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Anti-bacterial Activity of Withania Somnifera (Ashwagandha)

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Abstract

Withania somnifera, often known as ashwagandha, is renowned as the queen of Indian Ayurveda. Finding out which sections of the Ashwagandha are antibacterially active was the goal of this investigation. Numerous techniques have been used to examine antimicrobial susceptibility and find new antimicrobial agents in plants and other natural sources. The roots of Ashwagandha, as well as its leaves and fruits, which are used less frequently, have all been utilized to make herbal remedies. The plant has a range of pharmacological properties, including antibacterial properties. For the evaluation of the antibacterial activity of extracts and purified components of different plant sections of Ashwagandha, a wide variety of bacterial species have been used as test microorganisms. The information about Withania somnifera's antibacterial activity was compiled and discussed in this article.

Keywords: Antibacterial activity, Ashwagandha, Withania somnifera.

Introduction

Due to the rise in bacterial resistance to the common antibacterial drugs, bacterial infections are now recognized as a crucial clinical warning, with considerable allied illness and death (1,2).As a result, methods for determining the susceptibility of bacteria to different antibacterial drugs have been widely used and are still being developed (3). After the revolution in the ‘golden era’, when nearly all groups of important antibiotics (penicillin,aminoglycosides, tetracyclines, and macrolides) were discovered and the key difficulties of chemotherapy were resolved in the 1960s, the history repeats itself these days and these prevailing compounds are at risk of losing their effectiveness due to the increase in microbial resistance (3,4). Ashwagandha (W. somnifera), a medicinal plant, is sometimes referred to as "Indian Ginseng," "Indian Winter Cherry," and "Queen of Ayurveda" (5,6,7,8,9). The root's Indian name, "Ashwagandha," derives from the fact that it smells like a horse (9). The Latin name "somnifera" means "sleep inducer," which reflects the plant's pharmacological capacity to relieve stress. Over 3000 years have passed since the usage of ashwagandha (W. somnifera) in traditional Indian medicine (10,11,12). In traditional medicine, ashwagandha serves as a beneficial ingredient in more than 100 formulas (13). The commercially available supplement variants of this herb are widely known for their ability to increase energy (14).Earlier research have revealed Ashwagandha's antibacterial properties (15,16,17). We examined eight distinct Ashwagandha extracts using seven species of medically significant illnesses to add to the knowledge gained from past research (Methicillin resistant S. aureus, P. aeruginosa, Klebsiella, Salmonella, Enterococcus, E coli, and Candida)

Figure No 1: Ashwagandha

- Botanical Name : Withania Somnifera
- Family Name : Solanaceae
- Common Name : Withania, Indian Winter, Indian Ginseng.
- Wildlife: Grown in India's drier regions
- Goods available: Root

The plant is an upright branching low-lying shrub that grows in desert and subtropical areas and grows to around 1.50 m in height. It can only be found in arid subtropical environments. Madhya Pradesh, Gujarat, Maharashtra, Rajasthan, Punjab, Haryana, and Uttar Pradesh are the key Ashwagandha-producing states in the country. In Madhya Pradesh alone, it is grown on over 5000 hectares.Thought to be produced in India annually in excess of 1500 tonnes, although 7000 tonnes are needed, increasing cultivation and production is necessary (18).

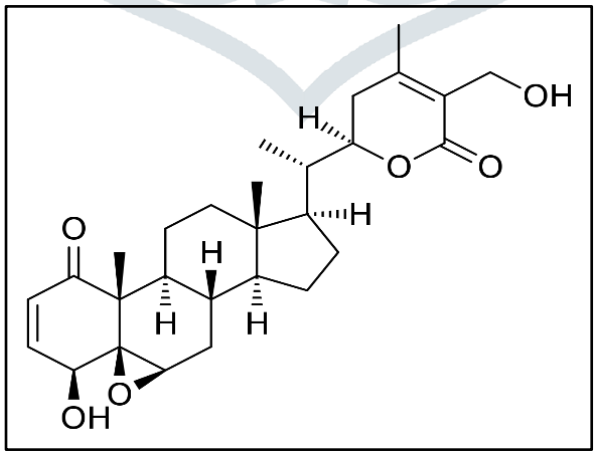

Figure No 2: Structure Of withaferin A

Chemical Constituents :

The Ashwagandha root and leaf extracts in methanol, hexane, and diethyl ether were discovered. Roots contain an alkaloid content ranging from 0.13 to 0.31%. The roots of Withania somnifera are well recognised to provide a variety of therapeutic effects, including alterative, aphrodisiac, deobstruent, diuretic, narcotic, sedative, and restorative. It is believed that the root's pharmacological activity is caused by alkaloids and steroidal lactones (18).

Even while certain areas have reported noticeably larger yields (up to 4.3%), it has been shown that Indian varieties' roots contain a total alkaloid content that varies between 0.13 and 0.3. The chemically heterogeneous alkaloids include isopelletierine, choline, tropanol, pseudotopanol, cuscokygrene, 3-tigioyloxytropana, and many more steroidal lactons. Research on the plant's roots has isolated 35 withanolides, 12 alkaloids, and seven sitoindosides (18).

Withanolide, a physiologically active component with a glucose molecule at carbon 27, is referred to as a sitoindoside. Withaferin A and withanolide D are the two primary withanolides thought to be responsible for Indian ginseng's pharmacological effects. According to reports, leaves contain withaferin-A, a withanolide that is therapeutically effective. The roots are also said to include starch, reducing sugars, glycosides, dulcitol, withancil, an acid, and a neutral substance in addition to alkaloids. Aspartic acid, glycine, tyrosine, alanine, glutamic acid, and cysteine are among the amino acids reported from the roots (18).

Description :

A small shrub (35-75 cm), it has a central stem from which branches radiate outward in the shape of stars (stellate), and it is covered in a dense matting of woolly hairs (tomentose). The fruit is mature when it is orangered and contains milk-coagulating characteristics, while the blooms are tiny and green. The plant's long, brown tuberous roots are utilised in medicine (18).

Figure No 3 : Ashwagandha's Health Benefits

Materials and Methods

- ***Plant materials collection***

Under the advice of the herbal garden's management (a botanist), Bandaranaike Memorial Ayurvedic Research Institute (BMARI), Colombo, Sri Lanka The roots of W. somnifera that are sold commercially were purchased in Colombo from a reputable retailer of Ayurvedic goods (19).

- ***Plant extracts can be prepared***

The entire plant was stripped of its leaves, stem, and roots before being cut into small pieces. The plant material was dried at 40 C for a week in order to maintain a constant weight. The extraction was conducted separately using two distinct solvents (methanol and EtOAc: water 1:1 v/v); the volume of the added solvent was five times the weight of the dried plant material. The plant material was divided into two halves, one of which was submerged in methanol and the other in an EtOAc-water mixture. Using a mechanical shaker, the solvents and plant materials were mixed for 48 hours at room temperature (32 C). The plant components' weights and the volume of the solvents employed in the extraction (19).

- ***Agar well technique***

To test the extracts' antibacterial activity, the agar well method was used. Each of the extracts was placed into a different well on the MHA plates, which had been prepared using the 0.5 McFarland standard and contained a microbial lawn. Overnight, the MHA plates were incubated and checked for a clear area surrounding the wells (19).

- ***Dilution process using an agar plate***

The agar plate dilution procedure was performed in accordance with the British Society of Antimicrobial Chemotherapy's instructions (BSAC). Each plant extract was created in MHA plates at different dilutions ranging from 256 mg/L to 16 mg/L. After incubation the plates at 37 C for an overnight period, the bacterial inoculum (density equivalent to 0.5 McFarland-standard) was spotted as a tiny drop on the surface of the agar and checked for visible growth (19).

- ***Identification of bacteria that live in roots***

The freshly extracted plant roots were rinsed in sterile water before being grown in a Muller Hinton Agar (MHA) plate. Both gramme staining and biochemical testing were used to identify the bacteria that had grown on the plate. 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 (19).

Traditional Uses of Withania Somnifera

Withania somnifera is a prominent botanical component of geriatric tonics in Indian medical systems. This plant is thought to have powerful aphrodisiac rejuvenative and life-extending effects according to the traditional medical system Ayurveda. It is used, among other things, to treat nervous exhaustion, conditions connected to memory, insomnia, difficulties with tiredness and potency, skin concerns, and coughing. It also has general energising and regenerative properties. Both learning capacity and memory are enhanced.Traditional uses of "Ashwagandha" included promoting energy, youth, endurance, strength, and health as well as fostering the body's temporal elements and enhancing the production of necessary fluids, muscle fat, blood, lymph, and semen. Chronic weariness, weakness, dehydration, bone thinning, loose teeth, thirst, impotence, early ageing, emaciation, debility, convalescence, and muscle tension are all combated by it (20,21).

Pharmacological Activity of Ashwagandha

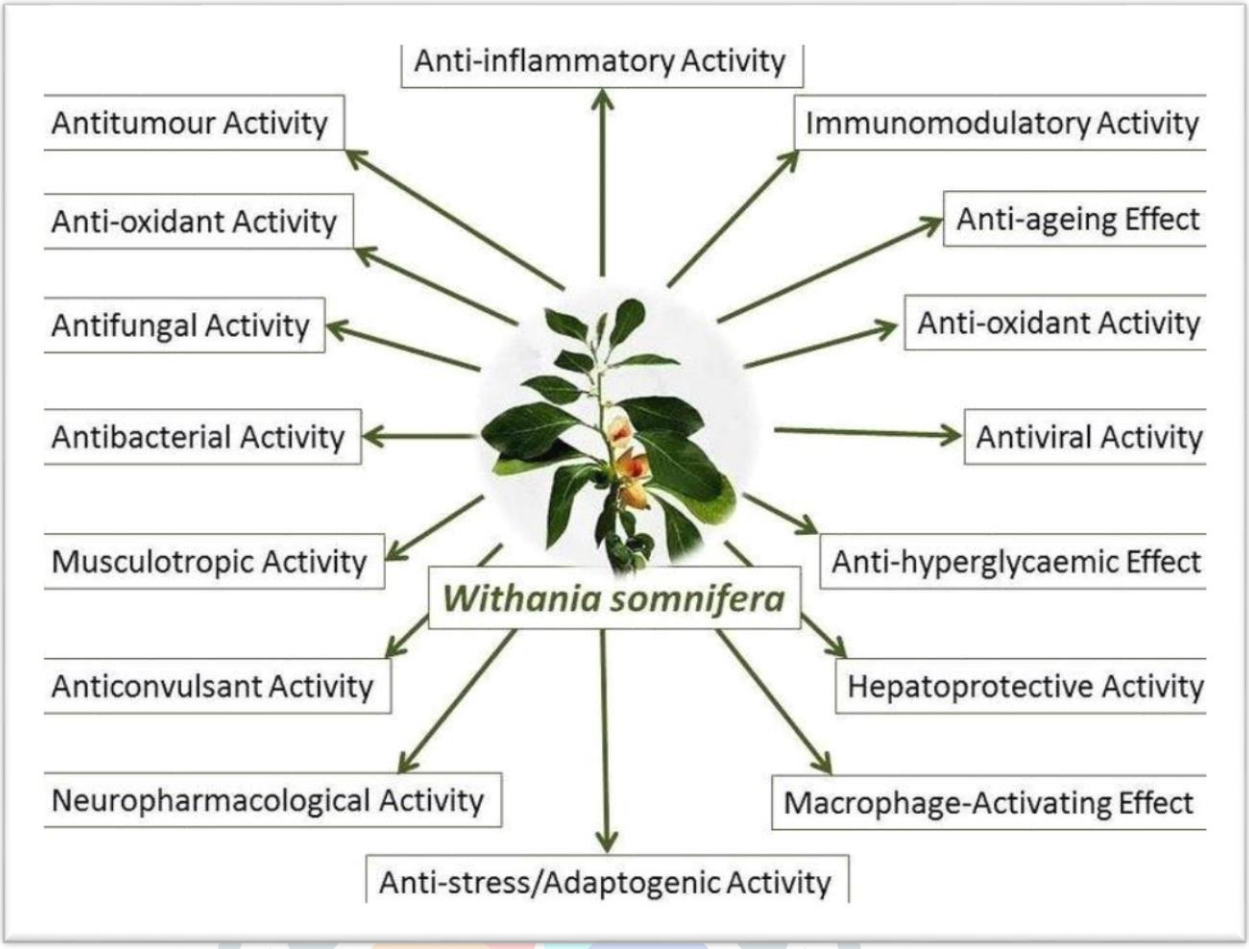

Fig No 4:Pharmacological activity of Ashwagandha

The pharmacological actions of W. somnifera are diverse. (Figure 4), including anti-inflammatory, anti-bacterial, anti-fungal, antiviral, antitumor, immunomodulatory, antistress /adaptogenic, anticonvulsant, neuropharmacological, muscular tonic, antioxidant, anti-ageing, anti-hyperglycemic, macrophage-activating, hepatoprotective, and morphine tolerance (22,23,24).

Anticancer activity

The anticancer action of this plant has been reported. This herb has been used to cure various cancer disorders for more than 2000 years. Lung, breast, prostate, pancreatic, colon, leukaemia, head, kidney, and neck cancer cells in humans can be inhibited by ashwagandha's anticancer capabilities. Recently, extensive research has been done on the anticancer potential of W. somnifera and its bioactive withanolides by numerous research teams from across the world.. As a result, many diverse mechanisms, including cell differentiation induction, cytotoxicity, COX-2 inhibition, cancer chemoprevention, and the ability to inhibit quinine reductase, have been identified. The chemical components of withanolides are what give ashwagandha its biological characteristics, particularly its anticancer action (25).

Anti-inflammatory activity

Rats' paw oedema caused by carrageenin was used to test the plant Withania somnifera's anti-inflammatory properties. Through antagonistic suppression of histamine, 5-hydroxytryptamine, and prostaglandins, the sequential involvement of inflammatory mediators is hypothesised. The resistance of 5-HT, histamine, and prostaglandins (0–2 hours) in the early phase and (0–4 hours) in the late phase of the inflammatory reaction in rats further emphasises the time period for the release of inflammatory mediators in the antiinflammatory activity of ashwagandha (26).

Anti-microbial activities

The anti-microbial effects of a hydro-alcoholic root extract from Withania somnifera were also examined. Antibacterial activity was tested on the test species S. aurens and E. coli. The research suggests that Withania Somnifera extract has significant anti-inflammatory, powerful antioxidant, and significant anti-microbial action against E. coli and S. aureus (27).

Antidiabetic activity

The diuretic, hypoglycemic, and hypocholesterolemic properties of W. somnifera's roots were examined in human test subjects. Significant increases in sodium, triglycerides, serum cholesterol, low density lipoproteins, and very low density lipoproteins were also observed, along with a striking decrease in cholesterol, suggesting that W. somnifera's root may be a source of diuretic, hypoglycemic, and hypocholestrolemic agents (28).

Antioxident activity

In-depth research is being done on the W. somnifera root powder's antioxidant and hypocholesteremic effects in male albino rats. Plasma levels of cholesterol, lipids, and triglycerides in hypocholesteremic animals showed a considerable drop when W. somnifera root powder was added to their diet. Additionally, lipid-peroxidation was significantly lower in these hypocholesteremic animals compared to their normal counterparts. However, the liver bile acid concentration, plasma HDL cholesterol levels, and HMG-CoA reductase activity were all significantly higher in these animals (29).

Diuretic activity

After being detoxified with chloroform and defatted with petroleum, albino rats were given an aqueous excretion of W. somnifera leaves to test for diuretic efficacy. The medication frusemide was used as usual. As a result, W. somnifera showed diuretic effect (perhaps as a result of the presence of polar chemicals in it) (30).

Antianxiety activity

The ability of W. somnifera to prevent sleep disorders in mice is investigated. Pretreatment of mice with W. somnifera root extract (100.200 mg/kg) and diazepam (0.5 mg/kg) significantly secured weight loss, improved mobility, and decreased anxiety levels. The biochemical analyses also demonstrate a significant reduction in glutathione levels associated with lipid peroxidation and an increase in catalase activity. According to preliminary findings, W. somnifera root extract can be used to control oxidative stress and sleep loss(31).

Hepatoprotective activity

The effects of W. were studied by Harikrishnan et al. somnifera root powder on urea, circulatory ammonia, lipid preoxidation products, and liver marker enzymes for its hepatoprotective effect in ammonium chloride-induced hyperammonemia (2008). The administration of ammonium chloride to experimental rats reveals a notable rise in the amounts of urea, transaminase, circulatory ammonia, asparatate, transaminase, alkaline phosphatase, alanine, thiobarbituric acid, and hydroperoxides. W. somnifera improves hepatoprotection by affecting the levels of lipid preoxidation in association with withanolides, alkaloids, flavonoids, etc. These alterations were dramatically reduced in rats given W. somnifera root powder and ammonium chloride therapy (32).

Anticonvulsant activity

When combined with exogenous GABA or diazepam or used alone, W. somnifera root extract reduced the threshold for pentylenetetrazol-induced seizures in mice, according to research by Kulkami SK et al (2008). The results supported the idea that the anticonvulsant properties of W. somnifera were due in part to GABA ergic regulation (33).

Cardioprotective activity

Mohanty IR et al. described W. somnifera's effects on ischemia and reperfusion (IR) injury in Wistar rats (2009). The post-ischemic reperfusion injury in the IR control group as compared to the sham group included significant apoptosis, necrosis, loss in antioxidant status, and rise in lipid preoxidation. With W. somnifera prior-treatment, the myocardial oxidant-antioxidant equilibrium was recovered. The cardioprotective effects of ashwagandha were aided by its antioxidant, anti-inflammatory, and anti-apoptotic capabilities (34).

Antifertility activity

An investigation of how stress affects male fertility and the efficacy of W. somnifera to treat stress-related male infertility. giving test subjects 5 g/day of root powder during a three-month period. The findings showed that a significant proportion of people benefit from W. somnifera in terms of reduced stress, increased antioxidant levels, and enhanced overall quality of semen. This treatment led to pregnancies in 14% of the patients' partners (35).

Antiepileptic activity

W.somnifera has historically been used to treat seizures and epilepsy. Preclinical in vitro and in vivo investigations have given enough support for the use of W. somnifera against different forms of epilepsy. Generally speaking, research using mice as the models demonstrates that W. somnifera is a bioactive

withanolide that can reduce seizures when combined with other medications The brain's seizure threshold is increased by combining a sub-effective dose of withania somnifera (50 mg/kg) with a sub-protective dose of either GABA (25 mg/kg) or diazepam (0.5 mg/kg). This is thought to be a result of the GABAA receptor modulation process.(33).

Anti-aging activity

A doubleblind clinical research was conducted to evaluate Ashwagandha's anti-aging abilities. Three grammes of the herb were administered daily for an entire year to 101 healthy guys between the ages of 50 and 59. Hemoglobin, the number of red blood cells, seated height, and hair melanin all exhibited considerable improvements. The serum cholesterol was reduced, and the calcium in the nails was conserved. Seventy percent of the study participants reported improved sexual performance (36).

Hypothyroid activity

Ashwagandha may have an impact on thyroid function, according to animal studies. For 20 days, mice received an aqueous extract of dried withania root every day. The plant appears to have a vitalizing impact at the glandular level as evidenced by the significant rise in serum T4 that was observed. Withania somnifera may, indirectly, modulate thyroid activity through its impact on cellular antioxidant mechanisms. Results showed that "Ashwagandha may be a beneficial herb in treating hypothyroidism."(37,38)

Antiparkisonian properties

A neurodegenerative disorder called Parkinson's disease is characterised by a specific loss of dopamine (DA) neurons in the substantia nigrapars compacta. Parkinson's disease treatment possibilities have been tested in animals using neuroleptic-induced catalepsy as an animal model.. By giving mice haloperidol or reserpine, large amounts of catalepsy have been generated. The considerable inhibition of reserpine- or haloperidol-induced catalepsy by WS offers hope for the treatment of Parkinson's disease (39) .

Antibacterial activity of Withania somnifera

In order to test the pure components of W. somnifera's extracts for antibacterial activity, numerous bacterial species were utilised.Baylyi Acinetobacter, Chlamydophila pneumonia, Citrobacter freundii, Corynebacterium diphtheriae, Agerobacterium tumefaciens, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Aerogene, Enterobacter aerogene Escherichia coli, Enterococcus faecalis pneumonia caused by Klebsiella Lactic Methicillin Resistance The bacteria Staphylococcus aureus, Neisseria gonorrhoea, Micrococcus luteus Proteus mirabilis, Proteus solanacearum, and Proteus vulgaris are all species of Proteus. Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Raoultella, Epidermis Staphylococcus, Streptococcus pyogenes, Xanthomonas axonopodis pv. malvacearum, Yersinia enterocolitica, and a few more are among the pathogens (22,23,24).

Table 1: Antibacterial activity of Ashwagandha (*W. somnifera*)

Sr. No.	Plant part used	Solvent used for extraction	Method Used	Test microorganisms	References
1.	Le	M, H, DEE	DD	*Escherichia coli and Salmonella typhimurium*	40
2.	Ro	E	AWD	*Typhimurium of salmonella*	41
3.	Le	M	AWD	*The gonorrhoea virus*	42
4.	Le	M	DD	*Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis, and Xanthomonas axonopodis pv. malvacearum*	43
5.	Ro	M	DD	*Escherichi coli, Proteus*	44

				vulgaris, Proteus solanacearum	
6.	Ro, St, Le, Ca	C	DD	*Escherichia coli, Staph aureus and Raoultella planticola*	45
7.	Ro	M,E,BU	AWD	*A streptococcal infection*	46
8.	Le	E	AWD	*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa*	47
9.	Ro,Fr,Le	M	AWD	*E. coli, Salmonella typhi, Citrobacter freundii, Pseudomonas aeruginosa, Klebsiella pneumonia*	48
10.	Le, Fl	E, A, IP, T, H	DD	*Escherichia coli, Staphylococcus aureus, Raoultella planticola, Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter aerogens*	49
11.	Fr, Ca	B, C, W	DD	*Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter aerogens*	50
12.	Le	E	AWD	*Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumonia, Staphylococcus aureus*	51
13.	St, Ro, Le	A	DD	*Bacillus subtilis,* Methicillin Resistance *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus feacalis, Pseudomonas aeruginosa and Escherichia coli, Klebsiella pnemoniae*	52
14.	Ro	H,PE,T,B,IP, C,EA,A,E, GAA,W	DD	*Agerobacterium tumefaciens, Proteus mirabilis, and Klebsiella pnemoniae*	53

15.	Ro	EA	AWD	*Methicillin Resistance, Staphylococcus aureus A. Staphylococcus*	54
16.	Ro	E	DD	*Escherichia coli, Bacillus subtilis, Klebsiella pnuemoniae, Pseudomonas aeruginosa, Salmonella typhi*	55
17.	Le	M	AWD	*Enterococcus species, Staphylococcus aureus*	56
18.	St	EA,H, M,DW	AWD	*Bacillus cereus, Pseudomonas aeruginosa, and Serratia marcescens,*	57
19.	Ro	W	TFSD	*Escherichia coli*	58
20.	Ro	M	AWD	*Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus luteus*	59
21.	Ro	M	DD	*Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermis, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli*	60
22.	Le	E,M,A,W	AWD	*Serratia species, Bacillus cereus, etc.*	61
23.	Le	C	DD	*Bacillus thuringiensis, Pseudomonas aeruginosa,and Corynebacteriu m diphtheriae Salmonella typhi, Chlamydophila pneumoniae*	62
24.	Le	M	AWD	*Escherichia coli, Bacillus*	63

				subtilis, Staphaylocuccus aureus, Pseudomonas fluorescens	
25.	Le	M	AWD	*Lactic acid bacterial (LAB) strains*	64
26.	Ro	M, DIW, C, H, EA	DD	*Klebsiella pneumonia, Proteus mirabilis, Acinetobacter baylyi, Streptococcus pyogenes, Pseudomonas aeruginosa*	65
27.	Le	E, C+H	DD	*Escherichia coli*	66

A: Acetone, B: Benzene, BU: Butanol, C: Chloroform, DIW: Deionised Water, DEE: Diethyl Ester, DW: Distilled Water, E: Ethanol, EA: Ethyl Acetate, GAA: Glacial Acetic Acid, H: Hexane, IP: Isopropanol M: Methanol, PG: Purified Glycoprotein, PE: Petroleum Ether, W: Water, AWD: Agar Well Diffusion, DD: Disc Diffusion, PFT: Poison Food Technique, Ca: Calyx, Fr: Fruit, Le: Leaves, Fl: Flower, Ro: Root, St: Stem

Toxicity and side effects

When used in the recommended dosage range, Ashwagandha is usually thought to be secure. Large doses have been reported to cause gastrointestinal distress, diarrhoea, and vomiting (67).

Conclusion

The herb ashwagandha (W. somnifera) has a wide range of therapeutic benefits, including antibacterial action. For testing the antimicrobial effects of extracts and purified chemicals from different Ashwagandha plant sections, a variety of test microorganisms have been employed.Still, there are many scopes of the research or the identification and isolation of antimicrobial agents from Ashwagandha. The information provided in this article will provide the platform for the researchers to select plants, plant parts, solvent system, test microorganisms, method of evaluation and other related factors affecting the analysis.

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