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Phytochemical Profile and Antioxidant Activity of Root and Leaf Extracts of Ashwagandha (*Withania somnifera*)

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Abstract

The present study investigated the phytochemical composition and antioxidant potential of *Withania somnifera* (Ashwagandha) root and leaf extracts prepared using different solvent systems. A 2 × 4 factorial experiment was conducted at VISTAS, Chennai, during 2024-2025, with plant part (roots and leaves) and extraction solvent (aqueous, 70% ethanol, 70% methanol, and acetone:water 70:30 v/v) as factors. Extract yields, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities were evaluated. Among the extraction methods, 70% methanol and 70% ethanol yielded significantly higher extract recovery (13.00 ± 0.40% and 12.40 ± 0.39%, respectively) than aqueous and acetone: water extractions. The highest TPC (72.64 ± 2.18 mg GAE/g) and TFC (34.11 ± 1.26 mg QE/g) were recorded in leaf extracts obtained with 70% methanol, followed by 70% ethanol. Antioxidant assays demonstrated that methanolic and ethanolic extracts exhibited the strongest radical scavenging and reducing power, as indicated by lower DPPH IC_{50} (92.68 ± 2.41 µg/mL and 98.47 ± 2.58 µg/mL), higher ABTS TEAC (224.65 ± 5.10 and 210.23 ± 4.78 µmol Trolox/g), and elevated FRAP values (302.14 ± 5.62 and 291.75 ± 5.24 µmol Fe^{2+}/g). The results indicate that 70% methanol is the most efficient extraction solvent for recovering bioactive constituents from *W. somnifera* leaves, suggesting its potential for developing natural antioxidant formulations and phytopharmaceutical applications.

***Key words*:** *Withania somnifera*, Ashwagandha, Phytochemicals, Antioxidant activity, Withanolides, Solvent extraction

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Indian ginseng, is a perennial shrub belonging to the family *Solanaceae*. It has been used for centuries in Ayurvedic and traditional Indian systems of medicine as a rasayana (rejuvenating) herb that enhances vitality and longevity [1]). The plant exhibits a broad spectrum of pharmacological properties, including adaptogenic, anti-stress, anti-inflammatory, immunomodulatory, neuroprotective, and antioxidant activities [2-3]. The therapeutic potential of *W. somnifera* is attributed to its rich composition of bioactive secondary metabolites, particularly withanolides, which are steroidal lactones structurally similar to ginsenosides. In addition to withanolides, the plant contains alkaloids, flavonoids, saponins, phenolic acids, and tannins [4-5]. These compounds play critical roles in mitigating oxidative stress and inflammation, thereby contributing to the plant's overall pharmacological efficacy [6]. Oxidative stress results from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, leading to cellular and molecular damage that underlies several chronic diseases such as diabetes, cancer, and neurodegeneration [7]. Natural antioxidants from medicinal plants have been widely recognized for their ability to neutralize ROS and enhance cellular antioxidant status [8]. Several studies have demonstrated that *W. somnifera* extracts can scavenge free radicals, reduce lipid peroxidation, and elevate antioxidant enzyme levels, confirming their potential in managing oxidative stress-related disorders [9-10].

However, the efficacy of plant extracts depends greatly on the solvent system and plant part used for extraction. Extraction solvent polarity significantly influences the recovery of phenolic and flavonoid compounds, while different plant organs (roots, leaves, stems) vary in their phytochemical profiles [11-12]. Recent reports indicate that hydroalcoholic solvents (e.g., 70% ethanol or methanol) are superior to pure solvents or water in extracting phenolic antioxidants [13]. Nevertheless, systematic comparisons of different extraction methods and plant parts of *W. somnifera* remain limited. Therefore, the present study was undertaken to evaluate the phytochemical profile and antioxidant activity of *W. somnifera* root and leaf extracts using different solvent extraction methods. The goal was to identify the most efficient solvent system and plant part combination for maximizing withanolide recovery and antioxidant potential, thereby contributing to the development of natural antioxidant formulations and phytopharmaceutical applications.

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MATERIALS AND METHODS

The experiment was conducted at the farm of Vels Institute of Science, Technology and Advanced Studies, during the 2024 - 2025 growing season. Fresh *Withania somnifera* (Ashwagandha) plants were collected from the experimental field. Fully matured and healthy plants were selected, washed with distilled water, shade-dried to constant weight, and powdered (60–80 mesh). The samples were stored in airtight amber containers at 4 °C for further analysis. Two plant parts, roots (P_1) and leaves (P_2), were selected as Factor A, and four extraction solvents aqueous (E_1), 70% ethanol (E_2), 70% methanol (E_3), and acetone : water (70:30 v/v) (E_4) as Factor B. The experiment followed a 2 × 4 factorial completely randomized design (CRD) with three biological replicates, resulting in 24 samples. Analytical-grade reagents were used throughout [12], [14]. Each 5 g of powdered sample was extracted with 50 mL solvent (1:10 w/v) by maceration on an orbital shaker (150 rpm, 24 h, room temperature). Filtrates were combined and concentrated: aqueous extracts were lyophilized, while ethanol, methanol, and acetone–water extracts were concentrated under reduced pressure (≤40 °C) and lyophilized [11]. Dried extracts were weighed to determine yield (%) and stored at −20 °C.

Phytochemical analysis: Total phenolic content (TPC) was estimated by the Folin–Ciocalteu method using gallic acid as standard [15], expressed as mg GAE/g extract. Total flavonoid content (TFC) was determined by the aluminum chloride method using quercetin as standard [16], expressed as mg QE/g extract. Antioxidant activity was evaluated using DPPH [17], ABTS [18], and FRAP [19] assays. Results were expressed as IC_{50}, Trolox equivalent antioxidant capacity (TEAC), and µmol Fe^{2+} eq/g extract, respectively. All experiments were performed in triplicate, and data were expressed as mean ± SD. Two-way ANOVA was used to assess effects of plant part, solvent, and interaction, followed by Tukey's HSD test at $p < 0.05$. Correlation and principal component analyses (PCA) were performed using SPSS v27 or R software [20]. All procedures followed standard laboratory safety and biosafety guidelines.

RESULTS AND DISCUSSION

Extraction yield

The extraction yield of *Withania somnifera* roots and leaves varied significantly ($p < 0.01$) among the different solvent systems used (Table 1). Although plant part had no significant effect, solvent type strongly influenced extract recovery. The highest yields were obtained with 70% methanol (13.00 ± 0.40%) and 70% ethanol (12.40 ± 0.39%), followed by acetone : water (70:30) (10.02 ± 0.44%), while the lowest yield was observed in aqueous extracts (9.09 ± 0.32%). Leaf extracts consistently yielded more than root extracts under comparable solvent conditions. This can be attributed to the higher presence of polar compounds such as flavonoids and phenolics in the leaf tissue, which are more soluble in hydroalcoholic solvents [21-22]. Similar solvent-dependent extraction patterns were previously reported in *W. somnifera* and other medicinal species [11], [23]. The superior performance of methanol and ethanol mixtures is likely due to their intermediate polarity, facilitating efficient extraction of both hydrophilic and moderately lipophilic bioactive molecules [12].

Table 1 Extraction yield (%) of root and leaf extracts of *Withania somnifera* under different extraction methods

Plant part	Aqueous (E_1)	70% Ethanol (E_2)	70% Methanol (E_3)	Acetone:Water (70:30) (E_4)
Root (P_1)	8.42 ± 0.25a	11.56 ± 0.33b	12.12 ± 0.28b	9.84 ± 0.41a
Leaf (P_2)	9.76 ± 0.31a	13.25 ± 0.44b	13.88 ± 0.52b	10.21 ± 0.47a
Mean ± SD	9.09 ± 0.32	12.40 ± 0.39	13.00 ± 0.40	10.02 ± 0.44
F-test	Plant part (ns)	Extraction method ($p < 0.01$)	Interaction (ns)	

Values are mean ± SD (n = 3)
Means with different letters in the same row differ significantly at $p < 0.05$ (Tukey's HSD). ns = non-significant

Table 2 Total phenolic and flavonoid contents of root and leaf extracts of *Withania somnifera*

Plant part	Extraction method	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Root (P_1)	E_1 – Aqueous	36.24 ± 1.21a	18.45 ± 0.63a
	E_2 – 70% Ethanol	58.62 ± 1.87b	28.35 ± 0.92b
	E_3 – 70% Methanol	61.45 ± 2.11b	30.12 ± 1.14b
	E_4 – Acetone : Water (70:30)	49.83 ± 1.56a	24.28 ± 0.77a
Leaf (P_2)	E_1 – Aqueous	45.12 ± 1.54a	21.33 ± 0.73a
	E_2 – 70% Ethanol	69.42 ± 2.04b	32.47 ± 1.12b
	E_3 – 70% Methanol	72.64 ± 2.18b	34.11 ± 1.26b
	E_4 – Acetone : Water (70:30)	59.28 ± 1.84a	27.94 ± 0.91a
	F-test	Plant part ($p < 0.05$)	Extraction method ($p < 0.01$)

GAE = Gallic acid equivalent; QE = Quercetin equivalent

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) varied significantly with both plant part and solvent type ($p < 0.05$ and $p < 0.01$, respectively) (Table 2). Leaf extracts contained markedly higher phenolic and flavonoid levels than root extracts across all solvents, indicating that the leaves are richer sources of antioxidant polyphenols. Among extraction methods, 70% methanol yielded the highest TPC (72.64 ± 2.18 mg GAE/g) and TFC (34.11 ± 1.26 mg QE/g), followed by 70% ethanol (69.42 ± 2.04 mg GAE/g; 32.47 ± 1.12 mg QE/g). The enhanced recovery of phenolics and flavonoids with hydroalcoholic solvents aligns with earlier findings that moderate polarity enhances the solubility of polyphenolic compounds [16], [24]. Aqueous and acetone–water extractions produced lower yields, possibly due to limited solubility or degradation of phenolic compounds during extraction. These results corroborate previous studies that identified methanol as the optimal solvent for recovering antioxidant phenolics from *Withania* leaves [22], [25].

Antioxidant activity

Antioxidant assays revealed marked differences in activity among the extracts (Table 3). Lower IC_{50} values in the DPPH assay and higher Trolox equivalent antioxidant capacity (TEAC) and FRAP values indicate stronger antioxidant potential. Consistent with TPC and TFC results, methanolic and ethanolic leaf extracts exhibited the highest antioxidant activity, with DPPH IC_{50} values of 92.68 ± 2.41 µg/mL and 98.47 ± 2.58 µg/mL, respectively. Corresponding ABTS values were 224.65 ± 5.10 µmol Trolox/g and 210.23 ± 4.78 µmol Trolox/g, and FRAP values were 302.14 ± 5.62 µmol Fe^{2+}/g and 291.75 ± 5.24 µmol Fe^{2+}/g.

The strong antioxidant capacity of methanolic extracts is attributed to their higher polyphenol and flavonoid concentrations [26], [10]. Polyphenolic compounds act as hydrogen donors, singlet oxygen quenchers, and metal chelators, thereby neutralizing reactive oxygen species (ROS) [25]. Previous comparative studies on *Withania somnifera* have similarly demonstrated a positive relationship between total phenolic content and antioxidant activity [22], [27-28].

Table 3 Antioxidant activity of root and leaf extracts of *Withania somnifera*

Plant part	Extraction method	DPPH IC_{50} (µg/mL) ↓	ABTS TEAC (µmol Trolox/g) ↑	FRAP (µmol Fe^{2+}/g) ↑
Root (P_1)	Aqueous	198.45 ± 4.63a	105.26 ± 3.82a	185.64 ± 4.22a
	70% Ethanol	121.42 ± 3.15b	182.47 ± 4.51b	264.37 ± 5.10b
	70% Methanol	114.58 ± 3.02b	195.18 ± 4.83b	278.51 ± 4.98b
	Acetone:Water (70:30)	137.28 ± 3.87a	162.84 ± 4.25a	232.69 ± 4.33a
Leaf (P_2)	Aqueous	174.56 ± 3.96a	121.36 ± 3.74a	194.28 ± 4.61a
	70% Ethanol	98.47 ± 2.58b	210.23 ± 4.78b	291.75 ± 5.24b
	70% Methanol	92.68 ± 2.41b	224.65 ± 5.10b	302.14 ± 5.62b
	Acetone:Water (70:30)	120.45 ± 3.12a	176.54 ± 4.42a	251.36 ± 4.89a

↓ Lower IC_{50} and ↑ higher TEAC/FRAP values indicate stronger antioxidant activity

CONCLUSION

The study demonstrated that the extraction efficiency, phenolic and flavonoid content, and antioxidant activity of *Withania somnifera* are significantly influenced by the choice of solvent and plant part. Hydroalcoholic solvents, particularly 70% methanol and 70% ethanol, yielded the highest extract recovery along with superior total phenolic and flavonoid concentrations, resulting in strong antioxidant activities across all assays. Leaf extracts consistently outperformed root extracts, reflecting their richer content of polar bioactive compounds. The positive correlation between polyphenol levels and antioxidant potential confirms that methanolic leaf extract is the most effective system for recovering antioxidant constituents from *W. somnifera*, supporting its suitability for natural antioxidant and nutraceutical applications.

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