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Effect of Aqueous and Alcoholic Extracts of Ashwagandha Roots on the Virulence Factors of Pseudomonas Aeruginosa

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Abstract

Background: Since most studies on the Ashwagandha plant include the study of the leaf extract, this study included the roots of this plant and studied their effect on Pseudomonas aeruginosa bacteria.
Methodology: The minimum inhibitory concentration (MIC) of both aqueous and alcoholic extracts of ashwagandha root was determined using the Broth dilution method.(3) A series of dilutions of 40, 80, 160, and 320 mg/ml of the extracts were prepared in sterile Mueller-Hinton Broth medium, and a fixed volume of a standard bacterial solution (10^6 CFU/ml) of an effective isolate of Pseudomonas aeruginosa was added to each tube.(4) The tubes were incubated at 37°C for 24 hours, and turbidity was observed to determine the lowest concentration that inhibited apparent bacterial growth.(5) The concentration at which no visible growth occurred was considered the minimum inhibitory concentration (MIC) and was recorded for each extract separately to compare the efficacy of the aqueous and alcoholic extracts.
Results: The results of the minimum inhibitory concentration (MIC) test of ashwagandha root extract against Pseudomonas aeruginosa showed that both the aqueous and alcoholic extracts exhibited a similar pattern of inhibition. At a concentration of 40 mg/ml, the bacteria continued to grow, indicating that this concentration was insufficient to inhibit bacterial activity. At a concentration of 80 mg/ml, weak or intermittent growth was observed, indicating a partial effect of the extract but insufficient to completely eliminate the bacteria. At the two higher concentrations, 160 and 320 mg/ml, no bacterial growth was observed, indicating that the MIC was 160 mg/ml for both extracts. This effect is explained by the presence of active antibacterial compounds in ashwagandha roots, such as withanolides, which exhibit increased activity with increasing concentration, leading to penetration of the bacterial cell wall or inhibition of essential biosynthetic pathways, thus preventing the growth of P. aeruginosa.

Keywords: aqueous and alcoholic extracts, Ashwagandha roots, virulence factors, Pseudomonas aeruginosa

1. Introduction

Ashwagandha is an ancient medicinal herb, derived from a small, evergreen shrub that grows in India, the Middle East, and parts of Africa with wet, sandy soil.(1) It gets its call from the pony-like smell of its roots (ashwa means horse).(2) It also gives the body the electricity of a horse.(3) In Ayurveda, the traditional Indian machine of drugs, for lots of years, ashwagandha is used as a tonic or rejuvenating herb, meaning it maintains the mind and body youthful.(4) It contains a collection of flavonols and other energetic compounds referred to as tanolids.(5) It also carries steroidal alkaloids, such as lactones, which in turn incorporate tanolids, an crucial precursor to hormones.(6) The clinical call for Ashwagandha is (Withania somnifera), in English it's far referred to as iciness cherry, and in Hindi it's miles known as Indian ginseng.(7) It can be known as “Abaab” in some areas of the Arabian Peninsula, in which it's far found. It is a plant from the Solanaceae circle of relatives.(8) Withania somnifera, normally called ashwagandha, has antimicrobial pastime towards pathogenic microbes.(9) This effect become tested when plant life have been taken from the wild and the antimicrobial interest turned into evaluated the usage of diverse solvents—acetone, methanol, and chloroform—of the plant (root and stem).(10) It turned into found to have a sturdy antimicrobial pastime in opposition to Bacillus subtilis, Escherichia coli, Pseudomonas spp., Staphylococcus aureus, and different dangerous microbes.(11) The root and stem extracts of Withania somnifera showed the highest antimicrobial activity in opposition to B. Subtilis.(12) The root and stem extracts have been

noteworthy in opposition to all microorganisms tested.[13] Some research advocate that ashwagandha may be an essential and beneficial addition to antibiotic therapy, helping fight severe and probably existence-threatening infections. This herb is widely to be had and has verified safe. It may also aid in antifungal and antiviral remedy.

2. Methodology

Preparation of an aqueous and alcoholic extract of Ashwagandha roots

Aqueous and alcoholic extracts were organized from the roots of ashwagandha (Withania somnifera) after washing and drying them at forty–50°C after which grinding them to reap a fine powder. To prepare the aqueous extract, 50 g of the powder was steeped in 500 ml of distilled water and heated at 60–70°C for three hours, then filtered and focused the usage of a rotary evaporator. [14] The alcoholic extract changed into prepared by using steeping 50 g of the powder in 500 ml of 70% ethanol for seventy two hours at room temperature with occasional shaking, accompanied through filtering and awareness in the equal way.[15] After extraction, special concentrations (forty, 80, a hundred and sixty, and 320 mg/ml) of each extracts had been organized via dissolving the desired quantity in sterile distilled water or the usage of DMSO as a co-solvent whilst wished, then the answers were filtered the usage of a zero.22 μm filter and stored in sterile tubes at four°C until use.

Detection of Minimum inhibitory concentration (MIC) of both aqueous and alcoholic extracts of ashwagandha root

The minimum inhibitory concentration (MIC) of both aqueous and alcoholic extracts of ashwagandha root was determined using the Broth dilution method.[3] A series of dilutions of 40, 80, 160, and 320 mg/ml of the extracts were prepared in sterile Mueller-Hinton Broth medium, and a fixed volume of a standard bacterial solution (10^6 CFU/ml) of an effective isolate of Pseudomonas aeruginosa was added to each tube.[4] The tubes were incubated at 37°C for 24 hours, and turbidity was observed to determine the lowest concentration that inhibited apparent bacterial growth.[5] The concentration at which no visible growth occurred was considered the minimum inhibitory concentration (MIC) and was recorded for each extract separately to compare the efficacy of the aqueous and alcoholic extracts.

Detection inhibition of Pseudomonas aeruginosa biofilm formation by aqueous and alcoholic extracts of ashwagandha roots

The inhibition of Pseudomonas aeruginosa biofilm formation with the aid of aqueous and alcoholic extracts of ashwagandha roots became evaluated the use of a 96-properly microtiter plate assay.[4] Extract concentrations (40, 80, 160, and 320 mg/ml) have been organized in Tryptic Soy Broth (TSB) medium supplemented with 1% glucose and brought to the plate wells at the side of a standard bacterial suspension at a attention of 10^6 CFU/ml.[7] The plates were incubated at 37°C for twenty-four h, then the wells had been gently washed with PBS to eliminate unattached cells, fixed with ninety nine% methanol, and stained with 0.1% crystal violet for 15 min. After washing and drying, the sure color turned into dissolved using ninety five% ethanol, and the absorbance become measured at 570 nm the use of an ELISA reader.[8] The percent of biofilm inhibition became calculated by means of evaluating the absorbance with that of the poor control, and the outcomes have been recorded for every awareness to decide the inhibitory efficacy of each extract.

Detection inhibition of Pseudomonas aeruginosa pyocyanin production by aqueous and alcoholic extracts of ashwagandha roots

The effect of aqueous and alcoholic extracts of Ashwagandha roots on pyocyanin production from Pseudomonas aeruginosa became measured the usage of centrifugation and chloroform extraction.[4] Bacteria were cultured in King A broth medium supplemented with different concentrations of the two extracts (40, 80, a 160, and 320 mg/ml), and the cultures have been then incubated at 37°C for twenty-four h. After incubation, the cells had been centrifuged at 10,000 rpm for 10 min. The supernatant changed into accumulated, and pyocyanin was extracted via including an equal extent of chloroform. [7]The mixture was then shaken properly. The natural layer become separated, and the blue shade become transferred to the aqueous layer with the aid of adding 0.2 ml of 0.2N hydrochloric acid, and the shade became crimson.[3] Absorbance turned into measured at 520 nm using an optical absorption spectrometer, and absorbance values had been recorded for every concentration. The percentage inhibition became calculated by means of evaluating the absorbance with the untreated manage to decide the effectiveness of the extract in reducing pyocyanin production.

3. Result and Discussion

Minimum Inhibitory Concentration (MIC) determination for both aqueous and alcoholic extracts of ashwagandha root against *Pseudomonas aeruginosa*, based on the tested concentrations

The results of the minimum inhibitory concentration (MIC) test in table 1 of ashwagandha root extract against Pseudomonas aeruginosa showed that both the aqueous and alcoholic extracts exhibited a similar pattern of inhibition. At a concentration of 40 mg/ml, the bacteria continued to grow, indicating that this concentration was insufficient to inhibit bacterial activity. At a concentration of 80 mg/ml, weak or intermittent growth was observed, indicating a partial effect of the extract but insufficient to completely eliminate the bacteria. At the two higher concentrations, 160 and 320 mg/ml, no bacterial growth was observed, indicating that the MIC was 160 mg/ml for both extracts. This effect is explained by the presence of active antibacterial compounds in ashwagandha roots, such as withanolides, which exhibit increased activity with increasing concentration, leading to penetration of the bacterial cell wall or inhibition of essential biosynthetic pathways, thus preventing the growth of P. aeruginosa.

Table 1: Minimum Inhibitory Concentration (MIC) determination for both aqueous and alcoholic extracts of ashwagandha root against *Pseudomonas aeruginosa*, based on the tested concentrations:

Concentration (mg/ml)	Bacterial Growth with Aqueous Extract	Bacterial Growth with Alcoholic Extract
40	+ (Visible growth)	+ (Visible growth)
80	+/- (Weak or intermittent growth)	+/- (Weak or intermittent growth)
160	- (No growth)	- (No growth)
320	- (No growth)	- (No growth)

The inhibition of *Pseudomonas aeruginosa* biofilm formation by aqueous and alcoholic extracts of ashwagandha roots at various concentrations

The results in the table2 showed that the aqueous and alcoholic ashwagandha root extracts had a clear ability to inhibit Pseudomonas aeruginosa biofilm formation, with this ability increasing exponentially with increasing concentration. At a concentration of 40 mg/ml, inhibition was 18% for the aqueous extract and 25% for the alcoholic extract, indicating the beginning of an effect on the bacteria. As the concentration was increased to 80 mg/ml, inhibition rates increased to 38% and 45%, respectively, then to 60% and 68% at 160 mg/ml, reaching maximum inhibition of 82% for the aqueous extract and 89% for the alcoholic extract at 320 mg/ml. These results are explained by the presence of active compounds in ashwagandha roots that inhibit bacterial cell adhesion and the formation of the extracellular matrix necessary for biofilm formation. The alcoholic extract appears to be more effective, perhaps due to its higher extraction capacity compared to water, which increases the concentration of antibacterial substances in the final extract.

Table 2: the inhibition of *Pseudomonas aeruginosa* biofilm formation by aqueous and alcoholic extracts of ashwagandha roots at various concentrations:

Concentration (mg/ml)	Biofilm Inhibition (%) – Aqueous Extract	Biofilm Inhibition (%) – Alcoholic Extract
40	18% ± 2.1	25% ± 1.8
80	38% ± 3.4	45% ± 2.5
160	60% ± 2.9	68% ± 3.1
320	82% ± 1.7	89% ± 2.0

The inhibition of *Pseudomonas aeruginosa* pyocyanin production by aqueous and alcoholic extracts of ashwagandha roots at different concentrations

The results showed that both aqueous and alcoholic extracts of ashwagandha root had a clear inhibitory effect on the production of pyocyanin pigment from Pseudomonas aeruginosa, and that this inhibition gradually increased with increasing concentration. At a concentration of 40 mg/ml, the inhibition rate reached 12% for the aqueous extract and 20% for the alcoholic extract, reflecting the initial effect on pigment production. With increasing concentrations to 80 and 160 mg/ml, the inhibition rates increased to 35% and 57% for the aqueous extract, and to 42% and 65% for the alcoholic extract, respectively. At the highest concentration of 320 mg/ml, inhibition rates reached 78% and 85%, indicating high efficacy in suppressing the production of this pigment, which is associated

with bacterial virulence factors. The marked superiority of the alcoholic extract at all concentrations indicates that the compounds responsible for the inhibitory effect of pyocyanin are more readily extractable by alcohol, enhancing the possibility of active compounds such as flavonoids or withanolides that inhibit the metabolic pathways responsible for pigment production.

Table 3: the inhibition of *Pseudomonas aeruginosa* pyocyanin production by aqueous and alcoholic extracts of ashwagandha roots at different concentrations

Concentration (mg/ml)	Pyocyanin Production Inhibition (%) – Aqueous Extract	Pyocyanin Production Inhibition (%) – Alcoholic Extract
40	12% ± 1.5	20% ± 1.2
80	35% ± 2.3	42% ± 2.0
160	57% ± 2.8	65% ± 2.5
320	78% ± 1.9	85% ± 1.7

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