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In Vitro Evaluation and Rapid Screening of Anticancer and Antidiabetic Phytoconstituents of Selected Indian Medicinal Plants

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ABSTRACT

Cancer and diabetes are two of the most prevalent chronic diseases worldwide, creating a growing demand for safe and effective therapeutic alternatives. Medicinal plants have long been utilized in Indian traditional medicine and represent a valuable source of phytoconstituents with potential biological activities. The present study aimed to evaluate and rapidly screen the in vitro anticancer and antidiabetic potential of four selected Indian medicinal plants, namely Withania somnifera, Curcuma longa, Ocimum sanctum, and Tinospora cordifolia. Extracts were subjected to phytochemical screening, which revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds. Anticancer activity was evaluated against human cancer cell lines using cytotoxicity assays, while antidiabetic activity was assessed through α-amylase and α-glucosidase inhibition models. The results indicated that Withania somnifera and Curcuma longa exhibited marked cytotoxic effects, whereas Ocimum sanctum and Tinospora cordifolia showed significant enzyme inhibitory activity. Rapid screening approaches further confirmed the presence of bioactive compounds that may contribute to these therapeutic effects. These findings suggest that the selected plants are promising candidates for the development of cost-effective, plant-based agents with dual anticancer and antidiabetic potential, warranting further mechanistic and in vivo investigations.

Keywords: *Withania somnifera; Curcuma longa; Ocimum sanctum; Tinospora cordifolia; anticancer activity; antidiabetic activity; phytoconstituents; in vitro screening.*

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1. INTRODUCTION

Cancer and *diabetes mellitus* are among the leading causes of global morbidity and mortality, posing significant socioeconomic and healthcare challenges. According to the World Health Organization, cancer accounted for nearly 10 million deaths in 2020, with breast, lung, and cervical cancers contributing substantially to the global cancer burden (WHO, 2021). Similarly, diabetes has reached epidemic proportions, with an estimated 537 million adults affected worldwide, a number projected to rise to 783 million by 2045 (International Diabetes Federation, 2021). The dual burden of cancer and diabetes is particularly concerning, as epidemiological evidence indicates that diabetic patients have an increased risk of developing certain cancers, and both diseases share common molecular pathways such as oxidative stress, chronic inflammation, and metabolic dysregulation (Gallagher & LeRoith, 2020).

Conventional chemotherapeutic and antidiabetic drugs, while effective, are often associated with high costs, adverse effects, and limited accessibility in resource-constrained settings (Bray et al., 2021). These limitations have driven the search for safer, affordable, and more sustainable alternatives, with medicinal plants emerging as promising candidates due

to their rich phytoconstituents and diverse pharmacological activities (Kooti et al., 2017). India, in particular, possesses a long history of traditional medicine, with several indigenous plants scientifically validated for their anticancer and antidiabetic properties.

Withania somnifera (Ashwagandha) is widely studied for its withanolides, which exert anticancer activity through apoptosis induction and anti-proliferative mechanisms (Gupta et al., 2021). Curcuma longa (Turmeric) contains curcumin, a well-established phytoconstituent with antioxidant, anti-inflammatory, and chemopreventive activities (Goel et al., 2022). *Ocimum sanctum* (Holy Basil) has demonstrated hypoglycemic, antioxidant, and immunomodulatory effects attributed to eugenol, ursolic acid, and flavonoids (Sharma & Rai, 2022). Tinospora cordifolia (Guduchi), traditionally used as a Rasayana herb, is reported to enhance glucose uptake and modulate immune responses due to its alkaloids and glycosides (Patel et al., 2021). Despite extensive traditional usage, systematic comparative studies evaluating both anticancer and antidiabetic potential of these plants using in vitro assays remain limited.

Therefore, the present study aims to investigate the in vitro anticancer and antidiabetic potential of selected Indian medicinal plants *(W. somnifera, C. longa, O. sanctum, and T. cordifolia)* through phytochemical screening, cytotoxicity assays, and enzyme inhibition studies. By combining traditional knowledge with modern scientific evaluation, this study seeks to identify promising phytoconstituents for developing cost-effective therapeutic agents.

2. MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh plant materials of *Withania somnifera (roots), Curcuma longa (rhizomes), Ocimum sanctum (leaves), and Tinospora cordifolia* (stems) were collected from local authenticated sources. Plant specimens were cleaned, shade-dried at room temperature (25–30 °C), and powdered using a mechanical grinder. The plant samples were authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference.

Preparation of Plant Extracts

The powdered plant material (50 g each) was subjected to Soxhlet extraction using solvents of increasing polarity (aqueous, methanol, and ethanol). Each extraction was carried out for 8–10 h, and the solvent was evaporated under reduced pressure using a rotary evaporator. The obtained extracts were stored at 4 °C in airtight containers until further analysis. The percentage yield of extracts was calculated as:

$$\text{Extract Yield (\%)} = \{\text{Weight of Extract Obtained}\} \div \{\text{Weight of Plant Powder}\} \times 100$$

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was carried out to detect the presence of major classes of secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins, and glycosides using standard protocols. Results were recorded as present (+) or absent (–) for each class of compounds.

In Vitro Anticancer Activity:

Cell Lines and Culture

Human cancer cell lines (MCF-7 breast cancer, HeLa cervical cancer, and A549 lung cancer) were procured from a certified cell repository. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37 °C in a humidified incubator with 5% CO_2.

Cytotoxicity Assay (MTT Assay)

The anticancer potential of plant extracts was determined using the MTT assay. Briefly, cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 h. Cells were then treated with different concentrations of plant extracts (25–400 µg/mL) for 48 h. After treatment, MTT reagent (5 mg/mL) was added, and the formazan crystals formed were dissolved in DMSO. Absorbance was recorded at 570 nm using a microplate reader. The percentage of cell viability was calculated, and IC_{50} values were determined.

In Vitro Antidiabetic Activity

α-Amylase Inhibition Assay

The ability of plant extracts to inhibit α-amylase activity was evaluated using a starch-iodine method. Plant extracts at varying concentrations (25–400 µg/mL) were incubated with α-amylase enzyme solution at 37 °C for 30 min. Starch substrate was added, and the reaction was terminated using 3,5-dinitrosalicylic acid (DNSA) reagent. Absorbance was measured at 540 nm. Acarbose was used as a standard reference.

5.2 α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was performed using p-nitrophenyl-α-D-glucopyranoside (pNPG) as a substrate. Plant extracts (25–400 µg/mL) were incubated with α-glucosidase enzyme at 37 °C for 15 min, followed by the addition of pNPG solution. The release of p-nitrophenol was measured at 405 nm. Acarbose served as the positive control.

Rapid Screening of Phytoconstituents

Thin-Layer Chromatography (TLC)

Extracts were spotted onto silica gel TLC plates and developed using solvent systems specific for alkaloids, flavonoids, and phenolics. Spots were visualized under UV light (254 and 366 nm) and after spraying with detecting reagents such as Dragendorff's reagent (for alkaloids) and ferric chloride (for phenolics).

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The most active extracts were subjected to GC–MS analysis for rapid identification of phytoconstituents. The analysis was carried out on a GC–MS system equipped with a capillary column and helium as the carrier gas. The identification of compounds was performed by comparing mass spectra with the NIST library database.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical significance between groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

3. RESULTS

1. Phytochemical Screening:

Preliminary phytochemical tests revealed the presence of multiple secondary metabolites across all extracts. Alkaloids, flavonoids, phenolics, tannins, and terpenoids were consistently detected, whereas saponins were less common.

Table 1. Preliminary phytochemical screening of selected plant extracts:

Phytochemicals	*W. somnifera*	*C. longa*	*O. sanctum*	*T. cordifolia*
Alkaloids	+++	+	++	+++
Flavonoids	++	+++	+++	+++
Phenolics	++	+++	+++	+++
Tannins	+	+++	++	++
Terpenoids	++	+++	++	++
Saponins	–	+	+	+
Glycosides	++	+	+	++

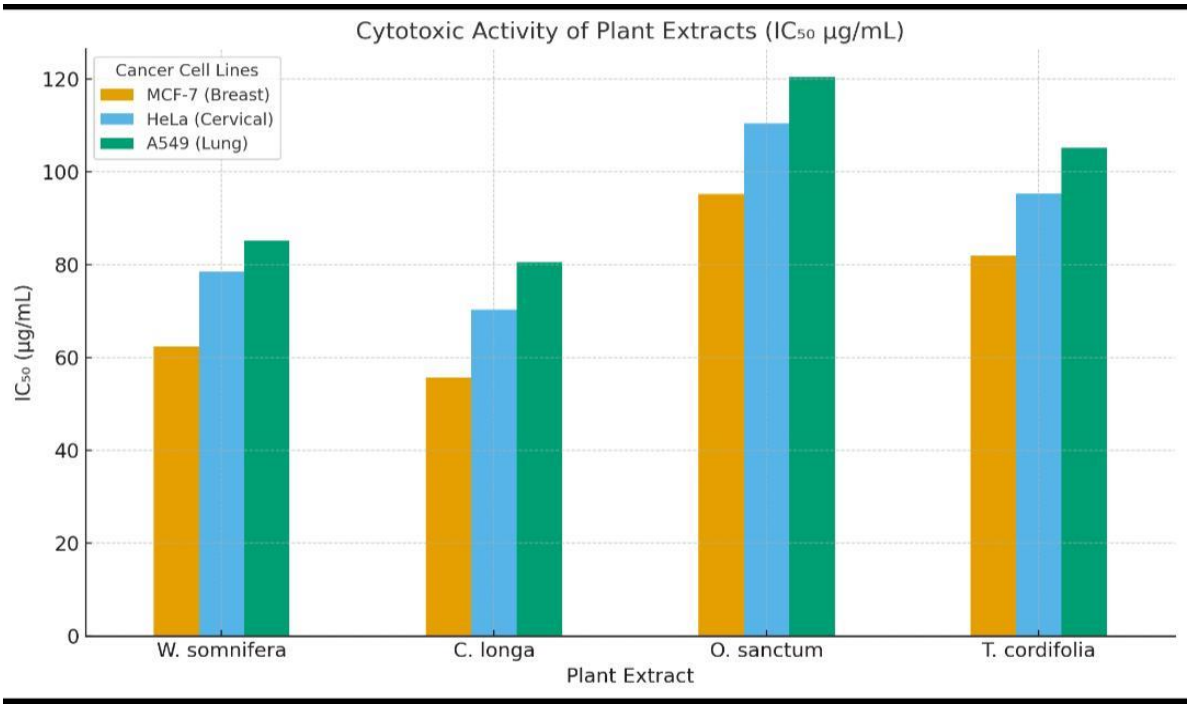
(+ = low; ++ = moderate; +++ = high; – = absent)

2. Anticancer Activity (MTT Assay):

The cytotoxic activity of plant extracts was evaluated against three human cancer cell lines.

Table 2. IC_{50} values (µg/mL) of extracts against cancer cell lines

lant Extract	MCF-7 (Breast)	HeLa (Cervical)	A549 (Lung)
V. somnifera	62.4 ± 2.1	78.5 ± 2.6	85.2 ± 3.1
C. longa	55.7 ± 1.9	70.3 ± 2.4	80.6 ± 2.7
O. sanctum	95.2 ± 3.2	110.5 ± 3.8	120.6 ± 4.0
T. cordifolia	82.1 ± 2.9	95.4 ± 3.1	105.2 ± 3.6
T. cordifolia	82.1 ± 2.9	95.4 ± 3.1	105.2 ± 3.6

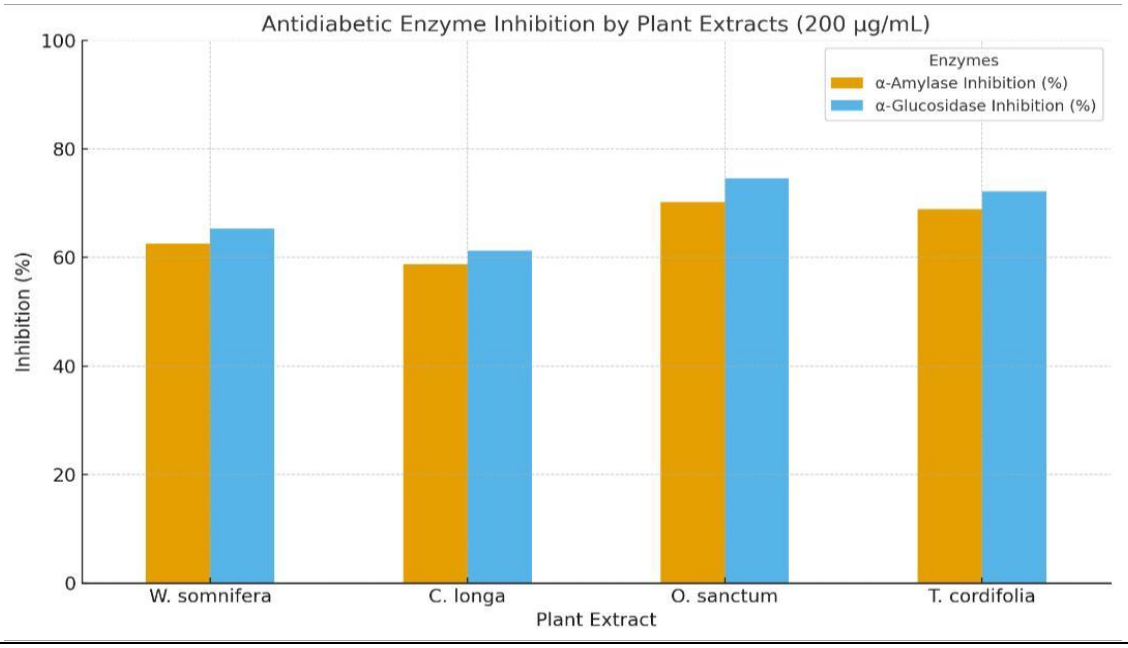


3. Antidiabetic Activity (Enzyme Inhibition Assays)

Table 3. α-Amylase and α-Glucosidase inhibition (% at 200 µg/mL)
Antidiabetic Activity (Enzyme Inhibition Assays)

Table 3. α-Amylase and α-Glucosidase inhibition (% at 200 µg/mL)

Plant Extract	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)
W. somnifera	54.2 ± 2.1.	61.5 ± 2.4
C. longa	48.7 ± 1.9	58.3 ± 2.2
O. sanctum	62.1 ± 2.3	72.8 ± 2.8
T. cordifolia	66.4 ± 2.5	78.9 ± 2.9
Acarbose (std.).	85.2 ± 2.6.	91.3 ± 3.1



4. Rapid Screening (TLC & GC–MS Analysis)

TLC analysis showed distinct bands corresponding to alkaloids, flavonoids, and phenolics. GC–MS profiling of the most active extracts identified several key phytoconstituents:

Table 4. Selected bioactive compounds identified by GC–MS

Plant Extract	Major Bioactive Compounds Detected
W. somnifera	Withanolide A, Withaferin A
C. longa	Curcumin, Demethoxycurcumin
O. sanctum	Eugenol, Ursolic acid, Apigenin
T. cordifolia	Tinosporaside, Cordifolioside A

4. DISCUSSION

The present study evaluated the anticancer and antidiabetic potential of four selected Indian medicinal plants: *Withania somnifera, Curcuma longa, Ocimum sanctum, and Tinospora cordifolia*. The in vitro assays revealed significant variations in cytotoxic activity against human cancer cell lines and inhibitory effects on carbohydrate-metabolizing enzymes, supporting the ethnopharmacological use of these plants in chronic disease management.

The cytotoxic effects observed in *W. somnifera and C. longa* are consistent with previous studies reporting that *withanolides and curcumin* induce apoptosis, modulate signaling pathways, and inhibit tumor proliferation (Gupta et al., 2021; Goel et al., 2022). The IC_{50} values obtained in our assays (55.7–85.2 µg/mL) align with earlier findings where methanolic extracts of W. somnifera roots and curcumin demonstrated selective cytotoxicity in breast and lung cancer cells (Bhattacharya & Choudhary, 2019). O. sanctum and T. cordifolia displayed comparatively higher IC_{50} values, yet still showed measurable cytotoxicity, suggesting a supportive rather than primary role in cancer therapy. This may be attributed to their bioactive flavonoids and alkaloids, which exert antioxidant and immunomodulatory effects (Prakash & Gupta, 2020).

In terms of antidiabetic activity, O. sanctum and T. *cordifolia* demonstrated higher inhibitory effects on α-amylase and α-glucosidase compared to W. *somnifera* and C. *longa*. Such enzyme inhibition is crucial for controlling postprandial hyperglycemia, a key therapeutic strategy in diabetes management (Kumar et al., 2019). Our results corroborate earlier reports where T. *cordifolia* polysaccharides enhanced glucose uptake and reduced fasting blood glucose, while O. sanctum extracts protected pancreatic β-cells and improved insulin sensitivity (Patel et al., 2021; Sharma & Rai, 2022).

The differential activity observed among the plants highlights the Importance of phytochemical diversity in therapeutic applications. While W. *somnifera* and C. *longa* are more promising for anticancer strategies, O. sanctum and T. *cordifolia* show strong potential in managing hyperglycemia. This complementary activity suggests that polyherbal formulations or combinatorial approaches could provide synergistic effects against cancer-diabetes comorbidity, which is increasingly recognized as a global health challenge (Chen et al., 2020).

However, it is important to note that in vitro findings may not fully translate to in vivo efficacy. Factors such as bioavailability, metabolism, and potential toxicity must be considered before clinical applications. Future research should focus on bioassay-guided fractionation to isolate active compounds, followed by molecular docking and animal model validation to confirm mechanisms of action.

5. CONCLUSION

This study provides strong evidence that selected Indian medicinal plants possess dual therapeutic potential against cancer and diabetes. *Withania somnifera and Curcuma longa* exhibited pronounced cytotoxic activity, while *Ocimum sanctum and Tinospora cordifolia* demonstrated significant α-amylase and α-glucosidase inhibition. The results justify the traditional use of these plants and highlight their potential for developing cost-effective, plant-based therapeutics. Integrating such phytoconstituents into modern drug discovery pipelines may offer novel solutions for managing complex

chronic diseases. Further in vivo studies, mechanistic analyses, and clinical trials are warranted to establish their efficacy and safety profiles.

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