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Antibacterial and antioxidant activity of different extracts obtained from *Withania somnifera* (*Ashwagandha*)

Research Article

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Abstract

Ashwagandha (*Withania somnifera*) is widely known as the queen of Indian *Ayurveda*. The objective of this study was to determine the antioxidant and antibacterial activity of the different parts of the *Ashwagandha*. The antioxidant activity of different extracts from different parts of *Ashwagandha* was determined using DPPH free radical scavenging method. *Ashwaghanda* root inhabiting bacteria was isolated using pour-plate technique. The agar well method and filter paper method were followed to assess the antibacterial activity of the extracts. Ethanol-water extracts (1:1 v/v) gave higher yield of extractable matter than methanol extracts. The antioxidant activity of fresh root extract was significantly higher than other parts of the plant (stem and leaves) as well as the plant material bought from open market. The minimal inhibitory concentration (MIC) values of Ethanol-water extract against MRSA, *Candida* Sp. and *Salmonella* Sp. were >256 mg/L. The commensal bacteria (*Bacilli*) inhabiting *Ashwagandha* roots displayed mild antibacterial activity against pathogenic *P. aeruginosa*.

Key Words: *Ashwaghanda,* Commensal bacteria, Antibacterial activity, Antioxidant activity, Crude extracts.

Introduction

The medicinal plant, *Ashwaganda* (*W. somnifera*) is commonly known as "Indian winter cherry" or "Indian Ginseng" or "queen of *Ayurveda*" (1-5). The root smells like a horse is the basis of its' Indian name, "*Ashwaganda*" (5). The meaning of the Latin word "*somnifera"* is "sleep inducer" which reflects the stress releasing pharmacological properties of the plant.

Ashwagandha (W. somnifera) has a history over 3000 years being used in Indian *Ayurveda* and indigenous medicine (6-8). *Ashwagandha* is a valuable component for over 100 formulations in traditional medicine (9). This herb is well known for its' energy boosting properties whereas the supplements forms are commercially available (10). According to *Ayurveda Ashwagandha* have various properties. Mainly *Ashwagandha* has effects of nourishing and strength promoters. It has been described in the *Vrunhaniya Gana* (Nourishing drugs) and *Balya Gana* (Strength promoting drugs) (11).

Ashwaghanda inherits wide range of pharmacological significance such as antitumor, antioxidant, anti−inflammatory and immunomodulator and for the treatment of the disorders of the central nervous system (1-4, 8, 12,13).

Antioxidant activity of *Ashwaghanda* roots has been observed previously (14). Ethanol-water mixture is a potential solvent for the extraction of compounds from the plant material for their biological activity whereas the biological properties of Ethanol-water (1:1 v/v) extract of *Ashwagandha* is lacking in previous work (14,15). Moreover, the biological properties of different parts of the plant are required to be compared with each other because of the root part is more frequently used in *Ayurveda* than stem and leaves. The aim of this study was to assess the biological properties of different parts of *Ashwaghanda* herb, particularly the antibacterial aspects which studied into a lesser extent, previously. Moreover, the antioxidant potential of commercially available herbal materials of *Ashwaghanda* (roots) is examined in comparison with fresh herb to obtain an insight on quality of commercially available products.

The antibacterial activity of *Ashwaghanda* has been previously mentioned in few studies (17, 18, 19). With a view of expanding the details given in previously conducted work, here we assayed different extracts of *Ashwaghanda* (8 different extracts) using 7 species of medically important pathogens (Methicillin resistant *S. aureus; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida*).

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Materials and Methods

Collection of plant materials

The plant materials *Ashwagandha* (*W. somnifera*) were obtained from the herbal garden at

Bandaranaike Memorial *Ayurvedic* Research Institute (BMARI), Colombo, Sri Lanka, under the consultation of manager (Botanist) of the herbal garden. The commercially available roots of *W. somnifera* were bought from a recognized Ayurvedic product seller shop in Colombo.

Preparation of plant extracts

The whole plants were separated into the leaves, stem and roots and those were chopped into small pieces. The plant material was dried in 40 C˚ for 1 week time until a constant weight was obtained. Two different solvents (methanol; EtOAc: water 1:1 v/v) were used for the extraction, separately and the volume of the solvent added, was 5 times the weight of the dried plant material used in the extraction. One portion of the plant material was dipped in methanol while the other portion was dipped in EtOAc-water mixture. The solvents and plant materials were shake together for 48 h in room temperature (32 C˚) using a mechanical shaker. The weights of plant materials and the volume of the solvents used in the extraction were recorded. The solvent portion of extracts were evaporated using a rotary-evaporator and the resulting material was freeze-dried to obtain solid-powder. Eight different extracts were prepared in total, and these extracts were used for antioxidant and antibacterial assays.

Antioxidant activity assay

Antioxidant activity of each extract was determined using DPPH free radical scavenging method. A concentrations series (500 mg/L 100 mg/L 10 mg/L) was prepared using extracts to assess the antioxidant potential of each extract. The extracts and DPPH reagent were added in 1:3 ratio and incubated for 30 minutes in the room temperature and in dark environment. The absorbance was obtained using spectrophotometer in 517 nm. The antioxidant activity was recorded as Percentage effect (E %) and calculated using following equation.

$$\text{Percentage effect (E \%)} = \frac{\text{Abs control-Abs sample} \times 100}{\text{Abs control}}$$

Antimicrobial assays of different extracts against human pathogens

For the initial screening of antimicrobial activity, agar well method was conducted. The agar plate dilution method was conducted to determine the individual minimum inhibitory concentration (MIC) values of extracts against medically important pathogenic bacteria.

Microbial cultures

The Microbial cultures (Methicillin resistant *S. aureus; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida*) were obtained from the culture collection at the Department of Microbiology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka. All the cultures were human clinical isolates and 4 different isolates (strains) from each species of bacteria were used for assays.

Agar well method

Agar well method was performed to screen the antibacterial activity of the extracts. The wells were prepared in the MHA plates containing a microbial lawn (Prepared using 0.5 McFarland standard) and each of the extracts were filled into different wells. The MHA plates incubated overnight and observed for a clear zone around the wells.

Agar plate dilution method

Agar plate dilution method was carried out according to the guidelines provided by British Society of Antimicrobial Chemotherapy (BSAC) (21). A dilution series, starting from 256 mg/L down to 16 mg/L was prepared in MHA plates using each extract of the plant. The bacterial inoculum (density equal to 0.5 McFarland-standard) was spotted as a small drop on the surface of the agar and observed for visual growth, after incubating the plates at 37 C˚, overnight.

Isolation of root inhabiting bacteria

The fresh roots obtained from the plant were rinsed by sterile water and the resulting water extract was cultured in a Muller Hinton Agar (MHA) plate. The bacteria grown in the plate was identified using gram staining and biochemical tests. A small piece of the root was stabbed into another MHA plate and the bacterial growth around the root was observed and growing bacterial species was identified.

Antimicrobial assays using isolated root inhabiting bacteria

The isolated root inhabiting bacteria (2.4.4) was used for antimicrobial assays against human pathogenic bacteria (Methicillin resistant *S. aureus* MRSA*; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida*) using filter paper technique. A piece of filter paper was soaked using the isolated bacterial inoculum from the roots and placed on the lawn of pathogenic bacteria, prepared in MHA plates. The plates were observed for an inhibition zone around the filter paper after incubating 37 C˚, overnight.

Results

Yield of the extractable matter

Table 1: Yield of extractable matter from different parts of the plant using the solvents, Ethanol water and methanol

Type of plant material	Ethanol-water extract (1:1; v/v); (% W/W)	Methanol extract (%; W/W)
Fresh Roots from the plant	2.7%	0.5%
Commercially available roots	7.8%	2.04%
Fresh Stem	3.14%	1.2%
Fresh Leaves	4.3%	2.5%

The weight of crude extract as a percentage of dry weight of the plant material is displayed in table 1. The highest percentage of extractable matter was obtained from commercially available roots using methanol-water as the solvent. The mean percentage of extractable matter in ethanol-water (4.48 %) extracts were higher than methanol extracts (1.56 %; Table 1).

Antioxidant activity of different extracts of the plant

The antioxidant activity of methanol extracts and ethanol-water extracts were highest in fresh roots obtained from the plant. The commercially available root showed significantly lesser antioxidant activity than fresh roots whereas the leaves showed the least antioxidant activity (Table 2). The comparison between methanolic and ethanol-water extract showed that the antioxidant potential was roughly similar in both the extract.

Table 2: Antioxidant activity of extracts obtained from different parts of the plant in Ethanol-water and methanol

Extract	Antioxidant activity (Percentage effect; E %)					
	Ethanol-water extract (1:1; v/v)			Methanol extract		
	500 mg/L	100 mg/L	10 mg/L	500 mg/L	100 mg/L	10 mg/L
Fresh roots	93.4	27	3.8	92.9	23.5	1.9
Commercially available roots	29.8	5.6	0.1	31.6	5.6	1.6
Fresh stem	18.4	5	1.1	52.9	6.3	2.2
Fresh leaves	17.1	4.3	0.7	21.4	6.3	0.3

3.3 Antibacterial activity of different extracts of the plant

The ethanol-water extract of fresh roots showed inhibitory zone (Diameter of the well 12 mm; Diameter of the inhibition zone including the well: 17 mm) in 5 mg/mL concentration against MRSA, *Candida and Salmonella*. Other extracts did not show any clear zone around the wells against assayed pathogens (Methicillin resistant *S. aureus; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida* [Table: 3]).

Table 3: Inhibitory activity of different ashwaghanda extracts against human pathogens

Organism	Antibacterial activity (Inhibition zone diameter) *		
	Roots (5 mg/ml)	Stem (5 mg/ml)	Leaves (5 mg/ml)
Methicillin resistant *S. aureus*	17 mm	No zone	No zone
P. aeruginosa	No zone	No zone	No zone
Klebsiella	No zone	No zone	No zone
Salmonella	17 mm	No zone	No zone
Enterococcus	No zone	No zone	No zone
E coli	No zone	No zone	No zone
Candida	17 mm	No zone	No zone

*Well diameter: 12 mm

Therefore, Agar Plate Dilution Method was conducted using only Ethanol-water extract. According to the Agar plate dilution method MRSA *Candida and Salmonella* showed MIC >256 mg/L.

Antibacterial activity of root inhabiting bacteria against pathogenic bacteria

The bacteria isolated from the roots of *W. somnifera* was identified as a gram-positive bacillus. The bacterium in the water which was used to rinse the fresh roots of the plant was similar to the bacteria grown around the stabbed root in the agar plate and both the isolates were pure cultures. This gram-positive bacterium showed mild antibacterial activity (clear zone of 3 mm width from of the filter paper) against *pseudomonas aeruginosa*. There was no inhibitory activity exhibited against other bacterial species/strains (Methicillin resistant *S. aureus; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida*).

Discussion

According to Indian Ayurveda, *Ashwagandha* has tastes of *Madhura* (sweet), *Tikta* (Bitter) and *Kashaya* (Astrigent). It has *Guna* (physico-pharmacological properties) of *Laghu* (light) and *Snigdha* (unctuous). It's *Vipaka* (final transformation) is *Madhura* and Veerya (powers that performs action in the body) is *Ushna* (hot). *Ashwagandha* helps for longevity and to restore the strength of the body (16).

In the extracting process, ethanol-water extract generated a better yield of crude material than methanol extract which has previously shown using other herbal plant species (15) (Table 1). The antioxidant activity of Ashwagandha root extracts is behind the gallic acid; however, roughly comparable with *Phyllanthus emblica* which is another potential antioxidant herb used in traditional medicine. Significantly, roots displayed higher antioxidant activity than other parts of the plant (stem and leaves). The plant material bought from open market showed inferior antioxidant activity than the fresh plant material (Table 2).

According to the results obtained by antimicrobial assays of crude extracts against medically important pathogens, MRSA *Candida* and *Salmonella* showed inhibition zone in 5 mg/mL concentration of the ethanol-water extract. Other extracts did not show any clear zone around the wells against the pathogens tested (Methicillin resistant *S. aureus; P. aeruginosa; Klebsiella; Salmonella; Enterococcus; E coli; Candida*). The minimum inhibitory concentrations (MICs) obtained by other studies against both gram negative and positive bacteria falls in the range of 0.78mg/mL-153 mg/Ml (17,18,19,20) where as some other herbal extracts such as tea catechins has shown 50-180 µg/mL MIC value against MRSA (21). The MICs of *Ashwagandha* extracts, in the range of 0.78mg/L-153 mg/L can be categorized as having mild to moderate antibacterial activity because of many highly potent antibacterial compounds and extracts display MICs bellow 128 µg/mL when British Society for Antimicrobial Chemotherapy (BSAC), agar plate dilution method is conducted for MIC determination (22). The mild to moderate potential of Ashwagandha in the bacteriology side is further reflected by the fact that major traditional medicine applications of this herb

basically target the neurological/psychology/energy metabolism aspects of the body (8, 22, 23, 24, 25, 27).

The commensal bacterium inhabiting the *Ashwagndha* roots was a Bacilli and surprisingly the microbial colony grown around the root was a pure culture of Bacilli and was same as the culture obtained from the root-rinsed-water. However, this commensal bacterium showed mild inhibitory activity only against *Pseudomonas aeruginosa* out of the 7 pathogens tested. All other pathogens did not show any inhibitory activity.

Conclusion

Ethanol-water is a better solvent for higher yield of active compounds than methanol. *Ashwagandha* root inhabiting bacteria (Bacilli) displayed mild antibacterial activity against pathogenic *P. aeruginosa*. The extracts of this herb showed mild to moderate antibacterial properties, overall. The fresh root extracts of *Ashwagandha* displayed an impressive antioxidant potential. The highly significant biological activities (antibacterial and antioxidant) were displayed by fresh roots extracts (Ethanol-water and Methanol extracts; Table: 2) compared to commercially available root extracts, fresh stem and fresh leaves. This fact is further evidenced in traditional medicine in-which roots are more frequently used for herbal preparations than leaves and the stem of *Ashwaghanda*.

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