SHORT COMMUNICATION

Phytochemical Characterization and Antioxidant Evaluation of *Withania somnifera*: Implications for Traditional Therapeutics

Preetam, Vijeyta Tiwari*

Abstract

The present study was conducted to evaluate some *in-vitro* phytochemical and antioxidative properties of *Withania somnifera* root extract for further accessing its potentiality as a hepatoprotective agent in *in-vivo* model. Qualitative tests for presence of various active principles like alkaloid, phenol, glycoside, saponin, tannin and resins were performed in an aqua methanolic extract of *Withania somnifera* root powder. Further, *Withania somnifera* root extract was also evaluated for its *in-vitro* antioxidant properties by DPPH and ABTS level measurements by standard methods. The results showed the presence of alkaloids, glycosides, phenols, resins, saponins, proteins and flavonoids in the extract sample. In the case of antioxidant markers, *Withania somnifera* root extract exhibited potent radicals scavenging property in a concentration dependent manner. From these results, it is concluded that Withania *somnifera* root extract possess good antioxidant potential. Thus *Withania spmnifera* could be a good choice of agent to evaluate its hepatoprotective potential in animals.

Key words: Hepatoprotective agent, Oxidative stress, *Withania somnifera*.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.4.29

Introduction

In recent years, oxidative stress and its implications in diverse toxic mechanisms have attracted considerable attention. Oxidative stress is implicated as a common pathologic mechanism contributing to the initiation and progression of hepatic damage in a variety of liver disorders. Oxidative stress is defined as a state in which the pro-oxidative processes overwhelm cellular antioxidant defense due to the disruption of redox signaling and adaptation (Ji and Yeo, 2021).

Medicinal plants are rich sources of natural antioxidants that can protect from oxidative stress, thus playing an important role in chemoprevention of diseases. *Withania somnifera* (L.) Dunal, also known as Ashwagandha is a plant in the *Solanaceae* or nightshade family (Mirjalili *et al*., 2009). The root of the plant possesses several biological activities like antibacterial, antiviral, anti-inflammatory, immunomodulatory, antihypertensive and hepatoprotective (Davis and Kuttan, 2002; Arora *et al*., 2004; Owais *et al*., 2005; Rasool and Varalakshmi, 2008). However, the various pharmacological activities of *Withania somnifera* vary in different parts of India according to the geographical factors like climate, soil, elevation for herbs and species of animal to be treated (Mishra *et al.,* 2000; Mahanta and Challa, 2022). Since oxidative stress plays a pivotal role in the pathogenesis of liver injury, preliminary evaluation of *in-vitro* antioxidant activity of *Withania somnifera* is essential before assessing hepatoprotective potential. Antioxidant screening will provide mechanistic insight, support traditional claims and will ensure regional sample standardization. Therefore, before screening the *in-vivo* hepatoprotective potency, firstly, we planned our study with some preliminary *in-vitro* studies to evaluate the suitability of *Withania somnifera* in animal models by evaluating its phytochemical characters and antioxidant properties in a sub-tropical area of Haryana region of India.

Department of Veterinary Pharmacology & Toxicology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, Haryana, India

Corresponding Author: Dr. Vijeyta Tiwari, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences, LUVAS, Hisar-125004, Haryana, India. e-mail: dr.vijeytatiwari@luvas.edu.in

How to cite this article: Preetam, & Tiwari, V. (2026). Phytochemical Characterization and Antioxidant Evaluation of *Withania somnifera*: Implications for Traditional Therapeutics. Ind J Vet Sci and Biotech, 22(4), 149-152.

Source of support: Nil

Conflict of interest: None

Submitted 20/03/2026 **Accepted** 29/04/2026 **Published** 10/07/2026

Materials and Methods

The present *in-vitro* study was carried out in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences, LUVAS, Hisar (India). The study being *in-vitro*, no ethical approval was sought.

Procurement and Preparation of Aqua-Methanolic Extracts of *W. somnifera* Roots

The roots of *Withania somnifera* were collected from Aromatic and Under-Utilized Plant Section, Department of Plant Breeding, CCSHAU, Hisar (Fig. 1, 2). Aqua-methanolic extract

of *Withania somnifera* roots (Fig.3) was prepared as per the method of Rosenthaler (1930). The percent extractability of aqua-methanolic extracts was calculated by the standard formula.

Phytochemical Study of *W. somnifera* Extract

The phytochemical study of aqua-methanolic extract of *W. somnifera* was done as per the methods described by Kokate (2005). The presence of alkaloids was tested by using Wagner's reagent, while presence of phenols and tannin was tested by Ferric chloride test and Glycosides by Fehling's test. The presence of resin was checked by turbidity test. Similarly, the presence of proteins, saponins and flavonoids was tested by using Xanthoprotein test, Foam test and NaOH test, respectively.

In-vitro Antioxidant Assay of *W. somnifera* Extract

The *Withania somnifera* root extract was screened *in-vitro* for its antioxidant activity by following assays.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging: The 96-well plate method, established by Kumar *et al*. (2019) was used to evaluate the antioxidant activity of the *Withania somnifera* root extract using ascorbic acid as the standard. After incubation, a decrease in DPPH radical was analyzed by measuring absorption at λ_{max} 517 nm.

2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS +) radical scavenging: The total antioxidant activity of *Withania somnifera* root extract sample was determined according to the method of Lee *et al*. (2015) based on ABTS radical cations ($ABTS^{\bullet+}$) scavenging assay. After incubation, the absorbance was measured at wave length 734 nm. The IC_{50} ABTS values (the concentration of sample required for inhibition of 50% of ABTS radicals) were obtained. The antioxidant activity was evaluated based on this IC_{50} value.

Results and Discussion

This study aimed to evaluate the phytochemical properties and antioxidant potential of *Withania somnifera* root extract, to assess its suitability as natural hepatoprotective agent. The prepared 50% aqueous-methanolic root extract of the *W. somnifera* was evaluated for extractability percentage, colour, and consistency. The percentage extractability recorded was 15.4 ± 0.34%, with the extract exhibiting a brownish colour and semi-solid consistency after evaporation of the solvent. The qualitative phytochemical screening of the aqua-methanolic extract of *W. somnifera* roots revealed the presence of several bioactive compounds. The results are summarized in Table 1.

The current 15.4±0.34 % extractability of aqua-methanolic extract of *W. somnifera* roots indicated good solvent

Fig. 1: *Withania somnifera* green plant

Fig. 2: Fresh roots of *Withania somnifera* plant

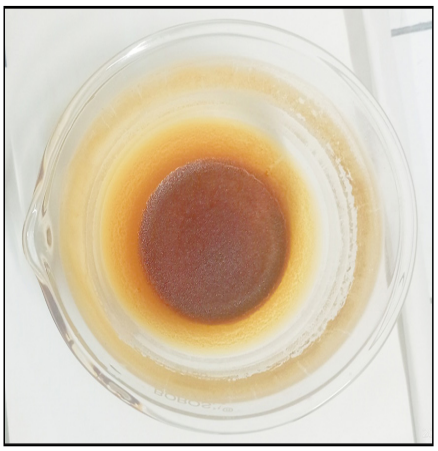

Fig. 3: Aqua-methanolic extract of *Withania somnifera*

Table 1: Phytochemical constituents of aqua-methanolic root extract of *Withania somnifera*

Active principle	Test used	Observation	Result
Alkaloids	Wagner's reagent	Formation of reddish precipitate	Present (+)
Phenols	Ferric chloride test	Development of blue colour	Present (+)
Glycosides	Fehling's reagent	Formation of brick-red colour	Present (+)
Tannins	Ferric chloride test	No colour change	Absent (–)
Resins	Alcohol test	Appearance of turbidity	Present (+)
Proteins	Xanthoprotein test	Yellowish precipitate formed	Present (+)
Saponins	Foam test	Formation of froth	Present (+)
Flavonoids	Sodium hydroxide test	Appearance of yellow-orange colour	Present (+)

efficiency for secondary metabolite recovery. It contained diverse bioactive phytoconstituents like alkaloids, phenolic compounds and flavonoids suggesting that the extract possesses significant established pharmacological potential (Letchuman *et al*., 2025).

Qualitative screening results of extract are in line with those reported by Mishra *et al*. (2000) and Mirjalili *et al*. (2009), who also identified similar phytoconstituents in *W. somnifera* roots. The presence of alkaloids such as withanine and somniferine, and steroidal lactones (withanolides), has been associated with the plant's adaptogenic and hepatoprotective effects. Researchers have also discovered that some of the chemicals found in *Withania somnifera* are powerful antioxidants. In one of the early studies, Attanayake *et al*. (2016) also claimed strong antioxidant activity of *Withania somnifera* extracts with relatively high content of flavonoids.

The antioxidant potential of the aqua-methanolic extract of *W. somnifera* roots was assessed using DPPH and ABTS radical scavenging assays and the results are presented in Table 2.

Table 2: IC_{50} values of different concentrations of aqua-methanolic root extracts of *Withania somnifera* and some standards (Trolox and ascorbic acid) for ABTS and DPPH radical scavenging activity (Mean ± SE)

Concentration of extract or standard (µg/ml)	Total antioxidant activity (ABTS) (µg/ml)	Free radical scavenging activity (DPPH) (µg/ml)
10	97.50 ± 2.04	457.70 ± 13.48
25	72.64 ± 1.59	424.46 ± 11.48
50	55.38 ± 3.52	387.60± 9.54
100	**23.30 ± 3.08**	**267.50 ± 8.78**
150	45.23 ± 2.79	302.60 ± 6.44
200	37.65 ± 1.39	349.25 ± 22.42
250	48.60 ± 1.50	487.25 ± 22.42
Trolox	11.17 ± 0.67	-
Ascorbic acid	-	7.95 ± 0.38

A lower value of IC_{50} indicates that a lower concentration of the sample is needed to reduce 50% of free radicals, meaning a higher antioxidant efficiency. In the present study, the plant extract exhibited variable IC_{50} values at different concentrations. The extract exhibited a concentration-dependent antioxidant response up to 100 µg/mL, with maximum activity observed with 100 µg/mL concentration with a minimum IC_{50} values of 23.30 ± 3.08 and 267.50 ± 8.78 µg/mL, respectively, in ABTS and DPPH assays. Beyond 100 µg/mL, a decline in antioxidant efficiency was noted as evidenced by increase in IC_{50} values. In ABTS assay, at a concentration of 150 µg/mL, the IC_{50} value was 45.23 ± 2.79 µg/mL. When the concentration was increased to 200 µg/mL, the IC_{50} observed was 37.65 ± 1.39, indicating enhanced activity at this level. However, at a higher concentration of 250 µg/mL, the IC_{50} value increased again to 48.60±1.50 µg/mL, suggesting a reduction in effectiveness at this concentration. Similar pattern of increasing in values of IC_{50} (ranging from 302.60 ± 6.44 to 487.25 ± 22.42 µg/mL) with higher concentrations of plant extract (150 to 250 µg/mL) were also noted in DPPH assay (Table 2). This decline in antioxidant efficiency with increasing the concentration of plant extracts is possibly due to pro-oxidant effects or saturation of reactive sites.

However, the antioxidant activity of the extract was lower compared to standard antioxidants trolox and ascorbic acid *i.e.* 11.17 ± 0.67 and 7.95 ± 0.38 µg/mL, respectively, in both the assays. The observed concentration-dependent increase in antioxidant activity up to 100 µg/mL suggests that the extract have potent free radical scavenging capacity, likely attributable to the presence of bioactive principles like phenols and flavonoids.

Conclusions

The present study demonstrates that *W. somnifera* root extract showed a moderate yield (15.4%) and contained multiple bioactive phytochemicals, notably alkaloids, phenols, glycosides, and flavonoids, which are associated with potent antioxidant and hepatoprotective properties. The *in-vitro* antioxidant assays (DPPH and ABTS) confirmed the free radical scavenging efficacy of the *W. somnifera* root extract, suggesting its potential role in attenuating oxidative stress and supporting its use as a natural hepatoprotective agent. Collectively, these findings provide a scientific rationale for the potential use of *W. somnifera* in developing herbal hepatoprotective formulations aimed at combating oxidative liver damage.

Acknowledgements

The authors gratefully acknowledge the institute Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar for providing all necessary facilities.

References

Arora, S., Dhillon, S., Rani, G., & Nagpal, A. (2004). The *in vitro* antibacterial synergistic activities of *Withania somnifera* extracts. *Fitoterapia*, *75*(3-4), 385-388.

Attanayake, A.P., Jayatilaka, K.A.P.W., & Malkanthi, B.M.A.S. (2016). Total flavonoid content, total antioxidant activities and phytochemical constituents of selected medicinal plant extracts used for oxidative stress related chronic diseases in Sri Lanka. *Journal of Medicinal Plants Studies*, *4*(6), 26-29.

Davis, L., & Kuttan, G. (2002). Effect of *Withania somnifera* on CTL activity. *Journal of Experimental and Clinical Cancer Research, 21*(2), 193-200.

Ji, L.L., & Yeo, D. (2021). Oxidative stress: an evolving definition. *Faculty Reviews*, *9(10)*, 13. doi: 10.12703/r/10-13.

Kokate, C.K. (2005). *A Textbook of Practical Pharmacognosy*. 5th edn., Vallabh Prakashan, Delhi, India, pp: 105-111.

Kumar, S., Lakshmi, P.K., Sahib, C., & Pawara, R.S. (2019). *Sida cordifolia* accelerates wound healing process delayed by dexamethasone in rats: Effect on ROS and probable mechanism of action. *Journal of Ethnopharmacology, 235,* 279-292.

Lee, K.J., Oh, Y.C., Cho, W.K., & Ma, J.Y. (2015). Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay. *Evidence Based Complement Alternative Medicine,* Article ID 165457, 13 pages.

Letchuman, S., Madhuranga, Madhurangi, H.D.T., Madhurangi, B.L.N.K., Premarathna, A.D., & Saravanan, M. (2025). Alkaloids unveiled: A comprehensive analysis of novel therapeutic properties, mechanisms, and plant-based innovations. *Intelligent Pharmacy, 3(4),* 268-276.

Mahanta, S.K., & Challa, S.R. (2022). Phytochemicals as pro-oxidants in cancer. In: S. Chakraborti, (Ed.) *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*. Springer, Singapore. pp 611-619.

Mirjalili, M.H., Moyano, E., Bonfill, M., Cusido, R.M., & Palazon, J. (2009). Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules, 14,* 2373-2393.

Mishra, L.C., Singh, B.B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. *Alternative Medicine Review, 5*(4), 334-346.

Owais, M., Akhtar, A., & Ali, M. (2005). Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicines, 2*(3), 229-235.

Rasool, M., & Varalakshmi, P. (2008). Antioxidant effect of *Withania somnifera* root powder on liver lipid peroxidation and antioxidant status of adjuvant induced arthritis in rats. *Research Journal of Medicinal Plants, 2*(1), 28-33.

Rosenthaler, L. (1930). *The Investigation of Plant.* 1st edn., Bell and Sons, London, p 36.