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Assessment of Phytochemical and Antimicrobial Activity of *Withania somnifera* (Ashwagandha)

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ABSTRACT

Withania somnifera L. (Dunal), a member of the Solanaceae family, has been used by Ayurvedic practitioners for thousands of years. The root of *Withania somnifera* contains flavonoids, alkaloids, steroids, and other useful functional components. It contains a high concentration of bioreactors, or secondary metabolites, such as steroidal lactones, alkaloids, and flavonoids, all of which have beneficial properties and are used in ninety Ayurvedic formulations. Ashwagandha has long been recognized as a potent rejuvenator, general health tonic, and treatment for a wide range of ailments. It is a sedative, diuretic, and anti-inflammatory herb that is well known for increasing energy and endurance while also acting as an adaptogen with high immune stimulatory and anti-stress properties. Ashwagandha is used to treat colds, cough, emaciation, diabetes, conjunctivitis, epilepsy, insomnia, senile dementia, leprosy, Parkinson's disease, mental disorders, rheumatic, arthritis, intestinal infections, bronchitis, asthma, impotence, and also used as HIV/AIDS suppressant. According to the Indian Herbal System, ashwagandha is one of the most important herbs and the best adaptogenic (Ayurveda). Withanolide is made up of steroidal molecules that are supposed to help with inflammation. Ashwagandha boosts the immune system, reduces inflammation, improves cognition, and promotes overall health and well-being. Ashwagandha is a shrub that promotes bone marrow and sperm production, as well as acting as an anti-aging. By using the agar well diffusion method, the antibacterial activity of different solvent extracts of *Withania somnifera* stem was assessed against the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. When tested against several test microorganisms, all of the extracts showed strong antibacterial activity. *E. coli* was the investigation's most vulnerable bacterium, and all of the plant extracts displayed zones of inhibition when used against it. The methanol extract of *W. somnifera* stem and leaf had the strongest antibacterial effect against *E.coli*, followed by *S. aureus* and *Pseudomonas*. All bacterial strains were least inhibited by DH_2O extract. The MIC of the extracts that were recorded ranged from 8 mm to 26 mm. In the current experiment, methanol extract had the lowest MIC value (8 mm) against *S. aureus* and *Pseudomonas*. Different phytochemicals were found in different extracts of *Withania* according to qualitative phytochemical analysis. Several studies have found that Ashwagandha has anti-tumor and anti-inflammatory properties. It has a significantly greater steroidal than hydrocortisone, which is a typical cancer treatment.

Key Words: *Withania somnifera*, Antibacterial activity, Antifungal activity, phytochemical analysis.

INTRODUCTION

Withania somnifera is a shrub belonging to the Solanaceae family and is one of the most commonly used herbal medicines. It is also known as "Ashwagandha" in Sanskrit means "the smell of horses". This name originated from the smell of its roots, which resembles a sweaty horse. The species name *somnifera* means sleeping pill indicating that it is assigned sedative properties but it has also been used for sexual activity (Panchal et al., 2015). It is an important perennial plant with many therapeutic applications in conventional and modern medicine (Datta et al., 2011). *W. somnifera* is gaining attention in various fields of research because it is best suited to current environmental conditions. The numerous antioxidant properties of *W. somnifera* and its capacity to improve memory are utilized (Gupta et al., 2007). Because of the medicinal properties of the roots, the species is also known as 'Indian Ginseng' (Gaurav et al., 2016). *Withania somnifera* L. (Dunal) is a member of the Solanaceae family and has been used for thousands of years by Ayurvedic practitioners. Flavonoids, alkaloids, steroids, and numerous active functional ingredients are found in *Withania somnifera* root (Gaurav et al., 2015). Anti-arthritic, anti-rheumatic, anti-inflammatory, abortifacient, adaptogenic, anti-stress, anti-tumor, immunomodulatory activity, anti-anxiety, anti-depression, and aphrodisiac properties are all present in the plant. Major biochemical elements such as alkaloids and steroids are responsible for Ashwagandha's therapeutic benefits. The plant's major tropane alkaloids withanolides have anti-tumor properties (Gaurav et al., 2016). Herbs have long been used as antimicrobials in almost every country, including Asia, Africa, Europe, and North America. Around 80% of prescriptions depend on traditional medication to treat their illnesses. Plant extracts, either crude or pure active components were used in almost all ancient medicines. The National Cancer Institute has recognized some plants as anticancer. These herbs belong to many families. There are 2300 species in the Solanaceae family, which is a huge family. To describe the diverse biological features of *Withania somnifera*, numerous pharmacological research has been conducted (Kumar et al., 2015). Active *W. somnifera* glycowithanolides (WSG) (10 and 20 mg/kg, i.p.) induced a dose-related increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activity on rat brain frontal cortex and striatum, which was statistically significant on days 14 and 21, except for the lower dose of WSG on GPX activity, which was evident only on the day. In terms of SOD, CAT, and GPX activities, the data were comparable to those induced by deprenyl (2 mg/kg/day, i.p.) by day 14. These findings are consistent with the Ayurvedic rasayana and medhya rasayana properties of *W. somnifera*. The antioxidant activity of *W. somnifera* active principles may explain, at least antistress, immunomodulatory, cognition-enhancing, anti-inflammatory, and anti-aging effects in experimental animals and clinical situations (Bhattacharya et al., 1997). The anticancer activities of *Withania* root extract have been reported (Rai et al., 2016). Burns, wounds, dermatological disorders, gastrointestinal disorders, respiratory system dysfunctions, asthma, bronchitis, cancer, and geriatric illnesses have all been reported to benefit from the plant (Singh et al., 2014; Winter. 2006). Humans have benefited from the medicinal compound known as secondary metabolites, which are found in the leaves, stems, roots, and sap of plants. Secondary metabolites from plants, such as saponins, alkaloids, flavonoids, glycosides, anthraquinone, steroids, and tannins, have also been used in modern medicine for their extensive therapeutic values (Panchal et al., 2015). Considering the good sized potentiality of plant life as resources with the aid of antibiotics are returning in new paperwork proof against antimicrobial tablets as regards to antibacterial sellers antibiotic healing procedures (Gaurav et al., 2016b; Gaurav et al., 2018a; Gaurav et al., 2018b).

MATERIAL AND METHODS

Collection of the plant materials

Fresh, disease-free leaves of the medicinal plant *Withania somnifera (L)* were obtained from the University garden. The plant's components (stems, leaves, and roots) were separated from one another and carefully cleansed with distilled water afterward. These were shade dried for a week at room temperature before being grind into a fine powder in a sterilized mixer grinder (Panchal et al., 2015). The powdered plant material was kept in air-tight glass vials out of direct sunlight until it was time to analyze it. All the glassware was completely cleaned and sterilized.

Preparation of plant extract

Extractions were carried out using solvents such as methanol and Distilled water. In a conical flask, leaves and stem powder were dissolved in solvents and maintained at room temperature in a rotating shaker for 48 hours after shaking put the filtrate into the water bath for evaporation with maintaining temperature 50^0C- 60^0C. After evaporation, the leftover crude extracts were weighed, scraped against the beaker's walls, and then dissolved in solvents (methanol and Distilled water). The extracts were then gathered in a beaker and chilled to 4°C. In 4 different conical flasks of 250 ml, grinded powdered stems and leaves were poured as given below.

DH_2O extract preparation	Methanol extract preparation
Stem (15gms)	150 ml of distilled water
Leaves (20gms)	150 ml of distilled water
Stem (5gms)	40 ml of methanol
Leaves (5gms)	30 ml of methanol

Antimicrobial activity of *Withania somnifera*

Bacterial Strains

Staphylococcus aureus, *E.coli*, *Pseudomonas* clinical isolates were collected from local hospitals. On the basis of morphological, cultural, and biochemical traits, the bacterial strains were re-identified. The bacterial strains were cultivated on nutrient agar media (NAM). Also, the Nutrient broth is prepared for these 3 bacteria that lack the solidifying agent i.e, Agar. Therefore, the broth will remain in liquid form at RT.

Microorganisms used

Microorganisms were received from the School of Basic and Applied Sciences, Department of Biotechnology, Shri Guru Ram Rai University, Dehradun.

Agar well diffusion Method

The antimicrobial activity of Methanol and Distilled water leaf and stem extracts were investigated using agar well diffusion (Perez et al.,1990; Perez et al., 1999; Bagamboula et al., 2004). 200 ml of bacteria were aseptically inserted and dispersed on the surface of gelled sterile Muller Hilton agar plates by using a sterile spreader. On each agar plate, a 10 mm diameter well was punched aseptically with a sterile well borer. In the wells of the plates, leaf or stem extracts were added with different concentrations. E.g., *S. aureus* stem with Methanol, 5 wells were punched aseptically (20µl, 30µl, 40µl, 50µl and Control 40µl respectively). After this different concentrations of leaf and stem extract were added to wells. As a positive control, an antibiotic (streptomycin for bacteria) was placed in the center of the agar plates. Plates were incubated at 37°C for 24 hours after being kept in laminar flow for 30 minutes to allow the extract to pre-diffusion. Next day, a scale was used to measure the zone of inhibition that resulted. By using the well diffusion method, the minimum inhibitory concentrations (MIC) of antimicrobial extracts of *Withania somnifera* (methanol and DH_2O) were determined.

Phytochemical Test

These methods described by Harborne (1978) for the identification of the presence of an active component in the test sample, phytochemicals are beneficial to raise immunomodulatory response and immunity against the disease. A phytochemical test was performed on a dry extract for proteins, carbohydrates, glycosides, flavonoids, saponins, phenol, and tannins (Baskaran et al., 2012).

Phytochemical screening of *Withania somnifera*

In order to investigate the types of secondary metabolites present in the plant species under investigation, the phytochemical analysis was carried out using the test according to the standard procedures of Proteins, carbohydrates, glycosides, phenolic compounds, and flavonoids found in *Withania somnifera* leaf extract, the crude extract of leaf was tested for phytochemical screening (Kokate et al., 2005).

Test for Proteins

Ninhydrin test

Add 2-5 drops of ninhydrin reagent prepared in acetone into 1 ml of crude leaf extract with methanol, violet colored appeared which indicate the presence of amino acid and proteins.

Millon'stest

Add 2-5 drops of Millan's reagent into 1 ml of crude leaf extract with DH_2O. Place the test tube in a boiling water bath, red color appeared to determine the presence of protein (Gaurav et al., 2016a).

Test for carbohydrates

Fehling'stest

Add 1 ml of Fehling reagent (mix equal amount of Fehling A and Fehling B reagent) into 1 ml of crude leaf extract of DH_2O shake properly and place the test tube in a boiling water bath. The presence of reducing sugar is indicated by the presence of a brick red ppt at the bottom of the test tube (Gaurav et al., 2016a).

Test for glycosides

Salkowski test

1ml of crude extract of leaf methanol mixed with 2ml of salkowski reagent and shake test tube properly, reddish-brown colour formations indicate the presence of steroidal ring. Dry coughs can be soothed and suppressed by glycosides. When consumed in tiny dosages, they have a beneficial calming and relaxing impact on the heart and muscles. They have a strong diuretic effect (Sharma et al., 2011).

Test for Flavonoids

Alkaline reagent test

1 ml of crude leaf extract mixed with 2 ml of 2% of NaOH, Reddish brown color appeared it turned color less after adding a few drops of dilute acid which determined the presence of flavonoids. Flavonoids from this plant have antioxidant properties, can strengthen capillary walls, reduce osteoporosis, improve blood cholesterol levels, and lower the risk of cancer and coronary heart disease (Middleton et al., 1998).

Test for phenols and tannins

1ml of crude leaf extract mixed with 2ml of FeCl3, black color ation indicates the presence of phenol and tannins. When applied to the skin, phenols are bioactive agents that cause irritation. Above all, phenols have a high affinity for metal chelation and scavenging free radicals in cells (Michalak et al., 2006). Many fungi, yeast, bacteria, and viruses are inhibited by the tannin compounds found in WS (Chung et al., 1998).

Test for saponins

Mix 1ml of crude extract of the leaf with 5ml of distilled water, and shake properly, the formation of stable foam is observed which indicates the presence of saponins. Saponins from plants have biological and pharmacological properties such as anti-inflammatory, anti-hepatotoxic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial, and antiviral properties (Rahaman et al., 2010).

RESULTS

Sample Collection: Plants were taken from the School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, and methanolic and DH2O plant leaf and stem extracts with 5 mg/ml concentrations were prepared (Figure 1, Figure 2, Figure 3a,b).

Figure 1: Collection of plant material from Nursery SGRR University Dehradun

Figure 2: A) Separated Leaf and Stem, B) Dried Powder of leafs and Stem.

Figure 3a: DH_2O Extract Preparation Figure

3b: Methanol Extract Preparation

Figure 4a: Inhibition zone of stem and leaf Figure 4b: Inhibition zone of stem and Leaf

Figure 4b: Inhibition zone of stem and Leaf extract against E.coli

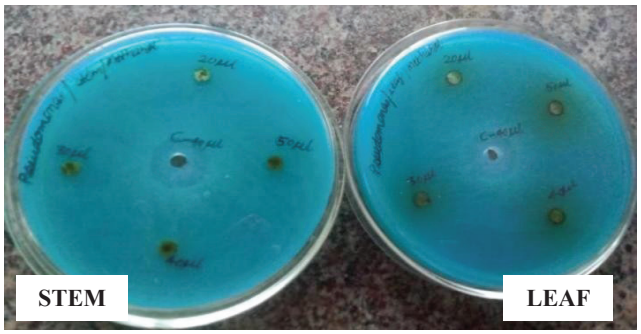

Figure 4c: Inhibition zone of stem and leaf methanol extract against Psedomonas

Figure 5a: Inhibition zone of stem and leaf DH2O extract against E.coli

Figure 5b: Inhibition zone of stem and Leaf DH2O extract against S. aureus

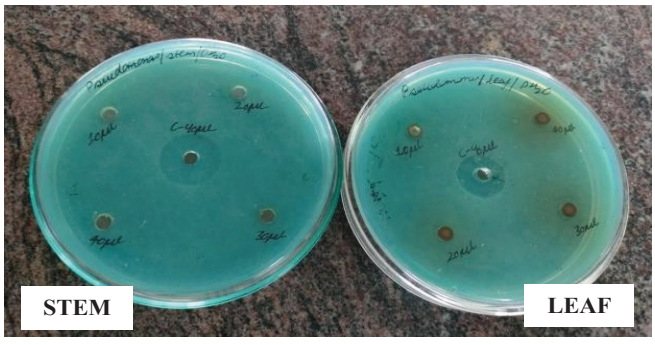

Figure 5c: Inhibition zone of stem and leaf DH2O extract against Pseudomonas

Antimicrobial activities

Mueller-Hinton Agar was used to test the antibacterial activity of isolated probiotics (*E. coli, S. aureus*) and antibiotic (*Pseudomonas*) bacteria (MHA). Using the agar well diffusion method methanol and DH_2O extract of *W. somnifera* was used for antimicrobial activity of stem and leaf against *E. coli, S. Pseudomonas, S. aureus* (Figure 4a,b,c; Figure 5a,b,c, table 1.2.3)

Table 1: Minimal Inhibitory Zone of selected bacterial strains by leaf extract (in Methanol) of *Withania somnifera* (L) as shown in figures 4a,b,c.

		Diameter of inhibition zone (mm)				
S. No.	**Bacteria type**	**Standard drug Streptomycin (10mg/10ml) CONTROL**	**Different concentrations of Leaf extract dissolved in Methanol were poured into different wells on agar plates.**			
		40µl	**10µl**	**20µl**	**30µl**	**40µl**
1	*E.coli*	22	14	16	18	20
2	S. aureus	9	15	18	20	21
3	*Pseudomonas*	9	8	9	10	12

Determination of Zone of Inhibition in methanolic extract of *Withania somnifera* and maximum zone of inhibition occurs in against gram-negative bacteria *E.coli.*

Table 2: Minimal Inhibitory Zone of selected bacterial strains by leaf extract (in DH_2O) of *Withania somnifera* (L) as shown in figures 5 a,b,c.

		Diameter of inhibition zone (mm)				
S. No.	**Bacteriatype**	**Standard drug Streptomycin (10mg/10ml) CONTROL**	**Different concentrations of Leaf extract dissolved in DH_2O were poured into indifferent wells on agar plates.**			
		40µl	**10µl**	**20µl**	**30µl**	**40µl**
1	*E.coli*	24	-	11	22	25
2	S. aureus	12	12	13	20	23
3	*Pseudomonas*	11	-	9	10	24

Determination of Zone of Inhibition in distilled water extract of *Withania somnifera* and maximum zone of inhibition occurs against gram-negative bacteria *E.coli*

Table 3: Minimal Inhibitory Zone of selected bacterial strains by leaf extract (in Methanol) of *Withania somnifera* (L) as shown in Figure 4

		Diameter of inhibition zone (mm)				
S. No.	**Bacteriatype**	**Standard drug Streptomycin (10mg/10ml) CONTROL**	**Different concentrations of Stem extract dissolved in Methanol were poured into different wells on agar plates.**			
		40µl	10µl	20µl	30µl	40µl
1	*E.coli*	22	10	11	13	15
2	S. aureus	11	8	9	10	12
3	*Pseudomonas*	9	-	-	-	-

Determination of minimal inhibitory in methanol extract of *Withania somnifera* and maximum zone of inhibition occurs against gram-negative bacteria *E.coli.* Determination of minimal inhibitory in distilled water extract of *Withania somnifera* and maximum zone of inhibition occurs against gram-negative bacteria *E.coli.* DH_2O extract had less antibacterial activity than the majority of the tested microorganisms, however, it was effective against *S. aureus*. The extract activity was comparable to that of the common antibiotic Streptomycin. Whereas, the antimicrobial activity of Methanol extract was lower against *Pseudomonas* and was best against *E.coli* (Figure 6,7).

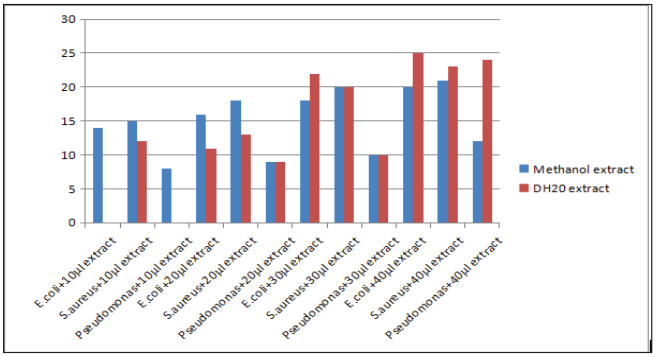

Figure 6: Anti-microbial activity of Methanol and DH_2O Leaf Extracts of *Withania somnifera* at different concentrations.

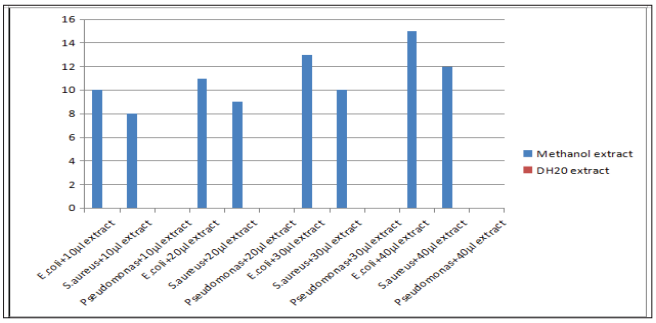

Figure 7: Anti-microbial activity of Methanol and DH_2O Stem Extracts of *Withania somnifera* at different concentrations

Phytochemical Tests

Proteins, carbohydrates, flavonoids, glycosides, phenol and tannins, and saponin were found in distilled water and methanol extracts of *Withania somnifera* leaf powder. The result determines the presence of the medicinally active compound in the plant.

Table 4: Phytochemical Screening of Leaf Extracts of *Withania somnifera* (L) Dunal.

S.No	Tests	Test type	Methanol leaf extract
1	Proteins	Ninhydrintest	-
2	Glycosides	Salkowskitest	+
3.	Saponins	Distilled water	+
4.	Carbohydrates	Benedict'stest	-
S.No	**Tests**	**Test type**	**DH_2O leaf extract**
1	Proteins	Millon'stest	+
2.	Carbohydrates	Fehling'stest	+
3.	Flavonoids	Alkaline reagent test	+
4.	Phenolsandtannins	FeCl3	+

'+' indicates presence of compound; '-' indicates absence of compound

In the above table, methanol leaf extract, indicates the presence of glycosides and saponins, whereas proteins and carbohydrates were not detected. In DH_2O leaf exact, proteins, carbohydrates, flavonoids, phenols, and tannins were detected shown in table 4.

DISCUSSION

Plants are an important source of potentially beneficial structures for the development of new chemotherapeutic marketers, and the in vitro antibacterial pastime assay is the first step toward this goal (Dharajiya et al., 2014). The current study may be valuable in supplementing information in terms of standardization and identification, as well as further research and application in the Ayurvedic medical system. These plants' use in folk medicine suggests that they could be a cost-effective and safe way to treat infectious disorders. According to the findings of this study, Methanol and DH_2O extracts of *Withania somnifera* contained the highest concentrations of flavonoids, glycosides, protein, phenols, and carbohydrates. Components produced from the *Withania* plant extract have been used to successfully treat Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, Huntington's disease, and Creutzfeldt-Jakob disease (CJD), which are some of the well-known age-related disorders. Despite the numerous current studies, neurodegenerative diseases are still incurable. The medications obtained from *Withania*, which are currently accessible for dementia and include donepezil, an acetylcholine inhibitor, are effective in the temporary treatment and cure of cognitive dysfunction or memory loss, but they do not prevent or reverse neurodegeneration. The plant is utilized to make over 200 formulations that are used to treat a variety of physiological ailments. It is used therapeutically as an immune stimulant in patients with low white blood cell counts as well as an adaptogen for individuals with nervous weariness, sleeplessness, and debility brought on by stress. Plant over-exploitation and reproductive failure are linked to species that are on the verge of extinction. The drugs obtain from *Withania,* currently available for dementia, such as donepezil, an acetylcholine inhibitor are effective in the temporary treatment and curing of memory dysfunction or memory loss, but the drug from *Withania* do not prevent or reverse the neurodegeneracy (Uddin et al., 2013*).*

CONCLUSION

The current study examines that the *Withania somnifera* plant has potent antibacterial properties. The findings indicate that the methanol extract of *W. somnifera* stem has the highest antibacterial activity observed by using Methanol extract. In comparison to methanol, leaf, and stem DH_2O extract has significantly reduced antibacterial activity. The leaf and stem of *W. somnifera* had more antibacterial activity when extracted with methanol. The findings of this study allow us to conclude that the leaf and stem extract of *W. somnifera* exhibited significant antibacterial activity, supporting its ethnobotanical significance in the treatment of a few diseases as a broad-spectrum antibacterial agent. These extracts can be utilized to treat infectious diseases, which is scientifically justified to separate and recognize the components responsible for having an antimicrobial interest However the crude extract must be further refined using antibacterial interest-guided fractionation. Phytochemicals were found in various parts of the plant and the results were compared. The flavonoids, glycosides, steroids, and carbohydrate compounds found in *Withania somnifera* were studied. Based on these findings, it can be concluded that the *Withania somnifera* extract in methanol and aqueous solution contained the highest concentrations of flavonoids, glycosides, steroids, phenols, and carbohydrates. The results of the current study can be applied in the future to the cost-effective formulation of the chemical active ingredients in natural medicines used to treat a range of neurological and inflammatory disease conditions.

Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

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