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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF LEAVES OF *Withania somnifera* (L.) Dunal

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ABSTRACT

Withania somnifera (L) Dunal popularly known as 'Aswagandha' has been an important herb in the Ayruvedic and indigenous medical systems for centuries in India. To validate this use, leaves of the plant was subjected to preliminary phytochemical analysis and *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella paratyphi* B. Acetone extract demonstrated highest antibacterial activity followed by ethanol extract. Aqueous extract showed minimal antibacterial activity against most of the test bacterial pathogens. Preliminary phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, phenolic compounds and flavonoids in extracts. Our findings suggest that an appropriate bioactive compound may be developed from leaves of *Withania somnifera* (L.) Dunal as an alternate to antibiotics.

INTRODUCTION

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by microbial pathogens has led to the screening of medicinal plants for their potential antimicrobial activity. *Withania somnifera* (L) Dunal, also known as Aswagandha, Indian ginseng, and Winter Cherry is a small medium under shrub belonging to the Solanaceae family. The plant has been found useful in the treatment of burns, wounds, dermatological disorders, gastrointestinal diseases, dysfunctions of the respiratory system, asthma, bronchitis, cancer and geriatric problems (Grierson and Afolayan, 1999; Bone, 1996).

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In the present study, we evaluated *in vitro* antibacterial activity of leaves of *Withania somnifera* using different extracts. Besides, phytochemical screening of the extracts was also carried out to asses the presence of different phytochemical in different extracts.

MATERIALS AND METHODS

Collection and Processing of plant materials

Fresh leaves of medicinal plant *Withania somnifera* (L) Dunal free from diseases were collected from Kolli hills of South India. The plant material was washed under running tap water; shade dried and powdered using mechanical grinder (Philips, India). The powdered form of plant material was stored in air tight glass bottles protected from sunlight until required for analysis.

Aqueous Extraction

For aqueous extraction, 10 g of leaf powder was dissolved in 100 ml of distilled water in a conical flask, boiled at 100ºC in a water bath (Servo Scientific Co. Ltd, India) for 6 hours and then filtered through Whatman No.1 filter paper. The filtrate was then condensed and stored at room temperature in screw capped bottles.

Alcoholic Extraction

For alcoholic extraction (ethanol and acetone) 10 g of leaf powder was dissolved in 100 ml of alcohol in a conical flask and kept at room temperature in a rotary shaker (Remi, India) for 48 hours. After 48 hours, it was filtered through Whatman No.1 filter paper; solvent was allowed to evaporate and stored at room temperature until when required for use.

Bacterial Strains

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella paratyphi* B were obtained from Gokulam Hospitals, Salem, South India. The bacterial strains were re-identified on the basis of morphological, cultural and biochemical characteristics (Cheesbrough, 2000).

Preliminary Phytochemical Analysis

The crude aqueous, ethanolic and acetone extract of leaves of Aswagandha was tested for the presence of phytochemicals using standard qualitative procedures (Harbone, 1973).

Test for Carbohydrates

A small quantity of extract was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. The filtrate was treated with 2 to 3 drops of 1% alcoholic α-napthol solution; 2ml of concentrated Sulphuric acid was added along the sides of the tubes. Appearance of violet colored ring at the junction of two liquid shows the presence of carbohydrates.

Test for Glycosides

A small portion of the extract was hydrolyzed with Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.

Test for Alkaloids

A small portion of the alcoholic extract was stirred separately with few drops of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

Test for Phytosterol

Salkowski test was done for the detection of phytosterols. In this test, 1 ml of concentrated Sulphuric acid was added to the plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols.

Test for Fixed Oil

Spot test was done for the detection of fixed oil. In this test, small quantity of alcoholic extract was pressed between two filter papers. Appearance of oil strain on the paper indicates the presence of fixed oil.

Test for Proteins

Small quantity of the extract was dissolved in few ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

Test for Phenolic Compounds

A small quantity of the extract was dissolved in few ml of water and subjected to $FeCl_3$ test. The dilute extract was treated with dilute $FeCl_3$ solution (5%) and appearance of violet colour shows the presence of phenolic compound and tannins.

Test for Flavonoids

The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavonones

Determination of Antibacterial activity

Antibacterial activity of the aqueous, ethanolic and acetone extracts of the plant sample was evaluated by the cup plate agar diffusion method (Aida *et al*., 2001). The test bacterial strain was inoculated in to Mueller-Hinton

broth (Hi-media, Mumbai) and incubated at 37ºC for 3 to 6 hours. After incubation, the test cultures were adjusted to 0.5 Mc Farland turbidity. Immediately after standardization, a sterile cotton swab was immersed in the bacterial suspension and swabbed aseptically on the surface of sterile Mueller-Hinton agar (Hi-media, Mumbai) plates. Wells of 8 mm diameter were punched in to the agar medium and filled with 100 µl of extracts (100 mg/ ml water). Wells loaded with 100 µl of Gentamicin (100 mg/ml water) served as positive control. The plates were incubated at 37ºC for overnight in a bacteriological incubator (Narang Scientific Co. Ltd., New Delhi). Antimicrobial activity was detected by measuring the zone of inhibition in mm around each well, excluding the diameter of well.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of aqueous, ethanolic and acetone extracts of leaves of *Withania somnifera* (L) Dunal revealed the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, phenolic compounds and flavonoids in extracts (Table 1.)

Table 1. Phytochemical Screening of Leaf Extracts of *Withania somnifera* (L) Dunal.

S.No	Phytochemicals / Test	Aqueous Extract	Ethanol Extract	Acetone Extract
1	Carbohydrates: Molisch's test	-	-	+
2	Glycosides: Fehling's test	+	+	+
3	Alkaloids: Dragandroff's test	+	+	+
4	Phytosterols: Salkowski test	+	+	+
5	Fixed oil: Spot test	+	+	+
6	Proteins: Xanthoprotein test	+	+	-
7	Phenolic compounds: $FeCl_3$ test	+	+	+
8	Flavanoids: Concentrated H_2SO_4 test	+	+	+

+ = Present, - = Absent

Table 2 shows the results of antibacterial activity of the extracts against clinical bacterial isolates. The presence of bioactive compounds in plants has been reported to confer resistance against microbial pathogens and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study (Srinivasan *et al*., 2001). The results of the present study revealed that acetone extract demonstrated highest antibacterial activity followed by ethanol extract. Aqueous extract showed less antibacterial activity against most of the test bacterial pathogens, but highest activity against *Proteus mirabilis*. The activity of the extracts was comparable to that of standard antibiotic gentamicin. In conclusion, bioactive compounds from *Withania somnifera* leaf extracts could be used as an alternate to antibiotics, considering the side effects and escalating levels of antibiotic resistance among microorganisms.

Table 2. Antibacterial Activity of Leaf Extracts of *Withania somnifera* (L) Dunal

S.No	Bacterial Pathogen	Diameter of Zone of Inhibition (mm)			
		Standard Drug	Aqueous Extract	Ethanol Extract	Acetone Extract
1	*Staphylococcus aureus*	18	7	12	16
2	*Escherichia coli*	18	4	-	10
3	*Klebsiella pneumoniae*	28	5	8	11
4	*Pseudomonas aeruginosa*	25	3	2	4
5	*Proteus mirabilis*	26	12	7	9
6	*Salmonella paratyphi* B	23	5	10	10

Values are mean of three replicates: Standard drug: Gentamicin (100 mg/ml)

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