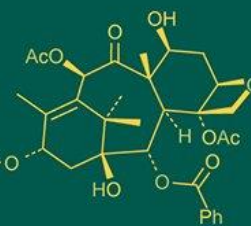
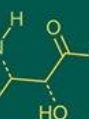
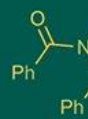


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In vitro antioxidant and antimicrobial potential of *Withania somnifera* root extracts and their phytochemical correlation

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Abstract

Ashwagandha, or *Withania somnifera* (L.) Dunal, is a well-known medicinal plant that is widely utilised in traditional medicine due to its antibacterial, adaptogenic, and antioxidant qualities. The goal of the current study was to assess the antibacterial and antioxidant properties of several solvent extracts of *W. somnifera* roots *in vitro* and to establish a relationship between these properties and their phytochemical makeup. Aqueous solution, methanol, and ethanol were used in making root extracts. The phytochemical screening identified alkaloids, flavonoids, phenolic, saponins, and withanolides from the root extract. Antioxidant tests include ferric reducing antioxidant power (FRAP), DPPH radical scavenging tests. The agar well diffusion method was utilised to demonstrate the antimicrobial activity of the effective compounds on certain strains of Gram-positive and Gram-negative bacteria. With a significant relation to the total phenolic/flavonoid content, methanolic root extracts displayed the most potent antioxidant and antibacterial activity. The study validates the potential of the roots of the *W. somnifera* as a natural reservoir of potent chemicals for the pharmaceutical industry.

Keywords: *Withania somnifera*, antioxidant activity, antimicrobial activity, phytochemical screening, medicinal plants

Introduction

Medicinal plants are part of the modern drug discovery process and have been and are essential for drug discovery and biological activities [1]. A large percentage of medication presently available is obtained or obtained indirectly from plant secondary metabolic beings due to their diversity and importance [2]. There has been renewed interest in plant-based substance research as an alternative substitute for man-made medications as cheaper and safer means of treating various infections as well as other kinds of illness [3].

Most of the pathological conditions, including cancer, cardiovascular disease, diabetes, and neurodegenerative disorders, are to a large extent caused by oxidative stress [4]. This results from an imbalance that exists between the antioxidant defense mechanism developed within the body and the generation of reactive oxygen species, ROS [5]. Excessive free radicals can cause damage to proteins, lipids, and DNA molecules within the cell, thus interfering with their efficient function [6]. It is an accepted fact that these free radicals can be easily neutralized by naturally present antioxidants like phenolic and flavonoid compounds, which provide protection to biological systems from oxidative damage [7].

At the same time, with increasing cases of antimicrobial resistance worldwide, this problem poses a challenge by lowering the efficacy of previously effective antibiotics with high occurrences of treatment failures [8]. The need for new antimicrobial medicines with unique modes of activity must always be met to counter the high adaptability of pathogenic bacteria that cause harm to human beings. The drugs that have acquired prominence as sources for antimicrobial activity with less toxicity consist of medicinal plants [9].

One of the widely used medicinal herbs in Ayurvedic medicine and other alternative medicine practices across the globe is identified as *Withania somnifera* (L.) Dunal, also known as ashwagandha or Indian ginseng. This plant belongs to the Solanaceae family [10]. Considerable interest exists pertaining to the rejuvenating effects of the roots of this plant owing to their inherent properties of being an adaptogen and anti-inflammatory or an immune-modulator.

According to studies concerning the chemical composition of this plant, its roots are rich in bioactive constituents like withanolides, alkaloids, flavonoids, tannins, and other phenolics^[11].

For the purpose of evaluating the antioxidant potential and antibacterial activity of variant extracts of the roots of *Withania somnifera* *in vitro* and to find a correlation between their qualitative constituents and efficiency in biological activities, this study was conducted. The potential results of this study can be useful in the potential applications of *Withania somnifera* in the development of natural antibacterials/oxidants and can provide a scientific foundation for its traditional employment^[12].

Materials and methodology

Collection and authentication of plant material

Fresh roots of *Withania somnifera* (L.) Dunal were gathered from a nearby medicinal plant production region in Jhajra, Uttarakhand, India. A voucher specimen was deposited for future reference after the authentication of the plant material by a competent taxonomist from the School of Agriculture, Dev Bhoomi Institute. After cleaning the sample properly in order to get rid of any remaining dust particles with the aid of distilled water, the collected roots were allowed to shade dry under normal conditions within ten to fourteen days. After drying the sample in powder form with the aid of a mechanical grinder machine, the powder was packed in containers until it was required.

Preparation of plant extracts

Aqueous, ethanolic, and methanolic media were employed in the sequential solvent extraction approach. Soxhlet technique was employed in the extraction process for 6-8 hours with 500 mL of each solvent employed in the process. This process was employed in the extraction process in obtaining 50 g of the dried root powder with the solvents employed. Rotary evaporation technique was employed in the evaporation process under low pressure following filtration through Whatman No. 1 filter paper. Water bath was employed in the evaporation process in the aqueous solution. Dry extracts were stored at 4 °C before further investigation following the measurement of the yield.

Chemicals and reagents

Every chemical and reagent utilised in the research was of analytical quality. Standard suppliers including Sigma-Aldrich and HiMedia Laboratories, India, provided DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, gallic acid, quercetin, TPTZ (2,4,6-tripyridyl-s-triazine), ferric chloride, methanol, ethanol, and nutrient agar.

Phytochemical Screening

Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, and withanolides were all obtained through qualitative phytochemical screening of all the extracts using established methods.

Estimation of Total Phenolic and Flavonoid Content

Investigation of the phenolic compounds, using the Folin-Ciocalteu method, resulted in the calculation of the total phenolic content, which was expressed as mg GAE/g extract. Similarly, the calculation of the flavonoid content,

using the aluminum chloride colorimetric method, led to the expression of the results as mg quercetin equivalents/g extract.

Antioxidant assays

The antioxidant assays used were the FRAP assay and the DPPH free radical scavenging tests. For the antioxidant tests, the IC₅₀ value was calculated after evaluating the DPPH scavenging activity at 517 nm. The antioxidant test results, which were done using the FRAP assay, were expressed as $\mu\text{mol Fe}^{2+}$ per g extract. The standard antioxidant was ascorbic acid.

Antimicrobial activity

Antibacterial activity was determined against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* by the agar well diffusion method. After incubation at 37 °C for 24 h, zones of inhibition were taken into consideration. Standard antibiotics were used as positive controls.

Statistical analysis

Each experiment was performed in triplicate, while mean $\pm$ SD was employed to show the findings of each result. The relationship between the constituents of PHYs and the exhibited bioactivities was calculated by employing Pearson's test at a significant level of $p < 0.05$.

Results

Qualitative phytochemical screening

All the solvent extracted roots of *Withania somnifera* had significant levels of secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, steroids, and withanolides. The preliminary phytochemical screening revealed that the phytoconstituents are found in the methanolic extracted roots followed by the ethanolic extracted roots. The aqueous extracted roots had a lower level of phytochemicals.

Table 1: Qualitative Phytochemical Screening of *W. somnifera* Root Extracts

Phytochemical	Aqueous	Ethanolic	Methanolic
Alkaloids	+	++	+++
Flavonoids	+	++	+++
Phenolics	+	++	+++
Tannins	+	++	+++
Saponins	+	++	+++
Steroids	+	++	+++
Withanolides	+	++	+++

Total flavonoid content (TFC) and total phenolic content (tpc)

The best extraction efficiency for bioactive components was demonstrated by the methanolic extract, which had the highest total phenolic and flavonoid content.

Table 2: TPC and TFC of *W. somnifera* Root Extracts

Extract	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Aqueous	42.6 $\pm$ 1.8	26.4 $\pm$ 1.2
Ethanolic	68.3 $\pm$ 2.4	44.7 $\pm$ 1.9
Methanolic	92.1 $\pm$ 3.1	63.5 $\pm$ 2.6

Antioxidant activity

Radical Scavenging Activity of DPPH

Table 3: DPPH Radical Scavenging Activity

Extract	IC ₅₀ (µg/mL)
Aqueous	94.7 ± 3.5
Ethanollic	61.2 ± 2.8
Methanolic	38.6 ± 1.9
Ascorbic acid	21.3 ± 1.2

FRAP assay

Methanolic extract exhibited significantly higher reducing power compared to other extracts.

Table 4: FRAP Activity

Extract	FRAP (µmol Fe ²⁺ /g extract)
Aqueous	310 ± 12
Ethanollic	468 ± 18
Methanolic	612 ± 22

Antimicrobial activity**Table 5:** Zone of Inhibition (mm)

Microorganism	Aqueous	Ethanollic	Methanolic	Ciprofloxacin
<i>S. aureus</i>	10 ± 0.6	14 ± 0.8	18 ± 1.0	24 ± 1.2
<i>E. coli</i>	8 ± 0.5	12 ± 0.7	16 ± 0.9	23 ± 1.0
<i>P. aeruginosa</i>	7 ± 0.4	11 ± 0.6	15 ± 0.8	22 ± 1.1

Correlation analysis

Antioxidant activity demonstrated a strong positive relationship ($r = 0.94$), as did antibacterial activity ($r = 0.91$), when correlated with total phenolic content. Total flavonoid content demonstrated a substantial relationship to values of FRAP ($r = 0.95$), as well as DPPH values ($r = 0.92$), to suggest that polyphenolic chemicals were of major importance in these findings.

Discussion

Bioactive compounds with considerable therapeutic activity have been reported to be present in large numbers in medicinal plants, and the polarity and particularly the extraction methods have been shown to greatly influence the efficacy of such compounds. Significant variance was observed in the various solvent extracts of *Withania somnifera* roots in relation to phytochemical constituents, antioxidant, and antibacterial potential in the present investigation [13].

All the extracts showed the presence of alkaloids, flavonoids, phenolics, saponins, and steroids, and the methanolic extract showed the highest concentration of these compounds compared to the other crude extracts. The enhanced concentration of the phytochemicals can also be explained by considering the fact that methanol is a good extractant for polyphenolic-type chemicals, and it is a good polarity series representative to extract these compounds.

The methanol extract was more effective in extracting phytochemicals compared to aqueous and ethanol extracts, according to the results from earlier research on *W. somnifera* [14].

These findings were further supported by the antioxidant assays. The methanolic extract demonstrated the highest ferric reducing antioxidant power and the strongest DPPH radical scavenging activity, demonstrating its superior capacity to neutralise free radicals and function as an efficient reducing agent. The hydrogen-donating and electron-transfer processes of phenolic and flavonoid chemicals are well known, and they play a major role in

antioxidant action. The poor solubility and extraction efficiency of certain polyphenols in water may be the reason for the relatively lower antioxidant capacity of aqueous extracts [15].

Methanol extract from *Withania somnifera* roots presented high antibacterial potential towards both Gram-positive and Gram-negative bacteria, inhibition being highest for *Staphylococcus aureus*. Gram-negative bacterial species probably have a naturally low sensitivity to different bioactive substances compared to Gram-positive bacterial species. APTs, such as phenolics, flavonoids, and alkaloids, harming bacterial membranes and interfering with their vital processes, could contribute to the antibacterial activity.

Conclusion

The current study shows that *Withania somnifera* root extracts have strong antibacterial and antioxidant properties, with the methanolic extract being more effective than the ethanollic and aqueous extracts. Correlation research shows that the methanolic extract's increased biological activity is closely linked to its higher phenolic and flavonoid component concentration.

The findings of the study confirm the medicinal value of *W. somnifera* as a natural source of antioxidants and antibacterial compounds and again emphasize the need for the polarity of the solvent in the extraction of potent bioactive phytochemicals. The findings of the study provide scientific evidence of the medicinal use of *W. somnifera* and suggest its possible use in the development of functional products and herbal medicine.

Further, as a prelude to the clinical relevance, more researches regarding the isolating the active ingredient, the mechanism, as well as the *in vivo* studies, are suggested.

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