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PHYTOCONSTITUENT SCREENING AND *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF *WITHANIA SOMNIFERA*

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ABSTRACT
Ashwagandha (*Withania somnifera*, family Solanaceae) commonly known as "Indian winter cherry" or "Indian Ginseng" enhances brain function, improves memory and function of the reproductive system. The presence of numerous secondary metabolites in the roots and leaves of *W. somnifera* is related to its biological activity. These include alkaloids, flavanol glycosides, steroidal lactones (withanolides), sterols, and phenolics. It was found from this study that ashwagandha has good antioxidant and antimicrobial potential. Ashwagandha root powder contains many active phytoconstituents including alkaloids, flavonoids, steroids (terpenoids), saponins and glycosides in the aqueous, chloroform, hexane and DMSO extract. 1,1-diphenyl-1-picryl-hydrazyl radical (DPPH) assay was performed to determine the antioxidant activity of different solvent extracts of ashwagandha root. DMSO and chloroform extracts showed strong antioxidant potential compared with standard ascorbic acid. The extracts showed antimicrobial activity against *Bacillus cereus, Shigella flexneri* and *Staphylococcus aureus* was also observed.

KEYWORDS: Ashwagandha, phytochemical analysis, antimicrobial, *Withania somnifera*, antioxidant.

1. INTRODUCTION

Medicinal plants and their extracts have been used as a rich source of bioactive agents of therapeutic importance due to the presence of secondary metabolic compounds.[1] Traditional medicinal systems like Ayurveda, Unani-Tibb system and Chinese traditional medicine utilise phyto-based medicines which can be attributed to their economic feasibility and fewer side-effects.[2,3] As a Rasayana (tonic), *Withania somnifera* (Ashawagandha) is a highly valued plant in the Indian Ayurvedic school of medicine.[2] It is used to treat a variety of ailments, but is best known as a nervine tonic. Ashwagandha enhances the body's immune system by boosting cell-mediated immunity. It also has strong antioxidant effects that aid in the prevention of cellular damage caused by free radicals.[4] Roots of ashwagandha are consumed as a powder (churna) or any other herbal formulation (Ashwagandharishta). It acts as an aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic, and stimulant, apart from treating old age debility, rheumatism, disturbed vata conditions, leucoderma, constipation, giotre, insomnia and neurological disorders. Biological activity of *W. somnifera* is related to the presence of a variety of secondary metabolites in its roots and leaves, including alkaloids, flavanol glycosides, steroidal lactones (withanolides), sterols, and phenolics. The two secondary metabolites responsible for bioactivity of *W. somnifera* are phytoconstituents Withanolide A and Withaferin A. *W. somnifera* is one of the most prominent medicinal plants cultivated in India to address the huge demand for pharmaceutical purposes.[5,6] Considering the importance of ashwagandha, there is a need to look into its phytoconstituent composition, its antimicrobial and antioxidant potential.[7] The present study could be used in future for the economical formulation of the active chemical ingredients in natural drugs against a variety of neurological and inflammatory diseases. Fig.1 depicts the various therapeutic effects of the phytochemicals present in *Withania somnifera*, including the antioxidant and antimicrobial effects of the same.

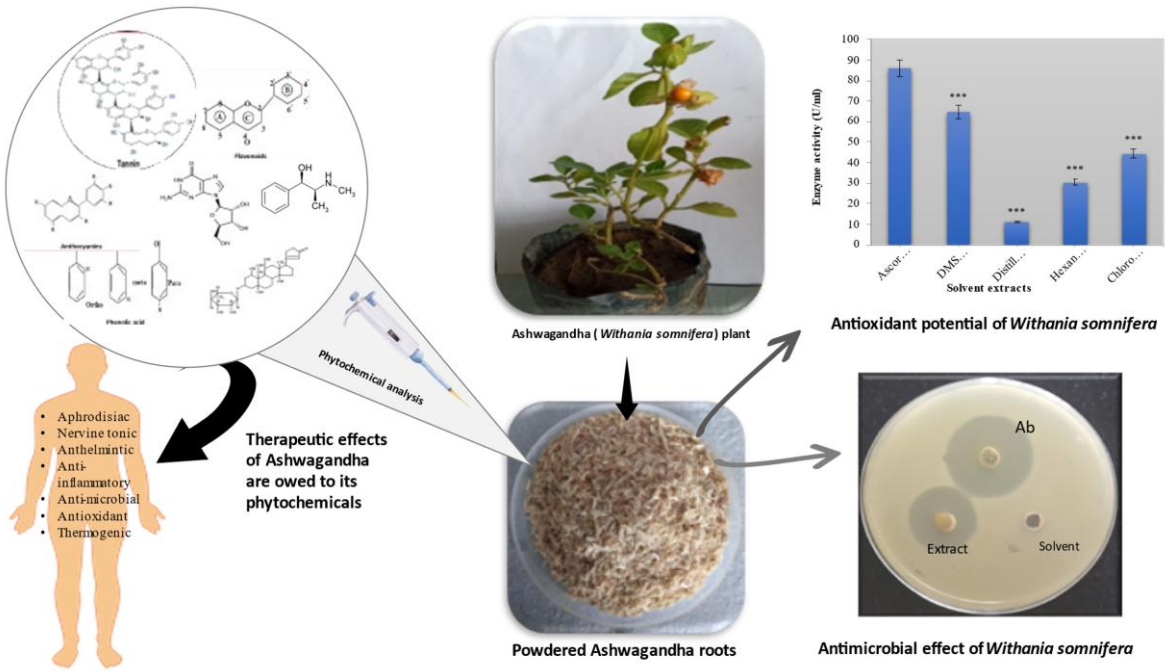


Fig. 1: Therapeutic effects of phytochemicals present in *Withania somnifera* and their antioxidant and antimicrobial effects.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The reagents and chemicals used in present study were of high purity and of analytical grade (AR/GR). NaCl, NaOH, DMSO, Acetone, Methanol, DPPH and others were purchased from Hi-Media and Merck, Mumbai, India; Sigma-Aldrich, Steinheim, Germany and Sigma chemicals Co., U.S.A.

2.2 Sample collection

The plant material was collected from Hamirpur District of Himachal Pradesh. Extracts of plant part were prepared in different solvents (distilled water, DMSO, chloroform, hexane).

2.3 Clinical isolates

The clinical isolates (*Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Shigella flexneri*) were procured from the Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla.

2.4 Plant material and preparation of extract

The roots were separated from the plant and dried in a hot air oven for 4 days at 37±2°C to remove excess moisture. The roots were ground to a fine powder, as shown in Fig. 2. Then, 5g of root powder was soaked in 50 ml of respective solvents and kept in capped reagent bottles for 36 hrs at 37°C and 140 rpm for maceration. As a result, phytochemicals were extracted into the solvent. The extract was filtered through Whatman filter paper No. 1. The filtrate was then collected and stored at 4°C until further use.

Fig. 2: Ashwagandha root powder.

2.5 Phytochemical analysis

2.5.1 Test for alkaloids

1 ml extract was mixed with equal volume of 1% HCl and a few drops of Mayor's reagent and Wagner's reagent were added. A creamish or milky precipitate indicated the presence of alkaloids.

2.5.2 Test for flavonoids

Heated equal volumes of extract and concentrated H_2SO_4 in a test tube. Formation of orange colour in the solution indicated presence of flavonoids.

2.5.3 Test for phenolics

1ml extract was taken and mixed with 1 ml of 1% ferric chloride. Formation of bluish-green or blackish colour confirmed the presence of phenolic compounds in the solution.

2.5.4 Test for tannins
To 1 ml of extract, few drops of 1% lead acetate solution were added. Yellow colour of the solution indicated presence of tannins.

2.5.5 Test for steroids (terpenoids)
2 ml extract and 2 ml of chloroform were taken in a test tube and mixed well. Conc. H_2SO_4 was carefully added to form a layer. Formation of reddish-brown colouration at the interface indicated the presence of terpenoids.

2.5.6 Test for amino acids
2 ml extract was mixed with 2 ml of 10% NaOH. 0.1% $CuSO_4$ was added dropwise to the mixture. Appearance of pink or purple colour showed the presence of amino acids.

2.5.7 Test for saponins
1 ml extract was mixed with 10 ml sterile distilled water. The mixture was shaken and allowed to stand for a while. Boiled for 5 minutes. Honeycomb froth indicated the presence of saponins.

2.5.8 Test for cardiac glycosides (Keller-Killani test)
2 ml extract was treated with 1 ml glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml conc. H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycosides.

2.5.9 Test for anthraquinones (Modified Borntrager's test)
2 ml sample was mixed with 5 ml dilute H_2SO_4 and few drops of 5% $FeCl_3$. The mixture was boiled. Upon cooling the solution was filtered and the filtrate was then mixed with diethyl ether. The organic layer was separated and to it equal volume of ammonia was added. Rose pink colouration indicated the presence of anthraquinones.

2.5.10 Test for carbohydrates (Molisch test)
1 ml extract was mixed well with 2 ml Molisch reagent. Conc. H_2SO_4 was poured along the sides of the test tube to form a layer. A violet ring at the interface indicated the presence of carbohydrates.

2.6 Anti-oxidant activity
Radical scavenging activity of each concentrate against the 1,1-diphenyl-1- picryl-hydrazyl radical (DPPH) was done by a slightly modified method.[8] Crude samples prepared in different solvents were used for the assay. Ascorbic acid (1 mg/ml) was used as standard. Absorbance was measured at 517 nm.

2.7 Antimicrobial activity
Antimicrobial activity of DMSO extract of ashwagandha root powder was tested against *E. coli, S. flexneri, B. cereus, P. aeruginosa, S. aureus* and *S. typhi* by agar well diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS).[13]

3.3 RESULTS
3.1 Phytochemical screening of different solvent extracts
Non-nutritive plant compounds with different degrees of disease-preventive effects are known as phytochemicals. They are significant raw material sources for both traditional and conventional medicine[9]. The results obtained from preliminary phytochemical examination of different extracts (chloroform, DMSO, hexane and distilled water) are summarized in Table 1.

Table 1: Phytochemical screening of different solvent extracts of ashwagandha root.

Phytochemical	Water extract	DMSO extract	Chloroform extract	Hexane extract
Alkaloids	++	++	+	+
Phenolic compounds	+	+	-	-
Tannins	+	+	-	-
Flavonoids	++	++	++	+
Cardiac glycosides	+	+	+	-
Steroids/terpenoids	++	++	+	+
Carbohydrates	+	+	+	-
Proteins	+	+	+	+
Saponins	-	+	+	-

'+' Present; **'-'** Absent

3.2 Antioxidant activity
The crude extracts of ashwagandha root powder in different solvents showed antioxidant activity, with varying percentage scavenging activities (Fig. 3). DMSO extract showed maximum scavenging activity of free radicals but less than that of ascorbic acid, while other extracts had even lower values. Phytochemical constituents like alkaloids, glycosides, tannins and flavonoids present in the extract were probably responsible for the antioxidant activity.

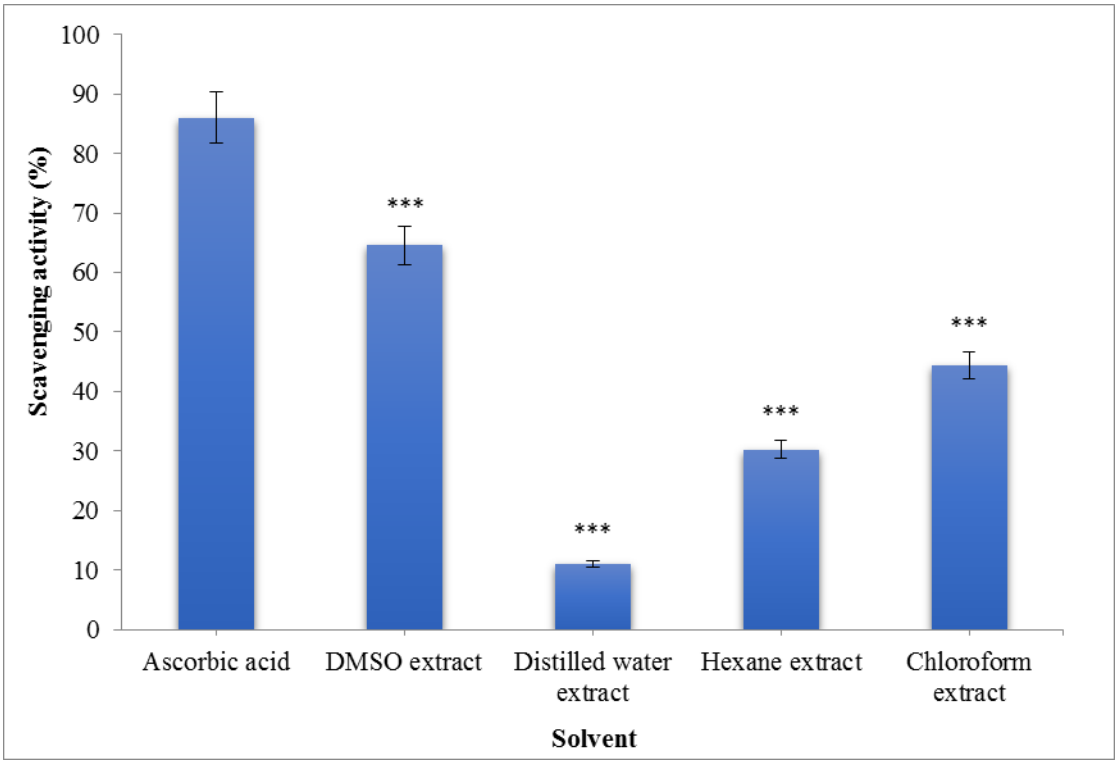
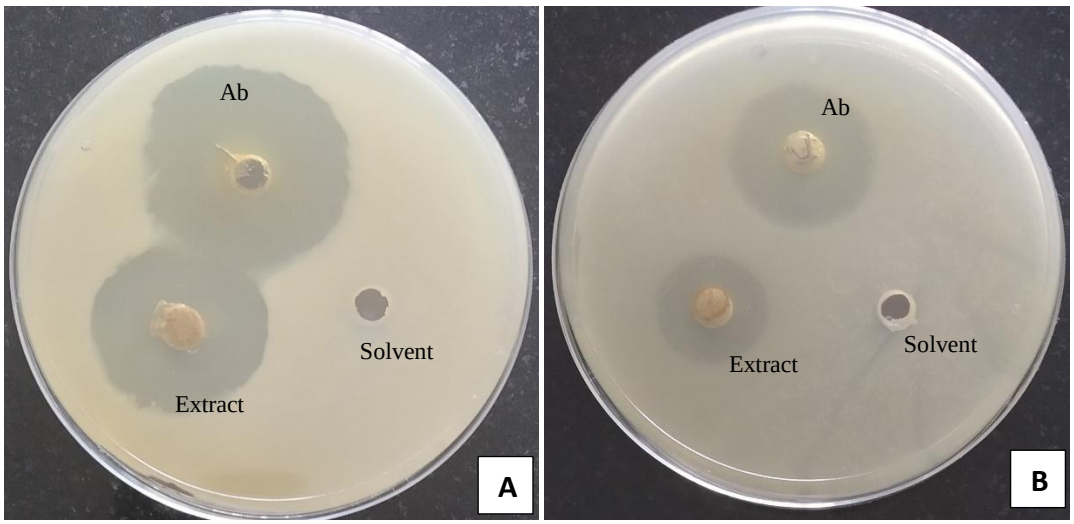


Fig. 3: Antioxidant potential of ashwagandha root extracts.

3.3 Antimicrobial activity

The antimicrobial activity of ashwagandha root in DMSO was studied against different bacteria (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Shigella flexneri*) and is shown in Fig. 4. The extract showed antimicrobial activity against *Bacillus cereus* followed by *Shigella flexneri* and *Staphylococcus aureus*. *Escherichia coli* was also inhibited by the extract. Least activity was shown against *Salmonella typhi* and *Pseudomonas aeruginosa*. This showed that the extracts inhibited growth of bacterial isolates, which gives an indication of the presence of substances that are active against bacterial species.



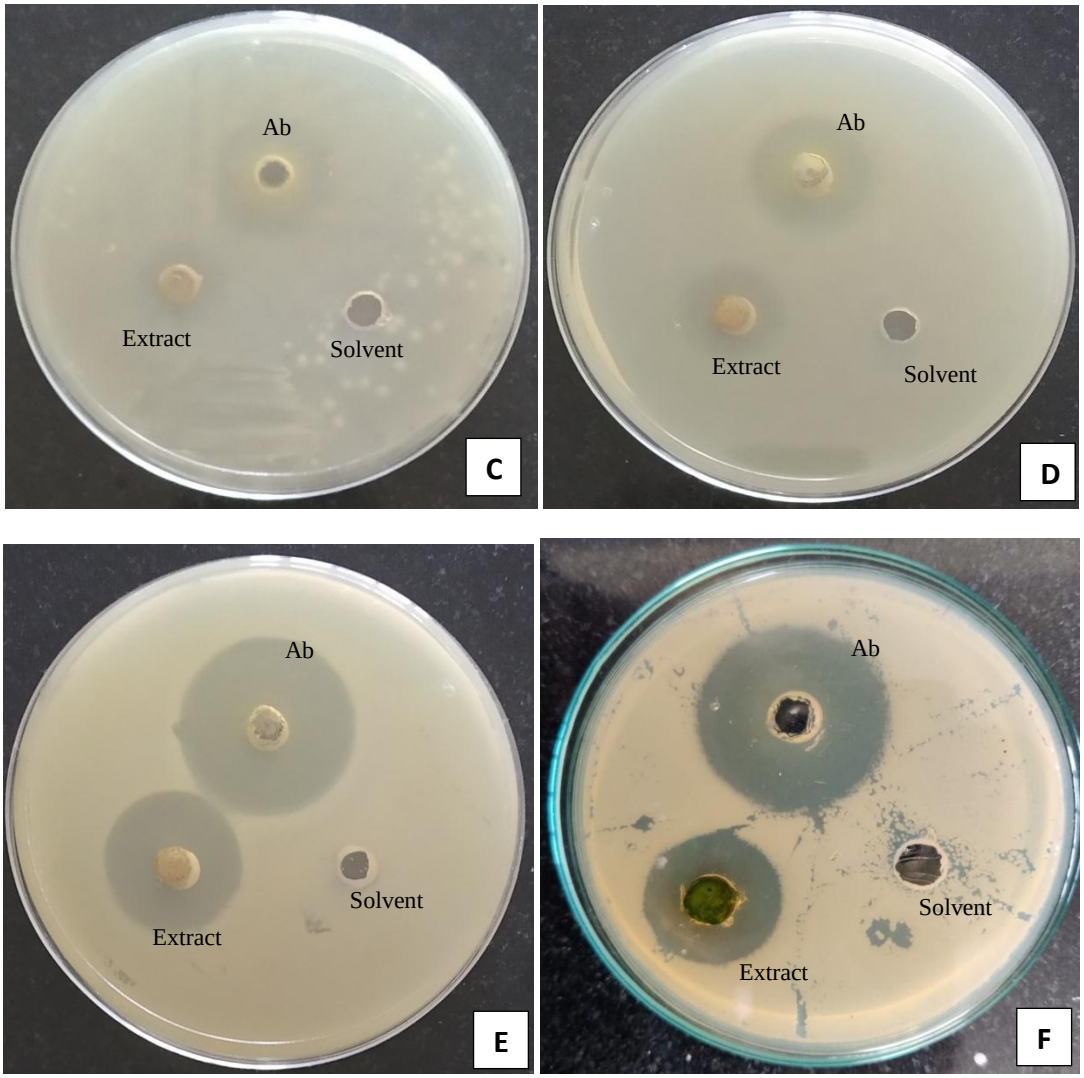


Fig. 4: Zone of inhibition shown by DMSO extract of ashwagandha root against pathogenic bacterial strains (A=*Bacillus cereus,* B=*Escherichia coli,* C=*Pseudomonas aeruginosa,* D=*Salmonella typhi,* E=*Staphylococcus aureus,* F=*Shigella flexneri*).

4. DISCUSSION

A similar phytochemical analysis of *Withania somnifera* has revealed the presence of active medicinal phytochemicals along with phlobatannins, reducing sugars, terpenoids, flavonoids and alkaloids.[10,11]

A previous study on the antioxidant activity of ashwagandha has shown significant scavenging effect on the DPPH free radical. The scavenging effect of sample was lower than that of ascorbic acid[12,13,14]

In a study performed to evaluate the antimicrobial activity of crude extract of aashwagandha, significant inhibitory effect on Gram positive bacteria like *S. aureus* and *B. subtilis* was observed. Among Gram negative bacteria ashwagandha root extract had antibacterial effect on *P. aeruginosa* and *E. coli*.[15]

5. CONCLUSION

The present study demonstrated that ashwagandha has good antioxidant and antimicrobial activity. Ashwagandha root powder contains many active phytoconstituents such as alkaloids, flavonoids, steroids (terpenoids), saponins and glycosides in the aqueous, chloroform and DMSO extract. DMSO and chloroform extracts showed strong antioxidant potential with DPPH assay. This can be owed to the presence of secondary metabolites such as flavonoids in these extracts. DMSO extract of ashwagandha root was tested for antimicrobial activity against different bacterial strains. Maximum antimicrobial activity was observed against *Bacillus cereus*, followed by *Shigella flexneri* and *Staphylococcus aureus.* Conclusions from this study could be employed in future for the inexpensive formulation of the active chemical constituents in natural medications against a variety of neurological and inflammatory illnesses.

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Conflict(s) of interest(s)

The authors declare that they have no conflict(s) of interest(s).

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