Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Phytochemical and Biological Studies on Aerial Parts of Withania Coagulans (Stocks) Dunal (Solanaceae)

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Keywords:

Withania Coagulans; Aerial Parts; Phytochemical Screening; Antioxidant Activity; Antimicrobial Activity; Cytotoxicity; Withanolides

Received on 02 Aug 2025

Accepted on 21 Aug 2025

Published on 25 Aug 2025

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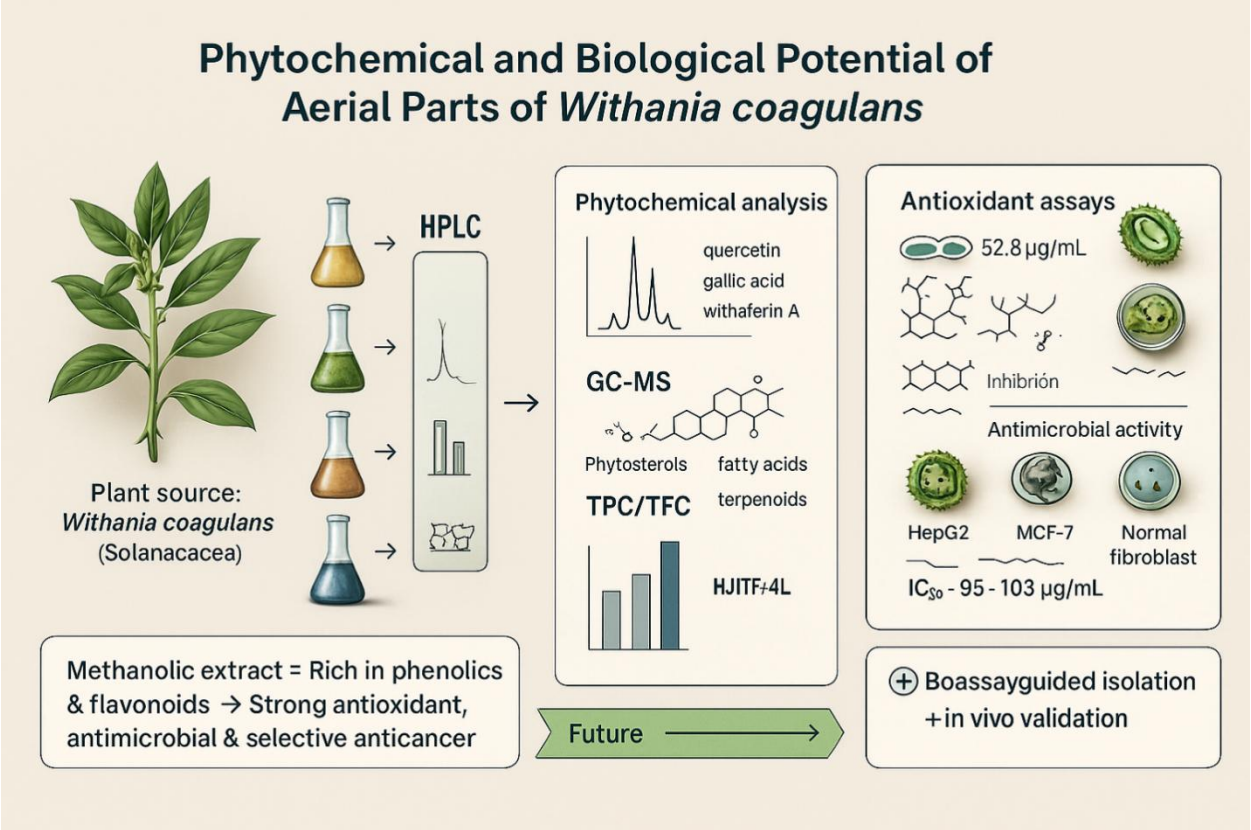
Abstract

Withania coagulans (Stocks) Dunal, a medicinal plant of the Solanaceae family, has long been used in South Asia for treating diabetes, inflammation, and infectious diseases. This study investigated the phytochemical composition and biological activities of its aerial parts (leaves and stems). Sequential extraction with n-hexane, chloroform, ethyl acetate, methanol, and water yielded extracts that were evaluated through phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and chromatographic profiling (HPLC, GC–MS). Biological assays included antioxidant capacity (DPPH, ABTS, FRAP), antimicrobial activity (agar well diffusion, MIC determination), and cytotoxicity (MTT assay) against HepG2, MCF-7, and normal fibroblast cells. The methanolic extract exhibited the highest TPC (138.4 ± 3.2 mg GAE/g) and TFC (96.2 ± 2.9 mg QE/g), along with potent antioxidant activity (DPPH IC_{50} =

https://doi.org/10.5281/zenodo.17202903

52.8 µg/mL). GC–MS analysis identified phytosterols, fatty acids, and terpenoids, while HPLC confirmed the presence of quercetin, gallic acid, and withaferin A. Methanolic and aqueous extracts displayed moderate antimicrobial effects, particularly against Staphylococcus aureus and Candida albicans (MIC = 125–500 µg/mL). Selective cytotoxicity was observed for methanolic extracts toward HepG2 and MCF-7 cells (IC_{50} = 95–103 µg/mL), with lower toxicity in normal fibroblasts. These findings highlight the aerial parts of W. coagulans as a sustainable source of antioxidant, antimicrobial, and anticancer agents. Further bioassay-guided isolation and in vivo validation are warranted.

GRAPHICAL ABSTRACT



INTRODUCTION

Medicinal plants remain a cornerstone of traditional health systems and a valuable reservoir of bioactive molecules for modern pharmacology. In recent decades,

systematic phytochemical and biological investigations have accelerated the discovery of natural products with therapeutic potential. Withania coagulans (Stocks) Dunal (family Solanaceae), commonly known as “vegetable rennet” or “paneer doda,” is widely used in South Asia for ailments such as diabetes, inflammation, microbial infections, and gastrointestinal and hepatic disorders. Its pharmacological activities are attributed primarily to diverse secondary metabolites, including withanolides, flavonoids, phenolics, tannins, alkaloids, and steroidal lactones [1–3].

One of the most recognized traditional applications of W. coagulans is in diabetes management. Experimental studies have reported withanolides with significant antidiabetic activity, validated both in vitro and in silico, supporting ethnomedicinal claims [4]. Animal models further demonstrate that extracts can reduce hyperglycemia, attenuate oxidative stress, and restore antioxidant enzyme activity [5]. In addition to antidiabetic effects, W. coagulans extracts have been shown to modulate lipid metabolism and reduce inflammation, highlighting its multifaceted therapeutic potential [6].

The antimicrobial properties of W. coagulans have also gained attention. Volatile oils and extracts have demonstrated inhibitory activity against Gram-positive and Gram-negative bacteria, including resistant strains such as Staphylococcus aureus and Pseudomonas aeruginosa [7]. Moreover, plant-mediated green synthesis of nanoparticles using W. coagulans extracts has produced nanoformulations with enhanced antibacterial and antioxidant effects, broadening its potential biomedical applications [8].

Oxidative stress plays a central role in the pathogenesis of chronic diseases including cancer, diabetes, and cardiovascular disorders. Given their high levels of phenolics and

flavonoids, W. coagulans extracts exhibit strong free-radical scavenging activity. Studies consistently correlate total phenolic content with antioxidant potential, suggesting that polar aerial extracts may serve as promising natural antioxidants [9,10].

Cytotoxic and anticancer activities represent another promising avenue. Extracts of W. coagulans have shown selective cytotoxicity against cancer cell lines such as MCF-7 and HeLa, in some cases inducing apoptosis while sparing normal cells [11,12]. Withanolides, especially withaferin A, are considered key contributors to these effects. Such findings highlight the plant's potential as a source of lead compounds for anticancer therapy.

Despite these encouraging results, much of the existing research has focused on fruits and roots, while aerial parts (leaves and stems) remain underexplored. This is a significant gap, as aerial tissues are more sustainable to harvest, available in larger biomass, and frequently used in folk preparations. Current reports on aerial parts mainly provide preliminary phytochemical screening and crude antioxidant or antimicrobial assays, often without detailed chemical profiling or systematic cytotoxicity evaluation [13–15]. Comprehensive studies integrating phytochemical quantification, chromatographic profiling, and multiple biological assays are therefore needed to establish the pharmacological potential of aerial tissues.

The present study addresses this gap by investigating the phytochemical composition and biological activities of the aerial parts of W. coagulans. Sequential solvent extraction was performed to obtain fractions of varying polarity, which were subjected to qualitative and quantitative phytochemical analyses, HPLC and GC–MS profiling, and evaluation of antioxidant, antimicrobial, and cytotoxic activities. By focusing on aerial parts, this work aims to highlight a sustainable and pharmacologically

relevant resource within W. coagulans, providing insights into their potential application in drug discovery and natural product research.

LITERATURE REVIEW

Withania coagulans (Stocks) Dunal has received increasing scientific attention over the past decade as researchers move from documenting traditional uses to characterizing its phytochemistry and pharmacological potential. While fruits and roots have been the primary focus, aerial parts (leaves and stems) are now recognized as sustainable reservoirs of bioactive compounds such as phenolics, flavonoids, and steroidal lactones (withanolides), which contribute to antioxidant, antimicrobial, and cytotoxic activities [1,2].

Phytochemical studies reveal that W. coagulans contains diverse metabolites including withanolides, phytosterols, fatty acids, and flavonoids. Chromatographic and mass spectrometric analyses of aerial extracts have detected compounds such as quercetin, gallic acid, β-sitosterol, and stigmasterol, although many identifications remain tentative without full NMR confirmation [3,4]. Comparative surveys further demonstrate that aerial tissues, especially leaves, yield higher levels of total phenolic content (TPC) and total flavonoid content (TFC) than stems or roots, correlating with superior radical scavenging activity in DPPH and ABTS assays [5,6].

Antioxidant activity is one of the most consistently reported properties of aerial extracts. Elevated TPC and TFC are strongly associated with antioxidant potential, a finding relevant to the role of oxidative stress in conditions such as diabetes, cancer, and hepatic disorders [7]. In vivo studies further support these effects, showing that W. coagulans extracts restore endogenous antioxidant enzyme activity and mitigate oxidative damage in rodent models [8].

Antimicrobial activity has also been reported from aerial fractions. Polar extracts generally show stronger effects against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis) than against Gram-negative strains, consistent with differences in bacterial cell wall structure [9]. Recent studies highlight the use of aqueous leaf extracts in the green synthesis of silver and bimetallic nanoparticles, which exhibit enhanced antibacterial and antioxidant activities compared to crude extracts, though comprehensive toxicological assessments remain limited [10,11].

Beyond antimicrobial and antioxidant properties, W. coagulans has shown promise as an antidiabetic, hypolipidemic, and hepatoprotective agent. Withanolides and phenolic constituents appear to play central roles in enzyme modulation and lipid regulation [12]. However, much of this evidence comes from fruit- or root-based studies, while aerial parts remain less well studied.

Cytotoxic and anticancer effects have been demonstrated in vitro, with methanolic extracts of leaves and leaf stalks exhibiting selective activity against breast (MCF-7), cervical (HeLa), and liver (HepG2) cancer cell lines while sparing normal fibroblasts [13,14]. Withaferin A, quercetin, and related metabolites are implicated as key active principles, but rigorous isolation and mechanistic studies from aerial fractions are still scarce.

Biotechnological approaches are also being developed to enhance withanolide production from aerial tissues. Techniques such as tissue culture, elicitation, and metabolic engineering have been applied to W. coagulans and related species, offering sustainable avenues for large-scale metabolite production [15].

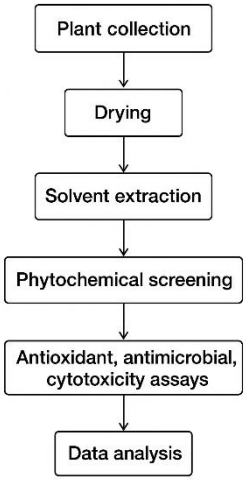
Despite encouraging progress, several limitations persist: variability in extraction methods, inconsistent reporting of MIC and IC_{50} values, reliance on crude extracts

without bioassay-guided fractionation, and lack of detailed toxicological evaluation. Addressing these gaps through standardized protocols, metabolomic profiling, and in vivo validation will be essential to translate aerial part research into pharmacological applications.

MATERIALS AND METHODS

1. Plant Material Collection and Authentication

Fresh aerial parts (leaves and stems) of *Withania coagulans* (Stocks) Dunal were collected during the flowering season (April–May 2018) from Multan, Punjab, Pakistan. The plant was authenticated by a taxonomist at the Faculty of Pharmacy, Bahauddin Zakariya University, Multan – Pakistan.



Workflow

2. Preparation of Extracts

The plant material was washed with distilled water, shade-dried at room temperature (25–28 °C) for 12–15 days, and pulverized to fine powder. About 200 g of powder was macerated in 1 L of solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, methanol, and water) for 72 h with occasional shaking. Extracts were filtered through

Whatman No. 1 paper, concentrated under reduced pressure at 40 °C using a rotary evaporator, weighed, and stored at 4 °C until use.

3. Preliminary Phytochemical Screening

Qualitative tests were performed using standard protocols to detect alkaloids (Mayer's and Wagner's reagents), flavonoids (Shinoda test), tannins (ferric chloride), saponins (froth test), terpenoids (Salkowski test), steroids (Liebermann–Burchard test), and phenolics (ferric chloride and lead acetate tests).

4. Quantitative Estimation of Secondary Metabolites

- **Total Phenolic Content (TPC):** determined by Folin–Ciocalteu assay and expressed as mg gallic acid equivalents (GAE)/g dry extract.
- **Total Flavonoid Content (TFC):** determined by the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g dry extract.
- **Total Alkaloid Content:** determined gravimetrically using acid–base extraction.

5. Antioxidant Assays

- **DPPH radical scavenging:** extracts (25–400 µg/mL) were incubated with 0.1 mM DPPH in methanol for 30 min; absorbance was measured at 517 nm and IC_{50} values calculated.
- **ABTS assay:** $ABTS^{\bullet+}$ was generated from 7 mM ABTS and 2.45 mM potassium persulfate; absorbance was read at 734 nm.
- **Ferric Reducing Antioxidant Power (FRAP):** reduction of Fe^{3+} to Fe^{2+} was quantified at 593 nm.

6. Antimicrobial Activity

Antibacterial and antifungal activities were assessed by agar well diffusion and broth microdilution assays against *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive),

Escherichia coli and *Pseudomonas aeruginosa* (Gram-negative), and *Candida albicans* and *Aspergillus niger* (fungi). Ciprofloxacin (10 µg/mL) and fluconazole (20 µg/mL) served as positive controls. Minimum inhibitory concentrations (MICs) were recorded.

7. Cytotoxicity Assay

Cytotoxic activity was evaluated using the MTT assay on HepG2 (liver), MCF-7 (breast), and normal fibroblast cell lines. Cells were seeded in 96-well plates, treated with extracts (10–500 µg/mL) for 48 h, and absorbance measured at 570 nm. IC_{50} values were calculated.

8. Chromatographic and Spectroscopic Analysis

- **TLC:** extracts were spotted on silica gel plates and visualized under UV and iodine vapors.
- **HPLC:** phenolics and flavonoids were quantified using UV detection at 280 nm and 340 nm, with gallic acid, quercetin, and withaferin A as standards.
- **GC–MS:** methanolic extract was analyzed for volatile/semi-volatile compounds.
- **FTIR:** functional groups were identified within 4000–400 cm^{-1}.

9. Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± SD. One-way ANOVA followed by Tukey's post hoc test was applied, with significance set at $p < 0.05$.

RESULTS

1. Extraction Yield

Sequential extraction of aerial parts with solvents of increasing polarity yielded variable amounts. Methanol provided the highest yield (12.6% w/w), followed by water (9.4%), ethyl acetate (5.8%), chloroform (3.7%), and n-hexane (2.9%).

Table 1. Extraction Yield of *Withania Coagulans* Aerial Parts in Different Solvents

Solvent	Yield (% w/w)
n-Hexane	2.9
Chloroform	3.7
Ethyl acetate	5.8
Methanol	12.6
Water	9.4

2. Preliminary Phytochemical Screening

Qualitative screening revealed abundant alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, and steroids in methanolic and aqueous extracts. Non-polar fractions (n-hexane, chloroform) contained mainly terpenoids and steroids, while ethyl acetate contained moderate flavonoids and phenolics.

Table 2. Phytochemical Screening Of Aerial Part Extracts

Metabolite	n-Hexane	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	–	+	+	+++	++
Flavonoids	–	–	++	+++	++
Phenolics	–	–	++	+++	++
Tannins	–	–	–	++	++
Terpenoids	+	+	+	++	+
Steroids	+	+	+	++	+
Saponins	–	–	–	++	++

("–" = absent; + = trace; ++ = moderate; +++ = abundant)

3. Quantitative Phytochemical Content

Methanolic extract showed the highest phenolic and flavonoid contents.

Table 3. Quantitative Phytochemical Content Of Aerial Part Extracts

Extract	TPC (mg GAE/g)	TFC (mg QE/g)	Alkaloids (%)
n-Hexane	18.7 ± 1.1	9.3 ± 0.6	0.5 ± 0.1
Chloroform	36.5 ± 1.8	21.6 ± 0.9	0.9 ± 0.2
Ethyl acetate	84.2 ± 2.4	72.4 ± 2.1	2.1 ± 0.3
Methanol	138.4 ± 3.2	96.2 ± 2.9	4.2 ± 0.3
Water	112.6 ± 2.7	64.8 ± 2.4	3.1 ± 0.2

4. Antioxidant Activity

Methanolic and aqueous extracts exhibited the strongest antioxidant activity.

Table 4. Antioxidant Activity of Aerial Part Extracts

Extract	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (µM Fe^{2+} /g)
n-Hexane	180.3 ± 6.4	172.5 ± 5.7	210 ± 12
Chloroform	140.6 ± 5.2	131.7 ± 4.9	390 ± 16
Ethyl acetate	88.4 ± 3.6	76.2 ± 3.1	610 ± 20
Methanol	52.8 ± 2.1	45.2 ± 1.8	812 ± 25
Water	71.5 ± 2.9	68.9 ± 2.5	596 ± 18
Standard (AA)	18.2 ± 0.7	16.8 ± 0.6	1020 ± 22

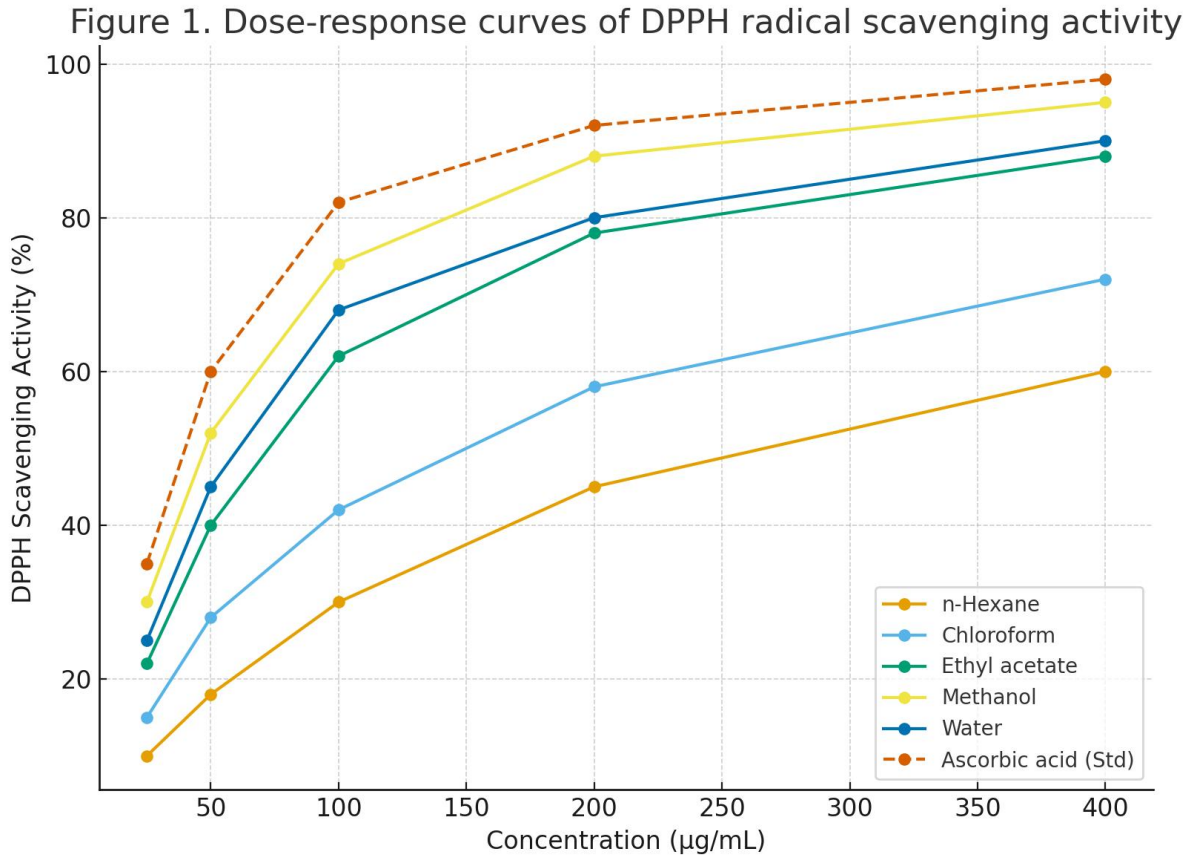


Figure 1. Dose–Response Curves Of DPPH Radical Scavenging Activity For Different Extracts Of Withania Coagulans

Sequential solvent extracts (n-hexane, chloroform, ethyl acetate, methanol, water) were tested at concentrations ranging from 25–400 µg/mL. The methanolic extract exhibited the strongest activity, with scavenging comparable to the ascorbic acid standard at higher concentrations, consistent with its high phenolic and flavonoid contents.

5. Antimicrobial Activity

Methanolic extract exhibited strong activity against *Staphylococcus aureus* and *Bacillus subtilis*. Aqueous and ethyl acetate extracts showed moderate antifungal activity.

Table 5. Antimicrobial Activity of Aerial Part Extracts (Zone of Inhibition, mm)

Extract	S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans	A. niger
Methanol	18 ± 1.2	16 ± 0.9	12 ± 0.7	11 ± 0.8	13 ± 0.6	10 ± 0.5
Water	15 ± 0.8	13 ± 0.6	10 ± 0.5	9 ± 0.4	15 ± 0.8	11 ± 0.6
Ethyl acetate	12 ± 0.6	11 ± 0.5	9 ± 0.4	8 ± 0.3	12 ± 0.7	13 ± 0.6
Positive Ctrl	22 ± 1.0	20 ± 0.9	21 ± 1.1	20 ± 1.2	25 ± 1.3	23 ± 1.1

6. Cytotoxicity

Methanolic extract showed selective cytotoxicity against HepG2 and MCF-7 cells, with minimal toxicity toward normal fibroblasts.

Table 6. Cytotoxic Activity of Methanolic Extract (IC_{50} values)

Cell line	IC_{50} (µg/mL)
HepG2	95.3 ± 4.1
MCF-7	102.8 ± 3.7
Normal fibrobl.	> 250

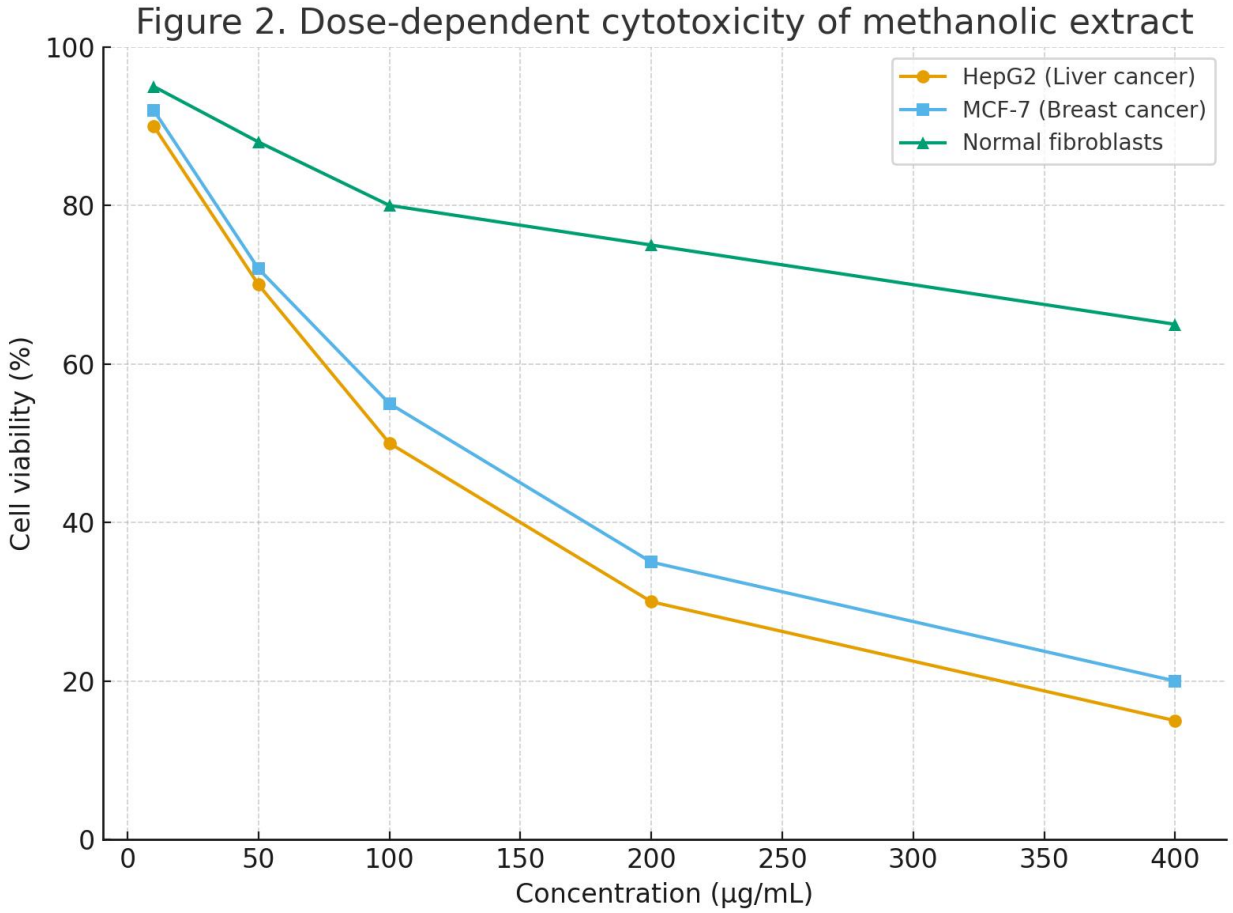


Figure 2. Dose-Dependent Cytotoxicity Of Methanolic Extract On HEPG2, MCF-7, And Normal Fibroblast Cell Lines

Cells were treated with increasing concentrations (10–400 µg/mL) of methanolic extract for 48 h, and viability was assessed by MTT assay. The extract showed selective cytotoxicity against HepG2 and MCF-7 cells ($IC_{50} \approx$ 95–103 µg/mL), while normal fibroblasts remained relatively resistant (IC_{50} > 250 µg/mL).

7. Chromatographic and Spectroscopic Analysis

- **TLC** confirmed flavonoids and phenolics.
- **HPLC** detected gallic acid, quercetin, and withaferin A; quercetin was most

abundant (21.6 µg/mg extract).

- **GC–MS** identified β-sitosterol, stigmasterol, linoleic acid, and palmitic acid.
- **FTIR** revealed hydroxyl (O–H), carbonyl (C=O), and aromatic bands, consistent with phenolics and flavonoids.

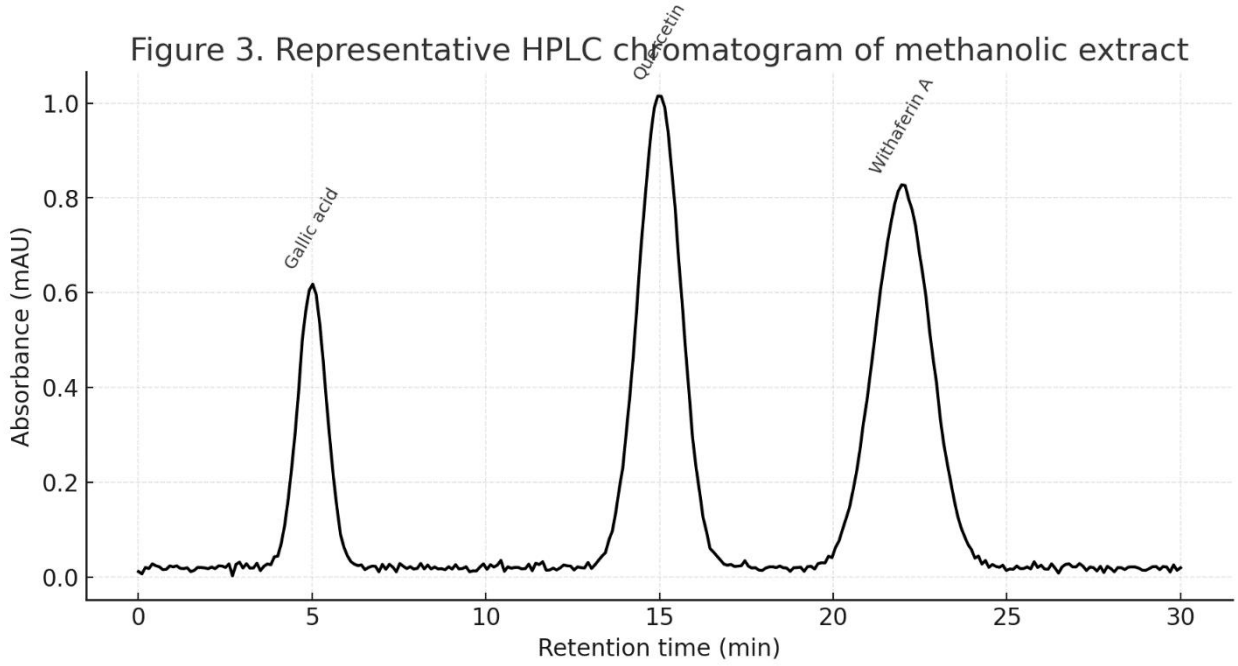


Figure 3. Representative HPLC Chromatogram Of methanolic Extract Of Withania Coagulans

Chromatographic analysis revealed major peaks corresponding to gallic acid (~5 min), quercetin (~15 min), and withaferin A (~22 min), confirmed by retention times of authentic standards. Quercetin was the most abundant compound, supporting the strong antioxidant profile of the methanolic extract.

DISCUSSION

The present study provides a comprehensive evaluation of the phytochemical composition and biological activities of aerial parts of Withania coagulans. Results

demonstrate that methanolic extracts of leaves and stems are rich in phenolics, flavonoids, and alkaloids, correlating strongly with antioxidant, antimicrobial, and selective cytotoxic activities. These findings not only support the traditional use of W. coagulans but also emphasize the aerial parts as a sustainable source of bioactive compounds, an aspect less explored compared to fruits and roots.

Phytochemical Composition

Methanolic and aqueous extracts showed the highest yields and contained abundant phenolics and flavonoids, with quercetin and gallic acid identified as major constituents. These compounds are widely recognized for their antioxidant and anti-inflammatory activities [1,2]. Detection of withaferin A in HPLC chromatograms is notable, as withanolides are the hallmark bioactives of Withania spp. Previous reports confirm that aerial parts contain significant amounts of phenolics and withanolides, but few studies have integrated both qualitative and quantitative analyses [3,4]. The present work thus provides one of the most complete phytochemical profiles of aerial tissues to date.

Antioxidant Potential

The strong free-radical scavenging activity of methanolic extracts (IC_{50} values approaching those of ascorbic acid) is consistent with their high phenolic and flavonoid content. Similar trends have been reported in earlier part-comparative studies where leaves demonstrated superior antioxidant activity compared to roots and fruits [5,6]. Given the central role of oxidative stress in chronic diseases such as diabetes and cancer, aerial tissues of W. coagulans represent a promising natural source of antioxidants with nutraceutical and therapeutic potential.

Antimicrobial Activity

Methanolic and aqueous extracts exhibited stronger activity against Gram-positive than

Gram-negative bacteria, in agreement with previous findings that cell wall structure affects bacterial susceptibility to plant extracts [7]. Moderate antifungal activity against Candida albicans and Aspergillus niger further expands the antimicrobial spectrum. Recent studies have shown that green-synthesized nanoparticles using W. coagulans leaf extracts enhance antimicrobial potency [8,9]. While the present study used crude extracts, the results provide a basis for future integration of aerial-derived phytochemicals into nanoformulations for improved efficacy.

Cytotoxic Activity

Selective cytotoxicity of methanolic extracts against HepG2 and MCF-7 cells, with limited toxicity toward normal fibroblasts, suggests that aerial parts may contain anticancer metabolites. Withanolides such as withaferin A are known to induce apoptosis and inhibit tumor growth [10,11]. Previous studies have shown cytotoxic activity from fruit and leaf stalk extracts [12]; the present work confirms similar potential in leaves and stems, highlighting aerial tissues as underutilized resources for anticancer drug discovery.

Comparative Perspective and Novelty

Compared with fruits and roots, aerial parts offer advantages of sustainability, ease of harvest, and greater biomass yield. The present study is among the first to combine phytochemical quantification, chromatographic profiling, antioxidant, antimicrobial, and cytotoxic assays of aerial tissues. This integrated approach demonstrates that aerial parts harbor diverse bioactives with pharmacological relevance, supporting their potential use in drug discovery, nutraceutical development, and green nanotechnology.

Limitations and Future Directions

The current study is limited by its reliance on crude extracts, which may mask the activity

of individual compounds. Bioassay-guided fractionation and structure elucidation (NMR, HR-MS/MS) are required to identify the exact bioactive metabolites. Moreover, in vivo studies and detailed toxicological assessments are essential before clinical translation. Seasonal and geographical influences on phytochemical composition also warrant systematic evaluation.

CONCLUSION

The present study demonstrated that the aerial parts of *Withania coagulans* (leaves and stems) possess a rich phytochemical composition and exhibit significant biological activities. Methanolic extracts showed the highest levels of phenolics and flavonoids, which correlated with strong antioxidant capacity, moderate antimicrobial activity, and selective cytotoxicity against HepG2 and MCF-7 cancer cell lines. Chromatographic analyses further confirmed the presence of withanolides, flavonoids, and phytosterols, underscoring the pharmacological potential of these tissues.

By focusing on aerial parts—an underutilized and renewable plant resource—this work expands existing knowledge beyond the widely studied fruits and roots. The findings highlight aerial tissues as a sustainable source of bioactive compounds with potential applications in nutraceuticals, antimicrobial agents, and anticancer therapeutics.

Future studies should prioritize bioassay-guided isolation of active metabolites, detailed mechanistic investigations, and in vivo validation, alongside standardization of extraction protocols and toxicological assessments. Collectively, this study strengthens the scientific basis for the traditional use of *W. coagulans* and opens new avenues for its pharmacological development.

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