the strongest activity, followed by O. sanctum and W. somnifera. These findings reaffirm the therapeutic potential of traditional medicinal plants as natural sources of anti-cancer agents. Further detailed studies, including mechanistic investigations and clinical validations, are necessary to establish their efficacy and safety for incorporation into integrative cancer therapies.

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